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REVIEW

Reactive oxygen signaling and abiotic stress

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Reactive oxygen species (ROS) play a dual role in plant biology acting on the one hand as important signal transduction molecules and on the other as toxic by-products of aerobic metabolism that accumulate in cells during different stress conditions. Because of their toxicity as well as their important signaling role, the level of ROS in cells is tightly controlled by a vast network of genes termed the 'ROS gene network'. Using mutants deficient in key ROS-scavenging enzymes, we have defined a signaling pathway that is activated in cells in response to ROS accumulation. Interestingly, many of the key players in this pathway, including different zinc finger proteins and WRKY transcription factors, are also central regulators of abiotic stress responses involved in temperature, salinity and osmotic stresses. Here, we describe our recent findings and discuss how ROS integrate different signals originating from different cellular compartments during abiotic stress.

Introduction

The deleterious effects of abiotic stresses on agricultural production in the United States are estimated at billions of dollar annually (Mittler 2006). Most types of abiotic stresses such as drought, salinity, flooding, heat and cold stresses disrupt the metabolic balance of cells, resulting in enhanced production of reactive oxygen species (ROS) (Mittler 2002). The accumulation of ROS, such as $^1O_2$, $O_2^{\bullet -}$, H_2O_2 and $HO^{\bullet}$, during abiotic stresses was long considered to be a by-product of stress metabolism as well as an overall unwelcome by-product of aerobic metabolism. Over the course of evolution, however, plants have developed an elaborate and efficient network of scavenging mechanisms that allowed them to overcome ROS toxicity and begun using some of these toxic molecules such as signal transduction mediators (Bailey-Serres and Mittler 2006, Mittler et al. 2004). In recent years, it became clear that ROS play a dual role in plants as both toxic compounds as well as key regulators of many biological processes such as growth, cell cycle, programmed cell death, hormone signaling, biotic and abiotic cell responses and development (Foyer and Noctor 2005, Fujita et al. 2006, Mittler et al. 2004).

The delicate balance between ROS production and scavenging that allows this duality in function to exist in plants is thought to be orchestrated by a large network of genes termed the 'ROS gene network', which includes more than 152 genes in Arabidopsis, tightly regulating ROS production and scavenging (Mittler et al. 2004). ROS signaling is therefore predominantly controlled by production and scavenging (Mittler et al. 2004). Organelles with a highly oxidizing metabolic activity or with an intense rate of electron flow, such as chloroplasts, mitochondria or microbodies, are a major source of ROS production in plant cells, and chloroplast and peroxisomes are thought to be the two major contributors to the oxidative load in plant cells during abiotic stress (Mittler et al. 2004). Thus, production of ROS by the Mehler

Abbreviations – APX, ascorbate peroxidase; AOX, alternative oxidase; CAT, catalase; CSD2, CuZn-superoxide dismutase 2; FSD1, iron superoxide dismutase 1; Hsf, heat shock transcription factor; MBF, multiprotein bridging factor; Rboh, respiratory burst oxidative homolog; RNAi, RNA interference; ROS, reactive oxygen species; SA, salicylic acid; tAPX, thylakoid APX.

reaction in chloroplasts, the glycolate oxidase reaction in peroxisomes and the electron transfer chain in mitochondria is enhanced by conditions limiting CO_2 fixation, such as drought, salt, heat and cold stress as well as the combination of these conditions with high light (Foyer and Noctor 2003, Mittler 2002, Moller 2001, Noctor et al. 2002).

In addition to the different proteins and enzymes that detoxify ROS, antioxidants such as ascorbate, glutathione and tocopherol play an important role in the regulation of the cellular ROS homeostasis. These antioxidants act as redox buffers and can influence gene expression associated with abiotic and biotic stresses (Foyer and Noctor 2005).

Many studies employing mutants and antisense lines for catalase (CAT) 1 and 2, ascorbate peroxidase (APX) 1, thylakoid APX (tAPX), mitochondrial alternative oxidase (AOX), CuZn-superoxide dismutase 2 (CSD2), 2-cysteine peroxiredoxin and various NADPH oxidases [respiratory burst oxidative homolog (Rboh)] have revealed a strong link between ROS and processes such as growth, development, stomatal responses and biotic and abiotic stress responses (Baier et al. 2000, Miller et al. 2007, Pnueli et al. 2003, Rizhsky et al. 2002, 2003, Torres and Dangl 2005, Umbach et al. 2005, Vanderauwera et al. 2005). The function of ROS, such as $^1O_2$, $O_2^{\cdot-}$ and H_2O_2, as signaling molecules involved in the expression of a large number of genes was studied using microarray experiments conducted on loss-of-function mutants that lack important ROS-scavenging enzymes (Davletova et al. 2005a, Lee et al. 2007, Pnueli et al. 2003, Rizhsky et al. 2003, Vandenabeele et al. 2004, Vanderauwera et al. 2005). Plants constantly sense and assess the level of ROS and reprogram their gene expression to optimally respond and acclimate to the changing conditions in their environment. The use of mutants that lack important ROS-scavenging enzymes such as APX1 and CAT enabled therefore the identification of key regulatory proteins involved in the sensing of ROS and the regulation of transcript expression by different ROS signals (Davletova et al. 2005a, Vandenabeele et al. 2004, Vanderauwera et al. 2005). The identification of ROS-generating enzymes such as the plant homolog of respiratory burst NADPH oxidases (Rboh) demonstrated that plant cells can initiate and most likely amplify ROS production for the purpose of signaling (Bailey-Serres and Mittler 2006). Furthermore, at least three different mechanisms were proposed for ROS sensing in plant cells: (1) yet unidentified receptor proteins, (2) redox-sensitive transcription factors such as heat shock transcription factors (Hsfs) and (iii) direct inhibition of phosphatases by ROS (Apel and Hirt 2004, Miller and Mittler, 2006, Mittler 2002, Mittler et al. 2004, Pieterse and Van Loon, 2004). This review aims at bringing some of the new developments in ROS signaling in response to abiotic stress conditions.

A model for ROS signaling developed from the study of plants lacking Apx1

In the past several years, we have been focusing our efforts on dissecting the response of plants that lack APX1 to light and oxidative stress (Fig. 1). Using a time-course microarray analysis we have identified several key regulators involved in ROS signaling in plants that lack APX1 and using different mutants we studied several of these proteins independently assessing their role in ROS signaling and responses to abiotic stress conditions (Ciftci-Yilmaz et al. 2007, Davletova et al. 2005a, 2005b, Mittler et al. 2006, Pnueli et al. 2003, Rizhsky et al. 2002, 2004a, Suzuki et al. 2005, 2008). The putative model presented in Fig. 1 suggests that ROS that accumulate in the cytosol could be sensed by different redox-response transcription factors such as HsfA4a and that these sensors function upstream to a cascade of different transcription factors that include members of the zinc finger protein Zat family (Zat12, 10 and 7) and members of the WRKY transcription factor family. Additional players in this pathway could include multiprotein bridging factor 1c (MBF1c) that is a transcriptional coactivator and RbohD that is likely to be involved in amplifying the ROS signal (Ciftci-Yilmaz et al. 2007, Davletova et al. 2005a, 2005b, Mittler et al. 2006, Pnueli et al. 2003, Rizhsky et al. 2002, 2004, Suzuki et al. 2005, 2008). The effectors of this pathway include antioxidative enzymes and different abiotic stress response proteins such as Lea proteins and could function in the scavenging of ROS and protection or repair of cells (Davletova et al. 2005b). Below, we discuss the different proteins identified by our analysis and compare our findings to studies from other labs focusing on the role of these proteins in ROS and abiotic stress signaling.

Heat shock transcription factors

Hsfs are modular transcription factors encoded by a large gene family in plants, having at least 21 recognized members in Arabidopsis and even more in rice and soybean (Kotak et al. 2007a). Hsfs are not only upregulated during heat stresses but respond to many other abiotic as well as biotic stresses. Transcriptional profiling of all Arabidopsis Hsfs, based on Genevestigator data, in response to several abiotic stresses, not only points to a high degree of redundancy within this family but also suggest a high degree of specificity in the function of certain Hsfs to particular stress conditions or

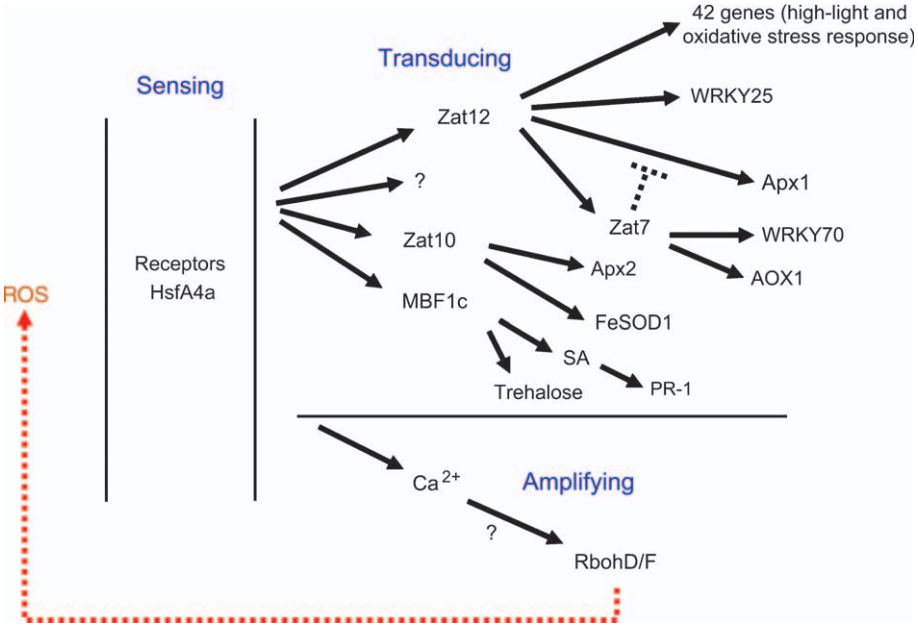

Fig. 1. A putative model showing the signal transduction pathway activated in knockout plants deficient in cytosolic ascorbate peroxidase (knockout Apx1). Three stages are proposed to exist in this signal transduction: (1) sensing of H_2O_2 by receptors and Hsfs; (2) transduction of the ROS signal by different zinc finger proteins, WRKY transcription factors and MBF1c and (3) amplification of the ROS signal by Rboh proteins. The model was constructed according to published results and data collected in our lab. Question marks (?) indicate work in progress.

developmental signals (Kotak et al. 2007b, Miller and Mittler 2006, von Koskull-Doring et al. 2007).

Several Hsfs have been shown to be involved in regulating the ROS gene network during abiotic stress conditions. HsfA1b-overexpressing plants exhibit increased levels of Apx1 and Apx2 during heat stress (Panchuk et al. 2002) and dominant negative lines for HsfA4a did not induce Apx1 during light stress (Davletova et al. 2005a). Considerable evidence supporting the possible role for plant Hsfs as direct sensors of ROS can be found in studies of mammalss, Drosophila and yeast. Recently, we discussed the possibility that oxidative stress responsive Hsfs such as HsfA2, HsfA4a and HsfA8, class A Hsfs that are capable of gene activation could act as H_2O_2 sensors (Miller and Mittler 2006), similar to the mammalian and yeast Hsfs (Kotak et al. 2007a).

Transcriptomic ROS footprinting revealed high specificity of HsfA2 and HsfA4a to oxidative conditions or to different ROS signals generated in mutants such as *csd2* (knockdown of chloroplastic CuZn-superoxide dismutase; CSD2), *apx1* (knockout of Apx1), *flu* and CAT2HP1 [RNA interference (RNAi) for CAT2; Gadjev et al. 2006 supplementary material]. The steady-state transcript level of HsfA4a and HsfA8 was greatly elevated in the knockout Apx1, which maintained increased H_2O_2 levels (Davletova et al. 2005a, Miller and Mittler 2006). Hsfs A2 and A7a are also H_2O_2 responsive genes that were upregulated in CAT RNAi Arabidopsis lines during high-light stress (Vanderauwera et al. 2005). HsfA5 is the most related Hsf to the A4 class (i.e. Arabidopsis HsfA4a, A4c and tomato HsfA4b), both share crucial similarities in structure and sequence in distinction from other members of the Hsf family (Baniwal et al. 2007, von Koskull-Doring et al. 2007). Recently, Baniwal et al. (2007) elegantly showed in reporter assays using tobacco mesophyl protoplast that HsfA5 specifically inhibits HsfA4 activity by interfering with its ability to form homotrimer. HsfA5 forms heterooligomers with HsfA4, interfering with its DNA-binding capacity, thus acting as a selective repressor regulating A4-type Hsf activity in plants. The formation of heterooligomers was shown to be preferable over formation of homooligomers in yeast two-hybrid and pull-down assays indicating the high affinity of this interaction (Baniwal et al. 2007). These findings suggested a strong repressor capability of HsfA5 over HsfA4 activity that may act in vivo as an off switch mechanism for HsfA4a-regulated genes. Normally and during stress conditions, HsfA5 mRNA has low abundance in Arabidopsis that is markedly lower than those of Hsfs A4a and A4c. There is only small increase in HsfA5 transcripts during oxidative stress, ozone treatment, salinity, heat and cold stresses, with no distinct correlation with A4 transcripts (Miller and Mittler 2006, von Koskull-Doring et al. 2007). However, keeping in mind

that it takes three monomers to create an active Hsf, even small increase in HsfA5 protein levels with its high affinity could effectively suppress HsfA4a-induced gene expression once stress is relieved. Future studies will reveal the different roles of specific Hsfs in regulating ROS and abiotic stress responses in plants. Nevertheless, the complexity of the Hsfs network would most likely require the study of double or triple mutants lacking specific Hsfs in different combinations.

Zat proteins

Transcriptional regulators play a key role in modulating defense and acclimation responses in plants (Ciftci-Yilmaz and Mittler 2008, Mittler et al. 2006). A subset of these transcriptional regulators belongs to the Zat family that contains more than 20 members in Arabidopsis. Members of this family, a Cys2/His2-type plant-specific zinc finger proteins, contain the EAR domain, that could function as a repressor (Ciftci-Yilmaz et al. 2007). Our analysis of knockout Apx1 plants identified three different members of the Zat family (Zat12, 10 and 7) as potentially involved in ROS signaling and responses to abiotic stress. Zat12 expression is transcriptionally enhanced during osmotic, drought, salinity, temperature, oxidative, high-light stress and wounding (Davletova et al. 2005b, Mittler et al. 2006). Zat12 was shown to be required for Apx1, Zat7 and WRKY25 expression during oxidative stress (Davletova et al. 2005a); however, overexpression of Zat12 did not induce Apx1 transcript level, suggesting that the relationship between Zat12 and Apx1 expression is complex (Davletova et al. 2005b). Knockout Zat12 Arabidopsis seedlings suffered of increased sensitivity to salinity, osmotic and heat stress. Conversely, Zat12 overexpressing plants showed higher tolerance to cold, oxidative and osmotic stresses (Davletova et al. 2005b, Rizhsky et al. 2004a, Vogel et al. 2005). Zat12 expression was also elevated in RNAi CAT2 lines during high light as well as during other stresses including heat, cold, drought and H_2O_2 treatments (Vanderauwera et al. 2005). Transcriptome profiling analysis of transgenic plants that overexpress Zat12 suggested that Zat12 controls a regulon of 42 different genes involved in responses to osmotic, oxidative stress and light stress (Davletova et al. 2005b; Fig. 1).

Zat10 was initially identified as a salt- and cold-response protein and was later shown to be induced in response to drought and osmotic stresses (Mittler et al. 2006, Sakamoto et al. 2004). Transgenic plants constitutively expressing Zat10 were found to be more tolerant to drought stress, osmotic stress, salinity and heat stress (Mittler et al. 2006, Sakamoto et al. 2004). However, Zat10 knockout and RNAi lines were also more tolerant to osmotic stress and salinity (Mittler et al. 2006). The overexpression as well as suppression of Zat10 conferred enhanced tolerances to salinity and osmotic stresses suggesting the possibility that Zat10 plays a dual role, as a repressor as well as an activator, in the control of plant acclimation (Mittler et al. 2006). The increased heat tolerance of Zat10 overexpressing plants but not the Zat10-deficient plants is in support of the hypothesis that different defense mechanisms were activated in gain and loss-of-function Zat10 plants. Apx1, Apx2 and iron superoxide dismutase 1 (FSD1) transcripts were constantly elevated in Zat10 transgenic plants and showed suppressed expression in knockout Zat10 plants during high-light stress. In contrast, other defense-related transcript including RD29a, RD29b, COR47 and COR15a were not altered in the Zat10 transgenic or knockout plant (Mittler et al. 2006). These findings imply that the Zat10-dependent increased tolerance to abiotic stresses could have been a result of specific activation of ROS-related defense genes. The finding that Zat12 transgenic plants did not have elevated expression of APX1, APX2 or FSD1 (Rizhsky et al. 2004a) suggested that Zat10 and Zat12 play different roles during stress (Mittler et al. 2006). Recently, it was shown that Zat12 and Zat10 have two phases of expression in response to high-light stress: an early phase that peaks between 1 min and 1 h and a later phase, which peeks between 6 and 24 h (Miller et al. 2007, Koussevitzky et al. 2007). Interestingly, the second but not the first stage required an intact chloroplast-to-nuclei retrograde signaling pathway (Koussevitzky et al. 2007).

In contrast to Zat12 and Za10 that respond to many different types of stress, the expression of Zat7 is more specific to salt stress and heat and is also elevated in knockout plants that lack APX1 (Ciftci-Yilmaz et al. 2007, Pnueli et al. 2003). Overexpression of Zat7 results in growth suppression, enhanced expression of defense transcripts such as WRKY70, AOX1, NHX1, Cor78 and dramatic tolerance to salinity and cold stresses (Ciftci-Yilmaz et al. 2007, Rizhsky et al. 2004a). Conversely, Zat7 RNAi lines showed decreased osmotic stress tolerance (Ciftci-Yilmaz et al. 2007). Interestingly, transgenic plants overexpressing the Zat7 protein with a mutated EAR motif, or entirely lacking the EAR motif, had the same growth retardation as plants expressing the intact Zat7 protein but were highly sensitive to salinity stress (Ciftci-Yilmaz et al. 2007). In addition, yeast two-hybrid assays showed that Zat7 directly interacts with WRKY70 and HASTY through EAR motif (Ciftci-Yilmaz et al. 2007). These results point to a key role for the EAR motif of Zat7 in mediating acclimation responses to salinity stress. Furthermore, the induction of Zat7 by H_2O_2 and methyl viologen (MV) was abolished in knockout

Zat12 plants suggesting that Zat7 expression is directly regulated by Zat12 (Rizhky et al. 2004). However, Zat12 overexpressing plants did not induce Zat7 expression under normal conditions indicating a more complex relationship, which could be oxidative stress dependent. In addition, plants expressing Zat7 were found to suppress Apx1 expression (Ciftci-Yilmaz et al. 2007).

The study of Zat responses to oxidative and abiotic stresses suggests a high degree of complexity and hierarchy in the response of different members of this family to cold, salinity and oxidative stress (Ciftci-Yilmaz et al. 2007, Davletova et al. 2005b, Koussevitzky et al. 2007, Mittler et al. 2006, Vogel et al. 2005). Although further studies are required to elucidate the Zat network in plants, it is already clear that Zat proteins are key regulators of ROS signaling and could function as integrators of ROS signaling and responses to different abiotic stresses.

WRKY family

The WRKY transcription factors gene family was recently suggested to play a key role in the response of plants to biotic, abiotic and oxidative stresses (Davletova et al. 2005a, Eulgem and Somssich 2007, Gadjev et al. 2006, Lee et al. 2007, Li et al. 2004, Ulker and Somssich 2004, Vandenabeele et al. 2004, Vanderauwera et al. 2005). WRKY proteins are conserved transcription factors containing the invariable WRKY amino acid signature that has a high affinity to the W-box *cis* element. The WRKY family is one of the largest plant transcription factors families consist of over 74 members in Arabidopsis and more than 90 in rice (Eulgem and Somssich 2007, Ulker and Somssich 2004). WRKY proteins are classified into three major groups according to the number of WRKY domains and zinc finger motifs (Ulker and Somssich 2004). In Arabidopsis, nearly all group III members respond to diverse biotic stresses, 49 of the 72 genes respond to bacterial infection or salicylic acid (SA) (Eulgem and Somssich 2007, Ulker and Somssich 2004), but there is a growing number of evidence for several WRKY genes that are highly expressed during abiotic stresses, especially oxidative stress, as well as developmental stages such as senescence (Davletova et al. 2005a, Gadjev et al. 2006, Rizhsky et al. 2004b, Ulker and Somssich 2004, Vanderauwera et al. 2005). In a comparison between eight oxidative stress-related microarray experiments, WRKY6 and WRKY75 were among the 27 transcripts elevated at least five-fold in six of the eight data sets' oxidative stress experiments (Gadjev et al. 2006).

WRKY25 responds to oxidative stress as well as to wounding, heat and osmotic stresses. WRKY25 induction during oxidative stress was shown to depend on Zat12; however, unlike Zat12 transgenic lines, its overexpression in Arabidopsis was insufficient in increasing oxidative stress tolerance (Rizhsky et al. 2004a). The transcript expression of WRKY70 was elevated in Zat7 transgenic Arabidopsis and conversely, repressed in plants expressing EAR-mutated Zat7 (Ciftci-Yilmaz et al. 2007). WRKY70 and HASTY that are co-expressed in knockout Apx1 plants (Davletova et al. 2005a, Pnueli et al. 2003) were shown to interact with Zat7 through the EAR motif in yeast two-hybrid assay (Ciftci-Yilmaz et al. 2007). HASTY is required for the biogenesis or stability of microRNAs and possibly involved in their export to the cytosol (Park et al. 2005). Accordingly, APX1 deficiency rendered salt stress tolerance (Ciftci-Yilmaz et al. 2007, Miller et al. 2007), suggesting the involvement of WRKY70, HASTY and Zat7 in this pathway (Ciftci-Yilmaz et al. 2007). Additional WRKY proteins expressed in knockout Apx1 include WRKY70, 40, 33 and 18. These are further upregulated in knockout Apx1 plants during light stress (Davletova et al. 2005a). WRKY30 was strongly induced in RNAi CAT2 Arabidopsis lines and was further upregulated together with WRKY 48 during high-light and cold stresses (Vanderauwera et al. 2005). Taken together, WRKY transcription factors are emerging as key regulators in abiotic stress defense responses and ROS stress and could function down- or upstream to Zat proteins.

Rboh genes

NADPH oxidases play a key role in ROS signaling and plant defense responses to pathogen infection. NADPH oxidase generate $O_2^{\cdot-}$ by oxidizing NADPH and transferring the electron to O_2. Rbohs are the plant homologs to the mammalian phagocyte NADPH oxidase subunit $gp91^{phox}$ (Torres and Dangl 2005). The Arabidopsis genome contains 10 classical Rboh genes, all of which contain a presumably cytosolic 300 amino-acid amino-terminal extension with two EF hands that bind Ca^{2+}, that could account for the direct regulation of these proteins (Torres and Dangl 2005). Another regulatory event that could control Rboh function is phosphorylation via a CDPK as was shown by Kobayashi et al. (2007).

AtRbohD and AtRbohF were found to be the two major NADPH oxidases expressed in guard and mesophyl cells. The hypersensitive response leading to cell death and plant immunity against pathogens requires activation of SA signaling. The oxidative burst generated by AtRbohD and AtRbohF were shown to antagonize SA and negatively regulate cell death expansion (Torres et al. 2005). Activation or amplification of the pathogen response pathway may also play a role in abiotic defense responses. AtRbohD-deficient plants were unable to

maintain the higher transcript level of Apx1 and CAT1 induced during high-light stress, suggesting that ROS generated by RbohD could act as an amplifying signal (Davletova et al. 2005a). Furthermore, the transient increase in AtRbohD transcript level in the knockout Apx1 mutant during light stress, which preceded the earlier induction in CAT1 (Davletova et al. 2005a), could indicate its responsiveness to H_2O_2. Another Rboh family member, AtRbohC regulates cell expansion during root hair formation. Analysis of the atrbohC mutant (also called root hair-defective 2) revealed that ROS produced by AtRbohC activate hyperpolarization of Ca^{2+} channels that are responsible for localized cell expansion during the root hair formation (Foreman et al. 2003). AtrbohD and AtrbohF have been implicated in the intracellular signaling and cell death that arises from O_3 exposure (Joo et al. 2005). The level of AtRbohD and RbohF mRNA is upregulated by ABA in guard cells and mesophyll cells (Kwak et al. 2003). ABA, a key stress hormone in plants is responsible for stomatal closure upon water stress. ABA-dependent stomatal closure was shown to be partially dependent on NADPH oxidase activity (Kwak et al. 2003, Pei et al. 2000). Thus, ABA-induced stomatal closure was reduced in the RbohF single mutant, whereas the RbohD mutant behaved like the wild-type. In the RbohD/F double mutant, the ABA stomatal closure was even more impaired than in the RbohF single mutant. ABA treatment failed to induce increases in ROS level in AtrobhD/F guard cells, suggesting that NADPH oxidases mediate ABA-induced ROS generation in guard cell and that RbohD and RbohF are the major catalytic subunits in this process (Kwak et al. 2003).

The study of Rboh function in abiotic stress responses is an ongoing effort in many labs. Rboh mutants were shown to be more sensitive to heat stress (Larkindale et al. 2005), suggesting that ROS generated by Rboh proteins is an essential part of the response of plants to different abiotic stresses. It was also suggested that Rboh proteins function as positive amplifiers enhancing the ROS signal generated during stress and maintaining this signal in an active state for a longer period of time (Mittler et al., 2004; Fig. 1)

Multiprotein bridging factor 1c

MBF1c is a highly conserved transcriptional coactivator that is involved in the regulation of diverse processes such as endothelial cell differentiation, hormone-regulated lipid metabolism, central nervous system development and His metabolism (Brendel et al. 2002, Liu et al. 2003, Takemaru et al. 1997). In Arabidopsis, MBF1c expression is elevated in response to pathogen infection, salinity, drought, heat, H_2O_2, ABA or SA (Rizhsky et al. 2002, 2004b, Suzuki et al. 2005, 2008, Tsuda and Yamazaki 2004, Vanderauwera et al. 2005). Constitutive expression of MBF1c in Arabidopsis enhanced the tolerance of transgenic plants to bacterial infection, osmotic stress, heat and the combination of osmotic and heat stresses, suggesting a broad function for MBF1c in response to biotic/abiotic stress. In contrast, the MBF1c knockout had increased sensitivity to heat stress, showing decreased basal thermosensitivity (Suzuki et al. 2005, 2008). *mbf1c* plants were unable to accumulate SA and trehalose during heat stress in contrast to the MBF1c overexpressing plants that showed increased levels of both (Suzuki et al. 2008). Exogenously applied trehalose or SA rescued the *mbf1c* heat sensitivity phenotype (Suzuki et al. 2008).

Analysis of *mbf1c* plants revealed that MBF1c functions as a key regulator of basal thermotolerance in Arabidopsis and acts upstream to SA, trehalose and ethylene signaling during heat stress (Suzuki et al. 2008). SA is an important signaling hormone in plants' defense responses to pathogen attack; however, there is also evidence that it functions in regulating plant responses to abiotic stresses (Dat et al. 1998, Fujita et al. 2006). The signaling networks that control pathogenesis-related responses and abiotic stress signaling were previously shown to partially overlap (Chung et al. 2004, Davletova et al. 2005a, ; Foyer and Noctor 2005, Fujita et al. 2006, Suzuki et al. 2005, 2008). NPR1 that plays a major role in SA signaling and activation of pathogenesis response genes is kept inactive in the cytosol through inter- or intramolecular disulfide-bridges and is converted to active monomers upon changes in redox state (Pieterse and Van Loon 2004). Interestingly, in ascorbic acid-deficient (*vtc1*) mature plants (older than 6 weeks), NPR1 accumulated in the nucleus, increased the expression of pathogenesis response genes and enhanced resistance to bacterial infection (Pavet et al. 2005).

Our analysis of MBF1c suggests that it could be a key regulatory hub linking ROS signaling with pathogen and abiotic stress responses. Further studies are, however, required to elucidate the signaling role of MBF1c in Arabidopsis.

Divergent ROS signals and their cross talk effect defense responses

The chemical identity and the subcellular source of the ROS that accumulate during abiotic stress could dictate the expression pattern of specific sets of genes and the induction of certain acclimation and defense mechanisms (Mittler et al. 2004). For example, HsfA4a and HsfA8 were elevated in knockout APX1 plants but did not respond to H_2O_2 generated in peroxisomes by CAT2 deficiency and vice versa for HsfA2 and HsfA7a (Davletova et al. 2005a, Pnueli et al. 2003, Vanderauwera

et al. 2005). Knockdown tAPX mutants exhibited altered expression of Zat12 in response to high-light stress and enhanced basal thermotolerance, suggesting that these phenotypes are dependent on a signal generated in the chloroplast (Miller et al. 2007). Koussevitzky et al. (2007) showed that signals from chloroplasts regulating nuclear gene expression converge to one retrograde pathway upstream to GUN1 in the chloroplast and leading to ABI-4 nuclear gene suppression. Consequently, retrograde signaling mutants *gun1* and *abi-4* are impaired in thermotolerance and showed altered expression pattern of Zat12 in response to high light (Koussevitzky et al. 2007, Miller et al. 2007). These observations suggest broadening the impact of the retrograde signaling pathway from mere coordination of nuclear and chloroplast gene activities required for chloroplast function (Lee et al. 2007), to include contributing to whole cell-stress-related defense responses.

Double or triple mutants deficient in key ROS network genes can serve as excellent tools to study cross talk between different types of ROS generated at different subcellular compartments. Tobacco plants deficient in the major H_2O_2-detoxifying enzymes cytoplasmic Apx1, or peroxisomal CAT, are constantly subjected to oxidative damages and are more sensitive to oxidative stress (Rizhsky et al. 2002). In contrast, deficiency in both cytosolic APX1 and peroxisomal CAT in tobacco caused plants to become tolerant to oxidative stress compared with the single mutants and even more tolerant than the wild-type (Rizhsky et al. 2002). It is possible that the integration of the two H_2O_2 signals from the cytosol and the peroxisomes induced cell reprogramming in the double mutant causing suppressed photosynthesis, induction of the pentose phosphate pathway, decreased expression of AOX1 relative to CAT antisense line and increased expression of the chloroplastic AOX (Rizhsky et al. 2002). Recently, we showed that Arabidopsis double mutants deficient in cytosolic APX1 and tAPX show several specific characteristic different from the single mutants, such as delayed flowering, increased tolerance of oxidative stress and decreased oxidative damages as well as increased anthocyanins level during high-light stress (Miller et al. 2007).

Recently, Giacomelli et al. showed that knocking out the chloroplast APX machinery (i.e. tAPX and stromal/mitochondrial APX) in Arabidopsis had no apparent effect on the whole plant sensitivity to high light intensity. However, introduction of the ascorbic acid-deficient *vtc2* allele rendered the triple mutant sensitive to high light causing necrosis of mature leaves (Giacomelli et al. 2007).

These observations are indicative of a complex relationship between divergent ROS signals that can result in cell reprogramming depending on the nature of the signals and their origin. Thus, different signals can integrate or collide resulting in different outcomes: (1) summation of the stress signals resulting in an additive effect, (2) integration between the different signals giving rise to a new signal and (3) an epistatic effect of one signal over another. All these possibilities could coexist in one double or triple mutant or even wild-type plants in the field, effecting the activation of many mechanisms and differently determining cellular coordination.

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References

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

Baier M, Noctor G, Foyer CH, Dietz KJ (2000) Antisense suppression of 2-cysteine peroxiredoxin in Arabidopsis specifically enhances the activities and expression of enzymes associated with ascorbate metabolism but not glutathione metabolism. Plant Physiol 124: 823–832

Bailey-Serres J, Mittler R (2006) The roles of reactive oxygen species in plant cells. Plant Physiol 141: 311

Baniwal SK, Chan KY, Scharf KD, Nover L (2007) Role of heat stress transcription factor HsfA5 as specific repressor of HsfA4. J Biol Chem 282: 3605–3613

Brendel C, Gelman L, Auwerx J (2002) Multiprotein bridging factor-1 (MBF-1) is a cofactor for nuclear receptors that regulate lipid metabolism. Mol Endocrinol 16: 1367–1377

Ciftci-Yilmaz S, Mittler R (2008) The zinc finger network of plants. Cell Mol Life Sci, doi: 10.1007/S00018-007-7473-4

Ciftci-Yilmaz S, Morsy MR, Song L, Coutu A, Krizek BA, Lewis MW, Warren D, Cushman J, Connolly EL, Mittler R (2007) The ear-motif of the C2H2 zinc-finger protein ZAT7 plays a key role in the defense response of Arabidopsis to salinity stress. J Biol Chem 282: 9260–9268

Chung E, Park JM, Oh SK, Joung YH, Lee S, Choi D (2004) Molecular and biochemical characterization of the Capsicum annuum calcium-dependent protein kinase 3 (CaCDPK3) gene induced by abiotic and biotic stresses. Planta 220: 286–295

Dat JF, Lopez-Delgado H, Foyer CH, Scott IM (1998) Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol 116: 1351–1357

Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005a) Cytosolic ascorbate peroxidase 1 is a central component of the

reactive oxygen gene network of Arabidopsis. Plant Cell 17: 268–281
Davletova S, Schlauch K, Coutu J, Mittler R (2005b) The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. Plant Physiol 139: 847–856
Eulgem T, Somssich IE (2007) Networks of WRKY transcription factors in defense signaling. Curr Opin Plant Biol 10: 366–371
Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, Davies JM, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422: 442–446
Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119: 355–364
Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17: 1866–1875
Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9: 436–442
Gadjev I, Vanderauwera S, Gechev TS, Laloi C, Minkov IN, Shulaev V, Apel K, Inze D, Mittler R, Van Breusegem F (2006) Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. Plant Physiol 141: 436–445
Giacomelli L, Masi A, Ripoll DR, Lee MJ, van Wijk KJ (2007) Arabidopsis thaliana deficient in two chloroplast ascorbate peroxidases shows accelerated light-induced necrosis when levels of cellular ascorbate are low. Plant Mol Biol 65: 627–644
Joo JH, Wang S, Chen JG, Jones AM, Fedoroff NV (2005) Different signaling and cell death roles of heterotrimeric G protein alpha and beta subunits in the Arabidopsis oxidative stress response to ozone. Plant Cell 17: 957–970
Kobayashi M, Ohura I, Kawakita K, Yokota N, Fujiwara M, Shimamoto K, Doke N, Yoshioka H (2007) Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. Plant Cell 19: 1065–1080
Kotak S, Larkindale J, Lee U, von Koskull-Doring P, Vierling E, Scharf KD (2007a) Complexity of the heat stress response in plants. Curr Opin Plant Biol 10: 310–316
Kotak S, Vierling E, Baumlein H, von Koskull-Doring P (2007b) A novel transcriptional cascade regulating expression of heat stress proteins during seed development of Arabidopsis. Plant Cell 19: 182–195
Koussevitzky S, Nott A, Mockler TC, Hong F, Sachetto-Martins G, Surpin M, Lim J, Mittler R, Chory J (2007) Multiple signals from damaged chloroplasts converge on a common pathway to regulate nuclear gene expression. Science 316: 715–719
Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J 22: 2623–2633
Larkindale J, Hall JD, Knight MR, Vierling E (2005) Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol 138: 882–897
Lee KP, Kim C, Landgraf F, Apel K (2007) EXECUTER1- and EXECUTER2-dependent transfer of stress-related signals from the plastid to the nucleus of Arabidopsis thaliana. Proc Natl Acad Sci USA 104: 10270–10275
Li J, Brader G, Palva ET (2004) The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell 16: 319–331
Liu QX, Jindra M, Ueda H, Hiromi Y, Hirose S (2003) Drosophila MBF1 is a co-activator for Tracheae Defective and contributes to the formation of tracheal and nervous systems. Development 130: 719–728
Miller G, Mittler R (2006) Could heat shock transcription factors function as hydrogen peroxide sensors in plants? Ann Bot (Lond) 98: 279–288
Miller G, Suzuki N, Rizhsky L, Hegie A, Koussevitzky S, Mittler R (2007) Double mutants deficient in cytosolic and thylakoid ascorbate peroxidase reveal a complex mode of interaction between reactive oxygen species, plant development, and response to abiotic stresses. Plant Physiol 144: 1777–1785
Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7: 405–410
Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11: 15–19
Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9: 490–498
Mittler R, Kim Y, Song L, Coutu J, Coutu A, Ciftci-Yilmaz S, Lee H, Stevenson B, Zhu JK (2006) Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress. FEBS Lett 580: 6537–6542
Moller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol 52: 561–591
Noctor G, Veljovic-Jovanovic S, Driscoll S, Novitskaya L, Foyer CH (2002) Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? Ann Bot (Lond) 89: 841–850

Panchuk II, Volkov RA, Schoffl F (2002) Heat stress- and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in Arabidopsis. Plant Physiol 129: 838–853

Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS (2005) Nuclear processing and export of microRNAs in Arabidopsis. Proc Natl Acad Sci USA 102: 3691–3696

Pavet V, Olmos E, Kiddle G, Mowla S, Kumar S, Antoniw J, Alvarez ME, Foyer CH (2005) Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis. Plant Physiol 139: 1291–1303

Pei ZM, 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

Pieterse CM, Van Loon LC (2004) NPR1: the spider in the web of induced resistance signaling pathways. Curr Opin Plant Biol 7: 456–464

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

Rizhsky L, Liang H, Mittler R (2003) The water-water cycle is essential for chloroplast protection in the absence of stress. J Biol Chem 278: 38921–38925

Rizhsky L, Davletova S, Liang H, Mittler R (2004a) The zinc finger protein Zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in Arabidopsis. J Biol Chem 279: 11736–11743

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R (2004b) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiol 134: 1683–1696

Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Arabidopsis Cys2/His2-type zinc-finger proteins function as transcription repressors under drought, cold, and high-salinity stress conditions. Plant Physiol 136: 2734–2746

Suzuki N, Rizhsky L, Liang H, Shuman J, Shulaev V, Mittler R (2005) Enhanced tolerance to environmental stress in transgenic plants expressing the transcriptional coactivator multiprotein bridging factor 1c. Plant Physiol 139: 1313–1322

Suzuki N, Bajad S, Shuman J, Shulaev V, Mittler R (2008) The transcriptional co-activator MBF1c is a key regulator of thermotolerance in *Arabidopsis thaliana*. J Biol Chem (Epub ahead of print)

Takemaru K, Li FQ, Ueda H, Hirose S (1997) Multiprotein bridging factor 1 (MBF1) is an evolutionarily conserved transcriptional coactivator that connects a regulatory factor and TATA element-binding protein. Proc Natl Acad Sci USA 94: 7251–7256

Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8: 397–403

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

Tsuda K, Yamazaki K (2004) Structure and expression analysis of three subtypes of Arabidopsis MBF1 genes. Biochim Biophys Acta 1680: 1–10

Ulker B, Somssich IE (2004) WRKY transcription factors: from DNA binding towards biological function. Curr Opin Plant Biol 7: 491–498

Umbach AL, Fiorani F, Siedow JN (2005) Characterization of transformed Arabidopsis with altered alternative oxidase levels and analysis of effects on reactive oxygen species in tissue. Plant Physiol 139: 1806–1820

Vandenabeele S, Vanderauwera S, Vuylsteke M, Rombauts S, Langebartels C, Seidlitz HK, Zabeau M, Van Montagu M, Inze D, Van Breusegem F (2004) Catalase deficiency drastically affects gene expression induced by high light in Arabidopsis thaliana. Plant J 39: 45–58

Vanderauwera S, Zimmermann P, Rombauts S, Vandenabeele S, Langebartels C, Gruissem W, Inze D, Van Breusegem F (2005) Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light-induced transcriptional cluster involved in anthocyanin biosynthesis. Plant Physiol 139: 806–821

Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF (2005) Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis. Plant J 41: 195–211

von Koskull-Doring P, Scharf KD, Nover L (2007) The diversity of plant heat stress transcription factors. Trends Plant Sci 12: 452–457

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