The Role of Reactive Oxygen Species in Hormonal Responses[1]

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Reactive oxygen species (ROS) are versatile molecules mediating a variety of cellular responses in plant cells, including programmed cell death (PCD), development, gravitropism, and hormone signaling. A picture showing how ROS function in signal transduction networks has started to emerge as the result of recent studies providing genetic, cell biological, and physiological evidence describing roles for ROS in signaling (Apel and Hirt, 2004; Laloi et al., 2004; Mittler et al., 2004; Mori and Schroeder, 2004). However, further efforts are necessary to characterize the targets and molecular functions of ROS, as well as the complex interplay of ROS-generating and ROS-scavenging mechanisms. Moreover, the interactions of nitric oxide with other ROS species in hormone signaling is a subject of interest (Desikan et al., 2004; Wendehenne et al., 2004; Guo and Crawford, 2005; Bright et al., 2006). Due to limited space, in this *Update* article we focus on recent progress made in understanding the roles of ROS in hormone signaling.

AUXIN, ETHYLENE, AND ROS

ROS have been implicated as second messengers in several plant hormone responses. Joo et al. (2001) showed that ROS are asymmetrically generated in roots by gravistimulation to regions of reduced growth. A function for ROS in root curvature was reported by inhibiting cell growth, thus contributing to tropisms. Auxin also induced ROS production in roots and the auxin transport inhibitor *N*-1-naphthylphthlamic acid did not inhibit hydrogen peroxide (H_2O_2)-induced root curvature, leading to the suggestion that ROS play a role downstream of transport in auxin signaling and gravitropism (Joo et al., 2001).

A pharmacological study suggested that ethylene and ROS are required for root nodule initiation and function as positive transducers downstream of the Nod factor response in a semiaquatic legume (D'Haeze et al., 2003). Additional studies have suggested roles of ethylene in either stomatal opening or closing, depending on the plant species (Giulivo, 1986). Recently, it was reported that H_2O_2-mediated stomatal closure is completely disrupted in the loss-of-function mutant of the ethylene receptor *etr1-7*, suggesting a role for ETR1 in guard cell ROS signaling and stomatal closure (Desikan et al., 2005). Interestingly, in another recent study, ethylene was proposed to counteract stomatal closure (Tanaka et al., 2005). Abscisic acid (ABA)-induced stomatal closure was inhibited by ethylene or the hormone precursor 1-aminocyclopropane-1-carboxylic acid and by the ethylene-overproducing mutation *eto1-1* (Tanaka et al., 2005). Moreover, this ethylene-induced inhibition of stomatal closure was suppressed in two ethylene-insensitive mutants, the dominant *etr1-1* allele, and *ein3-1* (Tanaka et al., 2005). Reverse transcription-PCR analysis with guard cell-enriched epidermal strips showed that *ETR1* is expressed in guard cells (Desikan et al., 2005). ATH1 and AG oligonucleotide-based (Affymetrix) microarray analyses of guard cell-expressed genes suggest that at least the *ETR1*, *ERS1*, and *EIN4* ethylene receptor genes are expressed in guard cells, with *ERS1* apparently showing the highest expression level in these experiments (Leonhardt et al., 2004; Heggie and Gray, 2005; J. Kwak, N. Leonhardt, Y. Yang, and J. Schroeder, unpublished data). As these receptors share overlapping redundant negative regulator functions (Hua and Meyerowitz, 1998), it would be interesting to analyze stomatal responses in *etr1ein4*, *etr1ers1*, and *ers1ein4* double mutants in addition to the recessive loss-of-function *etr1-7* line (Desikan et al., 2005). The dual functions of ethylene receptors proposed in these two studies—transduction of stomatal closure and inhibition of ABA-induced stomatal closure in Arabidopsis (*Arabidopsis thaliana*)—will require further analyses (Desikan et al., 2005; Tanaka et al., 2005).

ROLES OF ROS IN GA_3 SIGNALING IN THE ALEURONE LAYER AND CELL DEATH

ROS have been shown to play a central role in PCD of barley (*Hordeum vulgare*) aleurone cells, which offer

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a well-developed system for studying cell biological and physiological functions of GA_3 responses. GA_3 initiates cell death of aleurone cells, whereas ABA inhibits cell death (Wang et al., 1996; Appleford and Lenton, 1997). H_2O_2 was shown to represent a major reactive oxygen leading to cell death in aleurone cells (Bethke and Jones, 2001). Furthermore, transcript levels and activities of ROS-scavenging enzymes, including catalase, ascorbate peroxidase, and superoxide dismutase, were significantly down-regulated in GA_3-treated aleurone cells, thus rendering these cells sensitive to oxidative damage and cell death, whereas ABA caused increases in catalase activity and *CAT2* mRNA (Fath et al., 2001). Aleurone cells are devoid of functional chloroplasts (Jones, 1969), one of the ROS sources in plant cells. In aleurone cells, mitochondria are abundant and a cyanide-insensitive electron transport pathway switches to a cyanide-sensitive one in response to GA_3 that may contribute to ROS production (Fath et al., 2002). In addition, a reduction in the number of lipid-storing oleosomes correlates with increases in the enzyme activities of the glyoxylate cycle in aleurone cells, suggesting that mitochondria and glyoxysomes are major sources of ROS in aleurone cells (Fath et al., 2002).

ROS also play an important signaling role as regulators of PCD in response to pathogens (Levine et al., 1994). Two Arabidopsis NADPH oxidase genes, AtrbohD and AtrbohF, were reported to be a major source of pathogen-induced ROS generation (Torres et al., 2002). Interestingly, atrbohD/atrbohF double mutants showed reduced cell death in response to a bacterial pathogen, but enhanced cell death in response to a fungal pathogen. These opposite responses may derive from interaction with salicylic acid. Torres et al. (2005) reported that ROS produced by NADPH oxidases antagonize salicylic acid and suppress cell death in cells that are more distantly located from the cells at the site of infection. The cells at the site of infection undergo hypersensitive cell death. These results indicate that ROS play dual functions in both driving and suppressing PCD in different contexts in the pathogen response.

ROS AND ABA SIGNALING

Research on ABA signal transduction has characterized cell biological and genetic mechanisms upstream and downstream of ROS production, and we therefore describe these in further detail in this article. ROS were shown to induce increases in cytosolic Ca^{2+} and stomatal closure (McAinsh et al., 1996; Lee et al., 1999). Oligogalacturonic acid and chitosan treatments caused H_2O_2 production in guard cells, resulting in stomatal closure in tomato (*Lycopersicon esculentum*) and Commelina. These responses were suppressed by EGTA, catalase, and ascorbic acid (Lee et al., 1999). ABA activation of plasma membrane Ca^{2+}-permeable channels contributes to ABA-induced cytosolic Ca^{2+} increases in guard cells of Vicia and Arabidopsis (Hamilton et al., 2000; Pei et al., 2000), together with organellar Ca^{2+} release and Ca^{2+}-independent mechanisms (Schroeder et al., 2001; Hetherington and Woodward, 2003; Fan et al., 2004). Application of H_2O_2 to guard cells activates hyperpolarization-regulated Ca^{2+}-permeable (I_{Ca}) channels and produces simultaneous cytosolic calcium elevations, and this activation is impaired in the ABA-insensitive *gca2* mutant (Pei et al., 2000). ABA regulation of I_{Ca} channels requires cytoplasmic NADPH in whole-cell recordings of Arabidopsis guard cells (Murata et al., 2001). Furthermore, ABA enhances cellular ROS levels in Arabidopsis guard cells, in *Vicia faba* guard cells, and in maize (*Zea mays*) embryos (Guan et al., 2000; Pei et al., 2000; Zhang et al., 2001c). These studies define new ABA signal transduction events.

It was reported that H_2O_2 induces cytosolic alkalization in Vicia guard cells (Zhang et al., 2001a), whereas in another study, cytoplasmic alkalization preceded ROS production during stomatal responses to ABA and methyl jasmonate in Arabidopsis (Suhita et al., 2004). In *V. faba* guard cells, initial ABA-induced ROS increases were observed within 30 s of ABA application (Zhang et al., 2001c). The findings of cytosolic pH changes both before and after ROS may reflect the parallel and branched nature of the ABA-signaling network (Leung and Giraudat, 1998; Schroeder et al., 2001; Hetherington and Woodward, 2003; Fan et al., 2004). Nevertheless, further research into cellular ROS homeostasis and the timing of ROS production would be of interest and may require the development of time-resolved ratiometric reporters that show a high sensitivity to ROS, as have been developed for other second messengers (Allen et al., 1999; Zacharias et al., 2000; Zhang et al., 2002).

Ca^{2+}-permeable channels are activated by hyperpolarization in guard cells, root hair cells, Fucus rhizoids, root epidermal cells, and mesophyll cells (Gelli and Blumwald, 1997; Hamilton et al., 2000; Kiegle et al., 2000; Pei et al., 2000; Véry and Davies, 2000; Coelho et al., 2002; Demidchik et al., 2003; Foreman et al., 2003; Stoelzle et al., 2003). ROS regulation of these channels has been found in several systems (Pei et al., 2000; Coelho et al., 2002; Demidchik et al., 2003; Foreman et al., 2003; for review, see Mori and Schroeder, 2004). An open question is whether ROS directly regulate Ca^{2+}-permeable channels and/or upstream modulators (Mori and Schroeder, 2004). In vitro biochemical studies revealed that H_2O_2 directly inactivates the ABI1 and ABI2 type 2C protein phosphatase (PP2C) enzymes that function in ABA signaling (Meinhard and Grill, 2001; Meinhard et al., 2002). *ABI1* and *ABI2* function as negative regulators of ABA signaling (Merlot et al., 2001; Yoshida et al., 2006a). Knockout mutants for two other PP2Cs, *PP2C-HAB* and *PP2CA*, show strong hypersensitivity to ABA, showing that *PP2C-HAB* and *PP2CA* also negatively regulate ABA signaling (Leonhardt et al., 2004; Saez et al., 2004; Kuhn et al., 2006; Yoshida et al., 2006b). Thus, all four of these PP2Cs may be prime candidates as targets for ROS in mediating ABA responses.

ABA activation of Ca^{2+}-permeable channels is disrupted in the two dominant PP2C mutants, *abi1-1* and *abi2-1* (Murata et al., 2001). ABA-induced ROS production and ABA activation of I_{Ca} channels is impaired in the *abi1-1* mutant. The dominant *abi2-1* mutation causes disruption of both H_2O_2 activation of Ca^{2+}-permeable channels and H_2O_2-induced stomatal closing (Murata et al., 2001; Fig. 1). The ABA-activated protein kinase *OST1* was identified to function upstream of ROS production in ABA signal transduction because the *ost1* mutation disrupted ABA-induced ROS generation in guard cells (Mustilli et al., 2002; Fig. 1). OST1 is the Arabidopsis homolog of the Vicia ABA-activated protein kinase, AAPK (Li et al., 2000; Mustilli et al., 2002). Consistent with the disruption of ABA-induced ROS, increases in *abi1-1* and *ost1*, but not *abi2-1* (Murata et al., 2001; Mustilli et al., 2002), a recent study revealed that the ABI1 protein interacts with OST1 and the *abi1-1* mutation, but not *abi2-1* mutation, disrupts ABA activation of the OST1 protein kinase activity (Yoshida et al., 2006a). These data and the relatively strong ABA insensitivity of *abi1-1* and *ost1* mutants indicate that abi1-1 and OST1 may function upstream in the ABA-signaling network before the proposed branch points (Fig. 1).

An electrophysiological study provides evidence for a protein kinase-dependent and ROS-independent activation of I_{Ca} channels in *V. faba* guard cells (Fig. 1), showing that the PP1/PP2A protein phosphatase inhibitors, okadaic acid and calyculin A, enhanced I_{Ca} channel activity (Köhler and Blatt, 2002). In addition, phospholipids have been implicated in ABA-triggered cytosolic Ca^{2+} increases and stomatal closure (Staxen et al., 1999; Lemtiri-Chlieh et al., 2000). Treatment of epidermal strips with the phosphatidylinositol (PI)-3-kinase and PI-4-kinase inhibitors wortmannin and LY294002 inhibited PI kinase enzyme activities, enhanced stomatal opening, and impaired ABA-induced cytosolic Ca^{2+} increases, indicating that PI-3-P and PI-4-P modulate ABA signaling in guard cells (Jung et al., 2002). Furthermore, wortmannin and LY294002 inhibited ABA-triggered ROS production and stomatal closure, suggesting that PI-3-P (or products thereof) may function in the ABA-ROS-signaling branch in guard cells (Park et al., 2003).

Interestingly, outward K^+ channels are inhibited by H_2O_2, which could cause inhibition of ABA and/or ROS-induced stomatal closure (Torsethaugen et al., 1999; Zhang et al., 2001b; Köhler et al., 2003). Similarly, ozone causes secondary ROS production in guard cells and inhibits outward and inward K^+ channels (Torsethaugen et al., 1999; Joo et al., 2005). Ozone inhibits stomatal opening, but does not inhibit stomatal closing (Torsethaugen et al., 1999). The residual outward K^+ currents after ROS application may be sufficient to function in K^+ efflux during stomatal closing, as previous analyses have shown that roughly 25% of K^+ channel activities are sufficient for physiological K^+ fluxes during stomatal movements (Schroeder et al., 1984; Kelly et al., 1995; Kwak et al., 2001). In addition to inhibiting outward K^+ channels, H_2O_2 and ozone inhibited inward K^+ channels in guard cells, which correlates with ABA maintenance of stomatal closure (Torsethaugen et al., 1999; Zhang et al., 2001b; Köhler et al., 2003). Nevertheless, the dual down-regulation of both outward and inward K^+ channels by ROS either implicates localized oxidative bursts in guard cells or reversible regulation of ROS-dependent protein modifications during signaling.

NADPH OXIDASES AND CELL CALCIUM SIGNALING

Plant cells have a diverse array of enzymatic mechanisms responsible for ROS production (Apel and

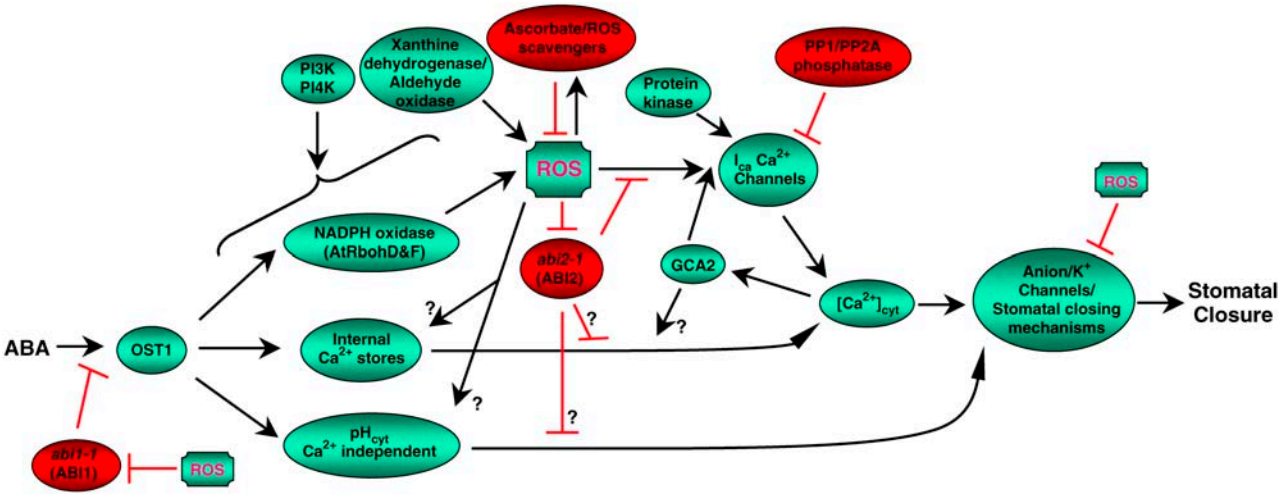

Figure 1. Simplified working model showing genetic interactions and putative functions and putative locations within the ROS-mediated ABA-signaling network in guard cells. Positive and negative regulators are shaded in green and red, respectively. PI3K, Phosphatidylinositol-3 kinase; PI4K, phosphatidylinositol-4 kinase.

Hirt, 2004; Mittler et al., 2004). Among those possible cellular mechanisms, NADPH oxidases have been suggested to function in ABA signaling (Pei et al., 2000; Murata et al., 2001; Jiang and Zhang, 2002, 2003). Genetic evidence for an important source of ABA-triggered ROS production in guard cells came from the analysis of two guard cell-expressed transmembrane NADPH oxidase catalytic subunit genes, *AtrbohD* and *AtrbohF* (Kwak et al., 2003). Analyses of stomatal movement responses show that ABA-induced stomatal closure is partially impaired in two independent alleles of the *atrbohD/atrbohF* double mutant (Fig. 2). Cellular events were impaired in *atrbohD/atrbohF* double-mutant guard cells, including ABA-induced ROS increases, ABA activation of I_{Ca} channels, and ABA-induced cytosolic Ca^{2+} increases (Kwak et al., 2003; Fig. 1). Exogenous application of ROS recovered I_{Ca} channel activation and stomatal closing in *atrbohD/atrbohF* double-mutant guard cells. The partial impairment in ABA-induced stomatal closing of *atrbohD/atrbohF* double mutants is consistent with models in which parallel pathways function in the early ABA-signaling network (Leung and Giraudat, 1998; Schroeder et al., 2001; Hetherington and Woodward, 2003; Fan et al., 2004; Figs. 1 and 2). These findings on *atrbohD/atrbohF* double mutants provide molecular genetic evidence for a function of ROS as a second messenger in ABA signaling in guard cells.

The AtrbohC NADPH oxidase was demonstrated to function in root hair growth and plays an important role in mediating the tip-focused Ca^{2+} gradient in Arabidopsis root hair cells (Foreman et al., 2003). Reactive oxygen also activated I_{Ca}-like channels in root hairs and mediated Ca^{2+} influx in the Fucus rhizoid, suggesting that ROS activation of Ca^{2+}-permeable channels is a more general signaling mechanism in plants (Coelho et al., 2002; Foreman et al., 2003). In animal cells, NADPH oxidases are regulated by cytosolic components, including Rho small G proteins (for review, see Bokoch and Knaus, 2003). Recent studies identified Rho small G proteins as regulators of ROS-generating enzymes in plants too. Carol et al. (2005) demonstrated that a RhoGTPase GDP dissociation inhibitor functions upstream of ROS produced by AtRbohC NADPH oxidase during root hair growth. Dominant mutations in the small G-protein ROP2 were shown to regulate ROS generation and phosphatidic acid-induced cell death (Park et al., 2004). Moreover, Joo et al. (2005) showed that heterotrimeric G-protein α- and β-subunits are differentially involved in ozone-triggered oxidative stress responses.

Two plant enzymes, xanthine dehydrogenase and aldehyde oxidase, could provide sources for ROS production during water stress and/or ABA signaling (Yesbergenova et al., 2005). Interestingly, in contrast to their animal counterparts, xanthine dehydrogenase is capable of producing only O_2^-, whereas aldehyde oxidase produced only H_2O_2 (Yesbergenova et al., 2005). A T-DNA insertional mutation in Arabidopsis xanthine dehydrogenase 1 showed loss of O_2^--producing activity. Furthermore, transcript levels of these enzymes were up-regulated upon ABA treatment and water stress (Yesbergenova et al., 2005). Additional ROS scavengers and/or ROS-producing enzymes are likely to function in ROS-signaling networks to ensure that a transient ROS burst does not trigger events that lead to cell damage and death.

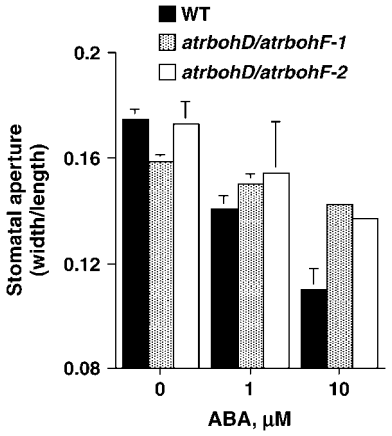

Figure 2. ABA-induced stomatal closure in two double-mutant alleles of the NADPH oxidases *atrbohD* and *atrbohF*. ABA-induced stomatal closure is partially impaired in the two independent alleles of *atrbohD/atrbohF* double mutants. *atrbohD/atrbohF-1* carries a *dSpm* transposon insertion in the fifth exon of *AtrbohD* (insertion D3) and a *dSpm* insertion in the first exon of *AtrbohF* (insertion F3; Torres et al., 2002). *atrbohD/atrbohF-2* carries a *dSpm* insertion in the second intron of *AtrbohF* (insertion F5; Torres et al., 2002) and a T-DNA insertion in the fourth intron of *AtrbohD* (Salk JP65_4B03L). Double-blind experiments were performed in which the ABA concentration and the genotype were unknown. Error bars represent SE of $n = 3$ experiments, 60 stomata per bar. Error bars are not visible when they are too small.

ROS SIGNALING REQUIRES REGULATION OF ROS-SCAVENGING MECHANISMS

The findings that oxidative bursts function in various cellular signaling responses in plants, highlighted in this issue of *Plant Physiology*, suggest that ROS-scavenging mechanisms will be important for mediating and controlling these responses (Mittler et al., 2004), as illustrated in barley aleurone cells (Fath et al., 2001). For example, a strong oxidative burst will cause cellular damage and death (Mittler, 2002; Apel and Hirt, 2004). Furthermore, constitutive ROS elevations, even if not very high, could cause malfunction or desensitization of ROS-dependent signaling responses. Several studies suggest that ROS scavenger proteins play central roles in ABA signaling. Several ROS scavenger mRNAs are regulated in response to GA_3, ABA, and oxidative stress (Desikan et al., 2001; Fath et al., 2001; Vranova et al., 2002; Vandenabeele et al., 2003).

The cellular redox state influences diverse cellular functions and enzyme activities. An antisense suppression

of catalase activity in transgenic tobacco (*Nicotiana tabacum*) plants resulted in increased ascorbate peroxidase and glutathione peroxidase levels and a 4-fold decrease in ascorbate (Willekens et al., 1997), a major H_2O_2-scavenging antioxidant in plant cells. Interestingly, transgenic tobacco plants in which both ascorbate peroxidase and catalase are suppressed were shown to be less sensitive to oxidative stress compared to single antisense plants suppressing either catalase or ascorbate peroxidase alone, suggesting that lack of H_2O_2-scavenging mechanisms might have turned on an alternative mechanism by which cells are protected (Rizhsky et al., 2002). A knockout mutation in ascorbate peroxidase 1 (*APX1*) caused the accumulation of H_2O_2 in Arabidopsis and decreases in the transcript levels of catalase and chloroplast superoxide dismutase and no significant changes in glutathione peroxidase transcript levels (Pnueli et al., 2003). Furthermore, stomates of the *apx1* knockout plants neither closed in response to darkness nor opened in response to light (Pnueli et al., 2003). APX1 was also shown to play a role in protecting the chloroplast from oxidative damage (Davletova et al., 2005). Arabidopsis plants with increased dehydroascorbate reductase, which generates ascorbate from dehydroascorbate, showed an increased ascorbate redox state and a reduction in H_2O_2 levels in guard cells. Consistent with guard cell-signaling analyses, this resulted in increased stomatal conductance and reduced sensitivity to H_2O_2 and ABA (Chen and Gallie, 2004). This result suggests that ABA/ROS signaling in guard cells is controlled by the ascorbic acid redox state.

FUTURE PERSPECTIVES

ROS mediate diverse functions in a variety of cellular processes. Many exciting findings have revealed roles of ROS in hormonal responses in the past few years and many new questions arise. What are the downstream protein targets that are modified by ROS during signal transduction, enabling stimulus-specific cellular responses? Among those mechanisms responsible for ROS generation in plant cells, which combination of cellular mechanisms mediates ROS production for specific signaling cascades, and how do ROS producers and scavengers interact with each other to regulate cellular ROS levels? What are the roles for the other seven *Atrboh* genes whose functions are waiting to be unveiled? How is the enzyme activity of NADPH oxidases regulated in plants? Surely there is a lot more to come in this stimulating field.

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LITERATURE CITED

Allen GJ, Kwak JM, Chu SP, Llopis J, Tsien RY, Harper JF, Schroeder JI (1999) Cameleon calcium indicator reports cytoplasmic calcium dynamics in *Arabidopsis* guard cells. Plant J **19:** 735–747

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol **55:** 373–399

Appleford N, Lenton J (1997) Hormonal regulation of α-amylase gene expression in germinating wheat (*Triticum aestivum*) grains. Physiol Plant **100:** 534–542

Bethke PC, Jones RL (2001) Cell death of barley aleurone protoplasts is mediated by reactive oxygen species. Plant J **25:** 19–29

Bokoch GM, Knaus UG (2003) NADPH oxidases: not just for leukocytes anymore! Trends Biochem Sci **28:** 502–508

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis. Plant J **45:** 113–122

Carol RJ, Takeda S, Linstead P, Durrant MC, Kakesova H, Derbyshire P, Drea S, Zarsky V, Dolan L (2005) A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. Nature **438:** 1013–1016

Chen Z, Gallie DR (2004) The ascorbic acid redox state controls guard cell signaling and stomatal movement. Plant Cell **16:** 1143–1162

Coelho SM, Taylor AR, Ryan KP, Sousa-Pinto I, Brown MT, Brownlee C (2002) Spatiotemporal patterning of reactive oxygen production and Ca^{2+} wave propagation in Fucus rhizoid cells. Plant Cell **14:** 2369–2381

Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005) Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. Plant Cell **17:** 268–281

Demidchik V, Shabala SN, Coutts KB, Tester MA, Davies J (2003) Free oxygen radicals regulate plasma membrane Ca^{2+}- and K^+-permeable channels in plant root cells. J Cell Sci **116:** 81–88

Desikan R, Cheung M-K, Bright J, Henson D, Hancock JT, Neill SJ (2004) ABA, hydrogen peroxide and nitric oxide signaling in stomatal guard cells. J Exp Bot **55:** 205–212

Desikan R, Hancock JT, Bright J, Harrison J, Weir I, Hooley R, Neill SJ (2005) A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. Plant Physiol **137:** 831–834

Desikan R, A-H-Mackerness S, Hancock JT, Neill SJ (2001) Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol **127:** 159–172

D'Haeze W, De Rycke R, Mathis R, Goormachtig S, Pagnotta S, Verplancke C, Capoen W, Holsters M (2003) Reactive oxygen species and ethylene play a positive role in lateral root base nodulation of a semiaquatic legume. Proc Natl Acad Sci USA **100:** 11789–11794

Fan L-M, Zhao Z, Assmann SM (2004) Guard cells: a dynamic signaling model. Curr Opin Plant Biol **7:** 537–546

Fath A, Bethke P, Beligni V, Jones R (2002) Active oxygen and cell death in cereal aleurone cells. J Exp Bot **53:** 1273–1282

Fath A, Bethke PC, Jones RL (2001) Enzymes that scavenge reactive oxygen species are down-regulated prior to gibberellic acid-induced programmed cell death in barley aleurone. Plant Physiol **126:** 156–166

Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JDG, et al (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature **422:** 442–446

Gelli A, Blumwald E (1997) Hyperpolarization-activated Ca^{2+}-permeable channels in the plasma membrane of tomato cells. J Membr Biol **155:** 35–45

Giulivo C (1986) Hormonal control of water transport in soil-plant-atmosphere continuum. Acta Hortic **179:** 385–393

Guan LM, Zhao J, Scandalios JG (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

Guo FQ, Crawford NM (2005) Arabidopsis nitric oxide synthase1 is targeted to mitochondria and protects against oxidative damage and dark-induced senescence. Plant Cell **17:** 3436–3450

Hamilton DWA, Hills A, Kohler B, Blatt MR (2000) Ca^{2+} channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. Proc Natl Acad Sci USA **97:** 4967–4972

Heggie L, Gray J (2005) Experiment: identification of genes controlling stomatal number in response to rising atmospheric carbon dioxide

levels. http://affymetrix.arabidopsis.info/narrays/experimentpage.pl?experimentid=29

Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. Nature **424:** 901–908

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. Cell **94:** 261–271

Jiang M, Zhang J (2002) Involvement of plasma-membrane NADPH oxidase in abscisic acid- and water stress-induced antioxidant defense in leaves of maize seedlings. Planta **215:** 1022–1030

Jiang M, Zhang J (2003) Cross-talk between calcium and reactive oxygen species originated from NADPH oxidase in abscisic acid-induced antioxidant defense in leaves of maize seedlings. Plant Cell Environ **26:** 929–939

Jones RL (1969) The fine structure of barley aleurone cells. Planta **85:** 359–374

Joo JH, Bae YS, Lee JS (2001) Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiol **126:** 1055–1060

Joo JH, Wang S, Chen JG, Jones AM, Fedoroff NV (2005) Different signaling and cell death roles of heterotrimeric G protein α- and β-subunits in the Arabidopsis oxidative stress response to ozone. Plant Cell **17:** 957–970

Jung JY, Kim YW, Kwak JM, Hwang JU, Young J, Schroeder JI, Hwang I, Lee Y (2002) Phosphatidylinositol 3- and 4-phosphate are required for normal stomatal movements. Plant Cell **14:** 2399–2412

Kelly WK, Esser JE, Schroeder JI (1995) Effects of cytosolic calcium and limited, possible dual, effects of G protein modulators on guard cell inward potassium channels. Plant J **8:** 479–489

Kiegle E, Gilliham M, Haseloff J, Tester M (2000) Hyperpolarisation-activated calcium currents found only in cells from the elongation zone of *Arabidopsis thaliana* roots. Plant J **21:** 225–229

Köhler B, Blatt MR (2002) Protein phosphorylation activates the guard cell Ca^{2+} channel and is a prerequisite for gating by abscisic acid. Plant J **32:** 185–194

Köhler B, Hills A, Blatt MR (2003) Control of guard cell ion channels by hydrogen peroxide and abscisic acid indicates their action through alternate signaling pathways. Plant Physiol **131:** 385–388

Kuhn JM, Boisson-Dernier A, Dizon MB, Maktabi MH, Schroeder JI (2006) The protein phosphatase AtPP2CA negatively regulates abscisic acid signal transduction in Arabidopsis, and effects of *abh1* on *AtPP2CA* mRNA. Plant Physiol **140:** 127–139

Kwak JM, Mori I, Pei Z-M, Leonhardt N, Torres MA, Dangl JL, Bloom R, Bodde S, Jones JDG, Schroeder JI (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis.* EMBO J **22:** 2623–2633

Kwak JM, Murata Y, Baizabal-Aguirre VM, Merrill J, Wang M, Kemper A, Hawke SD, Tallman G, Schroeder JI (2001) Dominant negative guard cell K^+ channel mutants reduce inward-rectifying K^+ currents and light-induced stomatal opening in Arabidopsis. Plant Physiol **127:** 473–485

Laloi C, Apel K, Danon A (2004) Reactive oxygen signalling: the latest news. Curr Opin Plant Biol **7:** 323–328

Lee S, Choi H, Suh S, Doo I-S, Oh K-Y, Choi EJ, Schroeder Taylor AT, Low PS, Lee Y (1999) Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and *Commelina communis.* Plant Physiol **121:** 147–152

Lemtiri-Chlieh F, MacRobbie EAC, Brealey CA (2000) Inositol hexakisphosphate is a physiological signal regulating the K^+-inward rectifying conductance in guard cells. Proc Natl Acad Sci USA **97:** 8687–8692

Leonhardt N, Kwak JM, Robert N, Waner D, Leonhardt G, Schroeder JI (2004) Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell **16:** 596–615

Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol **49:** 199–222

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

Li J, Wang X-Q, Watson MB, Assmann SM (2000) Regulation of abscisic acid-induced stomatal closure and anion channels by guard cell AAPK kinase. Science **287:** 300–303

McAinsh MR, Clayton H, Mansfield TA, Hetherington AM (1996) Changes in stomatal behavior and guard cell cytosolic free calcium in response to oxidative stress. Plant Physiol **111:** 1031–1042

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

Meinhard M, Rodriguez PL, Grill E (2002) The sensitivity of ABI2 to hydrogen peroxide links the abscisic acid-response regulator to redox signalling. Planta **214:** 775–782

Merlot S, Gosti F, Guerrier D, Vavasseur A, Giraudat J (2001) The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. Plant J **25:** 295–303

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

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci **9:** 490–498

Mori IC, Schroeder JI (2004) Reactive oxygen species activation of plant Ca^{2+} channels: a signaling mechanism in polar growth, hormone transduction, stress signaling, and hypothetically mechanotransduction. Plant Physiol **135:** 702–708

Murata Y, Pei Z-M, Mori IC, Schroeder JI (2001) Abscisic acid activation of plasma membrane Ca^{2+} channels in guard cells requires 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

Mustilli A-C, Merlot S, Vavasseur A, Fenzi F, Giraudat J (2002) Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. Plant Cell **14:** 3089–3099

Park J, Gu Y, Lee Y, Yang Z (2004) Phosphatidic acid induces leaf cell death in Arabidopsis by activating the Rho-related small G protein GTPase-mediated pathway of reactive oxygen species generation. Plant Physiol **134:** 129–136

Park KY, Jung JY, Park J, Hwang JU, Kim YW, Hwang I, Lee Y (2003) A role for phosphatidylinositol 3-phosphate in abscisic acid-induced reactive oxygen species generation in guard cells. Plant Physiol **132:** 92–98

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

Pnueli L, Liang H, Rozenberg M, Mittler R (2003) Growth suppression, altered stomatal responses, and augmented induction of heat shock proteins in cytosolic ascorbate peroxidase (Apx1)-deficient Arabidopsis plants. Plant J **34:** 187–203

Rizhsky L, Hallak-Herr E, Van Breusegem F, Rachmilevitch S, Barr JE, Rodermel S, Inze D, Mittler R (2002) Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J **32:** 329–342

Saez A, Apostolova N, Gonzalez-Guzman M, Gonzalez-Garcia MP, Nicolas C, Lorenzo O, Rodriguez PL (2004) Gain-of-function and loss-of-function phenotypes of the protein phosphatase 2C HAB1 reveal its role as a negative regulator of abscisic acid signalling. Plant J **37:** 354–369

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D (2001) Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol **52:** 627–658

Schroeder JI, Hedrich R, Fernandez JM (1984) Potassium-selective single channels in guard cell protoplasts of *Vicia faba.* Nature **312:** 361–362

Staxen I, Pical C, Montgomery LT, Gray JE, Hetherington AM, McAinsh MR (1999) Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. Proc Natl Acad Sci USA **96:** 1779–1784

Stoelzle S, Kagawa T, Wada M, Hedrich R, Dietrich P (2003) Blue light activates calcium-permeable channels in Arabidopsis mesophyll cells via the phototropin signaling pathway. Proc Natl Acad Sci USA **100:** 1456–1461

Suhita D, Raghavendra AS, Kwak JM, Vavasseur A (2004) Cytoplasmic alkalization precedes reactive oxygen species production during methyl jasmonate- and abscisic acid-induced stomatal closure. Plant Physiol **134:** 1536–1545

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S (2005) Ethylene inhibits abscisic acid-induced stomatal closure in Arabidopsis. Plant Physiol **138:** 2337–2343

Torres MA, Dangl JL, Jones JDG (2002) *Arabidopsis* $gp91^{phox}$ homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci USA **99:** 517–522

Torres MA, Jones JD, Dangl JL (2005) Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in Arabidopsis thaliana. Nat Genet **37:** 1130–1134

Torsethaugen G, Pell EJ, Assmann SM (1999) Ozone inhibits guard cell K^+ channels implicated in stomatal opening. Proc Natl Acad Sci USA **96:** 13577–13582

Vandenabeele S, Van Der Kelen K, Dat J, Gadjev I, Boonefaes T, Morsa S, Rottiers P, Slooten L, Van Montagu M, Zabeau M, et al (2003) A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. Proc Natl Acad Sci USA **100:** 16113–16118

Véry A-A, Davies JM (2000) Hyperpolarization-activated calcium channels at the tip of *Arabidopsis* root hairs. Proc Natl Acad Sci USA **97:** 9801–9806

Vranova E, Atichartpongkul S, Villarroel R, Van Montagu M, Inze D, Van Camp W (2002) Comprehensive analysis of gene expression in Nicotiana tabacum leaves acclimated to oxidative stress. Proc Natl Acad Sci USA **99:** 10870–10875

Wang M, Oppedijk BJ, Lu X, Van Duijn B, Schilperoort RA (1996) Apoptosis in barley aleurone during germination and its inhibition by abscisic acid. Plant Mol Biol **32:** 1125–1134

Wendehenne D, Durner J, Klessig DF (2004) Nitric oxide: a new player in plant signalling and defence responses. Curr Opin Plant Biol **7:** 449–455

Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, Van Montagu M, Inze D, Van Camp W (1997) Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. EMBO J **16:** 4806–4816

Yesbergenova Z, Yang G, Oron E, Soffer D, Fluhr R, Sagi M (2005) The plant Mo-hydroxylases aldehyde oxidase and xanthine dehydrogenase have distinct reactive oxygen species signatures and are induced by drought and abscisic acid. Plant J **42:** 862–876

Yoshida R, Umezawa T, Mizoguchi T, Takahashi S, Takahashi F, Shinozaki K (2006a) The regulatory domain of SRK2E/OST1/SNRK2.6 interacts with ABI1 and integrates ABA and osmotic stress signals controlling stomatal closure in *Arabidopsis.* J Biol Chem **281:** 5310–5318

Yoshida T, Nishimura N, Kitahata N, Kuromori T, Ito T, Asami T, Shinozaki K, Hirayama T (2006b) ABA-hypersensitive germination3 encodes a protein phosphatase 2C (*AtPP2CA*) that strongly regulates abscisic acid signaling during germination among Arabidopsis protein phosphatase 2Cs. Plant Physiol **140:** 115–126

Zacharias DA, Baird GS, Tsien RY (2000) Recent advances in technology for measuring and manipulating cell signals. Curr Opin Neurobiol **10:** 416–421

Zhang J, Campbell RE, Ting AY, Tsien RY (2002) Creating new fluorescent probes for cell biology. Nat Rev Mol Cell Biol **3:** 906–918

Zhang X, Dong FC, Gao JF, Song CP (2001a) Hydrogen peroxide-induced changes in intracellular pH of guard cells precede stomatal closure. Cell Res **11:** 37–43

Zhang X, Miao YC, An GY, Zhou Y, Shangguan ZP, Gao JF, Song CP (2001b) K^+ channels inhibited by hydrogen peroxide mediate abscisic acid signaling in Vicia guard cells. Cell Res **11:** 195–202

Zhang X, Zhang L, Dong F, Gao J, Galbraith DW, Song C-P (2001c) Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba.* Plant Physiol **126:** 1438–1448