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REVIEW PAPER

Spreading the news: subcellular and organellar reactive oxygen species production and signalling

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Abstract

As plants are sessile organisms that have to attune their physiology and morphology continuously to varying environmental challenges in order to survive and reproduce, they have evolved complex and integrated environment–cell, cell–cell, and cell–organelle signalling circuits that regulate and trigger the required adjustments (such as alteration of gene expression). Although reactive oxygen species (ROS) are essential components of this network, their pathways are not yet completely unravelled. In addition to the intrinsic chemical properties that define the array of interaction partners, mobility, and stability, ROS signalling specificity is obtained via the spatiotemporal control of production and scavenging at different organellar and subcellular locations (e.g. chloroplasts, mitochondria, peroxisomes, and apoplast). Furthermore, these cellular compartments may crosstalk to relay and further fine-tune the ROS message. Hence, plant cells might locally and systemically react upon environmental or developmental challenges by generating spatiotemporally controlled dosages of certain ROS types, each with specific chemical properties and interaction targets, that are influenced by interorganellar communication and by the subcellular location and distribution of the involved organelles, to trigger the suitable acclimation responses in association with other well-established cellular signalling components (e.g. reactive nitrogen species, phytohormones, and calcium ions). Further characterization of this comprehensive ROS signalling matrix may result in the identification of new targets and key regulators of ROS signalling, which might be excellent candidates for engineering or breeding stress-tolerant plants.

Key words: Acclimation, apoplast, organelle, photorespiration, reactive oxygen species, signalling.

Introduction

The predicted climate change and global warming will affect the biotic and abiotic environment of plants, with an impact on crop yields and biomass production (Lobell *et al.*, 2009; Schlenker and Roberts, 2009; IPCC, 2014). As plants are sessile, they evolved diverse strategies to combat environmental challenges. These strategies depend on the type, severity, and

Abbreviations: ABA, abscisic acid; *cat2*, *catalase 2* mutant; CDPK, calcium-dependent protein kinase; CRK, cysteine-rich receptor-like kinase; EDS1, ENHANCED DISEASE SUSCEPTIBILITY1; EX, EXECUTER; flg22, flagellin 22; FLS2, FLAGELLIN SENSING2; *flu*, *fluorescent* mutant; JA, jasmonic acid; LRR, leucine-rich repeat; PET, photosynthetic electron transport; RBOH, respiratory burst oxidase homolog; RET, respiratory electron transport; RLK, receptor-like kinase; ROS, reactive oxygen species; SA, salicylic acid.

combination of biotic and/or abiotic stress(es), and require specific and flexible combinations of signalling components to trigger adaptation and acclimation responses that may be applicable to more than one environmental cue, usually referred to as cross-tolerance (Suzuki *et al.*, 2012, 2014). The initiation and regulation of these processes mostly involve well-characterized signalling molecules, such as phytohormones (Pieterse *et al.*, 2012; Bartoli *et al.*, 2013), calcium ions (Ca^{2+}) (Stael *et al.*, 2015), reactive nitrogen species (Kocsy *et al.*, 2013), and reactive oxygen species (ROS) (Baxter *et al.*, 2014).

ROS, including the superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO·), as well as singlet oxygen ($^1O_2$), are formed in organelles during electron transport reactions in photosynthesis and respiration, and as by-products of enzymatic reactions in photorespiration and other metabolism (Das *et al.*, 2015). ROS are also produced enzymatically in the apoplast of lignifying cells and as a response to external stimuli (Demidchik, 2015). ROS were long regarded as unwanted and harmful by-products of normal cellular metabolism. However, during the last two decades our understanding of the role of ROS has greatly expanded from merely detrimental species causing oxidative stress to the view that they are essential messengers involved in redox signalling (Mittler *et al.*, 2011; Schieber and Chandel, 2014; Vaahtera *et al.*, 2014). ROS can initiate and integrate with signalling networks, thereby regulating a broad range of processes, such as growth, development, defence, and acclimation responses to biotic and abiotic stresses (Gapper and Dolan, 2006; Baxter *et al.*, 2014).

Each ROS has its own set of distinct chemical properties (Møller *et al.*, 2007) and may accumulate transiently in specific cellular microenvironments. Their production and scavenging systems are compartmentalized and/or need specific activation. ROS can react with apoplastic signalling components, cytosolic kinases, phosphatases, various redox-sensitive proteins, and transcription factors (Foyer and Noctor, 2013; Wrzaczek *et al.*, 2013), thereby initiating communication events that adjust plant growth, development, and stress responses according to environmental cues. ROS signalling is also strongly interconnected with the response to plant hormones, including salicylic acid (SA), jasmonic acid (JA), ethylene, abscisic acid (ABA), and auxin (Blomster *et al.*, 2011; Pieterse *et al.*, 2012; Bartoli *et al.*, 2013). These interactions can take place at the level of both hormone biosynthesis and hormone signalling; that is, proteins initially isolated for their role in hormone signalling also play signalling roles in ROS responses (Kangasjärvi *et al.*, 2005; Mittler *et al.*, 2011).

As all ROS have their own unique chemical properties and reactivity, they also possess their own specific set of targets and signalling routes (Vaahtera *et al.*, 2014). OH· is the most unstable ROS and rapidly reacts with all sorts of cellular components (e.g. lipids, DNA, carbohydrates, and proteins), as reflected by its half-life of only 1 ns. The $O_2^{\cdot -}$ and $^1O_2$ molecules are quite similar in terms of stability, with a half-life of 1 µs (Halliwell and Gutteridge, 2015). Nevertheless, they vary greatly in target specificity: whereas $O_2^{\cdot -}$ interacts mainly with protein Fe–S centres, $^1O_2$ may oxidize polyunsaturated fatty acids, guanine, and several amino acids. In contrast, H_2O_2 is much more stable (half-life of 1 ms) and can accumulate to relatively high concentrations (µM to mM) (Cheeseman, 2006; Møller *et al.*, 2007). However, these unique chemical properties of ROS do not include information about the subcellular production site.

The estimated subcellular H_2O_2 production rates in the light under photorespiratory conditions are 4030 nmol m^{-2} s^{-1} for chloroplasts, 10 000 nmol m^{-2} s^{-1} for peroxisomes, and <216 nmol m^{-2} s^{-1} for mitochondria (Foyer and Noctor, 2003), whereas the production rates of the more unstable $O_2^{\cdot -}$ and $^1O_2$ are difficult to estimate. Each ROS apparently propagates its signal through a distinct set of compartment-specific interaction partners. Hence, ROS signalling is probably linked to compartment-specific sensory systems that can connect them with other signalling pathways and transduce the signal downstream for compartment-specific or compartment-directed responses (Møller and Sweetlove, 2010). Thus, the signalling specificity of ROS results not only from the chemical characteristics, but also from spatiotemporal control of sensory mechanisms (Bailly *et al.*, 2008; Shapiguzov *et al.*, 2012; Foyer and Noctor, 2013; Baxter *et al.*, 2014; Sewelam *et al.*, 2014), and is affected by the distribution and relative position of the participating cellular organelles or subcellular compartments (Suzuki *et al.*, 2012) and by the developmental stage (Mhamdi *et al.*, 2010). This review is focused on ROS production and signalling in different subcellular compartments and organelles, and on the mechanisms involved in determining the ROS signal specificity and intercompartmental communication.

Apoplastic ROS production

The apoplast is the intercellular space outside the plasma membrane formed by the continuum of cell walls and the extracellular spaces. Diffusion through the apoplast is much faster than through the cytosol, thereby facilitating rapid cell–cell communication. The apoplast has a few special features that affect its redox properties (Potters *et al.*, 2009). First of all, the pH of the apoplast is lower than that of the cytoplasm (Gao *et al.*, 2004), which reduces the redox sensitivity of cysteine. Hence, the proteins in the apoplast should be less redox sensitive, but, compared with the cytosol, the apoplast also has lower amounts of low-molecular weight antioxidants, such as glutathione and ascorbate, and, thus, a lower antioxidant buffering capacity. Therefore, ROS can accumulate in the apoplast, enabling the activation of ROS signalling pathways that counteract the effect of low pH on the redox sensitivity of the apoplastic proteins (Fig. 1). Furthermore, changes in apoplastic ROS levels affect the ascorbate gradient and, consequently, lead to changes in cellular redox homeostasis (Foyer *et al.*, 2009; Munné-Bosch *et al.*, 2013). In addition, a large number of apoplastic proteins contain thiol groups that could be involved in or target redox regulation. Apoplastic h-type thioredoxins that interact with apoplastic ROS have been identified (Zhang *et al.*, 2011). A small cysteine-rich apoplastic pre-protein has been shown to be proteolytically processed by a metacaspase in a ROS-dependent manner to produce a ligand involved in ROS-dependent cell death (Wrzaczek *et al.*, 2015), whereas a group of cysteine-rich receptor-like kinases (CRKs) play primary and fine-tuning roles related to oxidative

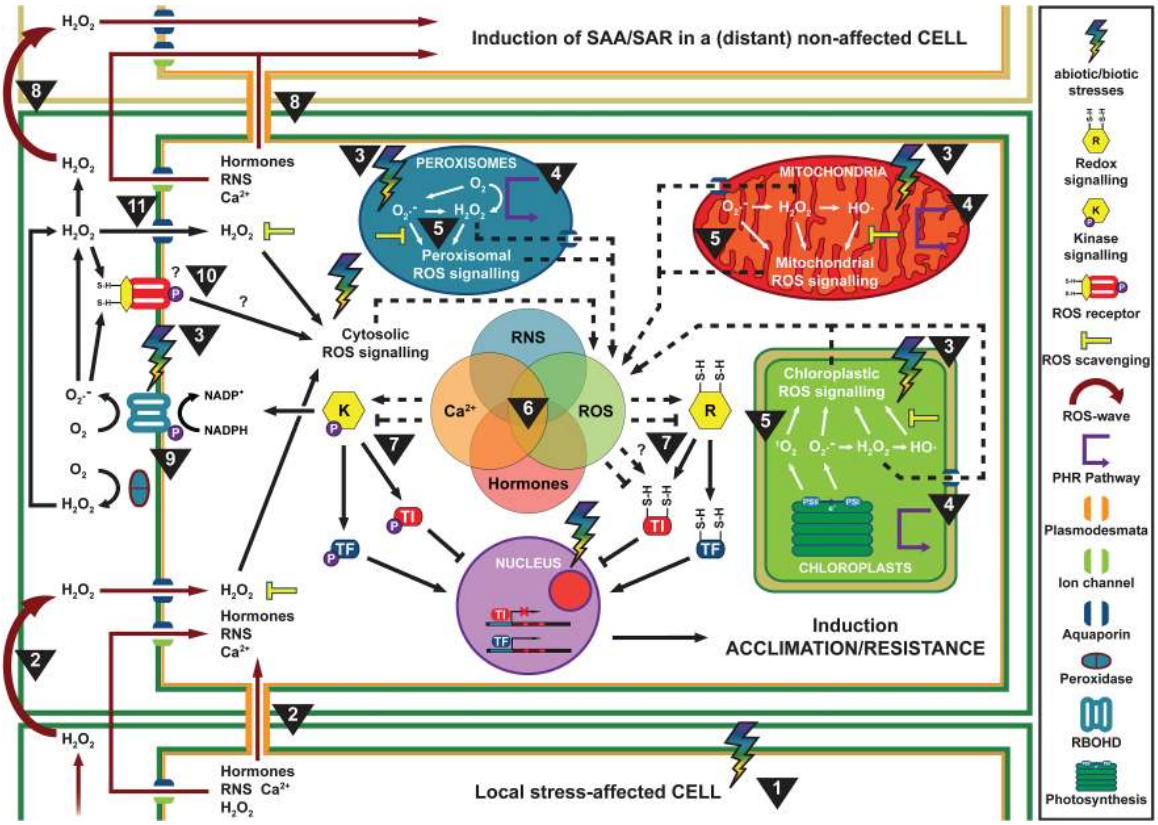

Fig. 1. ROS signalling-mediated local and systemic responses against (a)biotic stresses. Abiotic and biotic stresses lead to redox imbalances in different subcellular compartments, resulting in both local stress responses and systemic responses; that is, systemic acquired acclimation (SAA) and systemic acquired resistance (SAR). Information flow via intercompartmental ROS signalling is important for cellular homeostasis, hormonal balance, and co-ordinated stress responses. Crosstalk between different cellular signalling systems, resulting in stress responses and acclimation, is very complex, and many questions concerning ROS sensing, specificity, and regulation are still unanswered. ROS is produced continuously in different subcellular locations as part of cellular metabolism, but abiotic and biotic stresses induce additional ROS production in stress-specific subcellular locations (1, 3) that lead to stress recognition and activation of apoplastic and symplastic stress signalling pathways, including ROS, RNS, hormone, and Ca^{2+} signalling (2). In the case of a strong stress, ROS scavenging by antioxidants is not efficient enough to keep ROS under the threshold levels, causing redox imbalances that are sensed locally (4 and 5), but also in the cytosol. H_2O_2 can pass membranes through aquaporins (11), but otherwise it is not well understood how redox changes are sensed. Despite the complexity, plants are able to sort out stress signals and adjust their metabolism accordingly to maintain the optimal physiological status for growth (6), possibly involving signal transduction via kinase (K)- and/or redox (R)-based intermediates in the cytoplast (and apoplast) (7). Membrane lipids and redox-sensitive plasma membrane-located protein kinases, such as CRKs, have been suggested to sense apoplastic ROS produced by RBOHs and cell wall peroxidases (9) and to deliver the stress signals to the cytosol for further processing (10). Redox-sensitive protein kinases might even play a role in the propagation of the ROS wave that delivers the stress signal to the neighbouring cells (8). Arrows (→) represent stimulation/activation and bars (T) inhibition/deactivation. A question mark indicates assumed interactions or hypothetical signalling mechanisms. Dashed lines display temporal and variable ROS signalling contributions or interactions that depend on the type of cellular compartment and the characteristics of the (a)biotic stresses affecting these compartments. Red arrows symbolise systemic signalling or transport. TF, transcription factor; TI, transcription inhibitor; PHR, photorespiration; PS, photosystem; NADP(H), nicotinamide adenine dinucleotide phosphate (reduced); P, phosphorylated; S-H, cysteine thiol group.

stress (Bourdais *et al.*, 2015). However, specific proteins with specialized cysteine pairs as a direct target for ROS/redox regulation in the apoplast still await detailed elucidation.

Apoplastic ROS production, induced by extracellular stimuli, such as pathogens or ozone, is one of the first measurable events during different biotic and abiotic stresses (Wojtaszek, 1997). This ROS production is mediated by NADPH oxidases and class III cell wall peroxidases (Torres, 2010; Daudi *et al.*, 2012; O'Brien *et al.*, 2012). The NADPH oxidases, which are designated respiratory burst oxidase homologs (RBOHs) in plants, are integral plasma membrane proteins that utilize cytosolic NADPH as the electron donor to reduce extracellular O_2 to $O_2^{\cdot -}$. They are composed of six transmembrane domains, a C-terminal FAD-binding domain, and two N-terminal calcium-binding (EF-hand) domains, the activity of which is regulated both transcriptionally (Adachi *et al.*, 2015) and by various post-translational modifications, mostly targeting the cytosolic N-terminal domain. The N-terminal EF-hands can bind Ca^{2+}, and several amino acid residues are phosphorylated by various kinases, such as the OPEN STOMATA1 (OST1), calcium-dependent protein kinases (CDPKs), and calcineurin B-like (CBL)-interacting protein kinases, CIPKs (Marino *et al.*, 2012; Baxter *et al.*, 2014). The Ca^{2+}-dependent RBOH activation includes a conformational change of the EF-hand (Zhao *et al.*, 2005; Ogasawara *et al.*, 2008; Monshausen *et al.*, 2009) together with crosstalk with the phosphorylation events (Kimura *et al.*, 2012; Kadota *et al.*, 2014).

Apoplastic class-III peroxidases are a second set of enzymatic ROS producers that operate in various physiological processes, such as lignin polymerization (Passardi *et al.*, 2004),

but also during pathogen defence (Torres, 2010; Daudi *et al.*, 2012; O'Brien *et al.*, 2012). Class-III peroxidases are soluble and are bound ionically or covalently to the cell walls. Their reaction mechanism is based on a peroxidative and hydroxylic catalytic cycle (Passardi *et al.*, 2004, 2005; O'Brien *et al.*, 2012; Lüthje *et al.*, 2013). Whereas the latter couples the release of several ROS with the reduction of H_2O_2 or $O_2^{\cdot -}$, the peroxidative cycle combines the reduction of H_2O_2 to H_2O with the oxidation of various substrates, leading to $O_2^{\cdot -}$ production (Liszkay *et al.*, 2003; Passardi *et al.*, 2004). Hence, both cycles may regulate H_2O_2 production (Passardi *et al.*, 2004). Transgenic *Arabidopsis thaliana* plants expressing an antisense cDNA that targeted type-III peroxidases exhibited a reduced oxidative burst and an enhanced susceptibility to fungal and bacterial pathogens (Bindschedler *et al.*, 2006). Fungal elicitors trigger a type-III peroxidase and an RBOHD-dependent oxidative burst that initiates callose deposition and alters gene expression (Daudi *et al.*, 2012; O'Brien *et al.*, 2012).

Conveying the (apoplastic) message

Phospholipid membranes allow cells to build up physiologically relevant chemical or electrical gradients and to compartmentalize cellular pathways, nutrients, and metabolites. In combination with interplay between the diverse antioxidant and ROS-producing systems, these membranes enable cells to maintain a non-toxic steady-state level of ROS by allowing their transient accumulation in particular subcellular locations for signalling purposes (Mittler *et al.*, 2004; Foyer and Noctor, 2013). By separating the symplasm from the apoplasm, the plasma membrane is a key to cellular homeostasis and ROS signalling. For example, H_2O_2 cannot diffuse freely through the plasma membrane due to its polarity, and its transport is controlled by H_2O_2-permeable (or even specific) aquaporins, through which membranes are crossed in a regulated manner (Bienert and Chaumont, 2014; Grondin *et al.*, 2016). Thus, an apoplastic oxidative burst may result in enhanced oxidation of the apoplast, whereas the cytoplasm remains reduced. The redox gradient that is formed across the plasma membrane allows differential redox regulation of proteins at the cell surface, including the receptors and ion channels (Foyer and Noctor, 2013), possibly initiating a cascade of signalling pathways (Shapiguzov *et al.*, 2012; Foyer and Noctor, 2013; Wrzaczek *et al.*, 2013).

The apoplastic oxidative burst has been associated with extracellular signalling and cell–cell communication that may trigger systemic physiological responses and acclimation to environmental stresses (Mittler *et al.*, 2011; Suzuki *et al.*, 2013; Baxter *et al.*, 2014). Despite their immobility, RBOHD proteins can mediate long-distance signalling via dynamic autopropagating ROS waves that require each cell along the way to activate RBOHD proteins independently (Miller *et al.*, 2009, 2011; Suzuki *et al.*, 2013; Baxter *et al.*, 2014). This RBOH-related ROS generation in the adjacent cells probably involves the previously mentioned RBOH control mechanisms, including CDPKs, especially CPK5. The Arabidopsis NADPH oxidase RBOHD has been shown to be an *in vivo* target for CPK5. Phosphorylation of RBOHD by CPK5 activates it, resulting in ROS perception in the neighbouring cell, where Ca^{2+} signalling and CPK5 again are activated, spreading the RBOHD activation further (Dubiella *et al.*, 2013; Suzuki *et al.*, 2013). The resulting ROS wave has a systemic character, travelling from its local source at a rate of 8.4 cm min^{-1} through the apoplastic space to distant, non-stressed tissues, where it affects gene expression (Miller *et al.*, 2009). This ROS wave may be essential for the induction of systemic acquired resistance to biotic stresses and systemic acquired acclimation to abiotic environmental cues (Torres *et al.*, 2005; Miller *et al.*, 2009; Suzuki *et al.*, 2012, 2013; Baxter *et al.*, 2014). Furthermore, it acts in concert with hormonal (e.g. JA, SA, ethylene, and ABA), Ca^{2+}, and systemic electric signals (Hasegawa *et al.*, 2011; Mittler *et al.*, 2011; Karpiński *et al.*, 2013; Suzuki *et al.*, 2013; Baxter *et al.*, 2014) and may also trigger systemic responses related to wounding, metabolism, and plant development (Sagi *et al.*, 2004; Suzuki and Mittler, 2012; Baxter *et al.*, 2014).

Apoplastic ROS production is important not only for long-distance extracellular signalling, but also for the initiation of several intracellular signalling events and changes in the cellular redox homeostasis (Mittler *et al.*, 2011). One of the best understood processes in which extracellular ROS are involved in triggering intracellular responses is stomatal regulation. Genetic evidence indicates that the NADPH oxidases RBOHD and RBOHF are involved in the regulation of stomatal closure (Murata *et al.*, 2001; Zhang *et al.*, 2009). ABA-induced stomatal closure is impaired in the *rbohf* mutants and even more in the *rbohd rbohf* double mutants (Kwak *et al.*, 2003). Furthermore, application of the NADPH oxidase inhibitor diphenyliodonium produced a similar effect on ABA-induced stomatal closure (Zhang *et al.*, 2001). Similarly, high CO_2-induced stomatal closure is deficient in *rbohd rbohf* double mutants (Chater *et al.*, 2015). However, it is still unknown whether apoplastic ROS are active and perceived outside the plasma membrane, or whether H_2O_2 derived from the RBOH-produced $O_2^{\cdot -}$ is transported to the cytoplasm across the plasma membrane. The apoplastic H_2O_2 can move through the plasma membrane to the cytoplasm via aquaporins (Bienert and Chaumont, 2014; Bienert *et al.*, 2014), and thus alter the symplastic redox state, react with symplastic components, and induce further responses in cellular metabolism and gene expression. H_2O_2 may also diffuse symplastically from cell to cell via plasmodesmata or through the vasculature via the cells bordering the phloem and xylem (Suzuki *et al.*, 2011).

To affect both apoplastic and cytosolic signalling processes, the changes in ROS accumulation must be sensed in all subcellular compartments by means of location-specific systems. Different systems that perceive apoplastic ROS accumulation have been proposed, but no experimentally proven receptors or sensory systems have been identified in plants. Yet, indirect evidence points to their existence, and various components may be involved in ROS sensing (Shapiguzov *et al.*, 2012; Wrzaczek *et al.*, 2013). As many apoplastic proteins and peptides are rich in cysteine or contain conserved cysteines in their extracellular domain, they are suitable candidates as ROS sensors (Wrzaczek *et al.*, 2009; Murphy

et al., 2012). Moreover, the plasma membrane comprises several microdomains (i.e. lipid or membrane rafts) with various redox-sensitive systems and components (Lüthje *et al.*, 2013). In addition to the proposed receptor protein-related systems responsible for ROS perception, several other possible mechanisms that are affected by apoplastic ROS accumulation and cause downstream changes have been identified or might be involved in apoplastic ROS-sensory systems. ROS may also cause membrane lipid peroxidation (Farmer and Mueller, 2013), alter ion fluxes across the membrane by affecting redox-regulated ion channels (Garcia-Mata *et al.*, 2010), cause membrane depolarization (DeCoursey, 2003), result in functional conformational changes of proteins, and induce electric signals (Farmer and Mueller, 2013; Suzuki *et al.*, 2013; Wrzaczek *et al.*, 2013). These proposed apoplastic ROS sensors/receptors probably work in parallel, allowing fine-tuned and accurate intracellular signalling in response to apoplastic ROS (Wrzaczek *et al.*, 2013).

A significant number of receptor-like kinases (RLKs) are involved in plant responses to environmental cues (Chae *et al.*, 2009; Osakabe *et al.*, 2013; Sierla *et al.*, 2013; Kangasjärvi and Kangasjärvi, 2014). Given the central role of ROS, recent research on the involvement of RLKs in ROS sensing and signalling suggests that ROS play important roles in receptor activation. Apolastic ROS formed under stressful conditions can modify cell wall components and apoplastic proteins, whereas their breakdown products might act as receptor ligands. For example, a short peptide cleaved by metacaspase 9 from the extracellular GRIM REAPER (GRI) protein in a process that requires RBOHD-produced ROS acts as a ligand for the pollen-specific receptor-like kinase 5 (PRK5) protein, a leucine-rich repeat (LRR)-RLK involved in ROS-induced cell death (Wrzaczek *et al.*, 2009, 2015). ROS molecules have also been suggested to activate receptors by direct redox modifications of their extracellular domain, such as cysteine residues, leading to conformational changes and thus receptor activation and subsequent signal transduction. Several members of the CRK family, an RLK subfamily, appear to act in the sensory mechanisms that detect increased extracellular ROS (Idänheimo *et al.*, 2014; Bourdais *et al.*, 2015). An LRR-type RLK, GHR1, might also be involved in apoplastic ROS-related regulation of stomatal movements through conserved cysteine residues (Hua *et al.*, 2012). Thus, an RLK-related sensory system in which either CRKs (Bourdais *et al.*, 2015) or LRR-type RLKs (Hua *et al.*, 2012; Wrzaczek *et al.*, 2015) could be involved in the process induced or mediated by ROS. Similar apoplastic ROS signalling can be activated by exposure to a gaseous ROS molecule, such as ozone (O_3), that enters through stomatal pores and rapidly degrades into $O_2^{\cdot -}$ and H_2O_2 in the apoplast (reviewed in Vainonen and Kangasjärvi 2015).

The interaction of apoplastic ROS signalling with hormone signalling has mostly been studied with O_3 as elicitor. O_3 treatment increases biosynthesis of all major stress hormones (reviewed in Kangasjärvi *et al.*, 2005). The connections between hormones and ROS signalling can conveniently be analysed in Arabidopsis mutants or biosynthesis-deficient transgenic lines, such as *sid2*, deficient in ISOCHORISMATE SYNTHASE1 required for SA biosynthesis; *aos*, deficient in ALLENE OXIDE SYNTHASE and thus in JA biosynthesis; the *NahG* transgene, encoding a bacterial salicylate hydroxylase that degrades SA; and mutants involved in hormone signalling (*ein2*, ETHYLENE INSENSITIVE2; and *coi1*, CORONATINE INSENSITIVE1, encoding the JA receptor). To study the interaction between hormones, double and triple mutants that allow the study of two or three hormones simultaneously have been used (Kim *et al.*, 2014; Xu *et al.*, 2015). Treatment of these Arabidopsis mutants with O_3 has revealed a model in which JA is a negative regulator of apoplastic ROS-induced cell death, and SA and ethylene are positive regulators (Kangasjärvi *et al.*, 2005; Blomster *et al.*, 2011; Xu *et al.*, 2015). The most prominent interaction among the hormones is inhibition of JA signalling by ethylene, in contrast to the current dogma in pathogen signalling in which a synergistic interaction is assumed (Tuominen *et al.*, 2004; Kim *et al.*, 2014; Xu *et al.*, 2015). Intriguingly, with a similar set of mutants and O_3-induced changes in gene expression instead of cell death, several roles for these hormones have been observed, as positive and negative regulators, as well as antagonistic and synergistic hormonal interactors (Xu and Brosché, 2014; Xu *et al.*, 2015). Furthermore, in comparison with the wild type, a very large number of O_3-regulated genes had no altered expression in any hormone-related mutant (Xu *et al.*, 2015), overall emphasizing the context specificity of ROS signalling and the activation of multiple signalling pathways in response to apoplastic ROS.

Chloroplastic ROS production

Besides supplying photosynthesizing cells with energy, chloroplasts are also hubs for ROS signalling (Fig. 1). They produce significant and diverse pools of ROS in response to environmental changes, thereby initiating intraorganellar communication and affecting nuclear gene expression (Lee *et al.*, 2007; Shapiguzov *et al.*, 2012; Sierla *et al.*, 2013; Voss *et al.*, 2013). Exposure of chloroplasts to sudden high light intensities or abiotic stresses that decrease the maximal photosynthetic capacity provoke an excess of excitation/photon energy that over-reduces the photosynthetic electron transport (PET) components (Dinakar *et al.*, 2012; Adams *et al.*, 2013) and produce various ROS (Asada, 2006; Mubarakshina *et al.*, 2010; Foyer and Shigeoka, 2011; Fischer *et al.*, 2013). Chloroplastic $O_2^{\cdot -}$ can be delivered during photosynthesis in three ways. First, the partial oxidation of H_2O at the PSII electron donor side results in the formation of H_2O_2, which is then either reduced to $OH^{\cdot}$ or oxidized to $O_2^{\cdot -}$ (Pospíšil, 2009). Secondly, PET-related reduction of plastohydroquinone (PQH_2) at the cytochrome b_6f complex turns a plastoquinone (PQ) into a plastosemiquinone ($PQ^{\cdot -}$) that might generate $O_2^{\cdot -}$ upon interaction with oxygen (Cleland and Grace, 1999; Mubarakshina and Ivanov, 2010; Baniulis *et al.*, 2013). The $O_2^{\cdot -}$ formed can be reduced within the thylakoid membrane to H_2O_2 by PQH_2 (Mubarakshina *et al.*, 2006; Ivanov *et al.*, 2007; Pospíšil, 2009; Mubarakshina *et al.*, 2010), putatively involving the oxidation of the PSI components phylloquinone A_1 and the Fe–S centre S_X (Mubarakshina and Ivanov,

2010). Thirdly, within PSI, most electrons are promptly transferred from the reduced P700 reaction centre to the stromal Fe–S protein ferredoxin. As this water-soluble protein is a powerful reductant, oxygen reduction by reduced ferredoxins is probably responsible for most chloroplastic $O_2^{\cdot -}$ production (Asada, 2006). Under optimal circumstances, the chloroplastic $O_2^{\cdot -}$ is dismutated to H_2O_2 by thylakoid-bound and stromal superoxide dismutases and further oxidized to water by the ascorbate peroxidases and peroxiredoxins in the water–water cycle.

$^1O_2$ is produced at photosystem II (PSII) under specific conditions. Normally, an excited P680 will reduce its neighbouring electron acceptor molecule pheophytin and start a cascade of redox reactions via the members of the PET chain, resulting in the reduction of P700 of PSI and the establishment of an electrochemical proton gradient across the thylakoid membrane. However, whenever there is excess photon energy or a decreased CO_2 assimilation rate, over-reduction of the subsequent PET component will cause an excited triple state of the P680 and of chlorophyll antennae that may modify the electron configuration of neighbouring oxygen molecules and turn them into $^1O_2$ (Krieger-Liszkay, 2005; Asada, 2006; Krieger-Liszkay *et al.*, 2008; Fischer *et al.*, 2013), which will further react with different components in and around PSII.

Chloroplastic ROS and biotic stress

Together with other subcellular compartments, chloroplasts contribute to ROS production during the hypersensitive response (HR) in the defence against pathogens (Yao and Greenberg, 2006; Liu *et al.*, 2007; Zurbriggen *et al.*, 2009, 2010; Stael *et al.*, 2015). In the last few years, the involvement of chloroplasts in plant immunity has been increasingly recognized (Kangasjärvi *et al.*, 2012; Shapiguzov *et al.*, 2012; Sierla *et al.*, 2013; Serrano *et al.*, 2016). For example, the pathogen response differs between light and dark (Roden and Ingle, 2009; Hoeberichts *et al.*, 2013; Lozano-Durán and Zipfel, 2015), pathogen recognition triggers rapid transcriptional reprogramming of chloroplast-encoded transcripts, and some bacterial and viral elicitors interact with chloroplast-targeted proteins or are imported into chloroplasts (Padmanabhan and Dinesh-Kumar, 2010; de Torres Zabala *et al.*, 2016).

Chloroplasts also respond to ROS signals from other cellular compartments. For instance, when apoplastic ROS levels are high, this information is relayed to the chloroplasts by an as yet unknown mechanism, followed by chloroplastic ROS production. A short apoplastic ROS burst induces chloroplastic ROS production in guard cell chloroplasts (Vahisalu *et al.*, 2010). The flagellin 22 (flg22)/FLAGELLIN SENSING2 (FLS2) recognition-related immunity responses that trigger an apoplastic ROS burst have been shown to require chloroplastic ROS-dependent or -related processes (Nomura *et al.*, 2012; Göhre *et al.*, 2012; Sano *et al.*, 2014). Changes in gene expression profiles induced by flg22/FLS2-dependent processes in the chloroplasts resemble those provoked by $^1O_2$ (Nomura *et al.*, 2012; Stael *et al.*, 2015), suggesting that the processes initiated in the apoplast are followed by $^1O_2$ production and signalling in the chloroplasts. The chloroplastic responses in biotic interactions are reviewed in more detail by Serrano *et al.* (2016).

Chloroplastic $^1O_2$ signalling

To unravel which components of the signal transduction pathways are triggered by $^1O_2$, the *fluorescent* (*flu*) mutant of Arabidopsis (Meskauskiene *et al.*, 2001) has been the instrumental model system for years (op den Camp *et al.*, 2003; Ochsenbein *et al.*, 2006; Laloi *et al.*, 2007; Lee *et al.*, 2007; Kim *et al.*, 2012). In the dark, *flu* seedlings accumulate the chlorophyll precursor protochlorophyllide, resulting in $^1O_2$ production at dark-to-light transition. This subsequent, artificially produced burst of $^1O_2$ initiates pathways leading to chlorosis and cell death together with a profound reprogramming of nuclear gene expression (op den Camp *et al.*, 2003). The $^1O_2$ burst also activates a broad range of responses related to biotic and abiotic stresses, including the induction of ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1)-dependent accumulation of SA and the induction of the *PATHOGENESIS-RELATED PROTEIN1* (*PR1*) and *PR5* genes (Ochsenbein *et al.*, 2006; Lee *et al.*, 2007). EXECUTER1 (EX1) and EX2, two nuclear-encoded chloroplast proteins associated with thylakoid membranes with unknown function, are required for the $^1O_2$ -dependent chloroplast retrograde signalling (Wagner *et al.*, 2004; Lee *et al.*, 2007; Kim *et al.*, 2012). The biological effects related to $^1O_2$ might be antagonized by H_2O_2, because the overexpression of the thylakoid-bound ascorbate peroxidase in the *flu* mutant intensifies cell death, growth restriction, and $^1O_2$-specific nuclear gene expression (Laloi *et al.*, 2007). In the nucleus, TOPOISOMERASE VI is a regulator that can bind to promoters of $^1O_2$-responsive genes and act as an activator of $^1O_2$-regulated genes and as a repressor of H_2O_2-responsive genes (Šimková *et al.*, 2012)

Due to its reactivity and rapid quenching, $^1O_2$ cannot diffuse beyond the chloroplast envelope (Asada, 2006). Nonetheless, $^1O_2$ has been demonstrated to act as a potent retrograde signal (Triantaphylidès and Havaux, 2009; Laloi and Havaux, 2015) and to induce several stress-responsive pathways by producing an array of secondary messengers, such as reactive electrophile species, oxylipins, oxidized peptides, and the carotenoid cleavage compound β-cyclocitral (op den Camp *et al.*, 2003; Møller and Sweetlove, 2010; Farmer and Mueller, 2013; Fischer *et al.*, 2013; Laloi and Havaux, 2015). Genetic screens have revealed that EX1 and EX2 (Lee *et al.*, 2007; Kim *et al.*, 2012), the chloroplast thylakoid membrane-localized CALCIUM SENSING RECEPTOR protein CAS (Nomura *et al.*, 2012), the nuclear TOPOISOMERASE VI (Šimková *et al.*, 2012), and EDS1 (Ochsenbein *et al.*, 2006) act in $^1O_2$ signal transduction. EDS1 also processes chloroplastic $O_2^{\cdot -}$ signals and spatiotemporally co-ordinates chloroplastic and apoplastic ROS signals, probably in crosstalk with SA (Straus *et al.*, 2010).

Although the *flu* mutant is a convenient tool to produce $^1O_2$, some concerns have been raised as to whether its use

represents an artificial system for the study of $^1O_2$ signalling (Kim and Apel, 2013). Especially at later time points, the *flu* mutant displays excessive chloroplast leakage after dark-to-light transition. Whereas some of the early regulated genes (15 min) could be specific for $^1O_2$, the genes regulated after 2 h might correspond to genes responsive to many stresses (Kim and Apel, 2013). Hence, to get a full picture of $^1O_2$ signalling, additional mutants with increased $^1O_2$ accumulation and novel genetic screens are required to understand its role (Kim *et al.*, 2012; Kim and Apel, 2013; Ramel *et al.*, 2013). A screen for mutants defective in $^1O_2$ signalling in *Chlamydomonas reinhardtii* led to the identification of METHYLENE BLUE SENSITIVITY (MBS), a small zinc finger protein (Shao *et al.*, 2013). The corresponding proteins in Arabidopsis, MBS1 and MBS2, are needed for proper responses to high light, and they regulate the expression of genes responsive to $^1O_2$, but not to H_2O_2/O_2^- (Shao *et al.*, 2013). As indicated above, the response to $^1O_2$ is multifaceted, and several signalling pathways could probably act in parallel, including those regulated by EX1/EX2 and β-cyclocitral (Kim and Apel 2013).

Hormones and $^1O_2$ signalling

The signalling role of hormones from the chloroplast is obvious, given that biosynthesis of SA, JA, and ABA all start in the chloroplast. The exact role of hormones in ROS signalling initiated from the chloroplast has mainly been studied for $^1O_2$ signal transduction (i.e. in *flu*) or in gene expression experiments with treatments that increase chloroplastic ROS production (such as high light treatment). Increased $^1O_2$ production in *flu* promotes the synthesis of oxylipins, such as JA and *cis*-(+)-12-oxophytodienoic acid (OPDA) (Ochsenbein *et al.*, 2006). Cell death in *flu* and *chlorina1* (*chl1*), a mutant with reduced amounts of Chl *b* and, hence, increased $^1O_2$ production, decreases when crossed with JA-deficient mutants (Danon *et al.*, 2005; Ramel *et al.*, 2013). It is noteworthy that this cell death-activating effect of JA in $^1O_2$ signalling is opposite to the protective role of JA in apoplastic ROS-induced cell death (Kangasjärvi *et al.*, 2005; Blomster *et al.*, 2011). Oxylipin biosynthesis includes much more than only JA, and the precursor OPDA has been proposed to act antagonistically to JA in cell death regulation (Danon *et al.*, 2005). Whereas the bioactive JA-Ile conjugate is perceived through the receptor COI1, OPDA can be sensed through its own receptor CYCLOPHILIN 20-3 to regulate redox signalling (Park *et al.*, 2013). Hence, oxylipins and JA need to be considered as multiple signals rather than limited to the function of JA only.

In contrast to the opposite roles of JA in $^1O_2$ signal transduction versus apoplastic ROS signalling, the role of SA is more similar as a cell death promoter. Removal of SA through introduction of the *NahG* transgene or the *sid2* SA biosynthesis mutant into various ROS-sensitive backgrounds reduces cell death, regardless of whether the ROS signal is initiated from the apoplast, chloroplast, or peroxisome (Danon *et al.*, 2005; Kangasjärvi *et al.*, 2005; Kaurilind *et al.*, 2015). The effect of the $^1O_2$ signal molecule β-cyclocitral is at least partially regulated through SA in the regulation of damage and gene expression after high light treatment (Lv *et al.*, 2015). Consistent with mutant analysis, gene expression analysis from high-light-treated plants and *flu* reveals an increase in gene expression related to both SA and JA signalling (Ochsenbein *et al.*, 2006; Tikkanen *et al.*, 2014)

In contrast to $^1O_2$, H_2O_2 may diffuse from the thylakoid membrane (Davletova *et al.*, 2005; Mubarakshina and Ivanov, 2010) to the nucleus (Borisova *et al.*, 2012). These previous reports may be related to direct H_2O_2 translocation from the chloroplast to the nucleus via stromules that are induced in response to internal redox signals (Brunkard *et al.*, 2015) or during the ROS-induced programmed cell death in the HR (Caplan *et al.*, 2015). However, the signalling mechanisms triggered by organellar/plastid ROS that finally affect the nuclear gene expression remain largely elusive (Jaspers and Kangasjärvi, 2010; Shapiguzov *et al.*, 2012); these mechanisms are reviewed in detail by Kmiecik *et al.* (2016) and are not treated here.

Peroxisomal ROS production and signalling

Oxygenation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the chloroplasts initiates the intraorganellar photorespiration pathway that also leads to H_2O_2 production in the peroxisomes (Foyer and Noctor, 2009; Kangasjärvi *et al.*, 2012) (Fig. 1). The photorespiratory pathway consists of two interconnected cycles that functionally entangle chloroplasts, peroxisomes, mitochondria, and the cytosol: one cycle involved in glycolate carbon (C2) recycling and one in ammonium recycling (Foyer and Noctor, 2009; Bauwe *et al.*, 2010). The first step of the photorespiratory pathway in the peroxisomes is the oxidation of glycolate to glyoxylate that is catalysed by GLYCOLATE OXIDASE, generating H_2O_2 (Foyer and Noctor, 2009; Bauwe *et al.*, 2010).

Photorespiratory H_2O_2 production is considered to be the most significant oxidant source under light conditions in C_3 plants (Noctor *et al.*, 2002; Foyer and Noctor, 2003), because C_3 plants are prone to enhanced photorespiration, especially at high temperatures or under other stress conditions (Foyer and Noctor, 2009; Sage, 2013). Photorespiration is recognized as an important source of ROS signals when plants are exposed to various abiotic stresses (Osmond and Grace, 1995; Wingler *et al.*, 2000; Foyer and Noctor, 2009; Maurino and Peterhansel, 2010; Voss *et al.*, 2013; Weber and Bauwe, 2013). Catalases guard the peroxisomal H_2O_2 homeostasis in photosynthesizing tissues (Willekens *et al.*, 1997; Queval *et al.*, 2007; Du *et al.*, 2008; Mhamdi *et al.*, 2012). In loss-of-function mutants, such as the Arabidopsis *catalase2* (*cat2*) mutant, a conditional and non-invasive induction of peroxisomal H_2O_2 levels can be achieved through changes in growth conditions (Mhamdi *et al.*, 2010, 2012). Catalase-deficient plants have proven to be instrumental model systems to study the transcriptional responses provoked by the increased production and accumulation of peroxisomal H_2O_2 levels (Dat *et al.*, 2001; Vandenabeele *et al.*, 2004; Vanderauwera *et al.*, 2005;

Queval *et al.*, 2007, 2012). Increased H_2O_2 levels observed in *cat2* mutants have a direct impact on the cellular redox balance, as demonstrated by enhanced glutathione synthesis and decreased glutathione:glutathione disulfide ratios.

The responsiveness of the H_2O_2-induced genes may depend on the photoperiod rather than on the total light exposure or on the degree of oxidative stress (Queval *et al.*, 2007). The identified H_2O_2-responsive transcriptomes have revealed that expression levels of several defence-related genes increased, whereas expression of anthocyanin and some antioxidant-related genes decreased (Vandenabeele *et al.*, 2004; Vanderauwera *et al.*, 2005). One of the most rapidly and strongly induced genes was the UDP-glucosyltransferase-encoding gene *UGT74E2* (Vanderauwera *et al.*, 2005) that has been shown to enhance drought and salt tolerance and to modulate plant morphology by interacting with auxin homeostasis when overexpressed (Tognetti *et al.*, 2010). The connection between ROS and auxin appears to relate to the stress-induced morphogenic response that is responsible for the negative regulation of the optimal growth-related processes when stress acclimation is induced by ROS (Potters *et al.*, 2007; Teotia *et al.*, 2010; Blomster *et al.*, 2011; Brosché *et al.*, 2014).

The role played by stress hormones in cell death control in the *cat2* mutant has been studied via genetic approaches by means of double mutants (Chaouch *et al.*, 2010; Han *et al.*, 2013; Kaurilind *et al.*, 2015). SA and associated signalling components, including NONEXPRESSOR OF PR GENES1 and EDS1, are crucial determinants of *cat2*-driven cell death, whereas ethylene, a promoter of apoplastic ROS-mediated cell death, has seemingly no influence on *cat2*-mediated cell death (Kaurilind *et al.*, 2015), reinforcing the use of different signalling pathways for ROS from various subcellular sources (Sewelam *et al.*, 2014).

Mitochondrial ROS production and signalling

Mitochondrial ROS production takes place mostly in the oxidative phosphorylation process, the final stage of the aerobic respiration pathway. The oxidative phosphorylation couples the transfer of electrons that originate from NAD(P)H and flavin adenine dinucleotide (FAD) to their final acceptor O_2, with the transport of protons across a proton-impermeable inner mitochondrial membrane via several proton pumping enzyme complexes (I, III, and IV). This respiratory electron transport (RET) chain establishes an electrochemical proton gradient that is used in ATP synthesis. However, some RET chain components may leak electrons that can reduce O_2 to $O_2^{\cdot -}$ that, in turn, may yield H_2O_2 and $OH^{\cdot}$ via subsequent univalent reducing steps (Navrot *et al.*, 2007; Blokhina and Fagerstedt, 2010; Vanlerberghe, 2013). The RET chain sites from which most $O_2^{\cdot -}$ is released are the complexes I and III (Navrot *et al.*, 2007; Blokhina and Fagerstedt, 2010). Of crucial importance is the reduction state of ubiquinone, a small lipid-soluble electron and proton carrier located within the inner mitochondrial membrane; it serves as a regulatory cross-point for the mitochondrial RET-dependent $O_2^{\cdot -}$ production (Navrot *et al.*, 2007; Blokhina and Fagerstedt, 2010). Over-reduction of the ubiquinone pool at complex I can lead to a reverse RET activity and the release of $O_2^{\cdot -}$ into the mitochondrial matrix. At complex III, the hyper-reduced state of the ubiquinone pool induces a direct electron transfer to molecular O_2, followed by $O_2^{\cdot -}$ deposition in both the mitochondrial stroma and the matrix.

Alterations in the mitochondrial ROS homeostasis are involved in the retrograde communication between the mitochondria and the nucleus (Rhoads and Subbaiah, 2007; Woodson and Chory, 2008; Schwarzländer *et al.*, 2012; Shapiguzov *et al.*, 2012; De Clercq *et al.*, 2013). In Arabidopsis, this ROS-dependent signalling is reflected in the elevated expression of a set of genes known as the mitochondrial dysfunction regulon (De Clercq *et al.*, 2013). Amongst these genes, the ALTERNATIVE OXIDASE1-encoding gene *AOX1* (Vanlerberghe, 2013) is recognized as a hallmark responder to mitochondrial retrograde signalling events. AOX1 is generally activated under abiotic and biotic stresses and, more specifically, those deregulating respiratory metabolism (Clifton *et al.*, 2006; Li *et al.*, 2013). Under these conditions, AOX1 may dispatch an excess of electron energy from the RET as heat (Blokhina and Fagerstedt, 2010; Vanlerberghe, 2013), preventing over-reduction of the RET chain components and aggrevation of oxidative stress (Van Aken *et al.*, 2009; Cvetkovska and Vanlerberghe, 2012, 2013; Vanlerberghe, 2013). The ROS-inducible transcription factor WRKY15 represses *AOX1* by binding to its promoter (Vanderauwera *et al.*, 2012). This repression probably balances growth against salt/osmotic stress acclimation, because *WRKY15* overexpression renders plants sensitive to salt, osmotic, and oxidative stresses, and simultaneously enhances leaf growth and biomass production under control conditions (Vanderauwera *et al.*, 2012). Mitochondrially derived ROS may also affect the expression of several genes with the mitochondrial dysfunction motif in their promoters (De Clercq *et al.*, 2013). The *ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN13* (*ANAC013*) and *ANAC017* increase tolerance against oxidative stress in plants when overexpressed (De Clercq *et al.*, 2013; Ng *et al.*, 2013b). In addition, the homeostasis between mitochondrial ROS and antioxidant levels is essential to neutralize the excess of photosynthesis-reducing power, avoiding oxidative damage in the thylakoid membranes of chloroplasts (Noguchi and Yoshida, 2008; Dinakar *et al.*, 2010).

Interorganellar signalling

Although each organelle may individually manage its redox state and, thereby, contribute to the intracellular ROS homeostasis (Foyer and Noctor, 2013), ROS or related signalling intermediates are involved in interorganellar communication, as described for chloroplasts (Pesaresi *et al.*, 2007; Kopczewski and Kuźniak, 2013). Chloroplasts and mitochondria are tightly connected through metabolism, energy, and the redox state (Noguchi and Yoshida, 2008; Dang *et al.*, 2014;

Bailleul *et al.*, 2015), and co-operatively inform the nucleus of their developmental and functional states (Pesaresi *et al.*, 2007; Woodson and Chory, 2008; Kopczewski and Kuźniak, 2013). Mitochondrial ROS might be the common signals that are produced upon mitochondrial dysfunction (Rhoads and Subbaiah, 2007). A *cis*-regulatory element in the promoter region of several genes targeted by mitochondrial retrograde signalling, the mitochondrial dysfunction motif, is also responsive to H_2O_2 and recognized by transcription factors, such as ANAC013 and ANAC017 in Arabidopsis (De Clercq *et al.*, 2013; Ng *et al.*, 2013b). Upon mitochondrial redox imbalance, the endoplasmic reticulum-tethered ANAC013 and ANAC017 are proteolytically released from the endoplasmic reticulum and translocated into the nucleus to activate the transcription of several genes that encode mitochondria-targeted proteins. Incidentally, ANAC013 interacts with RCD1 (Jaspers *et al.*, 2009; O'Shea *et al.*, 2015), a nuclear protein involved as a co-regulator of several transcription factors, whereas absence of the interaction leads to high expression of genes with the mitochondrial dysfunction motif in their promoters, such as *AOX1* and *ANAC013* (Brosché *et al.*, 2014). The RCD1 protein is also involved in the acclimation response to chloroplastic redox imbalance and in the regulation of chloroplastic antioxidant and light-harvesting chlorophyll a/b-binding (LHCB) protein genes (Hiltscher *et al.*, 2014).

Another nuclear protein with known regulation of genes encoding both chloroplastic and mitochondrial-targeted components, such as AOX1 and LHCB, is the CYCLIN-DEPENDENT KINASE E1 (CDKE1) identified as the Arabidopsis *regulator of aox1* (*rao1*) mutant (Ng *et al.*, 2013*a*; Blanco *et al.*, 2014). CDKE1 acts in the kinase unit of the Mediator complex, which is a regulatory component between sequence-specific transcription factors and the general transcription factor TFIID required for RNA polymerase II binding. The C-terminal part of RCD1 contains an RST domain responsible for its interaction with transcription factors. This domain is also present in the Arabidopsis TAF4 (Jaspers *et al.*, 2010), a regulatory component of TFIID. Thus, the nucleus appears to contain proteins that respond to redox imbalances in both mitochondria and chloroplasts, downstream of the sequence-specific transcription factors, and to be involved in the regulation of transcriptional responses required for acclimation. However, very little is actually known about these aspects of interorganellar signalling and interactions. Identification of the additional components involved and elucidation of their mechanistic roles and interactions in the regulation of (interorganellar) signalling are one of the major questions for the future.

Interorganellar communication may use ROS redox-scavenging enzymes as well, such as poplar peroxiredoxin, thioredoxin, and glutaredoxin (Kopczewski and Kuźniak, 2013). ROS can be transported between different cellular compartments via intracellular vesicle trafficking (Leshem *et al.*, 2006, 2010). Furthermore, organelles are connected via metabolic pathways, such as the photorespiration pathway that encompasses chloroplasts, peroxisomes, mitochondria, and the cytosol. This pathway not only intertwines closely with the peroxisomal H_2O_2 production and signalling with chloroplastic photosynthesis rates, but it can also affect all other organelles by its integration into a paramount part of the plant metabolism and signalling cascades (Fernie *et al.*, 2013; Florian *et al.*, 2013; Sørhagen *et al.*, 2013). All the aspects of interorganellar connections are reviewed more thoroughly by Kmiecik *et al.* (2016).

In summary, ROS molecules can be produced in chloroplasts, peroxisomes, and mitochondria as a result of cellular metabolism and intentionally in the apoplastic region by enzymatic complexes (such as peroxidases and RBOHs) to serve diverse developmental or stress response-related purposes. Moreover, there are some indications that ROS might be generated within the cell nucleus (Ashtamker *et al.*, 2007; Mor *et al.*, 2014). However, unwanted, unfavourable stress conditions may intensify the regular ROS production and initiate the accumulation of toxic ROS doses in the plant cells (i.e. oxidative stress). Therefore, plants have developed several antioxidant strategies to prevent or control these harmful ROS levels, thus maintaining the redox homeostasis. Through consequent opportunities at the different subcellular compartments to allow transient increases in ROS, organellar and apoplastic ROS impact specifically (spatiotemporally) and differentially (positively or negatively) on the progression or inhibition of particular signalling cascades. Plants probably evolved such a complex and intertwined ROS-dependent interorganellar communication to capture the wide spectrum of distinct environmental stimuli that affect cellular homeostasis, allowing them to adapt their physiology accordingly. The ongoing assessment of this comprehensive ROS signalling matrix may result in the identification of new molecular targets implicated in ROS signalling that might be excellent candidates to develop novel technologies towards the breeding of more stress-tolerant plants.

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