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REVIEW PAPER

Reactive oxygen species and organellar signaling

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Abstract

The evolution of photosynthesis and its associated metabolic pathways has been crucial to the successful establishment of plants, but has also challenged plant cells in the form of production of reactive oxygen species (ROS). Intriguingly, multiple forms of ROS are generated in virtually every plant cell compartment through diverse pathways. As a result, a sophisticated network of ROS detoxification and signaling that is simultaneously tailored to individual organelles and safeguards the entire cell is necessary. Here we take an organelle-centric view on the principal sources and sinks of ROS across the plant cell and provide insights into the ROS-induced organelle to nucleus retrograde signaling pathways needed for operational readjustments during environmental stresses.

Keywords: Chloroplasts, metabolism, mitochondria, peroxisomes, photorespiration, photosynthesis, reactive oxygen species (ROS), retrograde signaling.

Introduction

Oxygenic photosynthesis converts solar energy into biochemical energy by splitting water and releasing oxygen (O_2). This process gradually modified the ancient anaerobic atmosphere [mainly composed of methane, nitrogen, and carbon dioxide (CO_2), with oxygen levels <0.1%] to the present more oxygen-enriched earth's atmosphere (Holland, 2006; Luo *et al.*, 2016), driving the evolution of aerobic lifestyles. The enrichment in atmospheric oxygen enabled the establishment of the ozone layer, which protects living organisms from harmful UV radiation. Moreover, the use of oxygen as the final electron acceptor of electron transport chains (ETCs) in cellular respiration boosted cellular energy production, yielding up to 15-fold more ATP from carbon sources, such as glucose (Halliwell, 2006).

The increased oxygen abundance within plant cells also came with a trade-off, namely the enhanced production of partially reduced or excited forms of oxygen, collectively known as reactive oxygen species (ROS) (reviewed by Noctor and Foyer, 2016; Waszczak *et al.*, 2018). ROS are highly reactive and can oxidize various biological molecules, such as DNA, lipid, and proteins, causing cellular damage (Van Breusegem and Dat, 2006). Changes in environmental conditions, such

Abbreviations: APX, ascorbate peroxidase; β-CC, β-cyclocitral; ETC, electron transport chain; GPX, glutathione peroxidase; MEcPP, methylerythritol cyclodiphosphate; $^1O_2$, singlet oxygen; $O_2^{\cdot -}$, superoxide anion; OH·, hydroxyl radical; PAP, 3′-phosphoadenosine 5′-phosphate; 2PG, 2-phosphoglycolate; 3PGA, 3-phosphoglycerate; PRX, peroxiredoxin; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; Trx, thioredoxin.

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as water availability, light intensity, temperature, and pathogen presence, trigger a surge in ROS production in organelles, such as chloroplasts and mitochondria, resulting in oxidative stress in plants. However, plants can also utilize the reactive properties of ROS to their benefit; for example, in a developmental context, by strategically increasing ROS concentrations at the apoplast to loosen the existing rigid cell wall for cell expansion during growth (Kärkönen and Kuchitsu, 2015) or to kill invading pathogens by launching an NADPH oxidase-dependent oxidative burst (Kadota *et al.*, 2015). A highly sophisticated regulatory network exists in plant cells to sense, respond to, detoxify, and utilize ROS. Intriguingly, recent discoveries highlight a prominent influence of organelle compartmentation on this ROS network. Therefore, this review takes on an organelle-centric view to examine the nature and mode of cellular acclimation to ROS.

ROS metabolism in plant cells

Unique and common aspects of ROS

Different ROS have distinct chemical reactivity characteristics that determine their half-lives and migration distance at the cellular level. For a useful summary of ROS types and characteristics, we refer the reader to Tripathy and Oelmüller (2012), Das and Roychoudhury (2014), and Mittler (2017). The hydroxyl radical (OH·) is the most reactive of all ROS and hence is the shortest lived (half-life of 1 ns). Singlet oxygen ($^1O_2$) and superoxide anions ($O_2^{\cdot -}$) are relatively longer lived ROS (half-life of 1–4 μs) and moderately reactive, although $O_2^{\cdot -}$ is rapidly converted to hydrogen peroxide (H_2O_2) either by superoxide dismutases (SODs) or via non-enzymatic dismutation (Bielski and Allen, 1977). Consequently $O_2^{\cdot -}$ has a relatively shorter migration distance range than $^1O_2$. H_2O_2 is considered the most stable ROS with the longest lifetime and can migrate through the cell (in the micromolar range).

With the exception of $^1O_2$, which is mostly generated at photosystem II (PSII) in thylakoid membranes of chloroplasts, the other three ROS are found across multiple subcellular compartments. Both $O_2^{\cdot -}$ and H_2O_2 are produced in mitochondria, chloroplasts, peroxisomes, and the apoplast. OH· is also produced in all of these compartments, because it derives from H_2O_2 in an Fe^{2+}-dependent reaction. Similarly, the removal of $O_2^{\cdot -}$, H_2O_2, and OH· shares similar mechanisms across different organelles. For example, each of the subcellular compartments contains at least one member of the SOD family for $O_2^{\cdot -}$ detoxification (Alscher *et al.*, 2002). H_2O_2 homeostasis across the different organelles is mainly determined by a core set of ubiquitous redox couples, including ascorbate (DHA/Asc), $NAD(P)^+$/NAD(P)H, and thiol (-SH, sulfhydryl group)-containing couples, such as thioredoxin (Trx), ferredoxin (Fd), and glutathione (GSH/GSSG). The reduction of these redox couples typically passes through a cycling process of thiol relays, with NAD(P)H feeding the cycle with electrons (Mittler *et al.*, 2004). Additionally, H_2O_2 can be detoxified by catalases (Mhamdi *et al.*, 2010). The most reactive OH· is probably scavenged non-enzymatically by nearby sugars and proteins to form less reactive oxidized sugar and protein radicals (Matros *et al.*, 2015; Kumar *et al.*, 2019).

Metabolism is a major ROS source in plants

Energy metabolism in plant cells not only links chloroplasts, mitochondria, and peroxisomes together in the channeling and conversion of carbon-containing compounds, but also generates ROS as by-products in all three organelles (Fig. 1), although in comparison with mammalian cells, chloroplasts and peroxisomes, rather than mitochondria, are considered the major ROS sources in illuminated photosynthetic cells (Noctor *et al.*, 2002). The high rates of electron and energy transfer within the plant energy organelles are crucial drivers that facilitate ROS production both at the thylakoid membrane of chloroplasts during photosynthetic light reactions and at the mitochondrial inner membrane ETC during cellular respiration (see below).

The second driver for ROS overproduction is the key player in carbon-fixing photosynthesis, Rubisco. The evolution of Rubisco as a CO_2-carboxylating enzyme preceded the aforementioned atmospheric oxygenation that occurred over a relatively shorter time scale (Iñiguez *et al.*, 2020). Consequently Rubisco has poor CO_2/O_2 discrimination and frequently incorporates O_2 rather than CO_2 to form 2-phosphoglycolate (2PG) instead of 3-phosphoglycerate (3PGA) in the Calvin–Benson cycle. 2PG can negatively impact on carbon fixation by (i) draining the carbon substrate from the Calvin–Benson cycle, when not recycled; and (ii) more importantly, inhibiting enzyme activities, including those of enzymes that function downstream of Rubisco in the Calvin–Benson cycle, such as triose-phosphate isomerase and sedoheptulose 1,7-bisphosphate phosphatase (Norman and Colman, 1991; Flügel *et al.*, 2017). To minimize these impacts, 2PG is rapidly degraded and ultimately converted back to 3PGA via photorespiration, a light-induced biochemical process that consumes O_2 and releases CO_2 (Hagemann *et al.*, 2016). Photorespiration spans across multiple cellular compartments (chloroplasts, peroxisomes, mitochondria, and cytosol), and ROS in the form of H_2O_2 are produced within the peroxisomes via the action of glycolate oxidase (Fig. 1). In cells of C_3 plants, photorespiration is estimated to account for ~70% of the H_2O_2 production (Noctor *et al.*, 2002). In contrast, in algae, the oxidation of glycolate is catalyzed either by a glycolate oxidase, as in plants, or by a glycolate dehydrogenase, which does not generate H_2O_2. The green microalga *Chlamydomonas reinhardtii* encodes both enzymes in its genome. Glycolate oxidation is performed by the mitochondria-localized glycolate dehydrogenase while the so-called glycolate oxidase is rather a lactate oxidase (Kern *et al.*, 2020).

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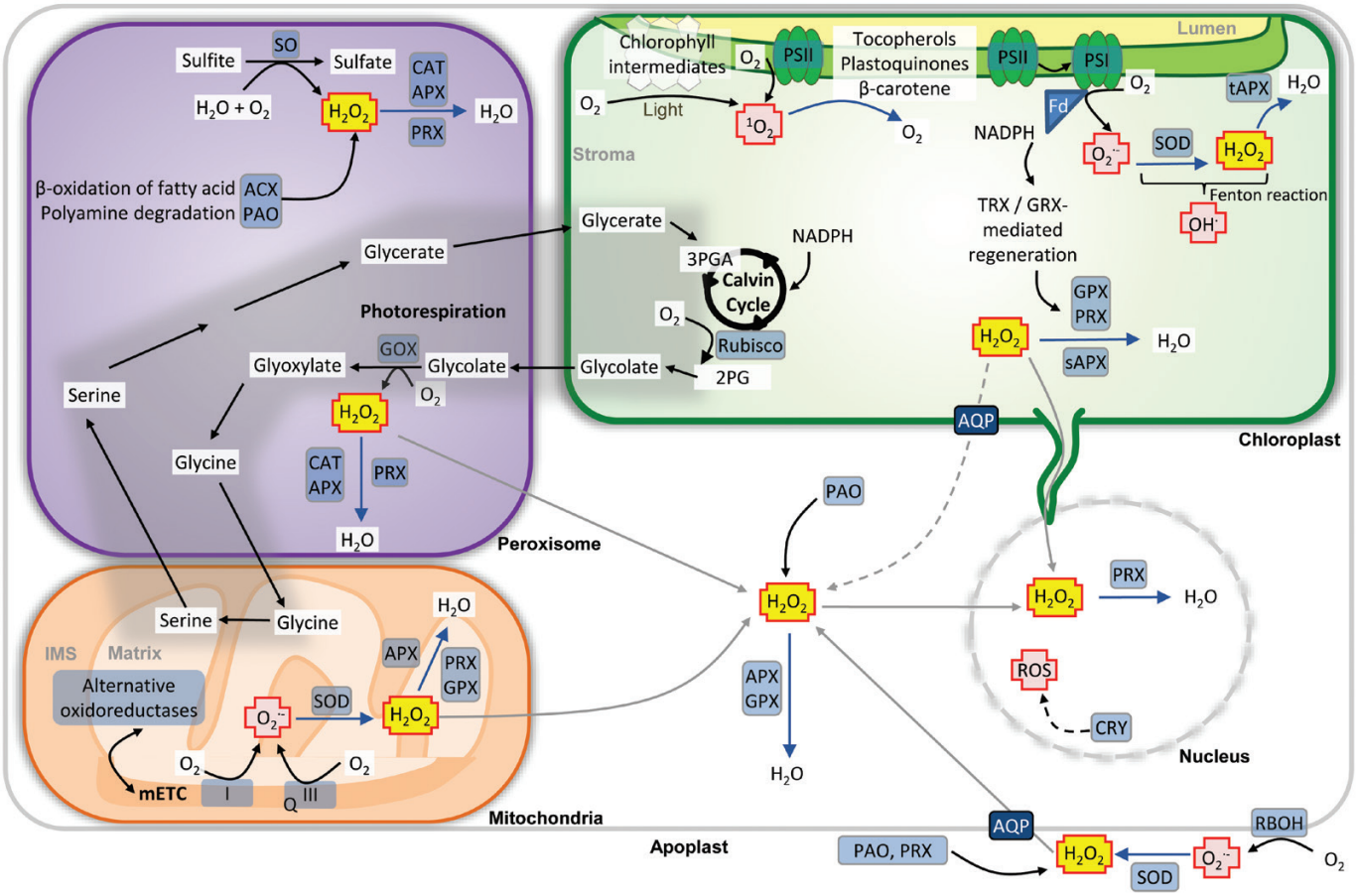


Fig. 1. Summary of the major ROS-producing and -quenching pathways in plant cells. In chloroplasts, the photosynthetic electron transport chain can produce reactive oxygen species (ROS) at both PSII and PSI. At PSII, excited chlorophyll molecules transfer energy to triplet oxygen to produce singlet oxygen ($^1O_2$), which can be quenched by multiple pigment molecules. $^1O_2$ can also be produced when the photosensitizing biosynthetic intermediates of chlorophyll are exposed to light. At PSI, electron leakage to O_2 results in superoxide ($O_2^{\cdot-}$) formation, which is converted to H_2O_2 either via the action of SODs or by spontaneous dismutation. H_2O_2 can attack the iron (Fe) cofactor of SODs to form hydroxyl radicals (OH·)—also known as the Fenton reaction. The incorporation of O_2 rather than CO_2 by Rubisco in chloroplasts leads to induction of the photorespiratory pathway; glycolate from chloroplasts is exported into peroxisomes where its conversion into glyoxylate is a major source of H_2O_2 in this compartment. Other enzymatic processes, such as sulfite and fatty acid oxidation, as well as polyamine degradation, also contribute to peroxisomal H_2O_2 production, Polyamine degradation by polyamine oxidases (PAOs) also produces H_2O_2 in the cytosol. In mitochondria, Complexes I and III of the mitochondrial electron transport chain (mETC) both produce $O_2^{\cdot-}$ which is then converted to H_2O_2. The apoplast is another major source of ROS; here the respiratory burst oxidase homolog (RBOH) proteins catalyze $O_2^{\cdot-}$ production. Similar to the other compartments, SODs are thought to be responsible for conversion of $O_2^{\cdot-}$ to H_2O_2 in the apoplast, with PAOs and peroxiredoxins (PRXs) also contributing to apoplastic H_2O_2 production. Cryptochromes (CRYs) have been implicated in ROS production in the nucleus, although the exact type of ROS being produced is unclear. H_2O_2 movement between subcellular compartments can be facilitated by aquaporins (AQPs). The detoxification of H_2O_2 to water is achieved via similar mechanisms in the different subcellular compartments, with ascorbate peroxidase (APX), glutathione peroxidase (GPX), and/or PRX being the main enzymes involved. GPX and PRX utilize redox-sensitive cysteines in their active site to convert H_2O_2 to water; the oxidized cysteines are regenerated by thioredoxins and glutaredoxins which are themselves regenerated by other reductases using reducing power from PSI. In the peroxisomes, catalase (CAT) is an additional, and indeed the dominant, H_2O_2 detoxification enzyme. In the mitochondria, multiple alternative oxidoreductases, such as alternative oxidase 1a (AOX1a) also contribute to decreasing $O_2^{\cdot-}$ and H_2O_2 production by removing excess electrons from the mETC. All black curved arrows denote electron transfer, straight black arrows indicate enzymatic reactions, gray arrows show movement, blue arrows indicate ROS detoxification steps, and dashed lines denote putative events with components/players yet to be experimentally demonstrated. Other abbreviations: acyl-CoA oxidases (ACX), ferredoxin (Fd), glycolate oxidase (GOX), intermembrane space (IMS), stromal ascorbate peroxidase (sAPX), sulfite oxidase (SO), thylakoidal ascorbate peroxidase (tAPX), ubiquinone/ubiquinol (Q), 3-phosphoglycerate (3-PGA), 2-phosphoglycolate (2-PG). Organelles are not drawn to scale.

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Chloroplastic ROS metabolism

When light intensity exceeds the photosynthetic capacity, photosynthetic organisms trigger different mechanisms to protect their photosynthetic ETC by minimizing production, or increasing the scavenging, of ROS (Müller *et al.*, 2001; Murchie and Ruban, 2020). One prominent set of short-term adaptations, named 'non-photochemical quenching' (NPQ), is associated with PSII (Horton *et al.*, 1996) and include processes such as (i) state transitions (qT), (ii) high energy-dependent quenching (qE), and (iii) photoinhibition (qI).

State transitions are important for the optimization of the energy distribution between PSI and PSII (Ruban and Johnson, 2009; Stirbet *et al.*, 2020). They are regulated by a kinase [state transition 7 (STT7) in *Chlamydomonas* and STN7 in higher plants (Rochaix, 2007)] the activation of which requires binding of plastoquinol to the cytochrome b_6f complex (Wollman, 2001). This kinase phosphorylates light-harvesting complex II (LHCII), and the phosphorylated LHCII disconnects from PSII and becomes a PSI antenna (state 2). When the plastoquinone pool is oxidized, the kinase is inactivated, the LHCII is dephosphorylated by a phosphatase (PPHI/TAP38), and moves back to PSII (state 1) (reviewed by Stirbet *et al.*, 2020). Interestingly, qT is very pronounced in cyanobacteria and algae, but is a weaker response in higher plants where it is not associated with a change in gene expression (Ruban and Johnson, 2009).

qE corresponds to the deactivation of the PSII antenna by heat and is induced by three factors: (i) an acidification of the pH in the lumen; (ii) the xanthophyll cycle that catalyzes the conversion of violaxanthin into antheraxanthin and zeaxanthin; and (iii) a PSII subunit, PsbS (Stirbet *et al.*, 2020). In algae, the qE extent depends on the LHC stress-related (LHCSR) proteins (Peers *et al.*, 2009) and to a lesser extent on PsbS (Correa-Galvis *et al.*, 2016).

qI occurs when the other mechanisms cannot alleviate the light stress. It corresponds to damage to D1, the PSII reaction center, produced by $^1O_2$ at the acceptor side of PSII or the production of long-lived highly oxidizing P680 at the donor site of PSII (Andersson and Aro, 2001).

It is noteworthy that qT, qE, and qI function at different time scales relative to ROS accumulation (reviewed in Dietz, 2015). Energy quenching via qE is activated within seconds of exposure to higher light intensity, and qT functions over a period of minutes thereafter, whereas qI occurs on a time frame of hours. Chloroplastic ROS accumulation has been mostly documented to occur within minutes to hours post-exposure to high light; thus qE and qT most probably prevent ROS accumulation while qI serves to alleviate damage by ROS overaccumulation. This is exemplified by the higher $^1O_2$ accumulation in high light-stressed *npq1*, deficient in the light-dependent violaxanthin to zeaxanthin interconversion component of qE, compared with the wild-type (Dall'Osto *et al.*, 2012).

As mentioned above, $^1O_2$ is commonly produced in the PSII center, when excited chlorophyll molecules react in a triplet state (^{3}Chl) with oxygen (Fischer *et al.*, 2013) rather than when its excess energy is quenched by nearby pigments, such as carotenoids (Fig. 1). $^1O_2$ can also be produced in the presence of biosynthetic intermediates of chlorophyll (protochlorophyllide or protoporphyrin IX), which are photosensitizers, upon light exposure (Wang *et al.*, 2016). Production of $^1O_2$ from chlorophyll intermediates can occur either on thylakoid membranes or in the prolamellar body of proplastids and etioplasts of developing seedlings when first exposed to light after germination in soil (Solymosi and Schoefs, 2010). These chlorophyll intermediates are normally kept at low levels under the tight regulation of chlorophyll production to minimize $^1O_2$ production in wild-type plants. In *Arabidopsis thaliana*, the *flu* mutant deficient in rapid light-responsive conversion of protochlorophyllide to avoid $^1O_2$ accumulation has served as a valuable tool to elucidate $^1O_2$ detoxification and signaling (discussed later). For example, use of *flu* as a conditional tool for light-induced $^1O_2$ accumulation showed that one of the first sites of damage is the grana margin, which is protected by the SAFEGUARD1 protein (L. Wang *et al.*, 2020). $^1O_2$ is also quenched by carotenoids, tocopherols, and plastoquinones at thylakoid membranes close to its production site, where the excess energy of $^1O_2$ is normally absorbed by these molecules to avoid lipid peroxidation and photoinhibition (Krieger-Liszkay and Trebst, 2006; Ramel *et al.*, 2012). Ubiquinol, ascorbate, and glutathione are also known to contribute to $^1O_2$ quenching (Laloi and Havaux, 2015).

Within the chloroplastic ETC, electrons can 'leak' toward oxygen, thereby generating $O_2^{\cdot -}$ (Roach and Krieger-Liszkay, 2014). Most of this electron leakage occurs at the level of PSI, where Fd conducts electrons toward O_2 instead of $NADP^+$, a process known as the Mehler reaction. This electron leak could be beneficial during CO_2-limiting conditions, particularly during environmental stresses, because it provides an alternative sink to sustain electron flow of photosynthesis (Ort and Baker, 2002; Sun *et al.*, 2020). In algae and cyanobacteria, the Mehler reaction functions as an important electron sink involving 10–50% of the photosynthetic electron flux (Badger *et al.*, 2000). However, in higher plants, especially in angiosperms, the Mehler reaction rate is limited from negligibly small to <10% of the linear electron flow (Shirao *et al.*, 2013; Mullineaux *et al.*, 2018). $O_2^{\cdot -}$ is rapidly neutralized by SODs to the less toxic species H_2O_2 (Fig. 1).

In the chloroplasts, iron (Fe)SODs and the membrane-bound copper/zinc (Cu/Zn)SODs are responsible for the conversion of $O_2^{\cdot -}$ to H_2O_2 (Alscher *et al.*, 2002; Pilon *et al.*, 2011), which is subsequently reduced to water by ascorbate and thiol peroxidases. Of the nine ascorbate peroxidases (APXs) in Arabidopsis that use ascorbate as an electron donor to reduce H_2O_2 to H_2O, two localize to the chloroplasts (see review by Maruta *et al.*, 2016 for detailed information on

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chloroplastic APXs). The thylakoid (t)APX isoform serves as a first-line detoxification system to remove H_2O_2 produced at PSI (Fig. 1). In addition, H_2O_2 diffusing throughout the stroma is scavenged by stromal (s)APX (Asada, 2000). Interestingly, although transgenic overexpression of these chloroplastic APXs promotes stress resistance in plants (Yabuta *et al.*, 2002), loss-of-function mutants do not display any severe phenotypes under laboratory settings (Giacomelli *et al.*, 2007; Kangasjärvi *et al.*, 2008; Maruta *et al.*, 2010; Caverzan *et al.*, 2014), highlighting the several layers of partially redundant ROS detoxification mechanisms in chloroplasts. For example, in addition to the glutathione–ascorbate cycle (Foyer and Halliwell, 1976; Asada, 2006), thiol peroxidases assist in the detoxification of chloroplastic H_2O_2. The chloroplasts also possess multiple Trx types, including TRXm, TRXf, TRXx, TRXy, TRXz, and NADPH-dependent TRX reductase C (NTRC), several of which are essential for ROS metabolism (Buchanan, 2016; Nikkanen and Rintamäki, 2019).

Mitochondrial ROS metabolism

Similar to chloroplasts, the mitochondrial (m)ETC generates ROS. During respiration, 1% of the oxygen consumption has been estimated to generate H_2O_2 in plants (Puntarulo *et al.*, 1988). $O_2^{\cdot -}$ radicals can be formed at complex I and complex III of the mETC, when oxygen is only reduced by a single electron (Fig. 1). Superoxides are produced in the flavoprotein region of NADH dehydrogenase (complex 1) in the mitochondrial matrix (Møller, 2001). At the complex III level, fully reduced ubiquinone (QH_2) passes electrons to cytochrome *c*, leaving an unstable ubisemiquinone radical that favors O_2 as an electron acceptor, thus forming $O_2^{\cdot -}$ radicals in both the matrix and the intermembrane space. Enzymes in the matrix could also contribute to $O_2^{\cdot -}$ and H_2O_2 production, either at their flavin site (such as 2-oxoacid dehydrogenase complexes) or at their quinone-binding site (such as mitochondrial 3-phosphate dehydrogenase) (reviewed in Larosa and Remacle, 2018). In the matrix, $O_2^{\cdot -}$ is rapidly neutralized to H_2O_2 by MnSOD and, hence, $O_2^{\cdot -}$ levels in the matrix are probably relatively low. However, it is unclear how $O_2^{\cdot -}$ is detoxified within the intermembrane space in plants. In yeast (Field *et al.*, 2003) and mammals (Iñarrea *et al.*, 2005), Cu/ZnSOD has been shown to localize to the intermembrane space to detoxify $O_2^{\cdot -}$. Given the evolutionarily conserved antioxidant machinery in mitochondria, a Cu/ZnSOD might also play a similar role in plants. In addition, $O_2^{\cdot -}$ might be scavenged by ascorbate (Halliwell and Foyer, 1976; Smirnoff, 2018), which is synthesized in the intermembrane space (Bartoli *et al.*, 2000). Both $O_2^{\cdot -}$ and its dismutation product H_2O_2 have been proposed to diffuse out to the cytosol, where they are subsequently detoxified or act as signaling molecules by altering local cytosolic redox states (Waszczak *et al.*, 2018). Besides the main mETC components, other enzymes also feed electrons to the mETC. Galactono-γ-lactone dehydrogenase (GLDH), in addition to its role in the structural regulation of the mETC complex I (Schimmeyer *et al.*, 2016; Soufari *et al.*, 2020), also catalyzes the last ascorbate biosynthesis step that provides an electron to cytochrome *c* (Bartoli *et al.*, 2000), as such contributing to an over-reduction of the QH_2 pool, and hence increasing the mitochondrial ROS levels.

Mitochondrial H_2O_2 is neutralized to H_2O by thiol and APXs, as well as by associated cycles similar to those in chloroplasts (Fig. 1). In fact, mitochondria and chloroplasts share most of the H_2O_2-scavenging enzymes, including APX, glutathione peroxidase (GPX), peroxiredoxin (PRX) II, monodehydroascorbate reductase (MDHAR), and glutathione reductase (Creissen *et al.*, 1995; Chew *et al.*, 2003; Rouhier *et al.*, 2005; Navrot *et al.*, 2006). In Arabidopsis, the stromal APX is dually targeted to chloroplasts and to the inner membrane facing the intermembrane space of mitochondria (Chew *et al.*, 2003), suggesting that H_2O_2 as well as the APX activity-formed monodehydroascorbate must migrate through the membrane, because the other enzymes of the ascorbate–glutathione cycle are located in the matrix (Edwards *et al.*, 1990; Creissen *et al.*, 1995; Jimenez *et al.*, 1997). In tomato (*Solanum lycopersicum*), however, APX isoforms have been detected both in the intermembrane space and in the matrix (Mittova *et al.*, 2004). Although the exact suborganellar localization has not been completely elucidated in plants yet, it is evident that an ascorbate–glutathione cycle is present in the mitochondria (Møller, 2001; Chew *et al.*, 2003). In contrast to chloroplasts, only GPX and PRXII, but not PRXQ or 2-Cys PRX, occur in mitochondria. Mitochondria also possess an NADP/Trx system which is governed by Trx-o1, Trx-o2, and NTRA, the latter obtaining electrons from NADPH (Laloi *et al.*, 2001; Meyer *et al.*, 2012).

Additional pathways are found in the mitochondria for the indirect regulation of ROS levels. The mETC in plants is highly branched, and extra 'alternative oxidoreductases' contribute to the electron flow, without producing energy (Fig. 1) (Møller, 2001; Rasmusson *et al.*, 2008; Schertl and Braun, 2014). These alternative paths for electron transport allow plants to adjust their metabolism as required (Rasmusson *et al.*, 2008). Type II NAD(P)H dehydrogenases (NDs) introduce electrons to the QH_2 pool, thereby by-passing complex I, and are located on both the internal (ND_{in}) and the external (ND_{ex}) sides of the inner membrane. Because NDs have a relatively low affinity for NAD(P)H compared with complex I, they are believed to act only during NADH excess (Møller *et al.*, 1993). However, because they can oxidize NAD(P)H originating from either the mitochondrial matrix or the cytosol (diffused to the intermembrane space), they play a role in redox homeostasis and signaling, particularly during stress (Rasmusson *et al.*, 2008; Podgórska *et al.*, 2018). In *Chlamydomonas*, the ND_{in} has been characterized: no phenotype is detected when it is inactivated, but when the mutation is combined with a complex I mutation, the phenotype is much more severe than that of a single complex I mutant,

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suggesting that ND_{in} also contributes to the NADH recycling for redox balance in the absence of stress conditions (Lecler *et al.*, 2012). Another alternative path, the alternative oxidase (AOX), bypasses complex III and VI by transferring electrons from the QH_2 pool to O_2, thus preventing over-reduction of the QH_2 pool and ROS production (Umbach *et al.*, 2005). Mutant studies have demonstrated that AOX inhibition of ROS formation is especially important during environmental stress, even when the mETC might not be the primary target of the stress (Umbach *et al.*, 2005; Giraud *et al.*, 2008; Wang *et al.*, 2011; Yoshida *et al.*, 2011; Vishwakarma *et al.*, 2015). The AOX pathway helps to maintain photorespiration in mitochondria by ensuring continuous consumption of NADH generated from the conversion of glycine to serine by complex I, thus contributing to PSII photoprotection in plants (Zhang *et al.*, 2017), and is beneficial in maintaining both mitochondrial and chloroplastic functions during drought stress (Dahal and Vanlerberghe, 2017).

Peroxisomal ROS metabolism

Peroxisomes house several metabolic processes, which directly produce ROS, the main one being the peroxisomal module of photorespiration (Fig. 1). As mentioned above, photorespiration is the process that converts 2PG, produced by the oxygenase activity of Rubisco, to 3PGA, the product of the carboxylase activity of Rubisco. This process extends over three organelles via multiple intermediate compounds, with the first step in the peroxisomes generating H_2O_2 through glycolate oxidase (GOX) activity. Under non-stressed conditions, ~25% of the Rubisco activity results in photorespiration and thereby H_2O_2 production, but greatly increases under CO_2-limiting conditions, for example after stomatal closure during stress. Mutants with perturbed photorespiratory pathways display accelerated photoinhibition phenotypes under excess light conditions, suggesting an important role for photorespiration in preventing photoinhibition under CO_2-limiting conditions (Wingler *et al.*, 2000; Takahashi and Badger, 2011).

Besides GOXs, other enzymes also catalyze reactions that result in H_2O_2 formation in peroxisomes (Fig. 1). In the first β-oxidation step that degrades fatty acids into acetyl-CoA, H_2O_2 is generated through acyl-CoA oxidases. In sulfur metabolism, the conversion of sulfite to sulfate by sulfite oxidase also results in the production of H_2O_2. Normally, H_2O_2 is rapidly converted to water by catalase (see below), but, under conditions of high sulfite levels, catalase is inhibited and the produced H_2O_2 assists in the sulfite detoxification by oxidizing sulfite to sulfate (Hänsch *et al.*, 2006). Two purine metabolism enzymes, xanthine oxidoreductase (XOD) and urate oxidase, are additional ROS sources. XOD can function as either a xanthine oxidase or a xanthine dehydrogenase, producing either $O_2^{\cdot -}$ or H_2O_2, respectively. However, until now, the presence of this enzyme in plant peroxisomes has only been confirmed in watermelon (*Citrullus lanatus*), castor bean (*Ricinus communis*), and pea (*Pisum sativum*) (Sandalio *et al.*, 1988; del Río and Donaldson, 1995; Corpas *et al.*, 2008). In other plants, such as Arabidopsis, only a cytosolic xanthine dehydrogenase isoform has been described (Corpas *et al.*, 2008; Zarepour *et al.*, 2010). Polyamine catabolism by FAD-dependent polyamine oxidases (PAOs) and copper-containing amine oxidases (CuAOs) also contributes to H_2O_2 production in peroxisomes, the cytosol, and apoplast (see reviews by Corpas *et al.*, 2019 and Wang *et al.*, 2019).

In pea, castor bean, and potato (*Solanum tuberosum*) tuber peroxisomes, a second source of $O_2^{\cdot -}$ is provided by the small ETC (del Río *et al.*, 2002; del Río and López-Huertas, 2016). Although the small ETC is embedded in the peroxisomal membrane, ROS are generated outside the membrane. In pea, three peroxisomal membrane polypeptides (PMPs) are responsible for $O_2^{\cdot -}$ production (López-Huertas *et al.*, 1999). Two PMPs (PMP18 and PMP32) use NADH as electron donor, whereas the other (PMP29) uses NADPH. Therefore, this small ETC is believed to be involved in the regeneration of NAD^+ and $NADP^+$ to sustain peroxisomal metabolism (del Río *et al.*, 2002). PMP29 displays cytochrome *c* reductase activity and is the main $O_2^{\cdot -}$ source. PMP18 and PMP32 are recognized as cytochrome *b* and MDHAR, respectively (López-Huertas *et al.*, 1999).

Peroxisomal H_2O_2 is mainly removed by catalase (Fig. 1), but peroxisomal PRX, APX, and the associated ascorbate–glutathione cycle also assist in H_2O_2 removal. However, catalase is considered the dominant enzyme in H_2O_2 removal in peroxisomes, based on the severe stress-sensitive phenotypes in catalase-deficient plants (Mhamdi *et al.*, 2010) that are not observed in peroxisomal APX-deficient plants (Narendra *et al.*, 2006). The high abundance of catalase in peroxisomes and the non-specific action of reductants, such as ascorbate or glutathione, might explain why catalase is more effective in peroxisomal H_2O_2 detoxification, despite its lower affinity for H_2O_2. For the dismutation of $O_2^{\cdot -}$ to H_2O_2, varying numbers and types of SOD isoforms in peroxisomes are in place (Nyathi and Baker, 2006). For example, whereas peroxisomes of watermelon possess both Cu/ZnSOD and MnSOD, Arabidopsis and sunflower (*Helianthus annuus*) peroxisomes possess only a Cu/ZnSOD (Bueno *et al.*, 1995; Corpas *et al.*, 1998; Rodríguez-Serrano *et al.*, 2007; Huang *et al.*, 2012).

Apoplastic ROS production

In contrast to the organellar ROS production, which occurs due to the nature of their resident metabolic pathways, in the apoplast ROS is produced in plants for growth, stomatal closure, and intercellular stress signaling (see reviews by Baxter *et al.*, 2014, Podgórska *et al.*, 2017, Chapman *et al.*, 2019, and Chen and Yang, 2020]. NADPH oxidases (respiratory burst oxidase homologs, RBOHs) are embedded in the plasma membrane and they transfer electrons from the cytoplasmic NADPH to the apoplastic oxygen, forming $O_2^{\cdot -}$ (Fig. 1). Of the 10 RBOH proteins in Arabidopsis, ROS production by RBOHD and RBOHF has been shown to be especially

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important for different processes in leaves, including rapid systemic signaling in response to environmental stresses (Miller *et al.*, 2009; Zandalinas *et al.*, 2020), plant–pathogen responses (Torres *et al.*, 2002; Morales *et al.*, 2016), and stomatal closure (Sierla *et al.*, 2016). RBOH activity is post-translationally regulated by several mechanisms including calcium binding at its EF-hand motif, N-terminal phosphorylation by a plethora of kinases including receptor-like cytoplasmic kinases such as Botrytis-induced kinase 1 (BIK1) (Kadota *et al.*, 2014) and calcium-dependent protein kinases (Dubiella *et al.*, 2013), and C-terminal phosphorylation by plasma membrane-bound receptor-like kinases (Kimura *et al.*, 2020). The superoxide radicals produced by RBOHs are subsequently dismutated to H_2O_2 spontaneously or by an apoplastic SOD. Although the presence of this apoplastic SOD has been proposed based on biochemical assays of apoplast extracts, its genetic identity is still unclear (Waszczak *et al.*, 2018).

In addition to their presence in peroxisomes, PAOs are also localized to the apoplast and thus provide another means for H_2O_2 production in this compartment (Fig. 1). They are responsible for the catabolism of polyamines, such as spermidine and spermine, which simultaneously generate H_2O_2. Interestingly, NADPH oxidases and PAOs form a feed-forward loop to increase ROS levels upon stresses (Gémes *et al.*, 2016). Because the apoplast requires ROS for cell wall loosening and hence growth, the apoplast has evolved to remain in an oxidized state and entails only a limited antioxidant capacity (Müller *et al.*, 2009; Noctor and Foyer, 2016). Indeed, in contrast to other organelles, in which peroxidases play an important role in the H_2O_2 detoxification, peroxidases in the apoplast perform the reverse reaction and are responsible for H_2O_2 and, subsequently OH· formation (Passardi *et al.*, 2005; Daudi *et al.*, 2012). These additional sources of apoplastic ROS are likely to contribute to the same processes that require RBOH-derived ROS, such as the regulation of stomatal closure (Sierla *et al.*, 2016).

H_2O_2 diffusion to the cytosol and nucleus

Cytosolic H_2O_2 originates from the apoplast and the organelles. Although H_2O_2 can diffuse through the membrane, the lipid bilayer forms a poorly permeable barrier (Bienert *et al.*, 2006). Mutant and heterologous expression studies demonstrated that plasma membrane aquaporins can be activated by kinases to assist in the transport of apoplastic H_2O_2 to the cytosol (Grondin *et al.*, 2015; Rodrigues *et al.*, 2017). A similar mechanism has been proposed for chloroplastic H_2O_2 based on chemical inhibition of aquaporins on isolated chloroplasts, although genetic evidence is still lacking (Borisova *et al.*, 2012) (Fig. 1). When H_2O_2 reaches the cytosol, it is rapidly neutralized by APXs and GPXs (Borisova *et al.*, 2012; Gaber *et al.*, 2012), which are subsequently reduced through the ascorbate–glutathione cycle and the Trx/Trx reductase cycle (Mittler *et al.*, 2004; Tripathy and Oelmüller, 2012). Because of its efficient antioxidant capacity, the cytosol has often been perceived as a 'buffering zone' that limits intracellular H_2O_2 movements. Nevertheless, recent studies using the H_2O_2 fluorescent protein sensor roGFP2-Orp1 show that some stimuli, such as pathogen elicitors and the signaling molecule H_2S, induce significant RBOH-derived H_2O_2 accumulation in the cytosol (Scuffi *et al.*, 2018; Nietzel *et al.*, 2019).

ROS production has also been detected in the nucleus. The first evidence to suggest that ROS are actively produced in the plant nucleus was provided by experiments with isolated nuclei in which increased H_2O_2 levels were measured upon treatment with calcium (Ashtamker *et al.*, 2007). Later, cryptochromes were proposed as a ROS source in plant nuclei (Consentino *et al.*, 2015; Jourdan *et al.*, 2015) because of a differential accumulation of nuclear ROS in overexpression and loss-of-function Arabidopsis mutants of cryptochrome 1 (CRY1) and CRY2 (El-Esawi *et al.*, 2017). Nonetheless, the physiological relevance or roles of this ROS source remain to be demonstrated in wild-type plants, and the molecular mechanisms behind the cryptochrome-mediated photoresponses are still being debated (Arthaut *et al.*, 2017; Wang and Lin, 2020). Whereas no nuclear aquaporin has been described in plants, nuclear H_2O_2 can also accumulate through diffusion, presumably via the large nuclear pores. Nuclear H_2O_2 can originate either from the cytosol or from the organelles directly. H_2O_2 transfer from organelles can be facilitated through the physical association of the organelles with the nucleus (Exposito-Rodriguez *et al.*, 2017). Organelles can also form extensions in the form of liquid tubules that could associate with the nucleus, called stromules (chloroplasts), matrixules (mitochondria), and peroxules (peroxisomes) (Noctor and Foyer, 2016). However, experimental evidence is only available for H_2O_2 transport via stromules into nuclei (Caplan Jeffrey *et al.*, 2015) (Fig. 1).

Control of the nuclear redox state is important for plant function. For example, in root cells of glutathione biosynthesis-deficient plants, the more oxidized redox state of their nuclei is accompanied by cell cycle arrest (Schnaubelt *et al.*, 2015). In addition to redox buffers, nuclear H_2O_2 is also enzymatically regulated. Whereas none of the H_2O_2 detoxification enzymes discussed above (e.g. catalase, APX, etc.) is localized to the plant nucleus endogenously (Martins *et al.*, 2018), H_2O_2 in the seed nucleus is neutralized by 1-Cys peroxiredoxins (Pulido *et al.*, 2009), and is recycled via the Trx/Trx reductase cycle. In Arabidopsis, four Trxs and eight Grxs have been assigned a nuclear localization, although they are often associated with the cytosol as well (Delorme-Hinoux *et al.*, 2016). Interestingly, their subcellular location can depend on environmental conditions. For example, GRXS17 resides in the cytosol under standard conditions, but accumulates in the nucleus upon heat treatment (Wu *et al.*, 2012).

ROS metabolism across organelles: some take-home messages

When considering current knowledge on ROS production and processing in the different organelles, some generalized principles are apparent. First, metabolic perturbation leading to

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electron leakage toward molecular oxygen (both enzymatically and non-enzymatically) represents a shared mechanism for ROS production in chloroplasts, mitochondria, and peroxisomes. These organelles coincidentally contribute to the bulk of ROS accumulation in plant cells during abiotic stress. Second, the different organelles utilize similar ROS detoxification strategies entailing ROS-quenching metabolites and multigene enzyme families. Intriguingly, organelle-specific differences remain in the types of enzyme(s) and metabolite(s) involved in both the production and detoxification of different ROS. Whether these differences are the result of specializations due to the unique biochemical environment of each organelle, or are simply outcomes of random gene evolution, would be interesting to explore. Further insights could come from systematically studying the conservation and/or diversification of these organellar ROS metabolism pathways across the evolutionary lineages.

In contrast to the organelles mentioned above, ROS production at the apoplast is initiated and controlled by the cell for stress responses rather than as consequences of metabolic perturbations (Kimura *et al.*, 2020; Zandalinas *et al.*, 2020). This difference between the apoplast and other organelles sets the scene of the dual nature of ROS as both harmful and useful metabolites, depending on their subcellular and metabolic context (Mittler, 2017). Collectively, these observations hint at the complex nature of evolution and specialization of ROS metabolism in plant cells.

ROS-induced organellar signaling

ROS accumulation is tuned to environmental stimuli that induce large-scale cellular responses, including transcriptomic reprogramming and metabolic adjustments. Different types and organellar origins of ROS can trigger specific nuclear transcriptomic responses as shown by genetic analysis of individual signaling pathways (De Clercq *et al.*, 2013; Dogra *et al.*, 2017) as well as a meta-analysis of transcriptome data from wild-type and mutant plants exposed to exogenous ROS or ROS-inducing treatments (Willems *et al.*, 2016). For example, PSII-derived $^1O_2$ induces a distinct subset of nuclear genes involved in the regulation of cell death, jasmonic acid signaling, and PSII repair, collectively termed singlet oxygen-responsive genes (SORGs; Chan *et al.*, 2016b), with two different $^1O_2$-responsive signaling pathways responsible for regulation of different SORG subsets depending on the severity of the imposed stress (further discussed below; Kim *et al.*, 2012; Ramel *et al.*, 2012; Dogra *et al.*, 2017). Another class of nuclear genes termed plastid redox-associated nuclear genes (PRANGs) comprise various stress-associated genes including antioxidant enzymes and respond primarily to PSI H_2O_2-associated signaling (Chan *et al.*, 2016b). Similarly, mitochondrial ROS-inducing treatments induce a distinct class of genes termed mitochondrial dysfunction stimulon (MDS) genes whose functions include mitochondrial ROS homeostasis (De Clercq *et al.*, 2013). Yet organellar ROS responses are not limited to the organelle in which ROS are generated: as further discussed below, chloroplast ROS-associated signals can have far-ranging impacts including on cytosolic ROS detoxification, endoplasmic reticulum (ER) protein homeostasis, and cell death.

With most of the ROS production occurring in the organelles and with the exception of the relatively longer lived H_2O_2, their short half-lives preclude ROS from being direct stress signals that travel (long distances) from their production sites to elicit stress-responsive gene expression induction in the nucleus. Although H_2O_2 can be transferred directly from the chloroplasts to the nucleus (Exposito-Rodriguez *et al.*, 2017), this mechanism on its own does not confer sufficient specificity, because the organellar origin of the nuclear H_2O_2 cannot be distinguished. Yet, the nuclear transcriptomic responses to peroxisomal and chloroplastic H_2O_2 are clearly distinct (Sewelam *et al.*, 2014), indicating organelle-specific ROS-induced signaling pathways and/or ROS-independent mechanisms. In the past decades, several signaling pathways emanating from organelles have been shown to be crucial for the adjustment and/or repair of the organelles during environmental stress. These pathways are termed organellar 'operational control' retrograde signaling [first described by Pogson *et al.* (2008); reviewed in Dietz *et al.*, 2016; Leister, 2017]. These signaling pathways share the common trait of being responsive to, or associated with, ROS.

Chloroplastic ROS-induced retrograde signaling

As described above, $^1O_2$ is commonly produced at PSII and quenched by pigments, including β-carotene bound to the reaction centers of photosystems embedded in the thylakoid membrane. β-Carotene itself is oxidized by $^1O_2$, giving rise to various cleavage products including β-cyclocitral (β-CC) and dihydroactinidiolide (Ramel *et al.*, 2012), which act as chloroplast retrograde signals during excess light stress (Fig. 2). Upon production, β-CC can induce $^1O_2$-specific stress responses, such as the up-regulation of the glutathione transferase class tau 5 (GSTU5) and mitogen-activated protein kinase kinase kinase 18 (MAPKKK18) (Ramel *et al.*, 2012). The β-CC signaling cascade proceeds at least partially via the RNA-binding protein methylene blue sensitivity 1 (MBS1) (Shumbe *et al.*, 2017) and intersects with various stress pathways, such as the xenobiotic detoxification response (D'Alessandro *et al.*, 2018) and the salicylic acid (SA) signaling (Lv *et al.*, 2015) for photooxidative stress acclimation. Interestingly, β-CC rapidly dissolves into β-cyclocitric acid (β-CCA), which only partially overlaps with the former in terms of regulated genes (Fig. 2), suggesting distinct functions for these two closely related metabolites (D'Alessandro *et al.*, 2019). Although multiple downstream effectors of β-CC have been characterized, direct sensor(s)/receptor(s) of β-CC are yet to be identified.

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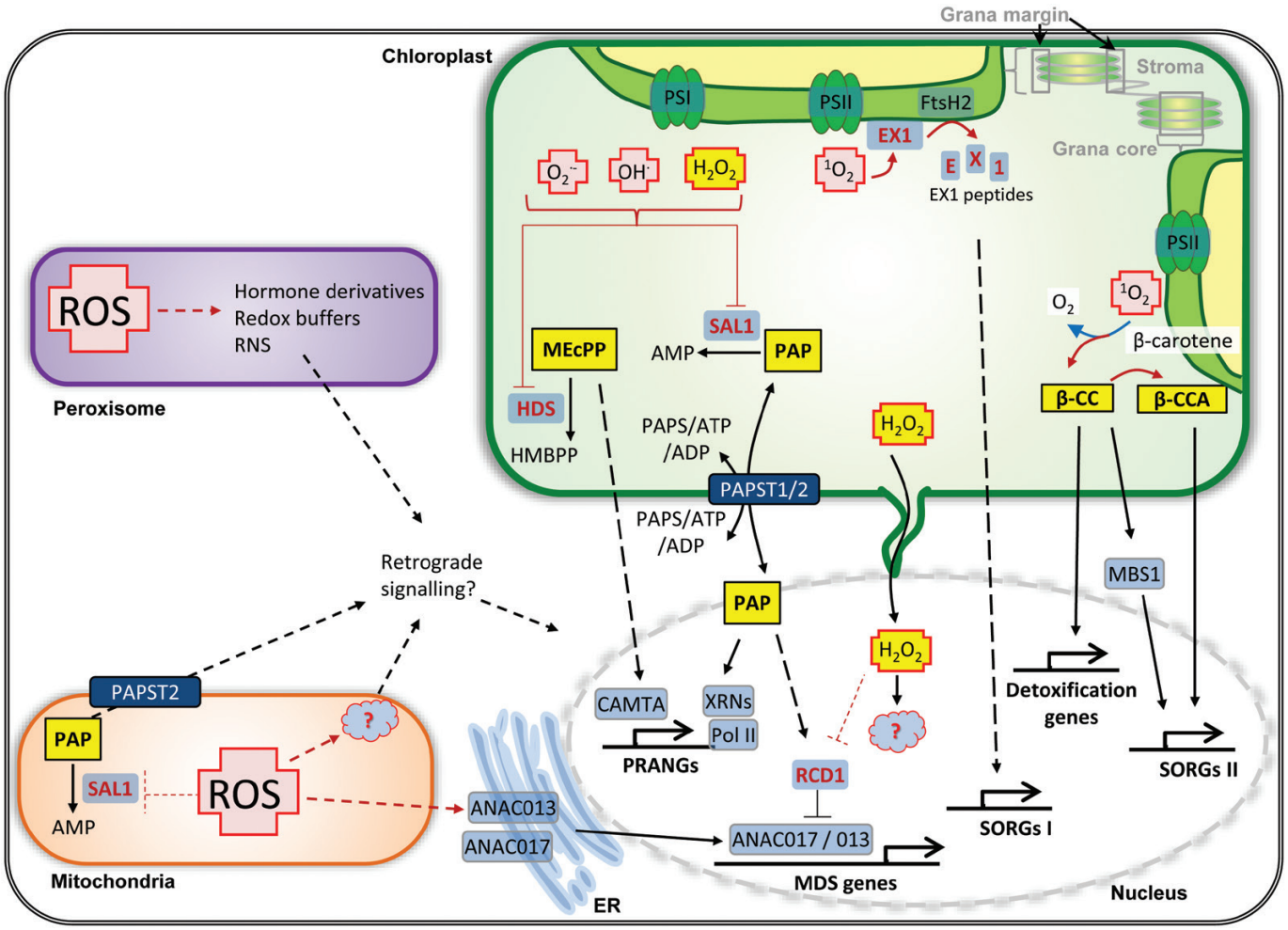


Fig. 2. ROS-induced organelle to nucleus retrograde signaling pathways. In the chloroplast, both PSII- and PSI-induced ROS are capable of activating different chloroplast to nucleus retrograde signals. Singlet oxygen ($^1O_2$) production at the grana margins leads to oxidation of the Executer 1 (EX1) protein, which is then cleaved by the thylakoidal FtSH2 protease. An unknown EX1 peptide fragment then initiates signaling to the nucleus to activate expression of a subset of singlet oxygen-responsive genes (SORGs I) associated with regulation of cell death. Conversely, $^1O_2$ production by the PSII reaction center at the grana core is sensed via oxidative cleavage of β-carotene to produce β-cyclocitral (β-CC) which is a volatile retrograde signal. β-CC can be rapidly converted to β-cyclocitric acid (β-CCA). Both β-CC and β-CCA regulate the expression of a second subset of SORGs (SORGs II), which is distinct from those regulated by EX1. The regulation of SORGs II by β-CC is dependent on the cytosolic protein methylene blue sensitive 1 (MBS1), whereas β-CCA functions independently of MBS1. β-CC also activates the expression of genes involved in xenobiotic detoxification responses. ROS produced from PSI are thought to decrease the activities of 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS) and SAL1 via redox regulation, thereby enabling accumulation of their respective substrates 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (MEcPP) and 3′-phosphoadenosine 5′-phosphate (PAP). Both of these metabolites function as retrograde signals which target a broad class of genes independent of $^1O_2$ but otherwise responsive to oxidative stress in chloroplasts termed plastid redox-associated nuclear genes (PRANGs). MEcPP-mediated signaling proceeds in the nucleus at least in part via the CAMTA transcription factor, but the mechanisms for intracellular transport and nuclear perception of MEcPP are still unknown. PAP transport between the chloroplast and the cytosol is mediated by the transporters PAPS/PAP transporter 1 (PAPST1) and PAPST2. In the nucleus, PAP binds to exoribonuclease enzymes (XRNs), which alters RNA polymerase II (Pol II) activity, thereby enabling up-regulation of a large number of PRANGs. Direct transfer of H_2O_2 from chloroplasts to the nucleus has been observed and is proposed to be a signaling mechanism, but nuclear receptors for H_2O_2 are currently unknown. ROS overproduction in mitochondria leads to activation of an unknown protease which releases the ANAC017/ANAC013 transcription factors from their ER tethering. The truncated ANAC017/ANAC013 proteins translocate to the nucleus, where they activate expression of mitochondrial dysfunction stimulon (MDS) genes including alternative oxidase 1a (AOX1a). This mitochondrial signaling pathway is normally suppressed in the nucleus by radical-induced cell death 1 (RCD1), but activated during mitochondrial stress due to redox-mediated inactivation of RCD1 (possibly by nuclear H_2O_2). Another proposed regulator for this mitochondrial pathway is PAP, although the mechanism is still unclear. Mitochondria also contain PAP and SAL1, but it is not clear if mitochondrial SAL1 is similarly redox regulated to enable PAP to function as a mitochondrial retrograde signal. Peroxisomes are sources of various potential signaling molecules, but their connection to peroxisomal ROS and hypothetical retrograde signaling from peroxisomes is still not clear. Red arrows/lines denote ROS-induced signaling events, blue arrow indicates ROS detoxification step, dashed lines denote putative events with components/players yet to be experimentally demonstrated, red fonts denote ROS sensors, and yellow boxes highlight signaling molecules. Other abbreviations: 4-hydroxyl-3-methyl-butenyl 1-diphosphate (HMBPP), 3′-phosphoadenosine 5′-phosphosulfate (PAPS), reactive nitrogen species (RNS). Organelles are not drawn to scale.

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Recently another $^1O_2$-specific stress sensor located on the thylakoid membrane of chloroplasts has been characterized, namely the EXECUTER 1 (EX1) protein (Wang *et al.*, 2016; Dogra *et al.*, 2019). EX1 is associated with PSII in the grana margin (Fig. 2) and can undergo a post-translational modification (PTM) by $^1O_2$ at the Trp643 residue of its singlet oxygen sensor (SOS) domain. This PTM step is critical for initiating EX1 degradation by the thylakoid membrane-bound FtsH2 metalloprotease, in which the protease domain faces the stromal side of thylakoids (Kato and Sakamoto, 2009) for activation of downstream $^1O_2$ signaling (Dogra *et al.*, 2017, 2019). It is possible that the as yet unidentified degradation product(s) of EX1 act(s) as a retrograde signal that triggers $^1O_2$-responsive gene activation in the nucleus, although experimental validation is still required. Although signals derived both from β-CC and EX1-FtsH2 are triggered by $^1O_2$, they originate from the grana core and margin, respectively. This spatial difference of $^1O_2$ generation may confer specificity in the cellular response, because β-CC and EX1 activate different sets of $^1O_2$-responsive genes in the nucleus, with only a minimal overlap between the gene sets (Dogra *et al.*, 2017). An alternative explanation is provided by the environmental context, since β-CC is associated with cell death-promoting high-light stress conditions while EX1-mediated signaling can occur at lower light intensities (Kim *et al.*, 2012).

Methylerythritol cyclodiphosphate (MEcPP; also abbreviated as MEcDP in some literature) is another chloroplastic stress retrograde signal that accumulates during wounding and high-light stresses (Xiao *et al.*, 2012). MEcPP is an intermediate metabolite of the methylerythritol phosphate (MEP) pathway for isoprenoid biosynthesis in plastids (Fig. 2). MEcPP accumulation is probably responsive to ROS, because the MEcPP-degrading enzyme 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS) contains a redox-sensitive iron–sulfur cluster at its active site (Seemann *et al.*, 2005) and bacterial HDS is known to be redox regulated (Ostrovsky *et al.*, 1998). Extensive work on the *hds* mutant shows that MEcPP activates various stress pathways via the nuclear transcription factor CAMTA3, including plastidial radical scavenging and the ER-unfolded protein response (Benn *et al.*, 2016). Overaccumulated MEcPP also impacts on growth-regulating pathways (de Souza *et al.*, 2017; Wang *et al.*, 2017; Jiang *et al.*, 2018, 2020). Intriguingly, quantification of MEcPP accumulation in isolated chloroplasts versus whole-leaf extracts of oxidatively stressed spinach (*Spinacia oleracea*) revealed that the bulk of the MEcPP remains in the chloroplasts (Rivasseau *et al.*, 2009). If this predominantly chloroplast-localized accumulation of MEcPP were evolutionarily conserved across plants, it could suggest that either (i) low-abundance MEcPP in the cytosol is rapidly and sensitively sensed by high-affinity receptor(s); or (ii) MEcPP is actually perceived within chloroplasts and its information of chloroplast stress relayed to the nucleus by unknown component(s). Examining these hypothetical possibilities could provide novel insights into this pathway.

Drought and high-light stresses both induce accumulation of the chloroplast retrograde signal 3′-phosphoadenosine-5′-phosphate (PAP) via oxidative inactivation of its catabolic enzyme SAL1 (Fig. 2) (Estavillo *et al.*, 2011; Chan *et al.*, 2016a). Interestingly, although PAP degradation by the redox-sensitive SAL1 may occur in both chloroplasts and mitochondria due to the dual localization of SAL1, PAP synthesis is thought to occur primarily in the cytosol and Golgi, based on the predicted localization of its biosynthetic enzymes, the sulfotransferases (SOTs). The SOTs transfer a sulfonyl group from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to other acceptor molecules, such as desulfoglucosinolates, thereby releasing PAP as a by-product (Chan *et al.*, 2019). The high polarity of PAP prevents it from crossing the organelle outer membrane by diffusion. Rather, PAP traverses the organellar membrane via two PAP transporters in Arabidopsis, namely the PAPS/PAP transporter 1 (PAPST1) that is localized to the plastid envelope membrane and thylakoids (Gigolashvili *et al.*, 2012), and PAPST2 in the plastidial and/or mitochondrial membranes, in a tissue-specific manner (Ashykhmina *et al.*, 2019). Both transporters are antiporters that can exchange any two of either PAPS, PAP, ATP, or ADP across the organelle membrane. PAPSTs probably play crucial roles in regulating the PAP-mediated signaling, because the PAP biosynthesis sites are uncoupled from those of its degradation. How these transporters fit into the PAP signaling framework is not fully understood (Chan *et al.*, 2019). The PAP movement from the cytosol into the nucleus is thought to proceed via passive diffusion (Estavillo *et al.*, 2011). In the nucleus, PAP inhibits the activity of 5′–3′ exoribonucleases (XRNs), which leads to altered RNA polymerase II activity, thereby inducing the up-regulation of many stress-responsive genes, including chloroplast- and cytosol-targeted APXs (Estavillo *et al.*, 2011; Crisp *et al.*, 2018). Interestingly, PAP also affects ROS metabolism at the apoplast of guard cells, most probably by influencing the activity of NADPH oxidases (Pornsiriwong *et al.*, 2017).

Are there other ROS-responsive/dependent chloroplast signals, whether protein or metabolite based? There is increasing evidence that some chloroplast-localized proteins can be directly translocated from the chloroplasts to the nucleus (Krupinska *et al.*, 2020). In response to the *Tobacco mosaic virus* effector protein p50, ROS accumulate in chloroplasts. Concurrently, the chloroplastic defense protein N receptor-interacting protein 1 (NRIP1) recognizes p50 and is translocated with p50 to the nucleus, possibly via stromules (Caplan Jeffrey *et al.*, 2015). In another example, plants expressing a tagged version of the DNA-binding protein WHIRLY1 specifically in the chloroplast genome, thus ruling out dual targeting of cytosolically translated WHIRLY1, also show plastidial WHIRLY1 translocation to the nucleus and coincided with the increased expression of pathogenesis-related genes, consistent with their promoters as targets for WHIRLY1 binding. WHIRLY1 translocation

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has been proposed to be regulated by changes in the chloroplast redox state, for example during pathogen infection (Foyer *et al.*, 2014) when ROS also overaccumulate (Caplan Jeffrey *et al.*, 2015). It will be important to fully elucidate the mechanism(s) by which ROS regulate the translocation of these proteins, given their crucial roles.

Similarly to how ROS can react with β-carotene to produce electrophilic β-CC, ROS can also react with phospholipids—key components of cellular and organellar membranes—to form lipid peroxides and/or oxylipins (Farmer and Mueller, 2013; Mano *et al.*, 2019). Lipid peroxides contribute to a variety of reactive electrophile species (RES) or reactive carbonyl species (RCS) that can further react with proteins and covalently modify them (Winger *et al.*, 2005, 2007). Lipid peroxides and their derivatives can trigger various physiological responses in plants and algae, including alteration in redox homeostasis, activation of programmed cell death, and changes in gene expression in the nucleus (Biswas and Mano, 2015; Roach *et al.*, 2018). Interestingly, treatment of plants and/or algae with moderate level of oxylipins/RES/RCS improved their tolerance toward stresses (e.g. light and heat) (Roach *et al.*, 2018; Monte *et al.*, 2020). Consequently, there is an increasing support for oxylipins/RES/RCS to be also considered as players in ROS/organelle retrograde signaling; however, conclusive evidence is still required (Farmer and Mueller, 2013; Alché, 2019; Mano *et al.*, 2019; Muñoz and Munné-Bosch, 2020).

Mitochondrial ROS-induced signaling

In contrast to chloroplast ROS-induced signaling pathways, which are fairly well characterized, detailed knowledge on equivalent mitochondrial pathways remains sparse. Only one major pathway has been elucidated to date, involving the Arabidopsis NAC domain-containing protein 13 (ANAC013) (De Clercq *et al.*, 2013), ANAC017 (Ng *et al.*, 2013; Meng *et al.*, 2019), and radical-induced cell death 1 (RCD1) (Shapiguzov *et al.*, 2019). Perturbations of the mETC lead to translocation of the ER-tethered ANAC013 and ANAC017 transcription factors to the nucleus via the action of unidentified proteases (Fig. 2). In the nucleus, ANAC013 and ANAC017 regulate the expression of MDS genes, which encode, among others, *AOX1a* that is crucial for mitochondrial ROS detoxification (reviewed in Waszczak *et al.*, 2018; Dourmap *et al.*, 2020; Mielecki *et al.*, 2020; Y. Wang *et al.*, 2020). RCD1 is a redox-sensitive WWE domain-containing nuclear protein. Under unstressed conditions, monomeric RCD1 physically interacts with and sequesters ANAC013/ANAC017. Under stress, ROS-induced oligomerization of RCD1 releases this inhibition, allowing ANAC013/ANAC017 to regulate the MDS gene expression (Shapiguzov *et al.*, 2019).

Interestingly, RCD1–ANAC013/017 were proposed to intersect with SAL1–PAP-mediated chloroplast retrograde signaling based on similarities in MDS gene expression and oxidative stress phenotypes of the respective mutants (Van Aken and Pogson, 2017; Shapiguzov *et al.*, 2019). Although the exact molecular mechanism behind this interaction remains a mystery, these findings suggest that chloroplast and mitochondrial retrograde signaling converge in the nucleus (Y. Wang *et al.*, 2020). One possible purpose of such convergence may be the coordination of transcriptional changes to fine-tune the overall cellular energy metabolism by readjusting the capacity of both organelles according to environmental cues.

Peroxisomal ROS-induced signaling

The existence of peroxisome-derived retrograde signaling has been proposed previously (Foyer and Noctor, 2003). This hypothesis is plausible because peroxisomes are the sources of various important signaling molecules, including β-oxidation-derived molecules, such as some hormone (jasmonic acid, auxin, and SA) derivatives, ROS, reactive nitrogen species (RNS), and components of redox buffers that convey the change in the peroxisomal redox state (Baker *et al.*, 2006; Nyathi and Baker, 2006). Peroxisomal ROS trigger specific transcriptional changes in the nucleus (Sewelam *et al.*, 2014). Reported changes in the peroxisomes under oxidative stress include altered morphology (shape and size), mobility, and numbers (biogenesis versus pexophagy/turnover) (Wang *et al.*, 2015; Jaipargas *et al.*, 2016; Rodríguez-Serrano *et al.*, 2016; Su *et al.*, 2019; Pan *et al.*, 2020; Sandalio *et al.*, 2021). These features hint at the possibility of peroxisomal ROS signaling, leading to changes in the nuclear gene expression that readjust peroxisome properties for acclimation, corresponding to the definition of retrograde signaling (Chan *et al.*, 2016b). However, no ROS-induced peroxisome to nucleus retrograde signaling pathway has been described to date (Fig. 2).

Whereas knowledge of peroxisomal ROS signaling is still fragmentary, in the past decade loss-of-function studies with the peroxisomal *catalase 2* (*cat2*) mutant have revealed candidate pathways and components. Conditional overproduction of peroxisomal ROS and enhanced cell death in *cat2* are associated with altered glutathione redox state and activation of the hypersensitive response via SA (Queval *et al.*, 2007; Chaouch *et al.*, 2010). Forward and reverse genetic screens for suppressors of the *cat2* photorespiratory ROS-related phenotypes also identified crucial roles for hormonal signaling and various transcription factors (Kaurilind *et al.*, 2015; Kerchev *et al.*, 2016; Waszczak *et al.*, 2016). Interestingly, the different processes mentioned above, albeit merely correlative, have also been linked with different chloroplast retrograde signaling pathways; for example, SA with β-CC signaling, auxin with both MEcPP and PAP, and glutathione redox state with PAP (Lv *et al.*, 2015; Jiang *et al.*, 2018; Phua *et al.*, 2018). The biggest challenge remains to identify the actual peroxisomal retrograde signal(s), if they exist, and elucidate the pathways in which they function. As part of this process, a starting point could be to examine the extent to which peroxisomal ROS diffuses into the cytosol and nuclei to affect redox couples and/or signaling proteins directly.

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Perspectives

In the past decade, although our understanding of ROS metabolism and signaling in different plant organelles has progressed, several questions remain. First, do the different retrograde signals function in concert with, independently of, or even antagonistically to, each other? Second, how do these signals intersect with the nuclear H_2O_2 perception directly transferred from chloroplasts? To address this question, the direct impact of the chloroplast to nucleus H_2O_2 transfer on the nuclear transcriptome needs to be determined as well. Third, which are the genes encoding the missing components in the organellar ROS signaling, including potential ROS/redox sensors in mitochondria and peroxisomes? In the specific case of ANAC013/017-mediated mitochondrial signaling, an important related question is how is mitochondrial ROS perception transduced to the ER, where the ANAC proteins reside. Fourth, are the different organelle ROS signaling networks interconnected in the same manner as the metabolic link between chloroplasts, mitochondria, and peroxisomes, and, if so, which are the missing components?

Each of these questions could be partially addressed by identifying signaling proteins responsive to ROS from the different organelles. Recent technological advancements enabled *in vivo* trapping and identification of oxidized cysteines in different subcellular compartments upon oxidative stresses in cultured Arabidopsis cells (De Smet *et al.*, 2019; Huang *et al.*, 2019; Wei *et al.*, 2020). These approaches revealed potentially crucial roles for ROS-mediated cysteine oxidation in RNA processing and amino acid metabolism (De Smet *et al.*, 2019; Huang *et al.*, 2019) and provide a complementary strategy to the conventional biochemical characterization of signaling proteins identified from genetic screens (Lee *et al.*, 2007; Estavillo *et al.*, 2011; Chan *et al.*, 2016a; Dogra *et al.*, 2019). Nevertheless, a major technical challenge lies in the improvement of the sensitivity of such cysteine-trapping approaches to capture novel organellar retrograde redox sensors/signals. Transfer of the trapping technology from cultured cells to intact plants will also enhance the physiological relevance of any identified ROS sensor(s).

Another possible avenue for future breakthroughs in ROS signaling is to break down the complexity of the studied biological system. A plant leaf is composed of several highly specialized cell types that can have diverse ROS signaling pathways, as evidenced by the contribution of apoplastic ROS production specifically by vascular cells, but not other cell types, to systemic stress signaling (Zandalinas *et al.*, 2020). It is noteworthy that transcriptomics of isolated single cells from a complex organ is now feasible for Arabidopsis (Wendrich *et al.*, 2020). Complementary approaches such as using cultured Arabidopsis cells derived from a single tissue type (Van Leene *et al.*, 2007) and/or shifting completely to a unicellular model organism that houses both chloroplasts and mitochondria, such as *C. reinhardtii*, could help identify missing ROS and organellar retrograde signaling pathways that are 'diluted' by whole-leaf analyses. For instance, Shao *et al.* (2013) successfully utilized *C. reinhardtii* to detect new players in ROS signaling in Arabidopsis, but in other cases the identified proteins had been lost in higher plants (Fischer *et al.*, 2012; Wakao *et al.*, 2014). As homologous proteins for the Arabidopsis organellar signaling proteins SAL1, HDS1, and EX1 are conserved in *C. reinhardtii*, it may be interesting to assess the extent to which the principles and components of ROS signaling, studied extensively in Arabidopsis, are actually maintained across the green lineage. In that respect, mutants affected in NPQ represent interesting tools to understand the response to light stress and signaling from the chloroplast. The photosynthetic response of mutants affected in qT (*lhcsr* mutants), qE (*stt7* mutants), or both pathways to various light conditions (high light or fluctuating light) has been characterized in detail (e.g. Peers *et al.*, 2009; Allorent *et al.*, 2013; Girolomoni *et al.*, 2019). Although the retrograde signaling leading to changes in the *LHCSR3* expression has not been elucidated yet, the involvement of chloroplastic Ca^{2+} variations and redox imbalance has been proposed (reviewed in Rea *et al.*, 2018). In addition, the availability of respiratory-deficient mutants (Salinas *et al.*, 2014), and the existence of double mutants affected in both respiration and photosynthetic activity (Cardol *et al.*, 2009; Massoz *et al.*, 2017; Larosa *et al.*, 2018) could help decipher retrograde signaling from mitochondria and its connection with the chloroplast.

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Conflict of interest

The authors declare no conflict of interest.

References

Alché JdD. 2019. A concise appraisal of lipid oxidation and lipoxidation in higher plants. Redox Biology **23**, 101136.

Allorent G, Tokutsu R, Roach T, *et al.* 2013. A dual strategy to cope with high light in *Chlamydomonas reinhardtii*. The Plant Cell **25**, 545–557.

Alscher RG, Erturk N, Heath LS. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Journal of Experimental Botany **53**, 1331–1341.

Andersson B, Aro E-M. 2001. Photodamage and D1 protein turnover in photosystem II. In: Aro E-M, Andersson B, eds. Regulation of photosynthesis. Dordrecht: Springer Netherlands, 377–393.

Arthaut LD, Jourdan N, Mteyrek A, *et al.* 2017. Blue-light induced accumulation of reactive oxygen species is a consequence of the *Drosophila* cryptochrome photocycle. PLoS One **12**, e0171836.

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Asada K. 2000. The water–water cycle as alternative photon and electron sinks. Philosophical Transactions of the Royal Society B: Biological Sciences **355**, 1419–1431.

Asada K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiology **141**, 391–396.

Ashtamker C, Kiss V, Sagi M, Davydov O, Fluhr R. 2007. Diverse subcellular locations of cryptogein-induced reactive oxygen species production in tobacco Bright Yellow-2 cells. Plant Physiology **143**, 1817–1826.

Ashykhmina N, Lorenz M, Frerigmann H, Koprivova A, Hofsetz E, Stührwohldt N, Flügge UI, Haferkamp I, Kopriva S, Gigolashvili T. 2019. PAPST2 plays critical roles in removing the stress signaling molecule 3′-phosphoadenosine 5′-phosphate from the cytosol and its subsequent degradation in plastids and mitochondria. The Plant Cell **31**, 231–249.

Badger MR, von Caemmerer S, Ruuska S, Nakano H. 2000. Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and rubisco oxygenase. Philosophical Transactions of the Royal Society B: Biological Sciences **355**, 1433–1446.

Baker A, Graham IA, Holdsworth M, Smith SM, Theodoulou FL. 2006. Chewing the fat: beta-oxidation in signalling and development. Trends in Plant Science **11**, 124–132.

Bartoli CG, Pastori GM, Foyer CH. 2000. Ascorbate biosynthesis in mitochondria is linked to the electron transport chain between complexes III and IV. Plant Physiology **123**, 335–344.

Baxter A, Mittler R, Suzuki N. 2014. ROS as key players in plant stress signalling. Journal of Experimental Botany **65**, 1229–1240.

Benn G, Bjornson M, Ke H, De Souza A, Balmond EI, Shaw JT, Dehesh K. 2016. Plastidial metabolite MEcPP induces a transcriptionally centered stress-response hub via the transcription factor CAMTA3. Proceedings of the National Academy of Sciences, USA **113**, 8855–8860.

Bielski BHJ, Allen AO. 1977. Mechanism of the disproportionation of superoxide radicals. Journal of Physical Chemistry **81**, 1048–1050.

Bienert GP, Schjoerring JK, Jahn TP. 2006. Membrane transport of hydrogen peroxide. Biochimica et Biophysica Acta **1758**, 994–1003.

Biswas MS, Mano J. 2015. Lipid peroxide-derived short-chain carbonyls mediate hydrogen peroxide-induced and salt-induced programmed cell death in plants. Plant Physiology **168**, 885–898.

Borisova MM, Kozuleva MA, Rudenko NN, Naydov IA, Klenina IB, Ivanov BN. 2012. Photosynthetic electron flow to oxygen and diffusion of hydrogen peroxide through the chloroplast envelope via aquaporins. Biochimica et Biophysica Acta **1817**, 1314–1321.

Buchanan BB. 2016. The path to thioredoxin and redox regulation in chloroplasts. Annual Review of Plant Biology **67**, 1–24.

Bueno P, Varela J, Gimeénez-Gallego G, del Río LA. 1995. Peroxisomal copper, zinc superoxide dismutase. Characterization of the isoenzyme from watermelon cotyledons. Plant Physiology **108**, 1151–1160.

Caplan Jeffrey L, Kumar Amutha S, Park E, Padmanabhan Meenu S, Hoban K, M odla S, Czymmek K, Dinesh-Kumar Savithramma P. 2015. Chloroplast stromules function during innate immunity. Developmental Cell **34**, 45–57.

Cardol P, Alric J, Girard-Bascou J, Franck F, Wollman FA, Finazzi G. 2009. Impaired respiration discloses the physiological significance of state transitions in Chlamydomonas. Proceedings of the National Academy of Sciences, USA **106**, 15979–15984.

Caverzan A, Bonifacio A, Carvalho FE, *et al.* 2014. The knockdown of chloroplastic ascorbate peroxidases reveals its regulatory role in the photosynthesis and protection under photo-oxidative stress in rice. Plant Science **214**, 74–87.

Chan KX, Mabbitt PD, Phua SY, *et al.* 2016*a*. Sensing and signaling of oxidative stress in chloroplasts by inactivation of the SAL1 phosphoadenosine phosphatase. Proceedings of the National Academy of Sciences, USA **113**, E4567–E4576.

Chan KX, Phua SY, Crisp P, McQuinn R, Pogson BJ. 2016*b*. Learning the languages of the chloroplast: retrograde signaling and beyond. Annual Review of Plant Biology **67**, 25–53.

Chan KX, Phua SY, Van Breusegem F. 2019. Secondary sulfur metabolism in cellular signalling and oxidative stress responses. Journal of Experimental Botany **70**, 4237–4250.

Chaouch S, Queval G, Vanderauwera S, Mhamdi A, Vandorpe M, Langlois-Meurinne M, Van Breusegem F, Saindrenan P, Noctor G. 2010. Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. Plant Physiology **153**, 1692–1705.

Chapman JM, Muhlemann JK, Gayomba SR, Muday GK. 2019. RBOH-dependent ROS synthesis and ROS scavenging by plant specialized metabolites to modulate plant development and stress responses. Chemical Research in Toxicology **32**, 370–396.

Chen Q, Yang G. 2020. Signal function studies of ROS, especially RBOH-dependent ROS, in plant growth, development and environmental stress. Journal of Plant Growth Regulation **39**, 157–171.

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. Journal of Biological Chemistry **278**, 46869–46877.

Consentino L, Lambert S, Martino C, *et al.* 2015. Blue-light dependent reactive oxygen species formation by Arabidopsis cryptochrome may define a novel evolutionarily conserved signaling mechanism. New Phytologist **206**, 1450–1462.

Corpas FJ, Del Río LA, Palma JM. 2019. Plant peroxisomes at the crossroad of NO and H_2O_2 metabolism. Journal of Integrative Plant Biology **61**, 803–816.

Corpas FJ, Palma JM, Sandalio LM, Valderrama R, Barroso JB, Del Río LA. 2008. Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisum sativum* L.) leaves. Journal of Plant Physiology **165**, 1319–1330.

Corpas FJ, Sandalio LM, Del Río LA, Trelease RN. 1998. Copper-zinc superoxide dismutase is a constituent enzyme of the matrix of peroxisomes in the cotyledons of oilseed plants. New Phytologist **138**, 307–314.

Correa-Galvis V, Redekop P, Guan K, Griess A, Truong TB, Wakao S, Niyogi KK, Jahns P. 2016. Photosystem II subunit PsbS is involved in the induction of LHCSR protein-dependent energy dissipation in *Chlamydomonas reinhardtii*. Journal of Biological Chemistry **291**, 17478–17487.

Creissen G, Reynolds H, Xue Y, Mullineaux P. 1995. Simultaneous targeting of pea glutathione reductase and of a bacterial fusion protein to chloroplasts and mitochondria in transgenic tobacco. The Plant Journal **8**, 167–175.

Crisp PA, Smith AB, Ganguly DR, Murray KD, Eichten SR, Millar AA, Pogson BJ. 2018. RNA polymerase II read-through promotes expression of neighboring genes in SAL1–PAP–XRN retrograde signaling. Plant Physiology **178**, 1614–1630.

Dahal K, Vanlerberghe GC. 2017. Alternative oxidase respiration maintains both mitochondrial and chloroplast function during drought. New Phytologist **213**, 560–571.

D'Alessandro S, Ksas B, Havaux M. 2018. Decoding β-cyclocitral-mediated retrograde signaling reveals the role of a detoxification response in plant tolerance to photooxidative stress. The Plant Cell **30**, 2495–2511.

D'Alessandro S, Mizokami Y, Légeret B, Havaux M. 2019. The apocarotenoid β-cyclocitric acid elicits drought tolerance in plants. iScience **19**, 461–473.

Dall'Osto L, Holt NE, Kaligotla S, Fuciman M, Cazzaniga S, Carbonera D, Frank HA, Alric J, Bassi R. 2012. Zeaxanthin protects plant photosynthesis by modulating chlorophyll triplet yield in specific light-harvesting antenna subunits. Journal of Biological Chemistry **287**, 41820–41834.

Das K, Roychoudhury A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science **2**, 53.

Daudi A, Cheng Z, O'Brien JA, Mammarella N, Khan S, Ausubel FM, Bolwell GP. 2012. The apoplastic oxidative burst peroxidase in Arabidopsis is a major component of pattern-triggered immunity. The Plant Cell **24**, 275–287.

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De Clercq I, Vermeirssen V, Van Aken O, *et al.* 2013. The membrane-bound NAC transcription factor ANAC013 functions in mitochondrial retrograde regulation of the oxidative stress response in Arabidopsis. The Plant Cell **25**, 3472–3490.

Delorme-Hinoux V, Bangash SA, Meyer AJ, Reichheld JP. 2016. Nuclear thiol redox systems in plants. Plant Science **243**, 84–95.

del Río LA, Corpas FJ, Sandalio LM, Palma JM, Gómez M, Barroso JB. 2002. Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. Journal of Experimental Botany **53**, 1255–1272.

del Río LA, Donaldson RP. 1995. Production of superoxide radicals in glyoxysomal membranes from castor bean endosperm. Journal of Plant Physiology **146**, 283–287.

del Río LA, López-Huertas E. 2016. ROS generation in peroxisomes and its role in cell signaling. Plant & Cell Physiology **57**, 1364–1376.

De Smet B, Willems P, Fernandez-Fernandez AD, Alseekh S, Fernie AR, Messens J, Van Breusegem F. 2019. In vivo detection of protein cysteine sulfenylation in plastids. The Plant Journal **97**, 765–778.

de Souza A, Wang JZ, Dehesh K. 2017. Retrograde signals: integrators of interorganellar communication and orchestrators of plant development. Annual Review of Plant Biology **68**, 85–108.

Dietz KJ. 2015. Efficient high light acclimation involves rapid processes at multiple mechanistic levels. Journal of Experimental Botany **66**, 2401–2414.

Dietz KJ, Turkan I, Krieger-Liszkay A. 2016. Redox- and reactive oxygen species-dependent signaling into and out of the photosynthesizing chloroplast. Plant Physiology **171**, 1541–1550.

Dogra V, Duan J, Lee KP, Lv S, Liu R, Kim C. 2017. FtsH2-dependent proteolysis of EXECUTER1 is essential in mediating singlet oxygen-triggered retrograde signaling in *Arabidopsis thaliana*. Frontiers in Plant Science **8**, 1145.

Dogra V, Li M, Singh S, Li M, Kim C. 2019. Oxidative post-translational modification of EXECUTER1 is required for singlet oxygen sensing in plastids. Nature Communications **10**, 2834.

Dourmap C, Roque S, Morin A, *et al.* 2020. Stress signalling dynamics of the mitochondrial electron transport chain and oxidative phosphorylation system in higher plants. Annals of Botany **125**, 721–736.

Dubiella U, Seybold H, Durian G, Komander E, Lassig R, Witte CP, Schulze WX, Romeis T. 2013. Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. Proceedings of the National Academy of Sciences, USA **110**, 8744–8749.

Edwards EA, Rawsthorne S, Mullineaux PM. 1990. Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.). Planta **180**, 278–284.

El-Esawi M, Arthaut LD, Jourdan N, d'Harlingue A, Link J, Martino CF, Ahmad M. 2017. Blue-light induced biosynthesis of ROS contributes to the signaling mechanism of Arabidopsis cryptochrome. Scientific Reports **7**, 13875.

Estavillo GM, Crisp PA, Pornsiriwong W, *et al.* 2011. Evidence for a SAL1–PAP chloroplast retrograde pathway that functions in drought and high light signaling in Arabidopsis. The 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. Nature Communications **8**, 49.

Farmer EE, Mueller MJ. 2013. ROS-mediated lipid peroxidation and RES-activated signaling. Annual Review of Plant Biology **64**, 429–450.

Field LS, Furukawa Y, O'Halloran TV, Culotta VC. 2003. Factors controlling the uptake of yeast copper/zinc superoxide dismutase into mitochondria. Journal of Biological Chemistry **278**, 28052–28059.

Fischer BB, Hideg É, Krieger-Liszkay A. 2013. Production, detection, and signaling of singlet oxygen in photosynthetic organisms. Antioxidants & Redox Signaling **18**, 2145–2162.

Fischer BB, Ledford HK, Wakao S, Huang SG, Casero D, Pellegrini M, Merchant SS, Koller A, Eggen RI, Niyogi KK. 2012. SINGLET OXYGEN RESISTANT 1 links reactive electrophile signaling to singlet oxygen acclimation in *Chlamydomonas reinhardtii*. Proceedings of the National Academy of Sciences, USA **109**, E1302–E1311.

Flügel F, Timm S, Arrivault S, Florian A, Stitt M, Fernie AR, Bauwe H. 2017. The photorespiratory metabolite 2-phosphoglycolate regulates photosynthesis and starch accumulation in Arabidopsis. The Plant Cell **29**, 2537–2551.

Foyer CH, Halliwell B. 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta **133**, 21–25.

Foyer CH, Karpinska B, Krupinska K. 2014. The functions of WHIRLY1 and REDOX-RESPONSIVE TRANSCRIPTION FACTOR 1 in cross tolerance responses in plants: a hypothesis. Philosophical Transactions of the Royal Society B: Biological Sciences **369**, 20130226.

Foyer CH, Noctor G. 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum **119**, 355–364.

Gaber A, Ogata T, Maruta T, Yoshimura K, Tamoi M, Shigeoka S. 2012. The involvement of Arabidopsis glutathione peroxidase 8 in the suppression of oxidative damage in the nucleus and cytosol. Plant & Cell Physiology **53**, 1596–1606.

Gémes K, Kim YJ, Park KY, Moschou PN, Andronis E, Valassaki C, Roussis A, Roubelakis-Angelakis KA. 2016. An NADPH-oxidase/polyamine oxidase feedback loop controls oxidative burst under salinity. Plant Physiology **172**, 1418–1431.

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 Molecular Biology **65**, 627–644.

Gigolashvili T, Geier M, Ashykhmina N, Frerigmann H, Wulfert S, Krueger S, Mugford SG, Kopriva S, Haferkamp I, Flügge UI. 2012. The Arabidopsis thylakoid ADP/ATP carrier TAAC has an additional role in supplying plastidic phosphoadenosine 5′-phosphosulfate to the cytosol. The Plant Cell **24**, 4187–4204.

Giraud E, Ho LH, Clifton R, *et al.* 2008. The absence of ALTERNATIVE OXIDASE1a in Arabidopsis results in acute sensitivity to combined light and drought stress. Plant Physiology **147**, 595–610.

Girolomoni L, Cazzaniga S, Pinnola A, Perozeni F, Ballottari M, Bassi R. 2019. LHCSR3 is a nonphotochemical quencher of both photosystems in *Chlamydomonas reinhardtii*. Proceedings of the National Academy of Sciences, USA **116**, 4212–4217.

Grondin A, Rodrigues O, Verdoucq L, Merlot S, Leonhardt N, Maurel C. 2015. Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. The Plant Cell **27**, 1945–1954.

Hagemann M, Weber APM, Eisenhut M. 2016. Photorespiration: origins and metabolic integration in interacting compartments. Journal of Experimental Botany **67**, 2915–2918.

Halliwell B. 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiology **141**, 312–322.

Halliwell B, Foyer CH. 1976. Ascorbic acid, metal ions and the superoxide radical. The Biochemical Journal **155**, 697–700.

Hänsch R, Lang C, Riebeseel E, Lindigkeit R, Gessler A, Rennenberg H, Mendel RR. 2006. Plant sulfite oxidase as novel producer of H_2O_2: combination of enzyme catalysis with a subsequent non-enzymatic reaction step. Journal of Biological Chemistry **281**, 6884–6888.

Holland HD. 2006. The oxygenation of the atmosphere and oceans. Philosophical Transactions of the Royal Society B: Biological Sciences **361**, 903–915.

Horton P, Ruban AV, Walters RG. 1996. Regulation of light harvesting in green plants. Annual Review of Plant Physiology and Plant Molecular Biology **47**, 655–684.

Huang CH, Kuo WY, Weiss C, Jinn TL. 2012. Copper chaperone-dependent and -independent activation of three copper-zinc superoxide dismutase homologs localized in different cellular compartments in Arabidopsis. Plant Physiology **158**, 737–746.

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Huang J, Willems P, Wei B, *et al.* 2019. Mining for protein S-sulfenylation in Arabidopsis uncovers redox-sensitive sites. Proceedings of the National Academy of Sciences, USA **116**, 21256–21261.

Iñarrea P, Moini H, Rettori D, Han D, Martínez J, García I, Fernández-Vizarra E, Iturralde M, Cadenas E. 2005. Redox activation of mitochondrial intermembrane space Cu, Zn-superoxide dismutase. The Biochemical Journal **387**, 203–209.

Iñiguez C, Capó-Bauçà S, Niinemets Ü, Stoll H, Aguiló-Nicolau P, Galmés J. 2020. Evolutionary trends in RuBisCO kinetics and their co-evolution with CO_2 concentrating mechanisms. The Plant Journal **101**, 897–918.

Jaipargas EA, Mathur N, Bou Daher F, Wasteneys GO, Mathur J. 2016. High light intensity leads to increased peroxule–mitochondria interactions in plants. Frontiers in Cell and Developmental Biology **4**, 6.

Jiang J, Rodriguez-Furlan C, Wang JZ, de Souza A, Ke H, Pasternak T, Lasok H, Ditengou FA, Palme K, Dehesh K. 2018. Interplay of the two ancient metabolites auxin and MEcPP regulates adaptive growth. Nature Communications **9**, 2262.

Jiang J, Xiao Y, Chen H, *et al.* 2020. Retrograde induction of phyB orchestrates ethylene–auxin hierarchy to regulate growth. Plant Physiology **183**, 1268–1280.

Jimenez A, Hernandez JA, Del Rio LA, Sevilla F. 1997. Evidence for the presence of the ascorbate–glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiology **114**, 275–284.

Jourdan N, Martino CF, El-Esawi M, Witczak J, Bouchet PE, d'Harlingue A, Ahmad M. 2015. Blue-light dependent ROS formation by Arabidopsis cryptochrome-2 may contribute toward its signaling role. Plant Signaling & Behavior **10**, e1042647.

Kadota Y, Shirasu K, Zipfel C. 2015. Regulation of the NADPH oxidase RBOHD during plant immunity. Plant & Cell Physiology **56**, 1472–1480.

Kadota Y, Sklenar J, Derbyshire P, *et al.* 2014. Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Molecular Cell **54**, 43–55.

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. The Biochemical Journal **412**, 275–285.

Kärkönen A, Kuchitsu K. 2015. Reactive oxygen species in cell wall metabolism and development in plants. Phytochemistry **112**, 22–32.

Kato Y, Sakamoto W. 2009. Protein quality control in chloroplasts: a current model of D1 protein degradation in the photosystem II repair cycle. Journal of Biochemistry **146**, 463–469.

Kaurilind E, Xu E, Brosché M. 2015. A genetic framework for H_2O_2 induced cell death in *Arabidopsis thaliana*. BMC Genomics **16**, 837.

Kerchev P, Waszczak C, Lewandowska A, *et al.* 2016. Lack of GLYCOLATE OXIDASE1, but not GLYCOLATE OXIDASE2, attenuates the photorespiratory phenotype of CATALASE2-deficient Arabidopsis. Plant Physiology **171**, 1704–1719.

Kern R, Facchinelli F, Delwiche C, Weber APM, Bauwe H, Hagemann M. 2020. Evolution of photorespiratory glycolate oxidase among Archaeplastida. Plants **9**, 106.

Kim C, Meskauskiene R, Zhang S, Lee KP, Lakshmanan Ashok M, Blajecka K, Herrfurth C, Feussner I, Apel K. 2012. Chloroplasts of Arabidopsis are the source and a primary target of a plant-specific programmed cell death signaling pathway. The Plant Cell **24**, 3026–3039.

Kimura S, Hunter K, Vaahtera L, *et al.* 2020. CRK2 and C-terminal phosphorylation of NADPH oxidase RBOHD regulate reactive oxygen species production in Arabidopsis. The Plant Cell **32**, 1063–1080.

Krieger-Liszkay A, Trebst A. 2006. Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre. Journal of Experimental Botany **57**, 1677–1684.

Krupinska K, Blanco NE, Oetke S, Zottini M. 2020. Genome communication in plants mediated by organelle–nucleus-located proteins. Philosophical Transactions of the Royal Society B: Biological Sciences **375**, 20190397.

Kumar A, Prasad A, Sedlářová M, Pospíšil P. 2019. Characterization of protein radicals in Arabidopsis. Frontiers in Physiology **10**, 958.

Laloi C, Havaux M. 2015. Key players of singlet oxygen-induced cell death in plants. Frontiers in Plant Science **6**, 39.

Laloi C, Rayapuram N, Chartier Y, Grienenberger JM, Bonnard G, Meyer Y. 2001. Identification and characterization of a mitochondrial thioredoxin system in plants. Proceedings of the National Academy of Sciences, USA **98**, 14144–14149.

Larosa V, Meneghesso A, La Rocca N, Steinbeck J, Hippler M, Szabò I, Morosinotto T. 2018. Mitochondria affect photosynthetic electron transport and photosensitivity in a green alga. Plant Physiology **176**, 2305–2314.

Larosa V, Remacle C. 2018. Insights into the respiratory chain and oxidative stress. Bioscience Reports **38**, BSR20171492.

Lecler R, Vigeolas H, Emonds-Alt B, Cardol P, Remacle C. 2012. Characterization of an internal type-II NADH dehydrogenase from *Chlamydomonas reinhardtii* mitochondria. Current Genetics **58**, 205–216.

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*. Proceedings of the National Academy of Sciences, USA **104**, 10270–10275.

Leister D. 2017. Piecing the puzzle together: the central role of reactive oxygen species and redox hubs in chloroplast retrograde signaling. Antioxidants & Redox Signaling **30**, 1206–1219.

López-Huertas E, Corpas FJ, Sandalio LM, Del Río LA. 1999. Characterization of membrane polypeptides from pea leaf peroxisomes involved in superoxide radical generation. The Biochemical Journal **337**, 531–536.

Luo G, Ono S, Beukes NJ, Wang DT, Xie S, Summons RE. 2016. Rapid oxygenation of Earth's atmosphere 2.33 billion years ago. Science Advances **2**, e1600134.

Lv F, Zhou J, Zeng L, Xing D. 2015. β-Cyclocitral upregulates salicylic acid signalling to enhance excess light acclimation in Arabidopsis. Journal of Experimental Botany **66**, 4719–4732.

Mano J, Biswas MS, Sugimoto K. 2019. Reactive carbonyl species: a missing link in ROS signaling. Plants **8**, 391.

Martins L, Trujillo-Hernandez JA, Reichheld JP. 2018. Thiol based redox signaling in plant nucleus. Frontiers in Plant Science **9**, 705.

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 Physiology **57**, 1377–1386.

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 Physiology **51**, 190–200.

Massoz S, Hanikenne M, Bailleul B, Coosemans N, Radoux M, Miranda-Astudillo H, Cardol P, Larosa V, Remacle C. 2017. In vivo chlorophyll fluorescence screening allows the isolation of a Chlamydomonas mutant defective for NDUFAF3, an assembly factor involved in mitochondrial complex I assembly. The Plant Journal **92**, 584–595.

Matros A, Peshev D, Peukert M, Mock H-P, Van den Ende W. 2015. Sugars as hydroxyl radical scavengers: proof-of-concept by studying the fate of sucralose in Arabidopsis. The Plant Journal **82**, 822–839.

Meng X, Li L, De Clercq I, *et al.* 2019. ANAC017 coordinates organellar functions and stress responses by reprogramming retrograde signaling. Plant Physiology **180**, 634–653.

Meyer Y, Belin C, Delorme-Hinoux V, Reichheld JP, Riondet C. 2012. Thioredoxin and glutaredoxin systems in plants: molecular mechanisms, crosstalks, and functional significance. Antioxidants & Redox Signaling **17**, 1124–1160.

Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G. 2010. Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. Journal of Experimental Botany **61**, 4197–4220.

Mielecki J, Gawroński P, Karpiński S. 2020. Retrograde signaling: understanding the communication between organelles. International Journal of Molecular Sciences **21**, 6173.

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Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R. 2009. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. Science Signaling **2**, ra45.

Mittler R. 2017. ROS are good. Trends in Plant Science **22**, 11–19.

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

Mittova V, Theodoulou FL, Kiddle G, Volokita M, Tal M, Foyer CH, Guy M. 2004. Comparison of mitochondrial ascorbate peroxidase in the cultivated tomato, *Lycopersicon esculentum*, and its wild, salt-tolerant relative, *L. pennellii*—a role for matrix isoforms in protection against oxidative damage. Plant, Cell & Environment **27**, 237–250.

Møller IM. 2001. Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annual Review of Plant Physiology and Plant Molecular Biology **52**, 561–591.

Møller IM, Rasmusson AG, Fredlund KM. 1993. NAD(P)H-ubiquinone oxidoreductases in plant mitochondria. Journal of Bioenergetics and Biomembranes **25**, 377–384.

Monte I, Kneeshaw S, Franco-Zorrilla JM, Chini A, Zamarreño AM, García-Mina JM, Solano R. 2020. An ancient COI1-independent function for reactive electrophilic oxylipins in thermotolerance. Current Biology **30**, 962–971.

Morales J, Kadota Y, Zipfel C, Molina A, Torres MA. 2016. The Arabidopsis NADPH oxidases RbohD and RbohF display differential expression patterns and contributions during plant immunity. Journal of Experimental Botany **67**, 1663–1676.

Müller K, Linkies A, Vreeburg RA, Fry SC, Krieger-Liszkay A, Leubner-Metzger G. 2009. In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. Plant Physiology **150**, 1855–1865.

Müller P, Li XP, Niyogi KK. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiology **125**, 1558–1566.

Mullineaux PM, Exposito-Rodriguez M, Laissue PP, Smirnoff N. 2018. ROS-dependent signalling pathways in plants and algae exposed to high light: comparisons with other eukaryotes. Free Radical Biology & Medicine **122**, 52–64.

Muñoz P, Munné-Bosch S. 2020. Oxylipins in plastidial retrograde signaling. Redox Biology **37**, 101717.

Murchie EH, Ruban AV. 2020. Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity. The Plant Journal **101**, 885–896.

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. Journal of Experimental Botany **57**, 3033–3042.

Navrot N, Collin V, Gualberto J, Gelhaye E, Hirasawa M, Rey P, Knaff DB, Issakidis E, Jacquot JP, Rouhier N. 2006. Plant glutathione peroxidases are functional peroxiredoxins distributed in several subcellular compartments and regulated during biotic and abiotic stresses. Plant Physiology **142**, 1364–1379.

Ng S, Ivanova A, Duncan O, *et al.* 2013. A membrane-bound NAC transcription factor, ANAC017, mediates mitochondrial retrograde signaling in Arabidopsis. The Plant Cell **25**, 3450–3471.

Nietzel T, Elsässer M, Ruberti C, *et al.* 2019. The fluorescent protein sensor roGFP2-Orp1 monitors in vivo H_2O_2 and thiol redox integration and elucidates intracellular H_2O_2 dynamics during elicitor-induced oxidative burst in Arabidopsis. New Phytologist **221**, 1649–1664.

Nikkanen L, Rintamäki E. 2019. Chloroplast thioredoxin systems dynamically regulate photosynthesis in plants. The Biochemical Journal **476**, 1159–1172.

Noctor G, Foyer CH. 2016. Intracellular redox compartmentation and ROS-related communication in regulation and signaling. Plant Physiology **171**, 1581–1592.

Noctor G, Veljovic-Jovanovic S, Driscoll S, Novitskaya L, Foyer CH. 2002. Drought and oxidative load in the leaves of C_3 plants: a predominant role for photorespiration? Annals of Botany **89**, 841–850.

Norman EG, Colman B. 1991. Purification and characterization of phosphoglycolate phosphatase from the cyanobacterium *Coccochloris peniocystis*. Plant Physiology **95**, 693–698.

Nyathi Y, Baker A. 2006. Plant peroxisomes as a source of signalling molecules. Biochimica et Biophysica Acta **1763**, 1478–1495.

Ort DR, Baker NR. 2002. A photoprotective role for O_2 as an alternative electron sink in photosynthesis? Current Opinion in Plant Biology **5**, 193–198.

Ostrovsky D, Diomina G, Lysak E, Matveeva E, Ogrel O, Trutko S. 1998. Effect of oxidative stress on the biosynthesis of 2-C-methyl-D-erythritol-2,4-cyclopyrophosphate and isoprenoids by several bacterial strains. Archives of Microbiology **171**, 69–72.

Pan R, Liu J, Wang S, Hu J. 2020. Peroxisomes: versatile organelles with diverse roles in plants. New Phytologist **225**, 1410–1427.

Passardi F, Cosio C, Penel C, Dunand C. 2005. Peroxidases have more functions than a Swiss army knife. Plant Cell Reports **24**, 255–265.

Peers G, Truong TB, Ostendorf E, Busch A, Elrad D, Grossman AR, Hippler M, Niyogi KK. 2009. An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. Nature **462**, 518–521.

Phua SY, Yan D, Chan KX, Estavillo GM, Nambara E, Pogson BJ. 2018. The Arabidopsis SAL1–PAP pathway: a case study for integrating chloroplast retrograde, light and hormonal signaling in modulating plant growth and development? Frontiers in Plant Science **9**, 1171.

Pilon M, Ravet K, Tapken W. 2011. The biogenesis and physiological function of chloroplast superoxide dismutases. Biochimica et Biophysica Acta **1807**, 989–998.

Podgórska A, Burian M, Szal B. 2017. Extra-cellular but extra-ordinarily important for cells: apoplastic reactive oxygen species metabolism. Frontiers in Plant Science **8**, 1353.

Podgórska A, Ostaszewska-Bugajska M, Borysiuk K, Tarnowska A, Jakubiak M, Burian M, Rasmusson AG, Szal B. 2018. Suppression of external NADPH dehydrogenase-NDB1 in *Arabidopsis thaliana* confers improved tolerance to ammonium toxicity via efficient glutathione/redox metabolism. International Journal of Molecular Sciences **19**, 1412.

Pogson BJ, Woo NS, Förster B, Small ID. 2008. Plastid signalling to the nucleus and beyond. Trends in Plant Science **13**, 602–609.

Pornsiriwong W, Estavillo GM, Chan KX, *et al.* 2017. A chloroplast retrograde signal, 3′-phosphoadenosine 5′-phosphate, acts as a secondary messenger in abscisic acid signaling in stomatal closure and germination. eLife **6**, e23361.

Pulido P, Cazalis R, Cejudo FJ. 2009. An antioxidant redox system in the nucleus of wheat seed cells suffering oxidative stress. The Plant Journal **57**, 132–145.

Puntarulo S, Sánchez RA, Boveris A. 1988. Hydrogen peroxide metabolism in soybean embryonic axes at the onset of germination. Plant Physiology **86**, 626–630.

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. The Plant Journal **52**, 640–657.

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylidès C, Havaux M. 2012. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proceedings of the National Academy of Sciences, USA **109**, 5535–5540.

Rasmusson AG, Geisler DA, Møller IM. 2008. The multiplicity of dehydrogenases in the electron transport chain of plant mitochondria. Mitochondrion **8**, 47–60.

Rea G, Antonacci A, Lambreva MD, Mattoo AK. 2018. Features of cues and processes during chloroplast-mediated retrograde signaling in the alga Chlamydomonas. Plant Science **272**, 193–206.

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Rivasseau C, Seemann M, Boisson AM, Streb P, Gout E, Douce R, Rohmer M, Bligny R. 2009. Accumulation of 2-C-methyl-D-erythritol 2,4-cyclodiphosphate in illuminated plant leaves at supraoptimal temperatures reveals a bottleneck of the prokaryotic methylerythritol 4-phosphate pathway of isoprenoid biosynthesis. Plant, Cell & Environment **32**, 82–92.

Roach T, Krieger-Liszkay A. 2014. Regulation of photosynthetic electron transport and photoinhibition. Current Protein & Peptide Science **15**, 351–362.

Roach T, Stöggl W, Baur T, Kranner I. 2018. Distress and eustress of reactive electrophiles and relevance to light stress acclimation via stimulation of thiol/disulphide-based redox defences. Free Radical Biology & Medicine **122**, 65–73.

Rochaix JD. 2007. Role of thylakoid protein kinases in photosynthetic acclimation. FEBS Letters **581**, 2768–2775.

Rodrigues O, Reshetnyak G, Grondin A, Saijo Y, Leonhardt N, Maurel C, Verdoucq L. 2017. Aquaporins facilitate hydrogen peroxide entry into guard cells to mediate ABA- and pathogen-triggered stomatal closure. Proceedings of the National Academy of Sciences, USA **114**, 9200–9205.

Rodríguez-Serrano M, Romero-Puertas MC, Pastori GM, Corpas FJ, Sandalio LM, del Río LA, Palma JM. 2007. Peroxisomal membrane manganese superoxide dismutase: characterization of the isozyme from watermelon (*Citrullus lanatus* Schrad.) cotyledons. Journal of Experimental Botany **58**, 2417–2427.

Rodríguez-Serrano M, Romero-Puertas MC, Sanz-Fernández M, Hu J, Sandalio LM. 2016. Peroxisomes extend peroxules in a fast response to stress via a reactive oxygen species-mediated induction of the peroxin PEX11a. Plant Physiology **171**, 1665–1674.

Rouhier N, Villarejo A, Srivastava M, *et al.* 2005. Identification of plant glutaredoxin targets. Antioxidants & Redox Signaling **7**, 919–929.

Ruban AV, Johnson MP. 2009. Dynamics of higher plant photosystem cross-section associated with state transitions. Photosynthesis Research **99**, 173–183.

Salinas T, Larosa V, Cardol P, Maréchal-Drouard L, Remacle C. 2014. Respiratory-deficient mutants of the unicellular green alga Chlamydomonas: a review. Biochimie **100**, 207–218.

Sandalio LM, Fernández VM, Rupérez FL, Del Río LA. 1988. Superoxide free radicals are produced in glyoxysomes. Plant Physiology **87**, 1–4.

Sandalio LM, Peláez-Vico MA, Molina-Moya E, Romero-Puertas MC. 2021. Peroxisomes as redox-signaling nodes in intracellular communication and stress responses. Plant Physiology doi: 10.1093/plphys/kiab060.

Schertl P, Braun HP. 2014. Respiratory electron transfer pathways in plant mitochondria. Frontiers in Plant Science **5**, 163.

Schimmeyer J, Bock R, Meyer EH. 2016. L-Galactono-1,4-lactone dehydrogenase is an assembly factor of the membrane arm of mitochondrial complex I in Arabidopsis. Plant Molecular Biology **90**, 117–126.

Schnaubelt D, Queval G, Dong Y, Diaz-Vivancos P, Makgopa ME, Howell G, De Simone A, Bai J, Hannah MA, Foyer CH. 2015. Low glutathione regulates gene expression and the redox potentials of the nucleus and cytosol in *Arabidopsis thaliana*. Plant, Cell & Environment **38**, 266–279.

Scuffi D, Nietzel T, Di Fino LM, Meyer AJ, Lamattina L, Schwarzländer M, Laxalt AM, García-Mata C. 2018. Hydrogen sulfide increases production of NADPH oxidase-dependent hydrogen peroxide and phospholipase D-derived phosphatidic acid in guard cell signaling. Plant Physiology **176**, 2532–2542.

Seemann M, Wegner P, Schünemann V, Bui BTS, Wolff M, Marquet A, Trautwein AX, Rohmer M. 2005. Isoprenoid biosynthesis in chloroplasts via the methylerythritol phosphate pathway: the (E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase (GcpE) from *Arabidopsis thaliana* is a [4Fe–4S] protein. Journal of Biological Inorganic Chemistry **10**, 131–137.

Sewelam N, Jaspert N, Van Der Kelen K, Tognetti VB, Schmitz J, Frerigmann H, Stahl E, Zeier J, Van Breusegem F, Maurino VG. 2014. Spatial H_2O_2 signaling specificity: H_2O_2 from chloroplasts and peroxisomes modulates the plant transcriptome differentially. Molecular Plant **7**, 1191–1210.

Shao N, Duan GY, Bock R. 2013. A mediator of singlet oxygen responses in *Chlamydomonas reinhardtii* and Arabidopsis identified by a luciferase-based genetic screen in algal cells. The Plant Cell **25**, 4209–4226.

Shapiguzov A, Vainonen JP, Hunter K, *et al.* 2019. Arabidopsis RCD1 coordinates chloroplast and mitochondrial functions through interaction with ANAC transcription factors. eLife **8**, e43284.

Shirao M, Kuroki S, Kaneko K, Kinjo Y, Tsuyama M, Förster B, Takahashi S, Badger MR. 2013. Gymnosperms have increased capacity for electron leakage to oxygen (Mehler and PTOX reactions) in photosynthesis compared with angiosperms. Plant & Cell Physiology **54**, 1152–1163.

Shumbe L, D'Alessandro S, Shao N, Chevalier A, Ksas B, Bock R, Havaux M. 2017. METHYLENE BLUE SENSITIVITY 1 (MBS1) is required for acclimation of Arabidopsis to singlet oxygen and acts downstream of β-cyclocitral. Plant, Cell & Environment **40**, 216–226.

Sierla M, Waszczak C, Vahisalu T, Kangasjärvi J. 2016. Reactive oxygen species in the regulation of stomatal movements. Plant Physiology **171**, 1569–1580.

Smirnoff N. 2018. Ascorbic acid metabolism and functions: a comparison of plants and mammals. Free Radical Biology & Medicine **122**, 116–129.

Solymosi K, Schoefs B. 2010. Etioplast and etio-chloroplast formation under natural conditions: the dark side of chlorophyll biosynthesis in angiosperms. Photosynthesis Research **105**, 143–166.

Soufari H, Parrot C, Kuhn L, Waltz F, Hashem Y. 2020. Specific features and assembly of the plant mitochondrial complex I revealed by cryo-EM. Nature Communications **11**, 5195.

Stirbet A, Lazár D, Guo Y, Govindjee G. 2020. Photosynthesis: basics, history and modelling. Annals of Botany **126**, 511–537.

Su T, Li W, Wang P, Ma C. 2019. Dynamics of peroxisome homeostasis and its role in stress response and signaling in plants. Frontiers in Plant Science **10**, 705.

Sun H, Yang YJ, Huang W. 2020. The water–water cycle is more effective in regulating redox state of photosystem I under fluctuating light than cyclic electron transport. Biochimica et Biophysica Acta **1861**, 148235.

Takahashi S, Badger MR. 2011. Photoprotection in plants: a new light on photosystem II damage. Trends in Plant Science **16**, 53–60.

Torres MA, Dangl JL, Jones JD. 2002. Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proceedings of the National Academy of Sciences, USA **99**, 517–522.

Tripathy BC, Oelmüller R. 2012. Reactive oxygen species generation and signaling in plants. Plant Signaling & Behavior **7**, 1621–1633.

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 Physiology **139**, 1806–1820.

Van Aken O, Pogson BJ. 2017. Convergence of mitochondrial and chloroplastic ANAC017/PAP-dependent retrograde signalling pathways and suppression of programmed cell death. Cell Death and Differentiation **24**, 955–960.

Van Breusegem F, Dat JF. 2006. Reactive oxygen species in plant cell death. Plant Physiology **141**, 384–390.

Van Leene J, Stals H, Eeckhout D, *et al.* 2007. A tandem affinity purification-based technology platform to study the cell cycle interactome in *Arabidopsis thaliana*. Molecular & Cellular Proteomics **6**, 1226–1238.

Vishwakarma A, Tetali SD, Selinski J, Scheibe R, Padmasree K. 2015. Importance of the alternative oxidase (AOX) pathway in regulating cellular redox and ROS homeostasis to optimize photosynthesis during restriction of the cytochrome oxidase pathway in *Arabidopsis thaliana*. Annals of Botany **116**, 555–569.

Wakao S, Chin BL, Ledford HK, Dent RM, Casero D, Pellegrini M, Merchant SS, Niyogi KK. 2014. Phosphoprotein SAK1 is a regulator of acclimation to singlet oxygen in *Chlamydomonas reinhardtii*. eLife **3**, e02286.

Wang J, Rajakulendran N, Amirsadeghi S, Vanlerberghe GC. 2011. Impact of mitochondrial alternative oxidase expression on the response of *Nicotiana tabacum* to cold temperature. Physiologia Plantarum **142**, 339–351.

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Wang JZ, Li B, Xiao Y, *et al.* 2017. Initiation of ER body formation and indole glucosinolate metabolism by the plastidial retrograde signaling metabolite, MEcPP. Molecular Plant **10**, 1400–1416.

Wang L, Kim C, Xu X, Piskurewicz U, Dogra V, Singh S, Mahler H, Apel K. 2016. Singlet oxygen- and EXECUTER1-mediated signaling is initiated in grana margins and depends on the protease FtsH2. Proceedings of the National Academy of Sciences, USA **113**, E3792–E3800.

Wang L, Leister D, Guan L, Zheng Y, Schneider K, Lehmann M, Apel K, Kleine T. 2020. The Arabidopsis SAFEGUARD1 suppresses singlet oxygen-induced stress responses by protecting grana margins. Proceedings of the National Academy of Sciences, USA **117**, 6918–6927.

Wang Q, Lin C. 2020. Mechanisms of cryptochrome-mediated photoresponses in plants. Annual Review of Plant Biology **71**, 103–129.

Wang W, Paschalidis K, Feng JC, Song J, Liu JH. 2019. Polyamine catabolism in plants: a universal process with diverse functions. Frontiers in Plant Science **10**, 561.

Wang X, Li S, Liu Y, Ma C. 2015. Redox regulated peroxisome homeostasis. Redox Biology **4**, 104–108.

Wang Y, Selinski J, Mao C, Zhu Y, Berkowitz O, Whelan J. 2020. Linking mitochondrial and chloroplast retrograde signalling in plants. Philosophical Transactions of the Royal Society B: Biological Sciences **375**, 20190410.

Waszczak C, Carmody M, Kangasjärvi J. 2018. Reactive oxygen species in plant signaling. Annual Review of Plant Biology **69**, 209–236.

Waszczak C, Kerchev PI, Mühlenbock P, *et al.* 2016. SHORT-ROOT deficiency alleviates the cell death phenotype of the Arabidopsis catalase2 mutant under photorespiration-promoting conditions. The Plant Cell **28**, 1844–1859.

Wei B, Willems P, Huang J, Tian C, Yang J, Messens J, Van Breusegem F. 2020. Identification of sulfenylated cysteines in *Arabidopsis thaliana* proteins using a disulfide-linked peptide reporter. Frontiers in Plant Science **11**, 777.

Wendrich JR, Yang B, Vandamme N, *et al.* 2020. Vascular transcription factors guide plant epidermal responses to limiting phosphate conditions. Science **370**, eaay4970.

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 Physiology **171**, 1720–1733.

Winger AM, Millar AH, Day DA. 2005. Sensitivity of plant mitochondrial terminal oxidases to the lipid peroxidation product 4-hydroxy-2-nonenal (HNE). The Biochemical Journal **387**, 865–870.

Winger AM, Taylor NL, Heazlewood JL, Day DA, Millar AH. 2007. The cytotoxic lipid peroxidation product 4-hydroxy-2-nonenal covalently modifies a selective range of proteins linked to respiratory function in plant mitochondria. Journal of Biological Chemistry **282**, 37436–37447.

Wingler A, Lea PJ, Quick WP, Leegood RC. 2000. Photorespiration: metabolic pathways and their role in stress protection. Philosophical Transactions of the Royal Society B: Biological Sciences **355**, 1517–1529.

Wollman FA. 2001. State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. The EMBO Journal **20**, 3623–3630.

Wu Q, Lin J, Liu JZ, *et al.* 2012. Ectopic expression of Arabidopsis glutaredoxin AtGRXS17 enhances thermotolerance in tomato. Plant Biotechnology Journal **10**, 945–955.

Xiao Y, Savchenko T, Baidoo EE, Chehab WE, Hayden DM, Tolstikov V, Corwin JA, Kliebenstein DJ, Keasling JD, Dehesh K. 2012. Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes. Cell **149**, 1525–1535.

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. The Plant Journal **32**, 915–925.

Yoshida K, Watanabe CK, Hachiya T, Tholen D, Shibata M, Terashima I, Noguchi K. 2011. Distinct responses of the mitochondrial respiratory chain to long- and short-term high-light environments in *Arabidopsis thaliana*. Plant, Cell & Environment **34**, 618–628.

Zandalinas SI, Fichman Y, Mittler R. 2020. Vascular bundles mediate systemic reactive oxygen signaling during light stress. The Plant Cell **32**, 3425–3435.

Zarepour M, Kaspari K, Stagge S, Rethmeier R, Mendel RR, Bittner F. 2010. Xanthine dehydrogenase AtXDH1 from *Arabidopsis thaliana* is a potent producer of superoxide anions via its NADH oxidase activity. Plant Molecular Biology **72**, 301–310.

Zhang ZS, Liu MJ, Scheibe R, Selinski J, Zhang LT, Yang C, Meng XL, Gao HY. 2017. Contribution of the alternative respiratory pathway to PSII photoprotection in C_3 and C_4 plants. Molecular Plant **10**, 131–142.

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