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ROS signaling: the new wave?

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Reactive oxygen species (ROS) play a multitude of signaling roles in different organisms from bacteria to mammalian cells. They were initially thought to be toxic byproducts of aerobic metabolism, but have now been acknowledged as central players in the complex signaling network of cells. In this review, we will attempt to address several key questions related to the use of ROS as signaling molecules in cells, including the dynamics and specificity of ROS signaling, networking of ROS with other signaling pathways, ROS signaling within and across different cells, ROS waves and the evolution of the ROS gene network.

Origins of the reactive oxygen species (ROS) network

It is easy to imagine how cells had to acquire different antioxidants and ROS scavenging/detoxifying enzymes during evolution to cope with the increased levels of atmospheric oxygen that accompanied the appearance of oxygen-evolving microorganisms on Earth billions of years ago [1–3]. It is nevertheless harder to imagine how ROS with their toxic potential can play such a current key signaling role in cells. When considering the evolution of ROS as important signaling molecules we can assume that once cells learned to deal with ROS toxicity, they were able to utilize ROS for signaling purposes (Box 1). Moreover, we can also assume that there are numerous advantages for using ROS as signaling molecules. What is it therefore that makes ROS such good signaling molecules (after all if cells evolved to use them as such they must have their advantages)?

Several possible advantages come to mind when considering the use of ROS as signaling molecules. These include the capacity of the cell to rapidly produce and scavenge different forms of ROS in a simultaneous manner, enabling rapid and dynamic changes in ROS levels (caused by simply tilting the balance between cellular production and scavenging rates). Another advantage could be a tight control over the subcellular localization of ROS signals in cells. If we assume a significant capacity of cells to detoxify/scavenge or buffer ROS throughout the cell, then local increases in ROS production can be limited to particular locations of the cell, such as a certain membrane patch, or an organelle, making the spatial control of ROS accumulation highly specific. Detection of oscillating ROS signals in root hairs clearly demonstrates such capability [4,5]. Another advantage of ROS is that they could be used as rapid long distance auto-propagating signals transferred throughout the plant. Each individual cell along the path of the signal could activate its own ROS producing mechanism(s) in an autonomous manner carrying a ROS signal over long distances. It was recently reported for example that such a signal could propagate at a rate of up to 8.4 cm/min in Arabidopsis (Arabidopsis thaliana) [6]. An additional signaling advantage of ROS is that different forms of ROS exist, with significantly different molecular properties. For example, superoxide is a charged molecule under most physiological conditions and could not passively transfer across a membrane. By contrast, superoxide could be easily converted into hydrogen peroxide (H_2O_2) that readily transfers across membranes passively or through water channels [7]. Superoxide and H_2O_2 can also mediate the formation of lipid peroxides that would be membrane soluble. Thus, ROS have the advantage of being versatile signaling molecules with regard to their properties and mobility within cells. Moreover, as part of a cellular signaling network, ROS could be integrated with several different signaling pathways. Links with calcium and protein phosphorylation networks have been extensively studied, for example in the case of the ROS-generating respiratory burst oxidase (RBOH) NADPH oxidase proteins that contain an EF-calcium binding as well as phosphorylation domain(s) [8,9]. In addition, ROS levels are linked with cellular redox networks, for example through thioredoxins, peroxiredoxins, glutaredoxins and/or NADPH [10–12].

Another key signaling advantage of ROS is their tight link to cellular homeostasis and metabolism. Almost any change in cellular homeostasis could lead to a change in the steady-state level of ROS in a particular compartment(s). Physiological conditions that favor photorespiration would for example cause enhanced production of ROS in peroxisomes [13]. It is easy to envision how a tight link between metabolism and ROS levels would make ROS good signals to monitor changes in cellular metabolism. It is

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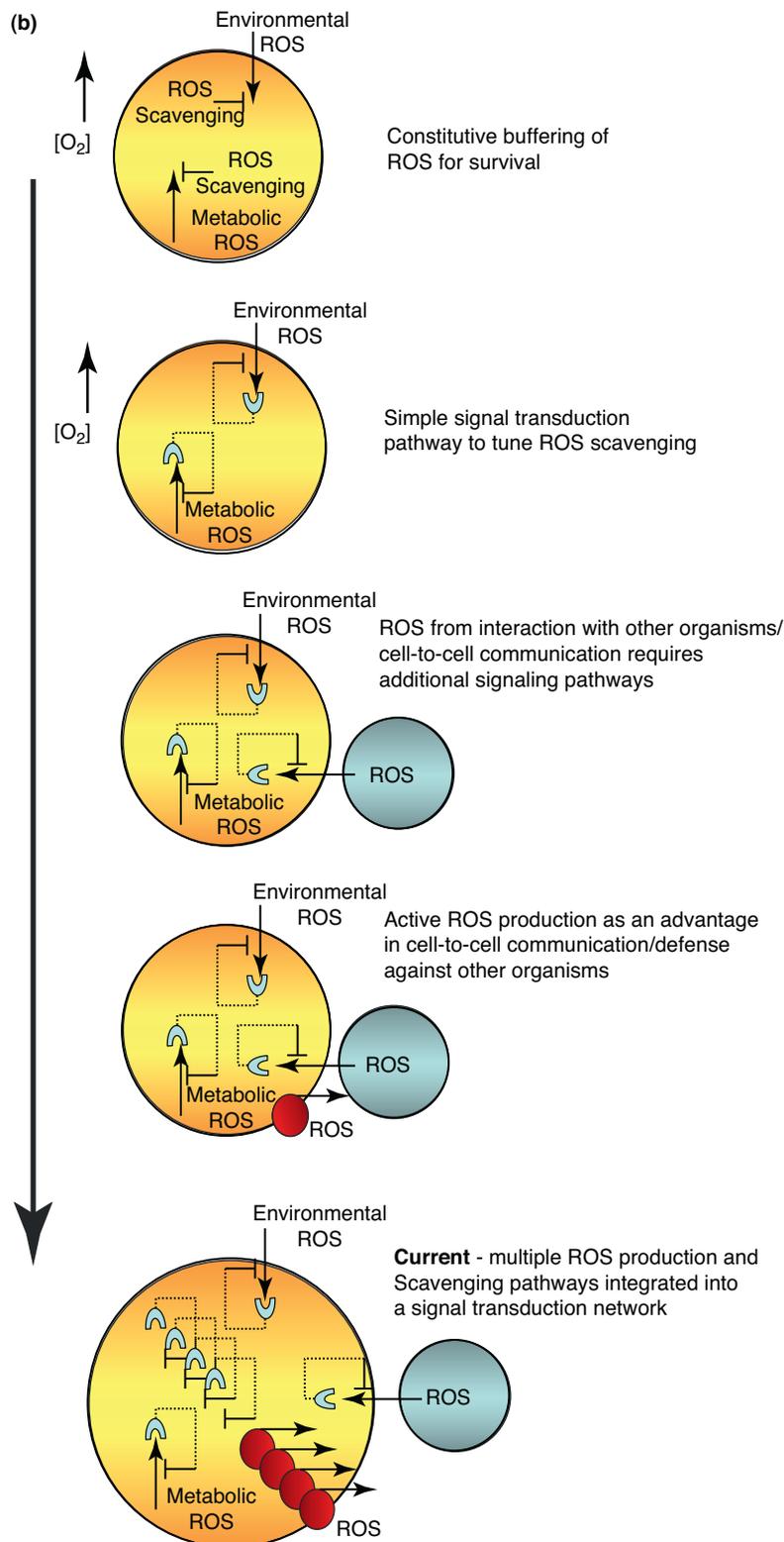## Box 1. An evolutionary view of the ROS gene network

An insight into the evolution of ROS signaling can be obtained by studying the ROS gene network of *Arabidopsis* [2] in a phylogenetic context, comparing it to several other representatives of the green plant lineage. In this regard, genome data of four dicots [*Arabidopsis*, poplar (*Populus trichocarpa*), grapevine (*Vitis vinifera*) and soybean (*Glycine max*)], four monocots [maize (*Zea mays*), rice (*Oryza sativa*), millet (*Sorghum vulgare*) and *Brachypodium distachyon*], a vascular non-seed plant (*Selanginella moellendorffii*), a moss (*Physcomitrella patens*) and four green algae (*Chlamydomonas reinhardtii*, *Volvox carteri*, *Micromonas* sp. *RCC299* and *Ostreococcus tauri*) were compared using the PLAZA platform [57]. Although the genomes of moss and green algae contain a smaller number of genes compared to flowering plants, they provide an excellent starting point to: (i) reconstruct the ancestral set of ROS-related genes at a certain time point during evolution and (ii) trace back the origin of newly acquired genes. Figure Ia summarizes the distribution of genes within the ROS-producing and ROS-scavenging families [2] across various species. (A complete list with gene identifier codes for each family and species can be viewed in Table S2 in the supplementary material online). This comparative analysis revealed that, except for catalase, all genomes of the green plant lineage encode members of each ROS-scavenging family. By contrast, a complete absence of the ROS-producing NAPDH oxidase gene family was observed in the algal genomes. Only from mosses on, plants appeared to have acquired genes to encode this gene family which then strongly expanded within the vascular plants. This family expansion might have been associated with the need for a more complex signaling network to coordinate multicellular growth, morphological complexity and biotic/abiotic stress responses. The evolutionary analysis of the ROS gene network in plants could therefore suggest that, at least with regard to the NADPH oxidase family, ROS-scavenging mechanisms were acquired before ROS-producing mechanisms, and that plants first learned to control their intracellular ROS levels and only then started using ROS for signaling purposes (Figure Ib).
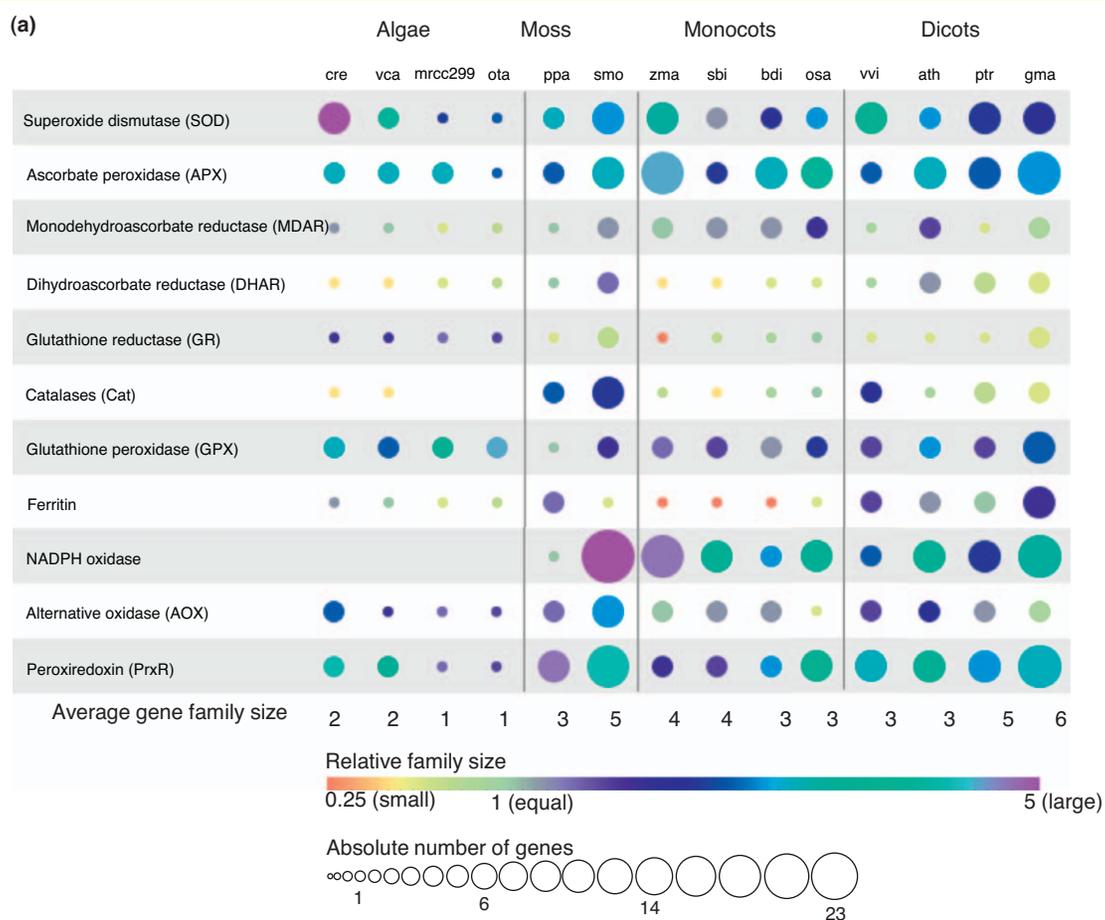
Figure I. An evolutionary framework of the ROS gene network within the green plant lineage. **(a)** Representation of ROS gene network genes in different species. For each ROS gene network family in *Arabidopsis* [2], orthologous genes in other species were determined using PLAZA 2.0, a comparative genomics resource to study gene and genome evolution in plants [57]. Starting from the *Arabidopsis* Genome Initiative codes, homologous gene families and phylogenetic profiles, reporting the gene distribution over the different species, were identified. Putative family members in other species were evaluated using PLAZA similarity heat maps, multiple sequence alignments, shared protein domain organization and phylogenetic trees. The absence of gene families in certain species was manually validated using dedicated sequence similarity searches against the genome sequence or assembled cDNA sequences. Circle size represents absolute number of genes in each family and species, whereas colors indicate relative family size (calculated as the ratio of the absolute number of genes divided by the average gene family size of each species). Abbreviations: ath, *Arabidopsis thaliana*; bdi, *Brachypodium distachyon*; cre, *Chlamydomonas reinhardtii*; gma, *Glycine max*; mrcc299, *Micromonas sp. RCC299*; osa, *Oryza sativa*; ota, *Ostreococcus tauri*; ppa, *Physcomitrella patens*; ptr, *Populus trichocarpa*; smo, *Selanginella moellendorffii*; sbi, *Sorghum bicolor*; vca, *Volvox carteri*; vvi, *Vitis vinifera*; zma, *Zea mays*. **(b)** A hypothetical model showing the different driving forces behind the ordered network evolution of ROS signaling in plants. A cell at an early evolutionary stage is shown as a circle at the top of the model. An increase in atmospheric pressure [O_2] is shown to drive the development of constitutive ROS buffering/scavenging systems ($\perp$). This is followed by the acquisition of simple signal transduction systems that include a sensing component (small semicircles linked with a dashed line to the buffering/scavenging systems) and finally ROS producing mechanisms (red circles), culminating in an advanced stage cell (bottom of model) that contains multiple ROS scavenging and ROS producing systems linked into a ROS signaling network.

(b)

Environmental ROS — Constitutive buffering of ROS for survival

Environmental ROS — Simple signal transduction pathway to tune ROS scavenging

Environmental ROS — ROS from interaction with other organisms/ cell-to-cell communication requires additional signaling pathways

Environmental ROS — Active ROS production as an advantage in cell-to-cell communication/defense against other organisms

Environmental ROS — **Current** - multiple ROS production and Scavenging pathways integrated into a signal transduction network

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Figure I. (*Continued*).

also possible that this was the initial evolutionary advantage to using ROS as signaling molecules, an advantage that led to further and future use of ROS to signal and control many different biological processes (Box 1). Because different organisms generate ROS at different levels and could leak or actively transport ROS such as H_2O_2 into their environment, it is possible that another advantage of ROS as signaling molecules in early stages of evolution was the sensing and/or communication between different organisms. Thus, the early need to sense and control internal (metabolic), as well as external (environmental/ other organisms/other cells), sources of ROS might have
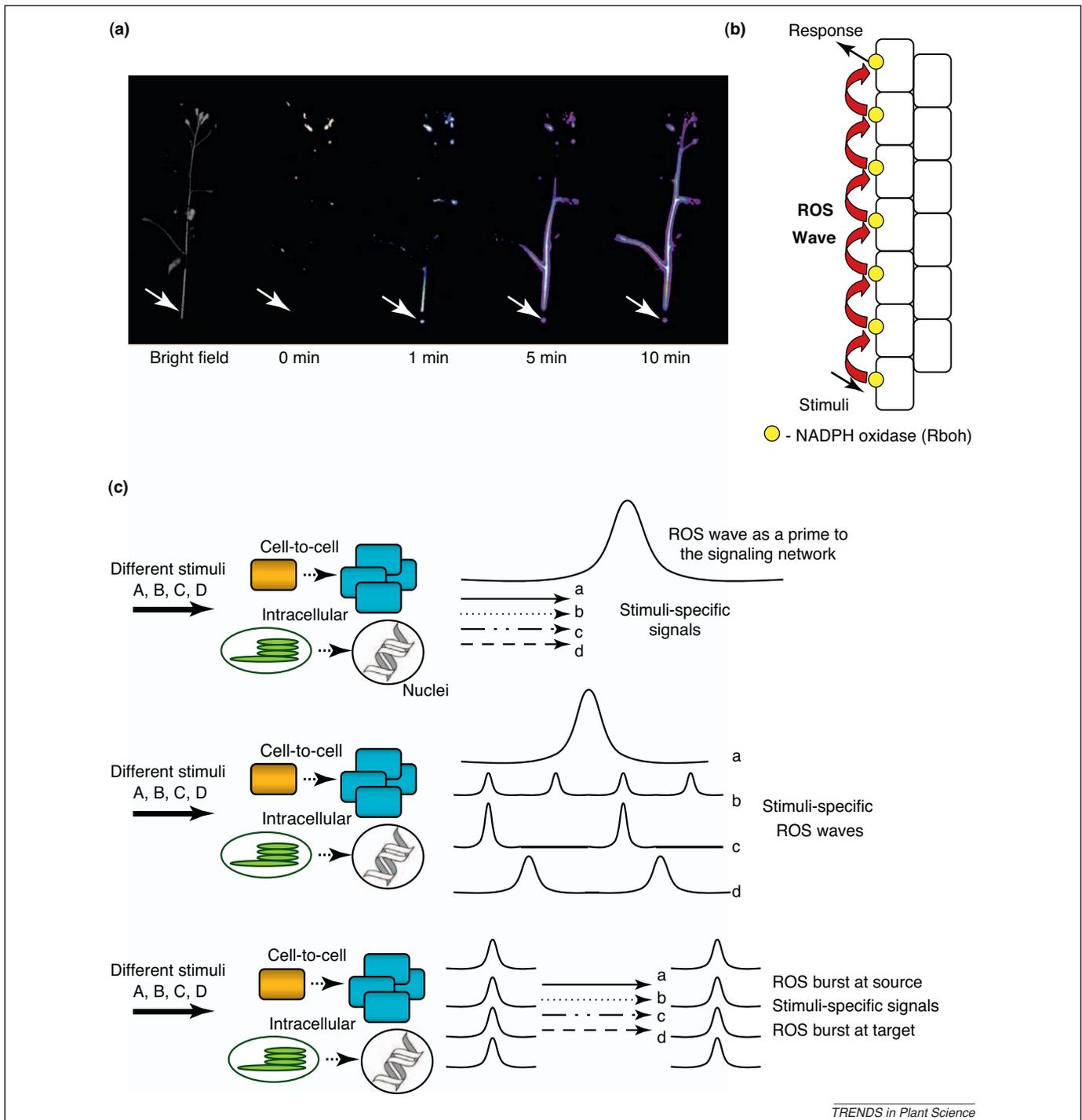
Figure 1. Dynamics and specificity in ROS signaling. **(a)** Time-lapse imaging of a ROS wave propagating through an *Arabidopsis* plant in response to wounding. Wounding site is indicated with a white arrow. Imaging of the ROS wave was obtained using a luciferase reporter gene fused to the promoter of the zinc finger protein ZAT12 as described in [6]. **(b)** Model for the extracellular propagation of the ROS wave [6]. Each cell along the path of the wave activates its NADPH oxidase (RbohD) and produces ROS in an autonomous manner resulting in an auto-propagating ROS wave. **(c)** Different models for ROS signaling specificity. Different signals (A, B, C, D) are transmitted between cells, or between different organelles within a cell, via ROS and other signaling pathways such as calcium, MAPK pathways and others.

contributed to the evolution of ROS as key signaling molecules (Box 1).

The dynamics of ROS signaling

The dynamic and rapid nature of ROS signaling in cells is a result of the contrasting processes of ROS production and scavenging [1–3,14]. Because these two processes always occur in cells in a simultaneous manner, tipping the balance between scavenging and production rates would

result in rapid alterations in ROS levels that will generate a signal. In many biological systems a burst of ROS, often occurring as two distinguished peaks, accompanies several different signaling events [15]. Nevertheless, new research using advanced imaging tools, such as a luciferase reporter gene expressed under the control of a rapid ROS-response promoter in plants [6], or a new H_2O_2/redox-GFP sensor in Zebrafish [16], revealed that the initial burst of ROS production could trigger a cascade of cell-to-cell communication

events that results in the formation of a ROS wave that propagates throughout the different tissues and carries the signal over long distances (Figure 1a; Movie S1 in the supplementary material online). Thus, the temporal concept of a ROS burst occurring in selected cells could now be modified into a temporal–spatial concept of a ROS wave. We can therefore envision ROS signaling as a dynamic process that occurs within cells between different organelles, as well as between cells over long distances.

Because plants have a high capacity to scavenge ROS, the long distance aspect of ROS signaling can only be explained by the continuous production of ROS in individual cells along the path of the ROS wave/signal. Such a mechanistic view will imply that the ROS wave is auto-propagating. It has recently been shown [6] that a ROS wave triggered by different stimuli can be blocked by the local application of catalase or an NADPH oxidase inhibitor, at distances that are up to 5–8 cm away from the signal initiation site. Moreover, the signal requires the presence of the NADPH oxidase *RbohD* gene and spreads throughout the plant in both the upper and lower directions [6] (Movie S1 in the supplementary material online). These experiments clearly demonstrate the auto-propagating nature of the ROS wave. The initiation of the wave in specific cells must therefore be associated with a long distance signal that causes individual cells along its path to activate ROS production via their own ROS producing mechanisms (Figure 1b).

The activation of systemic signals by local application of high light stress was recently shown to be accompanied by plasma membrane electrical signals in a light wavelength specific manner [17]. Because membrane potential could be directly affected by ROS and because the rate of certain electric signals in plants matches the rate of the ROS wave as previously reported [6], it is possible that the generation of a ROS wave affects the formation, amplitude and/or rate of the electrical signal. Further research is needed to address this intriguing possibility.

Examples of localized changes in ROS levels and/or ROS oscillation patterns were reported for root hairs, guard cells, pollen–stigma interactions and cells interacting with different pathogens or pests [4,15,18,19]. These examples, as well as the new reports of ROS functioning as long distance auto-propagating signals [6], strengthen our view of ROS as highly dynamic signaling molecules.

Specificity in ROS signaling: how can it be?

The question of ROS signal specificity has been the focus of considerable attention recently [20,21]. The main point of debate concerns how can a ROS signal, generated in a particular compartment or a particular cell, be specific for a particular stimulus? For example, an increase in ROS levels in the chloroplast or peroxisome could result from light intensity, heat or cold stresses. How then could this increase act as a specific signal to trigger an appropriate acclimation response? Likewise, a ROS signal can be generated in a group of cells in the plant in response to wounding, pathogen attack or a local abiotic stress, and be transferred to the entire plant, but how could it be specific? One possibility is that ROS are mainly used as a general signal to prime or activate the cellular signaling network of cells and that other signals function together

with ROS to convey specificity (Figure 1c, top panel). These other signals could be small peptides, hormones, lipids, cell wall fragments and others. A different possibility is that the ROS signal itself carries within it a decoded message, much like calcium signals that have specific oscillation patterns within defined cellular locations (Figure 1c, middle panel). The specific features of the signal (amplitude, frequency and/or localization) could then be perceived and decoded by specialized mechanisms to trigger specific gene expression patterns. A third possibility is that each cellular compartment or individual cell has its own set(s) of ROS receptors to decode ROS signals generated within it, which are then transferred by other networks such as calcium and/or protein phosphorylation (Figure 1c, lower panel). As is usually the case with different biological systems, it is most probable that a combination of the different mechanisms described above, as well as others, function in cells and that ROS signaling cannot be evaluated as a stand-alone network but as an integrated signaling pathway that functions together with many different signaling networks. In future studies it would be interesting to determine whether different subnetworks within the entire cellular signaling apparatus could be defined, and whether these could be linked in a hierarchic manner to provide detailed understanding of the ROS signaling process. A strong link is also likely to exist between ROS signaling, the redox network of cells and the different antioxidant pools in different cells, or in a different subcellular compartment of cells. Unraveling the different interactions between these interconnected networks would be the first step in linking these networks with other cellular signaling pathways.

An interesting example of high specificity in ROS signaling is the analysis of double mutants deficient in APX1 and CAT2 in *Arabidopsis*, and APX1 and CAT1 in tobacco (*Nicotiana tabacum*) [13,22]. Compared to wild type, and the single *apx* or *cat* mutants, these double mutants are surprisingly more tolerant to different environmental conditions [13,22]. It was recently found that the combined lack of APX1 and CAT2 in *Arabidopsis* causes a unique ROS signature in cells that triggers a novel acclimation response involving the activation of DNA repair, cell cycle control and antiprogrammed cell death mechanisms. A similar response is not found in the *apx1* or *cat2* single mutants demonstrating the need for a specific ROS signature for its activation, a signature that is only found in the double mutants [13].

Deciphering the complex mode of ROS signaling within cells would require the development and utilization of many more mutants deficient in ROS signaling and the combined use of these mutants with advanced subcellular-specific ROS imaging tools. Advances in the study of ROS gene network genes are summarized in Table S1 in the supplementary material online. A highly complex mode of interaction between ROS signaling and growth, development and stress responses in plants is revealed. Especially notable are the multiple mutants, lacking two or more ROS metabolizing/signaling enzymes, which often produce unexpected results [22,23] (Table S1 in the supplementary material online). These can provide new insights into how plants balance and mediate ROS signaling, as well as reveal new and yet unknown ROS producing or scavenging mechanisms.

Networking of ROS signaling with other signaling pathways

As indicated above, ROS signaling is integrated with many different signaling networks in plants. These include protein kinase networks, calcium signaling, cellular metabolic networks and redox responses. In some instances, ROS accumulation was found to precede the activation of signaling through these networks, whereas in other examples ROS accumulation was found to be a direct result of signaling through these networks. A good example for a ROS-activated signaling network is the mitogen-activated protein kinase (MAPK) cascade.

Many different MAPKs cascades can be activated following ROS accumulation. These include the ROS-responsive MAPKKK MEKK1, MPK4 and MPK6 [19,24,25]. The MEKK1 pathway is highly active during abiotic and oxidative stress conditions, and MEKK1 is an activator of two highly homologous MAPKKs (MKK1 and MKK2), which function upstream of the MAPKs MPK4 and MPK6 [24,25]. MEKK1 was suggested to be specifically required for the activation of MPK4 by H_2O_2 [26]. Transcriptome analysis of MEKK1, MPK4 and MKK1/2 deficient mutants identified 20 different transcription factors previously classified by Gadjev *et al.* [27] as highly responsive to multiple ROS-inducing conditions [28]. Two other ROS-responsive MAP kinases MPK3 and MPK6 that are activated by H_2O_2 were recently shown to depend on MKK9 for their activity [24,29]. The guard cell highly expressed MPK9 and MPK12 was recently implicated as required for abscisic acid (ABA)-induced stomata closure functioning downstream to ROS signals and necessary for the activation of anion channels. MPK12 activity was also shown to increase in response to both ABA and H_2O_2 application [19]. MAPK pathways are also implicated in the induction of nitric oxide (NO) and ROS bursts and signaling, which synergistically function in defense responses and were recently shown to confer resistance to pathogens [30]. Both NO and ROS were reported to be produced simultaneously through the MAPK cascade MEK2–SIPK (salicylic acid-induced protein kinase) [30,31].

In contrast to MAPKs, the NADPH oxidase complex is a good example for ROS production that is driven by a compilation of different cellular signaling pathways. Pathways that proceed NADPH oxidase activation include calcium signaling and protein phosphorylation [8,9,32]. *Solanum tuberosum* StCDPK4 and StCDPK5, have been shown to phosphorylate StrbohB in potato (*Solanum tuberosum*) [8], and the ABA-activated SnRK2 protein kinase open stomata 1 (OST1) (SRK2E/SnRK2.6) was recently shown to act upstream of ROS in guard cell ABA signaling. OST1 physically interact with AtRbohF and phosphorylates it on Ser13 and Ser174 [33]. More recently, SLAC1, a protein essential for guard cell anion channel functioning was shown to be a substrate for OST1 phosphorylation. SLAC1 is essential for stomatal closure in response to ABA, CO_2, O_3, light–dark transitions and humidity change, and by Ca^{2+}, H_2O_2 and NO [34]. AtRBOHD was shown to be directly phosphorylated *in vivo*, which together with Ca^{2+} binding synergistically activates its enzymatic activity [9]. Two MAPK cascades, MEK2–SIPK and MEK1–NTF6, were suggested to be involved in the induction of *NbRbohB* gene and protein activities in *Nicotiana benthamiana* [30].

Links between ROS signaling and cellular redox were proposed to be mediated by peroxiredoxins, NADPH, kinase activity or the detection of oxidized proteins and peptides [10–12,20]. Oxidation of methionine (Met) residues to Met sulfoxide (MetSO) in kinase substrate proteins, such as nitrate reductase, can inhibit the phosphorylation of nearby sites and thereby couple oxidative signals to changes in protein phosphorylation [35]. Direct coupling of ROS signaling with primary cellular metabolism is a key feature of ROS signaling in cells. ROS accumulation for example can cause an inhibition of the tricarboxylic acid cycle (TCA) in mitochondria and upregulation of glycolysis and oxidative pentose phosphate pathways [36–38]. In chloroplasts, ROS signaling is coupled to the redox state of the plastoquinone (PQ) pool and plays an important role in the response of plants to changes in environmental conditions [39–41]. Plants optimize their photosynthetic activity by regulating the association of light harvesting complexes with thylakoids, and by adjusting photosystem stoichiometry to rearrange the balance of excitation energy [42]. The STN7 kinase was recently identified as a key regulator of these responses, linking them to the PQ redox state [42]. A chloroplast sensor kinase was also recently shown to be required for the regulation of gene expression in chloroplasts in response to changes in redox state of electron carriers connecting the two photosystems [43]. These studies expand our understanding of how redox and ROS levels balance some of the key metabolic pathways in plants.

Links between ROS and hormonal signaling are summarized in Box 2. These demonstrate a complex interaction between ROS signaling, environmental conditions and plant development. Overall it appears as if the more we study ROS signaling, the more we discover how intertwined it is with almost all aspects of plant signaling, physiology and development. It is probable that unraveling the different interactions that tie the different signaling networks and pathways of plants will be a challenge for many years to come.

Imaging of ROS signaling

Recent developments in cellular imaging and real-time detection tools have advanced our understanding of ROS metabolism and cellular dynamics in human, animal and microbial systems. Among these are two-photon fluorescence microscopy, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) imaging, atomic force microscopy and optical tweezers force spectroscopy [44]. Although these techniques have proved valuable in measuring the level of ROS and other metabolites in different organisms, their use in plants is very limited. The main imaging tools that have been used in plants are the green fluorescent protein (GFP)-based redox probe roGFP, fluorescent dyes and luciferase [6,45–49]. Fluorescence-based probes provide an important means for non-invasive quantitative monitoring of redox changes and include redox-sensitive yellow fluorescent protein (rxYFP), roGFPs and the YFP-based probe HyPer [50–52], and have been successfully used to follow the cellular redox status in multiple biological systems. Application of some of the newer tools described above as well as development of

Box 2. Integration of ROS and hormonal signaling

ROS signaling is highly integrated with hormonal signaling networks (Figure I), thereby allowing plants to regulate developmental processes, as well as adaptive responses to environmental cues. Similar to ROS, salicylic acid (SA) is involved in defense and cell death responses [58] and increased ROS levels can cause SA accumulation [59]. Interestingly, ablation of SA biosynthesis could prevent ROS-induced defense responses and cell death without affecting redox state [60]. A ROS burst is involved in SA-induced stomatal closure [61] and the crosstalk between ROS and ABA was extensively reported [62]. Interactions between ROS, NO and ethylene signaling were described in both a stress responsive and a developmental context [63,64]. Gibberellin (GA) signaling is linked with ROS contributing to the fine-tuning of ROS levels by stimulating the destruction of the nuclear growth-repressing DELLA proteins that regulate transcript levels of antioxidant enzymes [65].

Root apical meristems (RAMs) are rapidly reorganized in response to environmental triggers. This plasticity is linked to a complex signaling module in which ROS, NOS and antioxidants operate in strict association with hormonal signaling pathways such as auxin, GA and cytokinin [66]. RAM responses are modulated by localized ROS production in quiescent centers and the GA–DELLA signaling pathway [66]. GA-induced genes include the plant-specific GAST1-like genes which encode small proteins with a conserved cysteine-rich

domain possibly involved in redox regulation [67]. The stress response of C19–GA2ox genes, a major GA inactivating pathway, is another example of ROS effect on hormone homeostasis integrating extrinsic signals with developmental programs [68].

The integration of ROS with auxin signaling networks, triggered by environmental factors, is known as the stress-induced morphogenic response. In this response, ROS and auxin metabolism interfere and lead to morphological changes that help avoid deleterious effects of environmental stress [69,70]. These morphological changes can be partly explained by ROS affecting auxin homeostasis at different levels including oxidative degradation by stress-induced peroxidases, increased auxin catabolism, auxin transport and redistribution caused by altered expression and cellular location of PIN proteins [71–74]. A cohort of auxin responsive genes are differentially expressed during various stresses, and oxidative stress related genes are regulated differentially by auxin [70,75]. Auxins are also known to induce a programmed and cell-specific ROS generation or to regulate the level of antioxidants [76–78]. Two key regulators of redox homeostasis: NADP-linked thioredoxin (NTRX) and glutathione were recently found to alter both auxin transport and metabolism [79]. It would be interesting in future studies to identify additional convergence points between ROS and hormone signaling pathways.
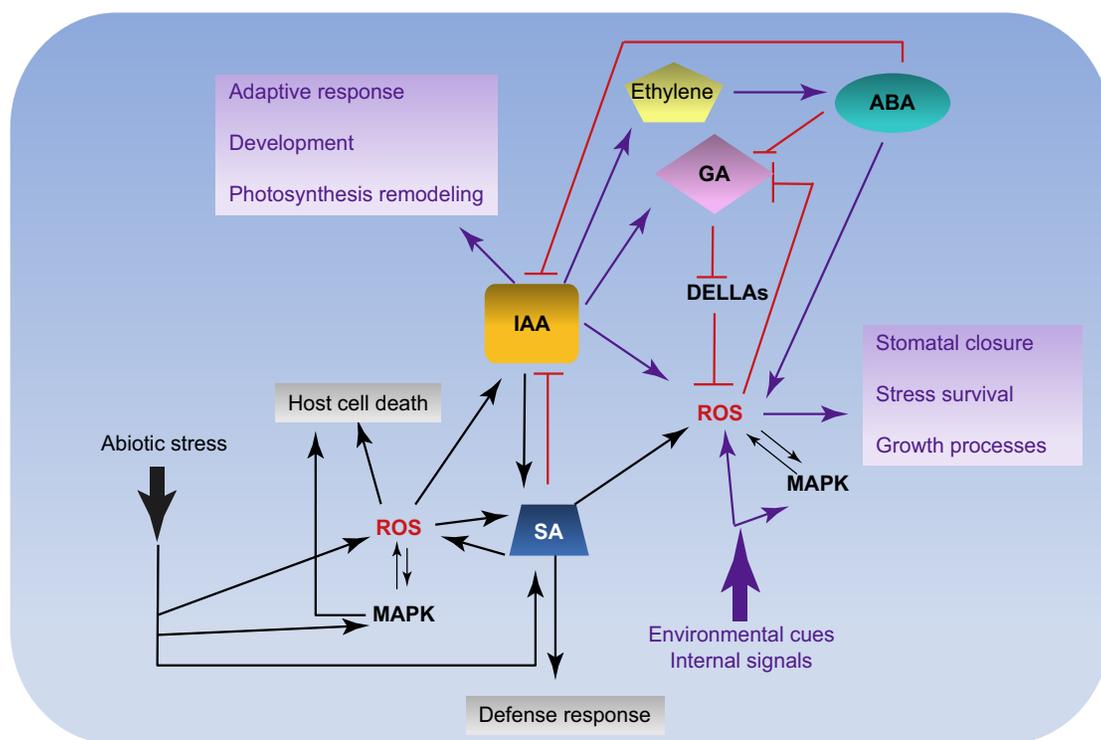
Figure I. ROS–auxin crosstalk in relation to other hormone signals. A schematic model showing coregulation and crosstalk between ROS and different hormonal signaling pathways in response to different environmental stimuli or developmental signals. Black and purple arrows indicate activation by abiotic or environmental and internal triggers, respectively, whereas red lines indicate repression. ROS can affect biosynthesis, degradation, metabolism, transport or perception of auxin. Abbreviations: ABA, abscisic acid; GA, gibberellins; IAA, auxins; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species; SA, salicylic acid.

new imaging tools in plants is highly needed to advance the study of ROS signaling in plants.

Future directions in ROS signaling research

Several possible research avenues come to mind when considering ROS signaling. The new concept of ROS waves, compared to ROS bursts, requires further research. How are these waves being propagated within and/or across

different cells? What is the degree of specificity communicated by these waves? How are they linked to changes in membrane potential? How are they even possible in light of the high capacity of cells to scavenge ROS?

The question of how ROS signals travel within or across different cells is also an intriguing one. It was previously reported that ROS can be formed within enclosed vesicles [53]. Is this a possible way to transfer ROS from one

subcellular location to another? Would these vesicles or other mechanisms transfer ROS signals along cytoskeleton fibers within cells? Are there yet unknown ROS producing enzymes/complexes that function within or on the surface of cells that could be associated with cytoskeleton components? Is the apoplastic space an easier conducting media for transferring ROS signals, compared with the cytosol or vacuole?

The view of ROS as being produced by specific complexes, such as the NADPH complex, is an acknowledged concept that suggests a high degree of cellular regulation by different proteins that associate with the complex. Signal-specific sensing and transducing complexes were reported in plant and animal cells. However, a sensor and/or signal transducer complex for ROS related proteins has not yet been identified in plants. The identification of such a complex would be of immense importance to the field of ROS signaling research.

What can we learn from animal systems? Several interesting papers have recently been published describing the dynamic nature of mitochondrial-to-mitochondrial communication in heart muscle cells. In heart cells mitochondria are found in a lattice-like arrangement and communicate with each other via waves of ROS-induced–ROS-release [54]. This ROS-induced–ROS-release mechanism might be similar to the mechanism(s) that propagates cell-to-cell ROS signaling across long distances in plants [6]. Similarities between animal and plant models for wound-induced ROS waves can also be found between Zebrafish and *Arabidopsis* [6,16]. ROS signaling could therefore have broad similarities across different kingdoms, as is expected from an evolutionary conserved signaling pathway.

A mostly unanswered question in plant ROS research has been: How do plant cells sense ROS? Several different systems involving 'single-Cys' and 'two-Cys' redox sensors, as well as different variations on metal sensing proteins that involve His oxidation, or two-component receptors have been proposed in bacteria, algae and animal cells [2,55]. It is unclear, however, whether similar systems function in plants, and how are they integrated into the ROS signaling network of plant cells. It is probable that genetic screens for mutants impaired in ROS sensing will reveal some of these mechanisms.

The development of advanced imaging tools, such as MALDI-MS imaging, would enable the detection of ROS and antioxidant pools in specific tissues and/or subcellular compartments. These would be of key importance to our understanding of ROS signaling. Dynamic changes in the nuclear pool of glutathione were recently reported in plants [56]. Subcellular changes in glutathione, as well as other antioxidants and/or ROS, could explain many different regulatory events in plants linking redox status with ROS signaling and enabling rapid responses to changes in environmental conditions or different developments cues.

Many unknown players in ROS signaling still exist. The vacuole, for example, could have a key buffering/signaling role in ROS metabolism. Organelle movement and the cytoskeleton could also play a central role in distributing ROS signals. Unknown ROS producing mechanisms could exist within plant cells as well as novel antioxidants. It is probable that the increased effort to sequence many different plant genomes, coupled with a more direct research into gene function and ROS metabolism, will unravel many of these mechanisms. Because ROS are linked to many biotic and abiotic responses, deciphering ROS signaling is likely to have a significant impact on agriculture and biotechnology in many countries and could lead to the development of crops with enhanced yield under suboptimal conditions. The future of ROS research is very promising!

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2011.03.007.

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