Ascorbate Peroxidases: Crucial Roles of Antioxidant Enzymes in Plant Stress Responses

Takanori Maruta and Takahiro Ishikawa

Abstract Ascorbate peroxidases (APXs) are, in general, photosynthetic eukaryote-specific enzymes, which catalyze the reduction of H_2O_2 using ascorbate as an electron donor. Considering the very low affinity of ascorbate with H_2O_2, the acquisition of APX was certainly an important event, allowing plants to use ascorbate for H_2O_2 metabolism. This also provides a plausible explanation for why plants accumulate a massive amount of ascorbate because this substrate is also required for stabilizing fragile APX enzymes (particularly chloroplastic isoforms). In higher plants, APXs are distributed in the cytosol, mitochondria, chloroplasts (both stroma and thylakoid membrane), and peroxisomes to modulate organellar and cellular levels of H_2O_2. Despite its potential toxicity, H_2O_2 is a relatively stable form of a reactive oxygen species, and consequently it can act as a key signaling molecule for plant stress responses. From this point of view, APXs also have a dual role, being antioxidant enzymes and H_2O_2 signaling regulators, and their balance is crucial for fine-tuning stress responses. In this chapter, we describe the physiological roles of APX isoforms in plants by overviewing the findings of biochemical, physiological, and genetic studies.

Keywords Ascorbate peroxidase · Oxidative stress · Oxidative signaling · Redox regulation · Stress response

1 Introduction

Suboptimal growth conditions caused by environmental changes, such as light, drought, and temperature, lead to yield losses in crops. Under these environmental stresses, enhanced production of reactive oxygen species (ROS) originates from

T. Maruta · T. Ishikawa (✉)
Department of Life Science and Biotechnology, Faculty of Life and Environmental Science, Shimane University, Matsue, Shimane, Japan
e-mail: ishikawa@life.shimane-u.ac.jp

M. A. Hossain et al. (eds.), *Ascorbic Acid in Plant Growth, Development and Stress Tolerance*, https://doi.org/10.1007/978-3-319-74057-7_4

photosynthesis, respiration, and photorespiration, or through several oxidases (Mittler et al. 2004; Shigeoka and Maruta 2014). ROS are potentially cytotoxic molecules that can oxidize any cellular component, causing oxidative damage. Among ROS, H_2O_2 is a relatively stable form and poorly oxidizes cellular components, such as nucleic acids, proteins, sugars, and lipids, with some exceptions (e.g., cysteine is a good target for this ROS). Thus, H_2O_2 itself is not very toxic for plants, as well as for other organisms (Mittler 2017). In the presence of free iron, however, the Fenton reaction occurs and converts H_2O_2 to a hydroxyl radical ($OH^{\bullet}$), which is the most reactive form of ROS and oxidizes any component randomly and rapidly (Mittler 2017). The very short half-life of $OH^{\bullet}$ (approximately 1 ns) indicates that the selective scavenging of this ROS is impossible in cells. Strict control of H_2O_2 levels is therefore essential in the prevention of oxidative damage from $OH^{\bullet}$.

It is well known that higher plants accumulate a large amount of ascorbate. The high accumulation occurs mainly in photosynthetic tissues, such as leaves. Because these tissues are the main targets of light-dependent oxidative stress (photooxidative stress) (Asada 1999), it is easy to imagine that ascorbate plays a key role in protecting cells from photooxidative damage. Although ascorbate is indeed a powerful antioxidant, this chemical itself does not efficiently interact with H_2O_2. Acquisition of ascorbate peroxidases (APXs), which convert H_2O_2 to water using ascorbate as an electron donor, during evolution has allowed plants to use ascorbate for H_2O_2 metabolism (Gest et al. 2013). In higher plants, APXs are distributed in the cytosol, chloroplasts, mitochondria, and peroxisomes (Mittler et al. 2004; Maruta et al. 2016), together with several layers of the ascorbate recycling system that supplies ascorbate for the APX reaction (Gallie 2013). The antioxidant ability of ascorbate in plant cells has been maximized by the evolution of ascorbate metabolism (Gest et al. 2013).

For the last few decades, physiological function of APXs, as well as that of other antioxidant enzymes, was analyzed based only on the oxidative stress theory, in which ROS are only cytotoxic molecules. However, it is now widely accepted that ROS, especially H_2O_2, have another face, functioning as signaling molecules to control a diverse range of physiological processes, such as stress responses, growth, and development. From this point of view, APXs also play a dual role, being antioxidant enzymes and H_2O_2 signaling regulators, and their balance is crucial for fine-tuning stress responses. In this chapter, we describe the physiological roles of APX isoforms in plants by summarized the findings of biochemical, physiological, and genetic studies.

2 Distribution of APX Isoforms in Plant Cells

APXs are heme peroxidases and members of Class I non-animal peroxidases, which also include cytochrome *c* peroxidases (CCPs) and bacterial catalase peroxidases (CPs) (Welinder 1992; Passardi et al. 2007). APXs are only found in plastid-containing organisms with some exceptions (Teixeira et al. 2004; Passardi et al.

2007; Nedelcu et al. 2008). As supported by genome-sequencing studies (Passardi et al. 2007), no *APX* gene has ever been found in cyanobacteria. In contrast, most eukaryotic algae analyzed possessed more than one *APX* gene (Maruta et al. 2016). Two types of hybrid peroxidases, atypical APX-CCP hybrid A1 and A2, were found in non-photosynthetic kinetoplastids and photosynthetic euglenids, respectively (Zámocký et al. 2014; Ishikawa et al. 2010). Mono-functional plant APXs are considered evolutionary descendants of hybrid A1, and they evolved in parallel with hybrid A2 (Zámocký et al. 2014).

All *APX* genes are nuclear encoded (Mittler et al. 2004). In higher plants, APX isoforms are distributed in the cytosol (cAPX), chloroplasts (chlAPX), mitochondria (mitAPX), and peroxisomes (pAPX), which are key sites for H_2O_2 production and/or scavenging (Fig. 1) (Shigeoka et al. 2002). Two chloroplastic isoforms, stromal sAPX and thylakoid membrane-bound tAPX, are found in chloroplasts of land plants (Maruta et al. 2016). They form the water–water cycle in a powerful ROS regulation system (see below; Asada 1999). The existence of an additional isoform in the chloroplast lumen (e.g., *Arabidopsis* At-APX4/TL29) was proposed in *Arabidopsis* (Kieselbach et al. 2000). The proposed isoform is highly conserved in other plant species, but the protein lacks some amino acid residues that are essential for APX activity. Indeed, its knockout has no effect on APX activity (Granlund et al. 2009), indicating TL29 is not a functional APX. This is apparently supported by a structural analysis (Lundberg et al. 2011).

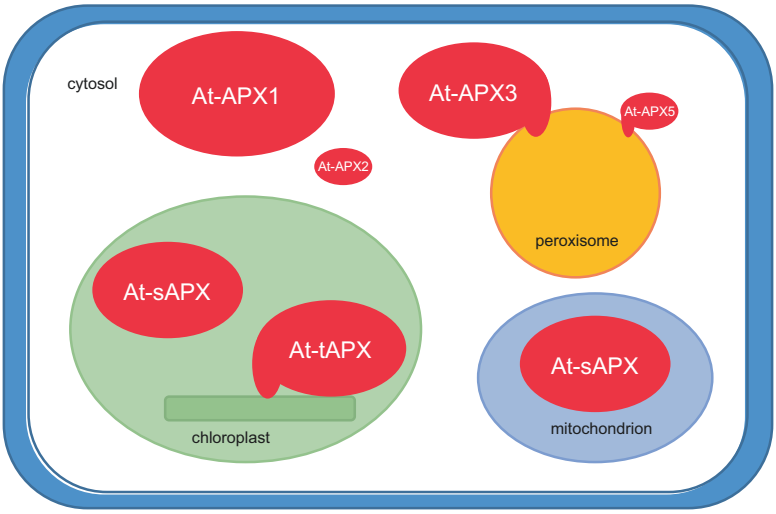

Fig. 1 Distribution of APX isoforms in *Arabidopsis* leaf cells. At-APX1 and 2 are cytosolic isoforms, while At-APX3 and 5 are peroxisomal. At-sAPX is a dual-targeting protein in both the chloroplast stroma and mitochondrial matrix. At-tAPX is solely distributed in chloroplasts and attached to thylakoid membrane. Size of red circles indicate expression levels of the enzymes. Expression level of At-APX2 is very low under normal growth conditions, but highly induced by high light

Previously, *Arabidopsis* was reported to have nine *APX* genes (Mittler et al. 2004), i.e., *At-APX1–7*, *At-sAPX*, and *At-tAPX*. Among them, At-APX6 and At-APX4 lack Arg-172, which is essential for the efficient use of ascorbate (Bursey and Poulos 2000), and At-APX6 is now annotated as an APX-related (APX-R) protein (Lazzarotto et al. 2011). In addition, At-APX7 (At1g33660) is described as a pseudogene in current databases, such as The Arabidopsis Information Resource (TAIR). Therefore, Arabidopsis has six functional *APX* genes. At-APX1 and 2 are cytosolic, whereas At-APX3 and 5 are peroxisomal (Mittler et al. 2004). At-tAPX is distributed throughout the thylakoid membrane although At-sAPX is a dual-targeting protein in both the chloroplast stroma and mitochondrial matrix (Chew et al. 2003; Maruta et al. 2016). In contrast to *Arabidopsis*, rice plants have 8 APX isoforms (Teixeira et al. 2004, 2006). Os-APX5 and Os-APX6 are targeted solely to mitochondria, and Os-APX7 (sAPX) and Os-APX8 (tAPX) to chloroplasts (Xu et al. 2013). Our recent comprehensive mining of *APX* genes in plant species whose genomes are already sequenced indicated that all monocot plants may have APX isoform(s) solely targeted to the mitochondria (Maruta et al. 2016). Although *Physcomitrella patens* APX (Pp-APX1), which is the most orthologous to *Arabidopsis* sAPX, is only targeted to chloroplasts, *Picea glauca* APX (Pg-APX1) is dual-targeted to both chloroplasts and mitochondria (Xu et al. 2013). Based on these findings, it was suggested that the dual-targeting ability of APX developed after the split between *Physcomitrella patens* and *Picea glauca* and was subsequently lost in rice following monocot divergence (Xu et al. 2013).

3 Expression and Regulation

3.1 Transcriptional Regulation

Cytosolic APXs are highly responsive to environmental stimuli, especially high irradiance, whereas other isoforms are not (Yoshimura et al. 2000). Expression of the *Arabidopsis At-APX2* gene has been thoroughly analyzed, and it is the representative stress marker gene. The first important finding was that high light-induced *At-APX2* is largely affected by the redox state of the photosynthetic electron transport (PET) chain (Karpinski et al. 1997). The PET inhibitors, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) block reduction and oxidation, respectively, of the plastoquinone pool. High light-induced *At-APX2* expression is almost completely inhibited by DCMU, whereas its expression is enhanced by DBMIB. This is also the case in the tobacco *cAPX* gene (Yabuta et al. 2004). These findings indicate that the plastoquinone redox state acts as a retrograde signal from chloroplasts to the nucleus for the regulation of *At-APX2* expression under high irradiance. Exogenous application of glutathione also suppresses *At-APX2* expression under high light intensity (Karpinski et al. 1997). Identification of an *Arabidopsis regulator of APX2 1–1* (*rax1–1*)

mutant, an allele of gamma-glutamylcysteine synthetase 1, consolidated the role of glutathione as a mediator of *At-APX2* induction (Ball et al. 2004). These signals are associated with photoelectrophysiological signaling (PEPS) in a light wavelength-specific manner (Szechyńska-Hebda et al. 2010).

Another *Arabidopsis* mutant, *altered expression of APX2* (*alx8*), provides an alternative retrograde signal from chloroplasts (Rossel et al. 2006). The *ALX8* gene encodes SAL1 that dephosphorylates 3′-phosphoadenosine 5′-phosphate (PAP) in chloroplasts. The PAP accumulated in chloroplasts of sal1 mutants can be transferred to the nucleus to inhibit 5′ to 3′ exoribonucleases (XRNs) that modulate thousands of mRNA expressions (Estavillo et al. 2011). This regulation is associated with the abscisic acid (ABA) pathway, and *alx8* as well as other *sal1* mutants accumulate ABA, being highly tolerant to drought stress (Rossel et al. 2006). Considering the fact that ABA is essential for *At-APX2* induction under high light intensity (Galvez-Valdivieso et al. 2009), a plausible explanation is that high irradiance stimulates the SAL1-PAP pathway, which in turn, activates ABA signaling for the gene expression; however, whether the SAL1-PAP pathway is active in wild-type plants exposed to high light intensity requires further validation.

H_2O_2 also acts as a signal for the regulation of *At-APX2* expression. Pre-infiltrating leaves with catalase, but not with superoxide dismutase, strongly inhibits the gene expression under high light intensity (Karpinski et al. 1999). Considering that catalase proteins cannot pass through the plasma membrane, the strong inhibition of *At-APX2* expression must be caused by a decrease in extracellular H_2O_2 levels. This is clearly supported by the finding that NADPH oxidases, which produce ROS in the apoplast, are essential for the full expression of *At-APX2* under high light intensity (Bechtold et al. 2008). A recent pioneering work using HyPer2, a genetically encoded fluorescent H_2O_2 sensor, demonstrated that photosynthesis-produced H_2O_2 is directly transferred from chloroplasts to nuclei and, then, induces tobacco cytosolic *APX* expression under high light stress (Exposito-Rodriguez et al. 2017). Thus, both intracellular and extracellular H_2O_2 can activate *cAPX* expression, possibly through the redox modification of heat-shock transcription factors (Jung et al. 2013).

Taken together, a variety of signals have been found to regulate cytosolic *APX* expression under high light intensity although it is still unclear how these signals are integrated or coordinated to fine-tune the gene expression in plant cells.

3.2 Post-transcriptional Regulation

In some plant species, sAPX and tAPX are encoded by a single gene, which produces both isoforms by alternative splicing in a tissue-specific manner. This regulation occurs in tobacco, spinach, pumpkin, and ice plants (Ishikawa and Shigeoka 2008). In the case of tobacco and spinach, chloroplastic APX pre-mRNA produces four types of mRNA variants, one *tAPX* and three *sAPX* forms (*sAPX-I*, *-II*, and *-III*).

The ratio of the level of *sAPX* mRNAs to *tAPX* is close to 1 in leaves, whereas the ratio in roots is largely elevated because of the increase in *sAPX-III* and decrease in *tAPX* (Yoshimura et al. 2002). The splicing regulatory cis element (SRE) sequence located between exons 12 and 13 of the *chlAPX* gene is required for tissue-specific splicing efficiency. Gel-shift assays revealed that SRE strongly interacts with nuclear protein(s) extracted from leaves, but not with those from roots (Yoshimura et al. 2002). Thus, SRE is anticipated to act as a splicing enhancer that regulates the tissue-specific alternative splicing of chloroplastic *APX* pre-mRNA.

3.3 Post-transcriptional Regulation

In addition to transcriptional regulation, cytosolic APX is also regulated at the post-translational level. This involves the redox modification of Cys-32, which is highly conserved in APXs. Nitric oxide and *S*-nitrosoglutathione react with Cys-32 of cytosolic APX to form *S*-nitrosylation. This modification has a positive effect on cytosolic APX activity in *Arabidopsis* (Yang et al. 2015), but an inhibitory effect in tobacco Bright Yellow-2 cells (de Pinto et al. 2013). *S*-nitrosylation also occurs in Cys-49 of At-APX1 although the modification has no effect on enzyme activity (Yang et al. 2015). Similarly, *S*-sulfhydration by hydrogen sulfide occurs at Cys-32 and activates APX activity (Aroca et al. 2015). This cysteine is a target of thioredoxins (Trxs), which are ubiquitous small disulfide oxidoreductases. Reduction of cytosolic APX by Trxs, as well as by reducing chemicals (such as DTT and glutathione), inactivates the peroxidase activity (Gelhaye et al. 2006).

One of characteristics of chloroplastic APXs is that these enzymes are extremely sensitive to H_2O_2 under low ascorbate levels compared to cytosolic and peroxisomal isoforms (Chen and Asada 1989; Miyake and Asada 1996). The half-inactivation time of chloroplastic APXs is 15 s when the concentration of ascorbate is less than 10 μM, whereas that of the cytosolic enzyme is more than 40 min (Kitajima 2008). The irreversible cross-linking of heme to the distal Trp-41 and radical formation in Cys-31 and Cys-125 are involved in this process. It should be noted that these amino acids are generally conserved in the stable cytosolic isoform (see Maruta et al. 2016). An insertion of amino acids specific to chloroplastic isoforms (chloroplastic domain 2) moves a loop structure, which is in the vicinity of the propionate side chains of heme, away from the propionate side chains. This structural property may facilitate the cross-linking process (Kitajima 2008). Triple mutations in the amino acids described above and deletion of the chloroplastic domain 2 have improved the H_2O_2 sensitivity of tobacco sAPX (Kitajima et al. 2008, 2010). Consequently, a rapid inactivation of chloroplastic APXs is observed in plants exposed to photooxidative stress (Miyake et al. 2006; Yoshimura et al. 2000). However, there have been difficulties with the elucidation of the exact relationship between the inactivation of these enzymes and ascorbate levels in vivo (see Maruta et al. 2016). It is possible that another unknown mechanism(s) may be involved in the inactivation process.

tAPX activity in vivo was also recently shown to be inactivated through direct phosphorylation by a specific kinase in wheat during pathogen infections (Gou et al. 2015). Indeed, phosphoproteomic studies have successfully identified *Arabidopsis* tAPX and sAPX as phosphorylated proteins (for example, Roitinger et al. 2015). A heme-containing APX-related (APX-R) protein (also referred to as At-APX6 in Arabidopsis) is located in chloroplasts and mitochondria, in which it physically interacts with APX, possibly to modulate its activity (Lazzarotto et al. 2011).

4 Role as Antioxidant Enzymes

4.1 Chloroplastic APXs

The APX reaction in chloroplasts is coupled with the photosynthetic electron transport chain to form the water–water cycle (Fig. 2). In this cycle, electrons excised from water in photosystem II (PSII) are transferred to oxygen by PSI, resulting in the formation of O_2^- (Asada 1999). Membrane-attached copper/zinc superoxide dismutase (Cu/Zn-SOD) converts O_2^- into H_2O_2, which is further reduced into water by tAPX. Even if they escaped from this system, ROS would be attacked by the second layer of ROS scavenging, consisting of iron SOD (Fe-SOD) and sAPX in the stroma. The oxidized form of ascorbate generated by the APX reaction is reduced by ferredoxin-, glutathione-, and NAD(P)H-dependent pathways. The water–water cycle acts as both an antioxidant system and a system for dissipating excess electrons from PET, i.e., an electron sink (Asada 1999).

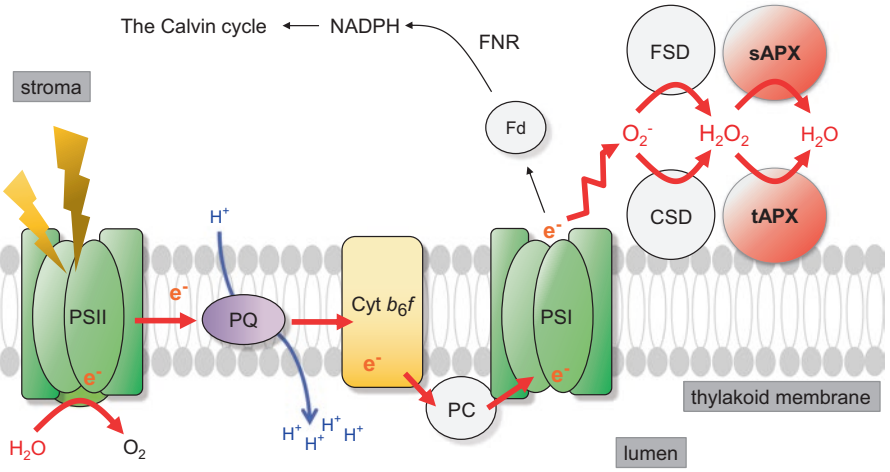

Fig. 2 The water–water cycle. Notes: CSD Cu/Zn-SOD, Cyt cytochrome, Fd ferredoxin, FNR Fd-NADP$^+$ reductase, FSD Fe-SOD, PC plastocyanin, PQ plastoquinone, PS, photosystem

Considering the fragile nature of chloroplastic APXs, it was a plausible hypothesis that APX activity in chloroplasts is a bottleneck for plant stress tolerance. This has been clearly supported by a number of findings showing that overexpression of peroxidases or catalases within chloroplasts results in enhanced stress tolerance in plants (Foyer and Shigeoka 2011). For example, overexpression of *Escherichia coli* catalase (KatE) with a chloroplast-targeting signal protects thiol-modulated enzymes in the Calvin cycle in tobacco plants, thereby mitigating the inhibition of photosynthesis under photooxidative stress (Shikanai et al. 1998; Miyagawa et al. 2000). One of the major causes of photoinhibition is the inhibition of D1 protein translation (Nishiyama et al. 2001). This inhibition is also alleviated in the KatE transgenic tobacco under a combination of salt and high light intensity (Al-Taweel et al. 2007). Overexpression of spinach tAPX has similar effects on tobacco stress tolerance (Yabuta et al. 2002). These lines of evidence strongly indicate that the inactivation of APXs is significant for photooxidative damage from abiotic stress in plant cells, and further suggested that the loss-of-function mutants of chloroplastic APXs should cause severe growth defects or lethality in plants under illumination (Yabuta et al. 2002).

Nevertheless, researchers have unexpectedly failed to find a "stress-sensitive phenotype" among loss-of-function mutants, at least in the laboratory conditions. In *Arabidopsis* mutants lacking sAPX and/or tAPX, the accumulation of H_2O_2 and decrease in PET activity are slightly pronounced after short-term application of high light intensity (more than 1000 μmol photons/m^2/s for up to 6 h) (Kangasjärvi et al. 2008; Maruta et al. 2010). However, no obvious phenotypic difference was found between these mutants and the wild type under short- and even long-term application of high irradiance (Giacomelli et al. 2007; Kangasjärvi et al. 2008; Maruta et al. 2010). A hexaploid wheat mutant S-SV8, which lacks one of three *tAPX* genes, was found to exhibit a growth retardation under mild light stress (Danna et al. 2003) although it is unclear whether *tAPX-6B* is the only gene absent in the mutant and responsible for its phenotype.

Compensation by other antioxidant enzyme(s) is a plausible explanation for the negligible phenotype of chloroplastic APX mutants under laboratory conditions. A complete double mutant lacking 2CPA and 2CPB, which are chloroplastic 2-Cys peroxiredoxins, exhibits growth retardation under a light intensity of 160 μmol photons/m^2/s, and this phenotype is further facilitated by an additional defect in At-tAPX (Awad et al. 2015). *Arabidopsis* chloroplastic glutathione peroxidases (GPX1 and GPX7) also provide an alternative route for the scavenging of H_2O_2 in the water–water cycle (Chang et al. 2009). Other mechanisms are also involved in regulating the production of ROS from photosynthesis. In addition to linear electron transport, cyclic electron transport (CET) around PSI via the proton gradient regulation 5 (PGR5)- and chloroplast NADH dehydrogenase-like (NDH) complex-dependent pathways largely contribute to the formation of a proton gradient across the thylakoid membrane (i.e., low pH in the lumen), which activates the xanthophyll cycle to dissipate excess light energy as heat (Shikanai 2014).

4.2 Cytosolic APXs

The cytosol is not a major site for ROS production. However, from the viewpoint of stress sensitivity of knockout mutants, cytosolic APX is likely to play a key role in cellular redox regulation. An *Arabidopsis apx1* mutant (ecotype Ws) exhibits a growth defect even under normal growth conditions, with an altered stomatal response and decreased photosynthetic activity because of cellular oxidative damage (Pnueli et al. 2003). These phenotypes might occur in an ecotype-dependent manner because our *apx1* mutant (ecotype Col-0) grows at wild-type levels under similar growth conditions (Maruta et al. 2012a). However, *apx1* (Ws) exhibits a severe sensitivity to high light intensity, methyl viologen-induced oxidative stress, and a combination of drought and heat (Davletova et al. 2005; Koussevitzky et al. 2008); *apx1* (Col-0) is also very sensitive to wounding (Maruta et al. 2012a). Thus, the lack of At-APX1 actually weakens plants against a wide range of stresses. Interestingly, oxidation of not only the cytosolic proteins, but also organellar ones is enhanced in the *apx1* mutants during stress (Davletova et al. 2005; Maruta et al. 2012a). The cytosol is in cellular compartments across organelles, such as chloroplasts, mitochondria, peroxisomes, and the nucleus. Thus, cAPX can protect organelles from oxidative stress by preventing H_2O_2 from flowing into one organelle from another. This is known as cross-compartment protection (Davletova et al. 2005).

4.3 Peroxisomal and Mitochondrial APXs

Peroxisomes are considered the most significant site for H_2O_2 production in C_3 leaves during photorespiration (Foyer and Noctor 2003) and therefore accumulate a large amount of catalase to scavenge and regulate H_2O_2 levels. Physiological importance of catalase has been demonstrated by knockout mutants of the *Arabidopsis CAT2* gene, which exhibit a severe bleaching phenotype under photorespiratory conditions (i.e., ambient air with high light intensity) (Mhamdi et al. 2012; Queval et al. 2007; Vandenabeele et al. 2004). In addition to catalase, APXs are also distributed in peroxisomes (Yamaguchi et al. 1995). These peroxisomal isoforms, for example, *Arabidopsis* At-APX3 and At-APX5, have a transmembrane domain with which they attach to the peroxisomal membrane, but their catalytic domain faces the cytosol (Ishikawa et al. 1998; Shen et al. 2010). Affinities for H_2O_2 are substantially different between catalase and APX, whose *K*m values for ROS are approximately 40–600 mM and 10–100 μM, respectively (Mhamdi et al. 2012; Shigeoka and Maruta 2014). Thus, peroxisomal APXs may react with a low concentration of H_2O_2, which escaped from the catalase reaction, to fine-tune the cellular H_2O_2 levels. Overexpression of peroxisomal APX is likely to enhance plant stress tolerance (for example, Wang et al. 1999). In contrast, knockout of At-APX3 had no effect on

plant tolerance to various abiotic stresses (Narendra et al. 2006). This might have been caused by compensation by catalase or another isoform At-APX5. Physiological significance of ascorbate metabolism in peroxisomes was indicated by Eastmond (2007), who showed that peroxisomal At-MDAR4 (monodehydroascorbate reductase) is essential for autotrophic growth although it is unclear if the At-MDAR4 function is coupled with the APX reaction. In contrast to that of peroxisomal APX, the catalytic domain of the MDAR isoform is in the peroxisomal matrix, which results in a question regarding how MDHA produced in the cytosol is reduced in the peroxisome matrix.

Mitochondria would be a significant site for H_2O_2 production, at least in non-photosynthetic tissues, such as roots. Nevertheless, H_2O_2 metabolism in the organelles and its physiological significance are poorly understood in plants. As described above, At-sAPX is a dual-targeting enzyme for both the chloroplast stroma and mitochondrial matrix (Chew et al. 2003). However, the knockout of the gene had little effect on plant tolerance for oxidative stress (Davletova et al. 2005; Maruta et al. 2010). Other thiol-dependent peroxidases, such as peroxiredoxin II F and glutathione peroxidase 6 in *Arabidopsis*, have been found to function in the mitochondria.

5 Role as Redox Signaling Regulators

H_2O_2 is currently recognized to act as a signal for regulating a wide range of physiological processes, including abiotic and biotic stress responses (Apel and Hirt 2004; Foyer and Shigeoka 2011; Mittler et al. 2011). Accumulating transcriptome data from plants subjected to oxidative stress or redox mutants, in which one or more antioxidant enzymes are knocked out/down, have revealed the existence of a production site- and type-specific pathways for ROS signaling (Gadjev et al. 2006; Vaahtera et al. 2014; Shigeoka and Maruta 2014; Willems et al. 2016). Although the mode of action of each pathway remains largely unclear, the integration and crosstalk of multiple pathways in plants have been considered to fine-tune stress responses. This must be based on the strict spatiotemporal control of ROS levels through a diverse set of antioxidant enzymes, including APXs, in various cellular compartments.

Because of the dual face of ROS actions, some redox mutants are paradoxically more resistant to some circumstances compared to the wild type. One of the clearest examples is that the photorespiratory oxidative stress phenotype of the *Arabidopsis cat2* mutant is largely mitigated by additional mutation in the cytosolic *At-APX1* gene (Vanderauwera et al. 2011). Specific activation of DNA damage response occurs in the *cat2 apx1* double mutant (Vanderauwera et al. 2011), probably through an interaction between cytosolic and peroxisomal H_2O_2 signals, leading to the stress-tolerant phenotype. A similar paradoxical phenotype is observed in the *apx1*

single mutants, which are highly tolerant to selenium and lead (Jiang et al. 2016, 2017). These observations clearly show that cAPX plays a key role in balancing the dual faces of ROS actions.

cAPX is also involved in plant immunity. Expression of tobacco cAPX is post-transcriptionally, but not transcriptionally, suppressed upon pathogen infection (Mittler et al. 1998). This has a negative correlation with enhanced ion leakage (cell death) and *pathogenesis-related 1* (*PR1*) gene expression. Hypersensitive response (HR) during pathogen attack is highly accelerated in transgenic tobacco plants with decreased cAPX expression (Mittler et al. 1999). There is also increasing experimental evidence for the involvement of chloroplastic APXs in plant immunity. Phosphorylative inactivation of tAPX through protein kinase wheat kinase start 1.1 (WKS1.1) occurs in wheat upon pathogen attack, resulting in enhanced H_2O_2 levels (Gou et al. 2015). In *Arabidopsis*, knockdown of tAPX enhances the accumulation of salicylic acid and subsequent transcriptional activation of defense-related genes without the application of any stress (Maruta et al. 2012b). These findings clearly indicate that cytosolic and chloroplastic APXs regulate immune responses by regulating H_2O_2 levels.

To identify chloroplastic H_2O_2-responsive genes, a conditional system for tAPX silencing in *Arabidopsis* has been developed using an estrogen-inducible RNAi method (Maruta et al. 2012b). Although no obvious oxidative stress symptom was observed in the tAPX-silenced plants, 365 and 409 genes were at least two-fold ($P < 0.05$) up- and down-regulated, respectively, in response to tAPX silencing. Interestingly, these genes rarely included typical marker genes for oxidative stress, which have been identified by comparing the transcriptomic data of several ROS-related mutants and plants treated with ROS-producing agents. Indeed, these genes only slightly overlapped with genes whose expression was affected by cytosolic and peroxisomal H_2O_2 (i.e., in the *apx1* and *cat2* mutant, respectively) (Maruta et al. 2012b: Queval and Foyer 2012). Classification and comprehensive analysis of these genes have indicated a regulatory role for tAPX in metabolic pathways related to abiotic stress acclimation in plants. For example, tAPX silencing enhances γ-amino aminobutyric acid (GABA) and anthocyanin metabolisms, which may protect chloroplastic APXs mutants from photooxidative stress (Maruta et al. 2013, 2014).

How does chloroplast-produced H_2O_2 act as signal to modulate expression of nuclear genes? A recent finding using HyPer2 revealed the mode of action of chloroplastic H_2O_2 signaling in *Nicotiana benthamiana* epidermal cells under high irradiation. Exposito-Rodriguez et al. (2017) found that chloroplast-produced H_2O_2 is directly transferred to nuclei, avoiding the cytosol. Nuclear H_2O_2 accumulation and subsequent high light-responsive gene expression were critically attenuated by sAPX overexpression or DCMU treatment, but not by cAPX overexpression. This clearly indicates the involvement of chloroplastic APXs in chloroplast-to-nucleus H_2O_2 signaling.

6 Conclusion and Future Perspectives

More than three decades have passed since the APX enzyme was first characterized in Euglena (Shigeoka et al. 1980). During this period, basic information on APX isoforms in higher plants has accumulated in the context of their enzymological properties, distribution, and functions as antioxidant enzymes. However, this is largely restricted to model plants, such as *Arabidopsis* and rice, and there are still more questions than answers. For example, the physiological significance of organellar isoforms in plant stress tolerance remains largely unclear. Furthermore, the validation of APXs as signaling modulators has just started. H_2O_2 has multiple signaling roles in a production site-specific manner. Because their crosstalk is believed to fine-tune plant stress responses, it will be interesting to clarify how APX isoforms functionally interact with each other under stressful conditions to achieve spatiotemporal tuning of H_2O_2 signaling pathways.

Acknowledgement This work was partially supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan [Grant-in-Aid for Scientific Research (B) (to T.I and T.M: 17H03807)].

References

Al-Taweel K, Iwaki T, Yabuta Y, Shigeoka S, Murata N, Wadano A (2007) A bacterial transgene for catalase protects translation of d1 protein during exposure of salt-stressed tobacco leaves to strong light. Plant Physiol 145:258–265

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

Aroca Á, Serna A, Gotor C, Romero LC (2015) S-sulfhydration: a cysteine posttranslational modification in plant systems. Plant Physiol 168:334–342

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

Awad J, Stotz HU, Fekete A, Krischke M, Engert C, Havaux M, Berger S, Mueller MJ (2015) 2-cysteine peroxiredoxins and thylakoid ascorbate peroxidase create a water-water cycle that is essential to protect the photosynthetic apparatus under high light stress conditions. Plant Physiol 167:1592–1160

Ball L, Accotto GP, Bechtold U, Creissen G, Funck D, Jimenez A, Kular B, Leyland N, Mejia-Carranza J, Reynolds H, Karpinski S, Mullineaux PM (2004) Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in *Arabidopsis*. Plant Cell 16:2448–2462

Bechtold U, Richard O, Zamboni A, Gapper C, Geisler M, Pogson B, Karpinski S, Mullineaux PM (2008) Impact of chloroplastic- and extracellular-sourced ROS on high light-responsive gene expression in *Arabidopsis*. J Exp Bot 59:121–133

Bursey EH, Poulos TL (2000) Two substrate binding sites in ascorbate peroxidase: the role of arginine 172. Biochemistry 39:7374–7379

Chang CC, Slesak I, Jordá L, Sotnikov A, Melzer M, Miszalski Z, Mullineaux PM, Parker JE, Karpinska B, Karpinski S (2009) *Arabidopsis* chloroplastic glutathione peroxidases play a role in cross talk between photooxidative stress and immune responses. Plant Physiol 150:670–683

Chen GX, Asada K (1989) Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol 30:987–998

Chew O, Whelan J, Millar AH (2003) Molecular definition of the ascorbate-glutathione cycle in *Arabidopsis* mitochondria reveals dual targeting of antioxidant defenses in plants. J Biol Chem 278:46869–46877

Danna CH, Bartoli CG, Sacco F, Ingala LR, Santa-Maria GE, Guiamet JJ, Ugalde RA (2003) Thylakoid-bound ascorbate peroxidase mutant exhibits impaired electron transport and photosynthetic activity. Plant Physiol 132:2116–2125

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

de Pinto MC, Locato V, Sgobba A, Romero-Puertas Mdel C, Gadaleta C, Delledonne M, De Gara L (2013) S-nitrosylation of ascorbate peroxidase is part of programmed cell death signaling in tobacco Bright Yellow-2 cells. Plant Physiol 163:1766–1775

Eastmond PJ (2007) MONODEHYROASCORBATE REDUCTASE4 is required for seed storage oil hydrolysis and postgerminative growth in *Arabidopsis*. Plant Cell 19:1376–1387

Estavillo GM, Crisp PA, Pornsiriwong W, Wirtz M, Collinge D, Carrie C, Giraud E, Whelan J, David P, Javot H, Brearley C, Hell R, Marin E, Pogson BJ (2011) Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in *Arabidopsis*. Plant Cell 23:3992–4012

Exposito-Rodriguez M, Laissue PP, Yvon-Durocher G, Smirnoff N, Mullineaux PM (2017) Photosynthesis-dependent H_2O_2 transfer from chloroplasts to nuclei provides a high-light signalling mechanism. Nat Commun 8:49

Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Planta 119:355–364

Foyer CH, Shigeoka S (2011) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol 155:93–100

Gadjev I, Vanderauwera S, Gechev TS, Laloi C, Minkov IN, Shulaev V, Apel K, Inzé D, Mittler R, Van Breusegem F (2006) Transcriptomic footprints disclose specificity of reactive oxygen species signaling in *Arabidopsis*. Plant Physiol 141:436–445

Gallie DR (2013) The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. J Exp Bot 64:433–443

Galvez-Valdivieso G, Fryer MJ, Lawson T, Slattery K, Truman W, Smirnoff N, Asami T, Davies WJ, Jones AM, Baker NR, Mullineaux PM (2009) The high light response in *Arabidopsis* involves ABA signaling between vascular and bundle sheath cells. Plant Cell 21:2143–2162

Gelhaye E, Navrot N, Macdonald IK, Rouhier N, Raven EL, Jacquot JP (2006) Ascorbate peroxidase-thioredoxin interaction. Photosynth Res 89:193–200

Gest N, Gautier H, Stevens R (2013) Ascorbate as seen through plant evolution: the rise of a successful molecule? J Exp Bot 64:33–53

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

Gou JY, Li K, Wu K, Wang X, Lin H, Cantu D, Uauy C, Dobon-Alonso A, Midorikawa T, Inoue K, Sánchez J, Fu D, Blechl A, Wallington E, Fahima T, Meeta M, Epstein L, Dubcovsky J (2015) Wheat stripe rust resistance protein WKS1 reduces the ability of the thylakoid-associated ascorbate peroxidase to detoxify reactive oxygen species. Plant Cell 27:1755–1770

Granlund I, Storm P, Schubert M, Garcia-Cerdan JG, Funk C, Schroder WP (2009) The TL29 protein is lumenlocated, associated with PSII and not an ascorbate peroxidase. Plant Cell Physiol 50:1898–1910

Ishikawa T, Shigeoka S (2008) Recent advances in ascorbate biosynthesis and the physiological significance of ascorbate peroxidase in photosynthesizing organisms. Biosci Biotechnol Biochem 72:1143–1154

Ishikawa T, Yoshimura K, Sakai K, Tamoi M, Takeda T, Shigeoka S (1998) Molecular characterization and physiological role of a glyoxysome-bound ascorbate peroxidase from spinach. Plant Cell Physiol 39:23–34

Ishikawa T, Tajima N, Nishikawa H, Gao Y, Rapolu M, Shibata H, Sawa Y, Shigeoka S (2010) *Euglena gracilis* ascorbate peroxidase forms an intramolecular dimeric structure: its unique molecular characterization. Biochem J 426:125–134

Jiang L, Chen Z, Gao Q, Ci L, Cao S, Han Y, Wang W (2016) Loss-of-function mutations in the *APX1* gene result in enhanced selenium tolerance in *Arabidopsis thaliana*. Plant Cell Environ 39:2133–2144

Jiang L, Wang W, Chen Z, Gao Q, Xu Q, Cao H (2017) A role for *APX1* gene in lead tolerance in *Arabidopsis thaliana*. Plant Sci 256:94–102

Jung HS, Crisp PA, Estavillo GM, Cole B, Hong F, Mockler TC, Pogson BJ, Chory J (2013) Subset of heat-shock transcription factors required for the early response of *Arabidopsis* to excess light. Proc Natl Acad Sci U S A 110:14474–14479

Kangasjärvi S, Lepistö A, Hännikäinen K, Piippo M, Luomala EM, Aro EM, Rintamäki E (2008) Diverse roles for chloroplast stromal and thylakoid-bound ascorbate peroxidases in plant stress responses. Biochem J 412:275–285

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

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P (1999) Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. Science 284:654–657

Kieselbach T, Bystedt M, Hynds P, Robinson C, Schröder WP (2000) A peroxidase homologue and novel plastocyanin located by proteomics to the *Arabidopsis* chloroplast thylakoid lumen. FEBS Lett 480:271–276

Kitajima S (2008) Hydrogen peroxide-mediated inactivation of two chloroplastic peroxidases, ascorbate peroxidase and 2-cys peroxiredoxin. Photochem Photobiol 84:1404–1409

Kitajima S, Kitamura M, Koja N (2008) Triple mutation of Cys26, Trp35, and Cys126 in stromal ascorbate peroxidase confers H_2O_2 tolerance comparable to that of the cytosolic isoform. Biochem Biophys Res Commun 372:918–923

Kitajima S, Nii H, Kitamura M (2010) Recombinant stromal APX defective in the unique loop region showed improved tolerance to hydrogen peroxide. Biosci Biotechnol Biochem 74:1501–1503

Koussevitzky S, Suzuki N, Huntington S, Armijo L, Sha W, Cortes D, Shulaev V, Mittler R (2008) Ascorbate peroxidase 1 plays a key role in the response of *Arabidopsis thaliana* to stress combination. J Biol Chem 283:34197–34203

Lazzarotto F, Teixeira FK, Rosa SB, Dunand C, Fernandes CL, Fontenele Ade V, Silveira JA, Verli H, Margis R, Margis-Pinheiro M (2011) Ascorbate peroxidase-related (APx-R) is a new heme-containing protein functionally associated with ascorbate peroxidase but evolutionarily divergent. New Phytol 191:234–250

Lundberg E, Storm P, Schröder WP, Funk C (2011) Crystal structure of the TL29 protein from *Arabidopsis thaliana*: an APX homolog without peroxidase activity. J Struct Biol 176:24–31

Maruta T, Tanouchi A, Tamoi M, Yabuta Y, Yoshimura K, Ishikawa T, Shigeoka S (2010) *Arabidopsis* chloroplastic ascorbate peroxidase isoenzymes play a dual role in photoprotection and gene regulation under photooxidative stress. Plant Cell Physiol 51:190–200

Maruta T, Inoue T, Noshi M, Tamoi M, Yabuta Y, Yoshimura K, Ishikawa T, Shigeoka S (2012a) Cytosolic ascorbate peroxidase 1 protects organelles against oxidative stress by wounding- and jasmonate-induced H_2O_2 in *Arabidopsis* plants. Biochim Biophys Acta 1820:1901–1907

Maruta T, Noshi M, Tanouchi A, Tamoi M, Yabuta Y, Yoshimura K, Ishikawa T, Shigeoka S (2012b) H_2O_2-triggered retrograde signaling from chloroplasts to nucleus plays specific role in response to stress. J Biol Chem 287:11717–11729

Maruta T, Ojiri M, Noshi M, Tamoi M, Ishikawa T, Shigeoka S (2013) Activation of γ-aminobutyrate production by chloroplastic H_2O_2 is associated with the oxidative stress response. Biosci Biotechnol Biochem 77:422–425

Maruta T, Noshi M, Nakamura M, Matsuda S, Tamoi M, Ishikawa T, Shigeoka S (2014) Ferulic acid 5-hydroxylase 1 is essential for expression of anthocyanin biosynthesis-associated genes and anthocyanin accumulation under photooxidative stress in *Arabidopsis*. Plant Sci 219–220:61–68

Maruta T, Sawa Y, Shigeoka S, Ishikawa T (2016) Diversity and evolution of ascorbate peroxidase functions in chloroplasts: more than just a classical antioxidant enzyme? Plant Cell Physiol 57:1377–1386

Mhamdi A, Noctor G, Baker A (2012) Plant catalases: peroxisomal redox guardians. Arch Biochem Biophys 525:181–194

Mittler R (2017) ROS are good. Trends Plant Sci 22:11–19

Mittler R, Feng X, Cohen M (1998) Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. Plant Cell 10:461–473

Mittler R, Herr EH, Orvar BL, van Camp W, Willekens H, Inzé D, Ellis BE (1999) Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. Proc Natl Acad Sci U S A 96:14165–14170

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

Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K, Gollery M, Shulaev V, Van Breusegem F (2011) ROS signaling: the new wave? Trends Plant Sci 16:300–309

Miyagawa Y, Tamoi M, Shigeoka S (2000) Evaluation of the defense system in chloroplasts to photooxidative stress caused by paraquat using transgenic tobacco plants expressing catalase from *Escherichia coli*. Plant Cell Physiol 41:311–320

Miyake C, Asada K (1996) Inactivation mechanism of ascorbate peroxidase at low concentrations of ascorbate; hydrogen peroxide decomposes compound I of ascorbate peroxidase. Plant Cell Physiol 37:423–430

Miyake C, Shinzaki Y, Nishioka M, Horiguchi S, Tomizawa K (2006) Photoinactivation of ascorbate peroxidase in isolated tobacco chloroplasts: *Galdieria partita* APX maintains the electron flux through the water-water cycle in transplastomic tobacco plants. Plant Cell Physiol 47:200–210

Narendra S, Venkataramani S, Shen G, Wang J, Pasapula V, Lin Y, Kornyeyev D, Holaday AS, Zhang H (2006) The *Arabidopsis* ascorbate peroxidase 3 is a peroxisomal membrane-bound antioxidant enzyme and is dispensable for *Arabidopsis* growth and development. J Exp Bot 57:3033–3042

Nedelcu AM, Miles IH, Fagir AM, Karol K (2008) Adaptive eukaryote-to-eukaryote lateral gene transfer: stress-related genes of algal origin in the closest unicellular relatives of animals. J Evol Biol 21:1852–1860

Nishiyama Y, Yamamoto H, Allakhverdiev SI, Inaba M, Yokota A, Murata N (2001) Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. EMBO J 20:5587–5594

Passardi F, Bakalovic N, Teixeira FK, Margis-Pinheiro M, Penel C, Dunand C (2007) Prokaryotic origins of the non-animal peroxidase superfamily and organelle-mediated transmission to eukaryotes. Genomics 89:567–579

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

Queval G, Foyer CH (2012) Redox regulation of photosynthetic gene expression. Philos Trans R Soc Lond Ser B Biol Sci 367:3475–3485

Queval G, Issakidis-Bourguet E, Hoeberichts FA, Vandorpe M, Gakière B, Vanacker H, Miginiac-Maslow M, Van Breusegem F, Noctor G (2007) Conditional oxidative stress responses in the *Arabidopsis* photorespiratory mutant *cat2* demonstrate that redox state is a key modulator of

daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H_2O_2-induced cell death. Plant J 52:640–657

Roitinger E, Hofer M, Köcher T, Pichler P, Novatchkova M, Yang J, Schlögelhofer P, Mechtler K (2015) Quantitative phosphoproteomics of the ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia-mutated and rad3-related (ATR) dependent DNA damage response in *Arabidopsis thaliana*. Mol Cell Proteomics 14:556–571

Rossel JB, Walter PB, Hendrickson L, Chow WS, Poole A, Mullineaux PM, Pogson BJ (2006) A mutation affecting *ASCORBATE PEROXIDASE 2* gene expression reveals a link between responses to high light and drought tolerance. Plant Cell Environ 29:269–281

Shen G, Kuppu S, Venkataramani S, Wang J, Yan J, Qiu X, Zhang H (2010) ANKYRIN REPEAT-CONTAINING PROTEIN 2A is an essential molecular chaperone for peroxisomal membrane-bound ASCORBATE PEROXIDASE3 in *Arabidopsis*. Plant Cell 22:811–831

Shigeoka S, Maruta T (2014) Cellular redox regulation, signaling, and stress response in plants. Biosci Biotechnol Biochem 78:1457–1470

Shigeoka S, Nakano Y, Kitaoka S (1980) Metabolism of hydrogen peroxide in *Euglena gracilis* Z by L-ascorbic acid peroxidase. Biochem J 186:377–380

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K (2002) Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot 53:1305–1319

Shikanai T (2014) Central role of cyclic electron transport around photosystem I in the regulation of photosynthesis. Curr Opin Biotechnol 26:25–30

Shikanai T, Takeda T, Yamauchi H, Sano S, Tomizawa K, Yokota A, Shigeoka S (1998) Inhibition of ascorbate peroxidase under oxidative stress in tobacco having bacterial catalase in chloroplasts. FEBS Lett 428:47–51

Szechyńska-Hebda M, Kruk J, Górecka M, Karpińska B, Karpiński S (2010) Evidence for light wavelength-specific photoelectrophysiological signaling and memory of excess light episodes in *Arabidopsis*. Plant Cell 22:2201–2218

Teixeira FK, Menezes-Benavente L, Margis R, Margis-Pinheiro M (2004) Analysis of the molecular evolutionary history of the ascorbate peroxidase gene family: inferences from the rice genome. J Mol Evol 59:761–770

Teixeira FK, Menezes-Benavente L, Galvão VC, Margis R, Margis-Pinheiro M (2006) Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. Planta 224:300–314

Vaahtera L, Brosché M, Wrzaczek M, Kangasjärvi J (2014) Specificity in ROS signaling and transcript signatures. Antioxid Redox Signal 21:1422–1441

Vandenabeele S, Vanderauwera S, Vuylsteke M, Rombauts S, Langebartels C, Seidlitz HK, Zabeau M, Van Montagu M, Inzé 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, Suzuki N, Miller G, van de Cotte B, Morsa S, Ravanat JL, Hegie A, Triantaphylidès C, Shulaev V, Van Montagu MC, Van Breusegem F, Mittler R (2011) Extranuclear protection of chromosomal DNA from oxidative stress. Proc Natl Acad Sci U S A 108:1711–1716

Wang J, Zhang H, Allen RD (1999) Overexpression of an *Arabidopsis* peroxisomal ascorbate peroxidase gene in tobacco increases protection against oxidative stress. Plant Cell Physiol 40:725–732

Welinder KG (1992) Superfamily of plant, fungal and bacterial peroxidases. Curr Opin Struct Biol 2:388–393

Willems P, Mhamdi A, Stael S, Storme V, Kerchev P, Noctor G, Gevaert K, Van Breusegem F (2016) The ROS wheel: refining ROS transcriptional footprints. Plant Physiol 171:1720–1733

Xu L, Carrie C, Law SR, Murcha MW, Whelan J (2013) Acquisition, conservation, and loss of dual-targeted proteins in land plants. Plant Physiol 161:644–662

Yabuta Y, Motoki T, Yoshimura K, Takeda T, Ishikawa T, Shigeoka S (2002) Thylakoid-membrane bound ascorbate peroxidase is a limiting factor of antioxidative systems under photo-oxidative stress. Plant J 32:915–926

Yabuta Y, Maruta T, Yoshimura K, Ishikawa T, Shigeoka S (2004) Two distinct redox signaling pathways for cytosolic APX induction under photooxidative stress. Plant Cell Physiol 45:1586–1594

Yamaguchi K, Mori H, Nishimura M (1995) A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal membranes in pumpkin. Plant Cell Physiol 36:1157–1162

Yang H, Mu J, Chen L, Feng J, Hu J, Li L, Zhou JM, Zuo J (2015) S-nitrosylation positively regulates ascorbate peroxidase activity during plant stress responses. Plant Physiol 167:1604–1615

Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S (2000) Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. Plant Physiol 123:223–234

Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S (2002) Identification of a cis element for tissue-specific alternative splicing of chloroplast ascorbate peroxidase pre-mRNA in higher plants. J Biol Chem 277:40623–40632

Zámocký M, Gasselhuber B, Furtmüller PG, Obinger C (2014) Turning points in the evolution of peroxidase-catalase superfamily: molecular phylogeny of hybrid heme peroxidases. Cell Mol Life Sci 71:4681–4696