This is a preprint of an article published in Nature Reviews Molecular Cell Biology. The final authenticated version is available online at: https://doi.org/10.1038/s41580-022-00499-2

Reactive oxygen species (ROS) signalling in plant stress responses

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Abstract | Reactive oxygen species (ROS) are key signalling molecules that enable cells to rapidly respond to different stimuli. In plants, ROS play a crucial role in abiotic and biotic stress sensing, integration of different environmental signals and activation of stress-response networks, thus contributing to the establishment of defense mechanisms and plant resilience. Recent advances in the study of ROS signalling in plants include the identification of ROS receptors and key regulatory hubs that connect ROS signalling with other important stress-response signal transduction pathways and hormones, as well as new roles for ROS in organelle-to-organelle and cell-to-cell signalling. Our understanding of how ROS are regulated in cells by balancing production, scavenging and transport has also increased. In this Review, we discuss these promising developments and how they might be used to increase plant resilience to environmental stress.

Introduction

Pathogens, insects and different abiotic stresses such as flooding, prolonged droughts and heat waves, result in heavy losses to agricultural production and threaten global food security[1,2]. The alarming increase in the frequency and intensity of these stresses, an outcome of global warming and climate change[3,4] highlights the importance of understanding the mechanisms that increase plant resilience against such stresses. Reactive oxygen species (ROS) play key roles in stress sensing, the integration of different stress-response signalling networks and the activation of plant defense mechanisms and acclimatization. Dissecting and understanding how ROS orchestrate plant responses to stress will allow us to improve plant tolerance to stress and increase our ability to mitigate crop damage when exposed to harsh environmental conditions[4].

The term ROS describes a group of molecules derived from molecular oxygen (O_2). Whereas O_2 is generally nonreactive towards most cellular components, ROS can cause the oxidation of lipids, proteins, RNA, DNA and many small molecules in cells. The high reactivity of ROS towards these cellular components is due to their altered chemistry, compared to O_2, that allows them to donate an electron or transfer an excited energy state to an acceptor molecule[5]. The major forms of ROS in cells, which vary greatly in their properties and chemical reactivity, include hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($HO^{\cdot}$) and various forms of organic and inorganic peroxides (FIG 1a; Supplementary Table 1)[5–9]. As ROS are highly reactive, and independently produced in all or most cell compartments, their levels are kept under control to prevent unintended cellular oxidation. This is achieved by balancing of ROS production, scavenging and transport, which together keeps ROS at low concentrations, as well as controls ROS signalling reactions and their outcomes (FIG 1b).

Several hundred genes encode for the different proteins and enzymes that regulate ROS metabolism and signalling in plants (Supplementary Table 2)[6–8]. ROS are produced 'passively', by house-keeping enzymes or as byproducts of metabolic pathways (for example, photosynthesis and respiration), or 'actively', by dedicated oxidases that generate ROS for the purpose of signalling — for example, RESPIRATORY BURST OXIDASE HOMOLOGs (RBOHs), which are the functional equivalents of mammalian NADPH OXIDASEs (NOXs)[5–9]. At the same time, ROS are scavenged by an array of enzymatic and non-enzymatic antioxidants also found in most or all cell compartments (Supplementary Table 2; Supplementary Box 1)[5-9]. In addition, ROS can be transported between different compartments (for example, by AQUAPORINs; AQPs)[10], or to other cells and tissues, for the purpose of signalling, removal, or accumulation. Thus, ROS can function where they are produced, or at a distance.

ROS accumulation in cells during stress affects the redox state of many different proteins, including enzymes, receptors, and small molecules, activating, modifying, or integrating multiple stress-response

signal transduction pathways (FIG 1b). These alter gene expression and enhance the resilience of plants to stress[11–21]. Recent advancements in our understanding of these important processes include the identification of specific ROS sensors and regulatory hubs that connect ROS signalling with other stress-response signal transduction pathways and hormones, the use of artificial intelligence-driven tools to dissect the different regulatory networks triggered by ROS sensing, and the identification of new roles for ROS in organelle-to-organelle and cell-to-cell stress signalling.

In this Review, we first describe our current understanding of the mechanisms that control ROS production, scavenging, sensing and transport in plants. We then discuss how plants integrate ROS signalling with different hormone, retrograde, calcium, phosphorylation, and other stress-response signal transduction mechanisms to regulate gene expression and induce stress resilience. We focus mainly on H_2O_2, as it has a prominent role in the regulation of biological activity in cells.

ROS production and scavenging during stress

Cellular homeostasis is characterized by a baseline level of ROS that depends on the plant developmental stage, circadian clock, environmental and physiological conditions, and interactions with its microbiome. Different biotic and abiotic stresses can disrupt this homeostasis, uncouple metabolic pathways and lead to the accumulation of ROS in different cell compartments.

For example, during excess light stress, when the flux of photons overcomes the plant energy needs to fix CO_2, $O_2^{\cdot-}$ and $^1O_2$ are primarily produced in the chloroplasts by photosystems I and II, respectively, and if photorespiration is activated (*e.g.*, in C3 plants), H_2O_2 will also be produced in peroxisomes[22–24]. The production of ROS could be further elevated during drought stress when CO_2 availability is limited due to the closure of stomata, and the excess energy absorbed by the photosynthetic apparatus cannot be channeled into CO_2 fixation[25–27]. During heat stress when membrane complexes involved in different electron transfer chains are disrupted, $O_2^{\cdot-}$ and H_2O_2 are produced in mitochondria and chloroplasts, and increased levels of ROS accumulate in the cytosol and nucleus (Supplementary Box 1)[28,29].

A different pattern of ROS accumulation appears during responses to pathogens. $O_2^{\cdot-}$ and H_2O_2 are primarily produced in the apoplast due to the activation of specific oxidases such as RBOHs (Supplementary Box 1), as well as in chloroplasts as a consequence of the disruption and imbalance of metabolic pathways[30–34]. In contrast, virus infection was recently shown to cause the suppression of peroxisomal ROS production due to interactions of viral proteins with glycolate oxidase[35].

Recent advancements in the use of genetically encoded ROS sensors and dyes revealed that during different stresses different types of ROS accumulate in different compartments of the cell[25,28,29,36–45]. Therefore, different patterns or signatures of ROS accumulation in cells are induced in a stress-specific manner (FIG 2). Moreover, recent studies have revealed that ROS can be transported in or out of different compartments and/or trigger different retrograde and anterograde signalling pathways between different cell compartments and the nucleus[10,34,39,46–49]. The different ROS and other signals produced in the different cell compartments in response to different stimuli could trigger stress-specific signal transduction pathways that activate stress-specific acclimatization and defense mechanism (FIG 2). The findings that different stresses result in the formation of different ROS signatures can serve as a working platform for future studies on how specificity in plant responses to stress is achieved.

When studying ROS signalling in plant cells it is also important to consider that aerobic life evolved in the presence of ROS (Supplementary Box 2)[50,51], suggesting that most cells are able to prevent ROS toxicity, and that ROS are primarily used for stress-sensing and signalling purposes[6].

To understand how the transient or continuous accumulation of ROS in different compartments during stress triggers defense responses, it is first important to understand how ROS are sensed in cells.

ROS perception and redox regulation

Unlike most 'classical' signal transduction molecules such as hormones or peptides that have a defined set of receptors, changes in ROS levels in cells can alter the structure and function of multiple proteins and therefore impact on many different signal transduction pathways. This 'multiple-pathway' signalling property of ROS is primarily mediated through oxidative post-translational modifications (oxi-PTMs)[52–56] and allow ROS to be broad and dynamic regulators of multiple responses to stress (FIG 3).

Oxi-PTMs of different proteins during stress. Thiols in Cysteine (Cys) and Methionine (Met) residues of many proteins are susceptible to oxidation as they are intrinsically nucleophilic. However, their protein microenvironment, such as the presence of positively charged residues or hydrogen bonds, influences their reactivity[57]. The first ROS-induced oxidation intermediate of the Cys thiol is sulfenic acid (-SOH) which is highly reactive and reversible (FIG 3a). Sulfenic acid can be further oxidized to sulfinic ($-SO_2H$) and sulfonic (SO_3H) acids, both of which are considered to be mostly irreversible modifications triggering protein degradation ($-SO_2H$ can in some cases be reversed through the action of sulfiredoxin)[58–60]. Most common in the context of ROS signalling events are the reactions of sulfenic acids with proximal

proteinaceous thiols that are either inter- or intra-molecular (*i.e.*, mixed disulfides), or with small molecules such as glutathione (GSH; *i.e.*, S-glutathionylation)[61,62] (FIG 3a).

In addition to ROS, other reactive electrophilic species can modify Cys thiols. For example, nitric oxide (NO) can trigger the formation S-nitrosothiols (–SNO), whereas hydrogen sulfide (H_2S) can react with –SOH to form persulfides (–SSH). Methionine residues of proteins can also undergo oxidation to form Met sulfoxides (MetO) that can be reduced back to Met by Met-sulfoxide reductases[63]. If they are not reduced back, Met-sulfoxide can be further converted into Met sulfone ($MetO_2$).

Recent studies have shown that the majority of the oxi-PTMs described above cause protein conformation changes (for example in kinases, phosphatases and transcription factors). ROS can thus induce changes in the properties of these proteins, including their activity, specificity and localization, which can activate or suppress stress-response signal transduction processes.

Reversibility in ROS-induced oxi-PTMs as a key feature of ROS signalling. The ability to revert an oxi-PTM in a regulated manner adds plasticity to ROS signalling during stress, especially when it comes to integrating different stress or developmental signals, and/or recovering from stress. Glutathionylation events are typically reversed back to the original thiol by GLUTAREDOXINs (GRXs), whereas protein-disulfides are mostly reduced back by THIOREDOXINs (TRXs) (FIG 3a)[64–67]. TRXs contain at least one conserved redox-active dithiol and form a mixed disulfide bond with their target proteins, regulating their structure and function, whereas GRXs function as oxidoreductases that regulate the redox state of thiol groups or exchange a glutathionylated moiety with a protein. These reactions can be highly selective, adding an extra level of complexity to redox signalling during stress. Depending on the original context of the oxi-PTM, reversing it can reactivate or suppress protein function, which can activate, suppress or alter stress-response pathways.

A unique role for THIOL PEROXIDASEs (TPXs) in ROS signalling. Thiol-based peroxidases, such as GLUTATHIONE PEROXIDASEs (GPXs) and PEROXIREDOXINs (PrxRs) can reduce H_2O_2, peroxynitrites and different organic peroxides[68,69]. In addition to this peroxiredoxin activity, they can act as redox sensors transducing the H_2O_2 signal to different regulatory or enzymatic targets (FIG 3a). The high affinity of GPXs for H_2O_2, combined with their relatively low peroxidase activity, make some GPXs ideal candidates for these signalling functions. It was found, for example, that in yeast GPX3 conveys an H_2O_2 signal to the transcription factor YAP1 to regulate a multitude of H_2O_2 transcriptional responses[70]. In plants, a dual role of scavenging and signalling was proposed for AtGPXL3, as loss-of-function *gpxl3* mutants displayed higher sensitivity to H_2O_2 treatments and, *in vitro*, AtGPXL3 suppressed the activity of the 2C-type Ser/Thr protein phosphatase 2A (FIG 3b)[71].

GSH and the ascorbate-glutathione (ASC-GSH) cycle. The Foyer-Asada-Halliwell pathway (also known as the ASC-GSH cycle)[5,8,72] is an NADPH-driven H_2O_2-scavenging pathway found in many plant subcellular compartments (Supplementary Box 1). Although an integral part of the ASC-GSH cycle, GSH is also used by other pathways; for example, GSH is oxidized by PrxR and/or GPX. Although the ASC-GSH cycle was originally considered to be a potent first line of defense against excessive H_2O_2 accumulation, changes in the oxidation state of the GSH pool (that is, changes in the GSH/GSSG ratio), caused by the function of the ASC-GSH cycle, also act as a sensing mechanism for altered ROS levels and redox perturbations during stress[72–76]. ROS-induced changes in the GSH/GSSG ratio can induce oxi-PTMs of Cys residues of receptors, signal transducers, RBOHs, transcription factors and other proteins, potentially through S-glutathionylation (FIG 3a). In addition to directly oxidizing Cys residues, H_2O_2 can therefore impact the GSH/GSSG ratio in cells through the ASC-GSH cycle, PrxRs and GPXs, indirectly regulating GSH-driven oxi-PTMs.

Recent examples of ROS-induced oxi-PTMs involved in stress signalling in plants. ROS- and redox-driven oxi-PTMs regulate many metabolic reactions in plant cells (*e.g.*, the Calvin–Benson cycle), as well as the activity of different kinases, phosphatases, transcription factors and chromatin/RNA processing regulators, ion channels, and receptors during stress (FIG 3b-f).

Some of the most prominent examples include inhibition of protein phosphatases such as protein TYR PHOSPHATASE (PTP), CLASS 2 PROTEIN PHOSPHATASE (PP2A or PP2C; FIG 3b), the catabolic phosphatase SAL1, and the phosphatase STARCH-EXCESS 4 (SEX4)[56,77–79], involved in hormone, metabolic and retrograde signalling. In contrast, ROS-induced oxi-PTMs activate MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) cascades such as the MEKK1-MM1/2-MPK4/6 cascade, and Ser/Thr kinases, required for the full activation of MPK3 and MPK6[80–82], that play key roles in the induction of pathogen and stress responses.

Additional examples for important oxi-PTM targets during stress include transcriptional regulators such as NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1; FIG 3c), HEAT SHOCK TRANSCRIPTION FACTORs (HSFs), C-REPEAT BINDING FACTORs (CBFs), ANAC089, MYB30, and RADICAL-INDUCED CELL DEATH 1 (RCD1)[46,83–88], involved in pathogen, heat, cold and retrograde signaling, respectively. Although histones are not typically subjected to oxi-PTMs, chromatin and histone modifiers such as the methyltransferase PROTEIN ARGININE METHYLTRANSFERASE 5 (PRMT5), as well as the DICER proteins DCL3 and DCL4, and RNASE THREE LIKE 1 (RTL1; FIG 3d) are; linking ROS to gene regulation[89]. In addition, ion channels such as the STELAR K^+ OUTWARD RECTIFIER (SKOR) efflux channel, involved in drought and nutrient stress responses, were shown to undergo oxi-PTM[90].

ROS were also shown to induce the oxidation of BRASSINAZOLE-RESISTANT 1 (BZR1), which functions as a master regulator of brassinosteroid signalling in plants, causing it to bind DNA and alter stress responses (FIG 3e)[91]. Lastly, receptors such as the leucine-rich-repeat receptor kinase HYDROGEN-PEROXIDE-INDUCED CALCIUM INCREASES 1 (HPCA1; FIG 3f), were recently shown to undergo oxi-PTMs at their extracellular domains leading to autophosphorylation and subsequent activation of plasma membrane (PM)-localized Ca^{2+} channels[92], that trigger stomatal closure in response to stress. HPCA1 was also identified as CANNOT RESPOND TO DMBQ 1 (CARD1) involved in the signalling response of plants to quinones[93], required for the interaction of parasitic plants with their hosts.

A recent study has identified QUIESCIN SULFHYDRYL OXIDASE 1 (QSOX1) as a redox sensor that inactivates S-NITROSOGLUTATHIONE REDUCTASE (GSNOR), which leads to increased levels of S-nitrosoglutathione (GSNO), S-nitrosylation, and inactivation of RBOHs[94]. QSOX1 could therefore function as part of a negative feedback loop that decreases ROS production upon ROS accumulation in cells. A recent Cryo-EM analysis of the plant GLUTAMATE RECEPTOR LIKE (GLR) channel GLR3.4, that plays a key role in Ca^{2+} signalling, revealed that GSH regulates GLR3.4 channel activity by binding to Cys 205 in the amino-terminal domain of each subunit of the protein tetramer[95]. The redox level of the cell, reflected in the levels of free GSH could therefore impact Ca^{2+} signalling.

The potential of ROS to induce oxi-PTMs of so many different components of numerous signal transduction pathways, as well as different ion channels and other metabolic enzymes, highlights the important part that ROS play in stress sensing and signalling in plants. To understand and potentially modulate these roles, it is important to know how ROS levels are regulated across the different plant subcellular compartments, as discussed below.

ROS signalling pathways in plants

In the complex subcellular environment of plant cells, the sensing of ROS and activation of different signal transduction pathways can occur at different compartments (FIG 2). In general, ROS signalling can be divided into extrinsic (apoplast and cell wall), intrinsic (cytosol and nucleus) and organellar (chloroplast, mitochondria, peroxisomes and other compartments; FIG 4a). Recent studies revealed that these different routes can interact or remain separate during stress.

Extrinsic ROS signalling. The apoplast and cell wall contain multiple enzymes that scavenge or actively produce ROS, as well as several non-enzymatic antioxidants (Supplementary Table 2). RBOHs, AQPs, and cell wall-bound PEROXIDASEs (PRXs) have the greatest role in ROS signalling at the apoplast (FIG 4a).

RBOHs are highly regulated transmembrane proteins that use cytosolic NADPH to generate $O_2^{\cdot-}$ in the apoplast (converted to H_2O_2 spontaneously or by SUPEROXIDE DISMUTASEs; SODs)[6–8]. They are thought to reside at the PM in nano domains together with several ancillary proteins involved in their regulation[96–98]. ROS production by RBOHs can be regulated by the binding of Ca^{2+} to EF-hand domains in their cytosolic N-terminal region, phosphorylation/dephosphorylation of their cytosolic N- or C-terminals, binding of phosphatidic acid, and/or binding of RHO OF PLANTS (ROP) small GTP-binding proteins. Recent studies have shown that RBOHs are also regulated by ubiquitination, persulfidation, nitrosylation, glutathionylation, and/or endocytosis[99–113]. RBOHs have been called 'the engines of ROS signalling' and are turned 'on' or 'off' in response to many different stresses, and/or other stimuli, driving the formation of ROS signatures at the apoplast (FIG 4a)[30,114–116]. Cell-wall-bound PRXs can also produce or scavenge ROS under different conditions and have been shown to regulate apoplastic ROS levels in response to different stimuli[31,117]. Moreover, other oxidases localized to the apoplast produce ROS (Supplementary Table 2)[118].

ROS that accumulate in the apoplast can directly, or indirectly (potentially through redox-transducing proteins), react with different receptors (*e.g.*, HPCA1), oxidize different antioxidants, and/or regulate Ca^{2+} and/or K^+ channels (FIG 4a). However, to directly regulate intracellular pathways, ROS produced at the apoplast must enter cells via AQPs. AQPs are water channels that facilitate the transport of H_2O_2[10,119,120]. The opening and closing of AQPs is regulated by phosphorylation, acetylation and/or guanidinylation, linking different signalling processes with ROS transport[120–125]. ROS and/or entire complexes of RBOHs can also enter cells via endocytosis and impact cytosolic ROS levels[126]. As ROS production via RBOHs and ROS transport via AQPs are regulated processes, ROS levels in the apoplast and cytosol, and their signalling functions, can be actively controlled in response to different stresses. Moreover, because apoplastic ROS production and entry into the cytosol are regulated through PTMs of RBOHs and AQPs at their cytosolic side, and ROS accumulation at the apoplast can trigger cytosolic phosphorylation reactions via receptors and alter Ca^{2+} fluxes through plasma membrane channels, the apoplast–cytosol interface is emerging as a major hub for many ROS-associated signal transduction processes during stress (FIG 4a).

Intrinsic ROS signalling. The cytosol contains many ROS scavenging mechanisms, as well as a few ROS producing enzymes (Supplementary Table 2). These are thought to regulate ROS signals generated in the cytosol as well as ROS signals transported from the apoplast or the different organelles to the nucleus, via the cytosol (FIG 4a)[127,128]. In addition, the cytosol contains many different signalling hubs, such as MAPK cascades, CALCIUM-DEPENDENT PROTEIN KINASEs (CDPKs or CPKs), CALCINEURIN B-LIKE (CBL)-interacting protein kinases (CIPKs), ROP/RAC small GTPases, different phosphatases (PP2As, PP2Cs, PTPs)[129] and different redox sensing networks (*e.g.*, PrxRs, GRXs, TRXs) that integrate different ROS signals with other signalling molecules, such as Ca^{2+} and different hormones (FIG 4a).

As AQPs found at the PM and/or organelle membranes facilitate the transport of H_2O_2 in both directions, cytosolic H_2O_2 levels can impact H_2O_2 levels in other compartments and *vice versa*. In addition, retrograde and anterograde signals between organelles and the nucleus are relayed via the cytosol[46,47,49,56]. Indeed, manipulating the ability of the cytosol to scavenge ROS can change signalling in response to stress and alter acclimatization and/or defense responses, supporting a key role for the cytosol in regulating ROS signalling[127,128,130,131]. Furthermore, ROS gradients can form within cells, suggesting that cytosolic ROS scavenging mechanisms attenuate ROS signals[132]. Thus, the cytosol plays an important role in decoding and integrating different ROS signatures generated in different cell compartments, transferring the information stored in these signatures to the nucleus. Moreover, the ROS- and redox-dependent activation of many transcriptional regulators that control plant stress responses, such as NPR1, HSFA, and ANACs, occurs in the cytosol before these proteins enter the nucleus[46,133–136].

Compared to the cytosol, regulation of ROS and redox levels in the nucleus are poorly understood. The plant nucleus contains several ROS and redox regulating proteins, such as GRXs, TRXs, PrxRs and GPXs, as well as GSH (Supplementary Table 2)[137,138]. These can regulate oxi-PTMs of different transcription factors, as well as attenuate ROS signals in the nucleus[139–141]. The findings that many redox-responsive transcriptional regulators are activated in the cytosol before entering the nucleus suggests that ROS levels in the nucleus are maintained under control to prevent extreme fluctuations which could cause DNA damage and mutations. One of the most important questions related to intrinsic ROS signalling is how can different ROS signals, generated in the different subcellular compartments during different stresses, reach the nucleus through the cytosol without losing their specificity?[8,9] A possibility that has been put forward in recent studies[34,39,143–149], is the inclusion of a separate ROS signalling network, that of organelles.

Organelle ROS signalling network. The different plant cell organelles contain multiple ROS scavenging and producing mechanisms that regulate ROS signalling within each organelle as well as participate in organelle–to-organelle and organelle-to–nucleus communication (Supplementary Table 2)[6–8,72,142]. The levels of ROS in each compartment are determined by an interplay between three different processes: organelle-autonomous regulation, nucleus-controlled retrograde/anterograde regulation, and direct export/import (FIG 4b). Recent studies have shown that some ROS signals between organelles or from organelles to the nucleus do not cross the cytosol or cross the cytosol only over very short distances[34,39]. At least three different mechanisms are thought to play a role in this process: physical proximity between organelles (resulting in shorter distances and gradients), physical connections between different organelles and the nucleus, enabled by long tube-like extensions (*e.g.*, stromules, peroxules and matrixules), and organelle-to-organelle protein complexes that form membrane contact sites and may contain aquaporins (FIG 4b)[39]. Examples to these mechanisms include stress-response ROS signalling mediated by sub-

populations of chloroplasts found in close proximity to the nucleus, and formation of stromules that mediate ROS signals between chloroplasts and the nucleus in response to pathogens, excess light , H_2O_2 or salicylic acid (SA)[34,39,143–149]. The levels of ROS in one organelle could also impact the levels of ROS in another organelle or the nucleus through different intermediate metabolites, hormones and/or the mobilization of different proteins (FIG 4b)[46,56,83,134,150]. The concept of a subcellular network of organelles that can communicate with each other via ROS and other signals is therefore emerging (FIG 4c). Responses to stresses that primarily trigger extrinsic or intrinsic ROS signalling could be spatially and/or temporally (and therefore partially or completely) separated from responses to stresses mediated by this organelle-to-organelle or organelle-to-nucleus ROS signalling network (FIG 4c), and this separation could be a mechanism for ROS to convey specific information to the nucleus regarding the type of stress the plant encounters. While most studies have focused on ROS signalling in chloroplasts, mitochondria and peroxisomes, little is known about ROS signalling and metabolism in plasmodesmata, the endoplasmic reticulum (ER), and the vacuole (FIG 2; Supplementary Table 2). The ER and vacuole are thought to contain highly oxidized environments, and plasmodesmata have been recently found to play an important role in cell-to-cell ROS signalling[6–8,151].

The newly gained insights into how different ROS signatures are formed in cells during stress and how ROS levels at different compartments are linked with each other (FIGs 2; 4c), suggest that different stresses could generate different stress-specific ‘maps’ or ‘landscapes’ of ROS signatures across the entire cell. These could be decoded by multiple ROS sensors found in the different compartments (FIG 3). The sensing and triggering of any specific response to any particular stress should therefore be viewed as a response to a change in the entire ROS signalling ‘landscape’ of the cell rather than to a response to an isolated event occurring in a particular compartment. Moreover, because ROS can accumulate to high levels in some compartments and remain high for a long time without causing toxicity — for example, levels of ROS produced in the apoplast of Arabidopsis by RBOHD remained high for 3-6 hours following a 10 min excess light stress treatment)[152], some cell compartments could serve as a reservoir of ROS. Similar to calcium being stored in certain compartments such as the ER or mitochondria and used for signalling by opening or closing calcium channels, ROS could be kept at high levels in some compartments, for example by active production through RBOHs, and used for signalling by opening or closing of aquaporins (Fig. 4c).

Regulation of plant defense and acclimatization by ROS

Changes in ROS levels in different cell compartments and integration of such signals during stress activate defense and acclimatization responses.

Integration of stress sensing with ROS signalling. Plants have different sensors and receptors for changes in light, temperature and osmotic pressure. These include Ca^{2+}-permeable channels such as REDUCED HYPEROSMOLALITY, INDUCED Ca^{2+} INCREASE 1 (OSCA1) and MECHANOSENSITIVE CHANNEL OF SMALL CONDUCTANCE-LIKE 10 (MSL10) that detect osmotic changes; Ca^{2+}-permeable channels such as CYCLIC NUCLEOTIDE-GATED CHANNEL (CNGC) that detect heat stress; RECEPTOR LIKE KINASES (RLKs) and ROP proteins that detect osmotic changes; and photoreceptors such as PHYTOCHROME B (PHYB) and CRYPTOCHROME (CRY) that detect changes in light quality and intensity. Retrograde signalling and release of ROS and Ca^{2+} from chloroplasts are also thought to be involved in the sensing of light stress in plants, and PHYB also detects changes in temperature (FIG 5a)[152–159].

The physical proximity of some of these receptors to RBOH proteins, for example when they reside in the same nanodomains at the plasma membrane, or when chloroplasts are near the PM or nucleus, could facilitate ROS production during the initial stages of stress sensing and responses[34,39,96–98]. The initial sensing of abiotic stresses by plants through different receptors and sensors that leads to rapid changes in Ca^{2+} signalling and phosphorylation reactions could therefore be directly linked to ROS production (FIG 5a). This process is similar to the sensing of pathogens, whereby Ca^{2+}- and/or phosphorylation- dependent activation of RBOHs rapidly triggers ROS production[30,99–101,106–109,111–113], highlighting the evolutionary importance of ROS signalling for plants and the central role of RBOHs in these processes.

One of the most intriguing findings in recent years is that in the absence of certain RBOHs, light stress does not induce rapid ROS accumulation in plants[152,160]. This finding is surprising because it was traditionally thought that during light stress the excess ROS produced in chloroplasts diffuses to the cytosol through aquaporins[6–8], and raises the possibility that during light stress chloroplasts are capable of managing their internal ROS levels, and ROS accumulation in cells is predominantly the result of ROS production for signalling by RBOHs. It is also possible that two different populations of chloroplasts are involved in ROS signalling during light stress: (i) Nucleus-associated chloroplasts that mediate chloroplast to nucleus signalling, and (ii) Plasma membrane-associated chloroplasts that trigger RBOH-driven ROS signals (FIG 4c). $O_2^{\cdot-}$, H_2O_2, or $^1O_2$ accumulating in chloroplasts (even at low levels) during light stress could also trigger different retrograde signals that activate ROS production by RBOHs. Alternatively, PHYB could serve as the light sensor causing the activation of RBOHs during light stress (FIG 5a)[152].

The dynamics of ROS signalling during stress. Responses to stress can occur within seconds to minutes of stress perception and involve changes in the metabolome and transcriptome of plants. These early responses initiate slower responses that activate many different defense and acclimatization networks, enabling the plant to survive the stress and eventually recover from it[161–166]. It was recently shown that ROS

are involved in both early and late responses to stress and that this involvement is an outcome of both 'active' and 'passive' ROS production (FIG 5b)[163]. This new view of plant responses to stress suggests that different stresses are rapidly sensed by stress-specific receptors (FIG 5a) that trigger ROS production by RBOHs, and/or cause stress-specific imbalances that alter the levels of ROS and other stress-associated metabolites (FIG 5b). This process occurs within seconds to minutes of stress initiation and is coordinated with changes in redox, Ca^{2+} levels, phosphorylation and other signalling events that trigger stress-specific signal transduction pathways. The activation of these pathways is also accompanied by rapid increases in hormone levels, for example newly synthesized jasmonic acid (JA), or release of abscisic acid (ABA) and salicylic acid from conjugated forms[23,167–170].

The activation of acclimatization and defense networks resulting from these early signalling events further alters ROS signatures, increasing plant resilience to stress (FIG 5b)[11-21,163,169,171]. Some aspects of this heightened state of resistance can be long-lasting or transmitted to the next generation through ROS-associated epigenetic mechanisms[172]. ROS are thus involved in almost all stages of early and late responses to stress and are intimately linked with many of the pathways, networks and hormones required for plant survival during stress (FIG 5b).

ROS roles during exposure to multiple stresses. In nature, plants are often exposed to different stresses simultaneously, *e.g.*, a combination of drought, high light, and heat, which activates multiple signalling pathways; referred to as 'stress combination'. ROS were found to be essential for plant acclimatization to such conditions[4]. Indeed, mutants deficient in ASCORBATE PEROXIDASE 1 (APX1) are more sensitive to a combination of drought and heat stress, and mutants deficient in RBOHD are more susceptible to conditions of multifactorial stress combination[173,174]. During the integration of cold stress and pathogen responses, the ROS-regulated MPK3/6 and MPK4 cascades play antagonistic roles in the triggering of defense and acclimatization networks[175]. ROS thus have an important role in the integration of different signals generated during stress combination. Different stresses simultaneously or sequentially impacting a plant could induce different ROS signatures and the integration of these could attenuate or regulate the overall response of plants to complex environmental conditions. Integration of two different ROS signatures could also occur when a particular stress (*e.g.*, heat) occurs on the background of a particular developmental stage (*e.g.*, plant reproduction)[176], or during interactions with the plant microbiome[177]. Under such conditions, the overall levels of ROS are integrated to trigger a specific or broad state of plant tolerance or susceptibility to stress.

Induction of plant resilience through transcriptional regulation by ROS. Stress sensing by receptors and ROS-activated redox sensors triggers and modulates different transcription factor networks that enable the plant to respond to a wide spectrum of different conditions (FIG 6).

Transcriptional responses are regulated in plants by two distinct processes: (i) Stress- or ROS-derived changes in phosphorylation, Ca^{2+}-binding, SUMOylation and/or other signal transduction mechanisms that alter transcription factor function, and (ii) Direct or indirect ROS-induced redox-regulation[88,129,133–136,178–183]. These two processes are interlinked because ROS signalling and other signalling events (for example those mediated by Ca^{2+} and phosphorylation) are also interconnected, for example through RBOHs and aquaporins (FIG 4a; 6a).

Redox-dependent modulation of gene expression in response to stress is also achieved through other mechanisms. Subunits of the plant Mediator complex are redox regulated; and ROS can alter the levels and function of different miRNAs, as well as modulate mRNA splicing (FIG 6a)[184–186]. The effect of ROS on these mechanisms further tunes plant stress responses and connects them to cellular ROS levels. For example, an increase in ROS levels could inhibit the expression of groups of housekeeping genes that require extensive splicing, miRNA function or interactions with the mediator complex for their expression (*e.g.*, during heat stress)[181].

In addition to regulating transcription through genetic and/or epigenetic mechanisms during stress, ROS affect the translocation of different redox-regulated transcriptional regulators, such as NPR1, HSFA8/HSFA1A, MBF1c, and ANAC013/17/089, involved in responses to pathogens, heat and excess light, respectively, from the cytosol, or the outer membranes of the ER, to the nucleus following their activation (FIG 6b)[87,133–136,169]. The translocation of these transcriptional regulators into the nucleus then triggers gene expression networks and enhance plant resilience to stress. A recent study that used a supervised learning approach to generate a ROS-response integrated gene regulatory network (iGRN), using DNA motifs, open chromatin regions, transcription factor-binding and expression-based regulatory interactions, discovered several new ROS-regulated transcription factors and defined some of the regulatory networks and hubs they control[180]. Transcriptomic studies of mutants deficient in regulatory hubs such as RBOHs, MAPK cascades, HSFs and different Ca^{2+} signalling pathways also revealed how these hubs integrate ROS signals with other signal transduction networks activated during stress (FIGs 4a, 6). For example, a study examining the transcriptome response of the *rbohD* mutant to light stress revealed that RBOHD is required for the expression of many early response transcripts[164], including the transcription factor MYB30 that was found to be important in plant responses to oxidative stress[187]. Moreover, MYB30 functions upstream of many other transcription factors to regulate thousands of transcripts in response to light stress (FIG 6b)[188].

Transcriptional control of abiotic and biotic stress responses

Redox-regulated transcription factors have roles in the response to heat stress, pathogens and excess light, as discussed below.

Responses to heat stress. In response to increased temperatures, ROS accumulate in the cytosol and nuclei of plants[29]. The elevated levels of ROS cause the redox-dependent activation and translocation of HSFA1 and MBF1c from the cytosol to the nuclei (FIG 6b)[134,169]. Moreover, bZIP28 is activated and translocated from the ER to the nucleus[189]. HSFA and MBF1c cooperate in the transcriptional activation of many HEAT SHOCK PROTEINs (HSPs) and other transcription factors such as DEHYDRATION-RESPONSIVE ELEMENT BINDING FACTORS (DREBs), and are both required for the acquisition of thermotolerance (FIG 6b)[181]. bZIP28 cooperates with bZIP60 to transcriptionally activate the unfolded protein response (UPR) following heat stress[189]. Furthermore, the redox state of the chloroplast is important for the induction of heat stress tolerance, suggesting that chloroplasts also play a part in these responses[190].

Pathogen responses. Responses to pathogen infection following pathogen recognition, for example by plasma membrane-localized PATTERN RECOGNITION RECEPTORs (PRRs), are often initiated by a transient oxidative burst, mediated by RBOHs or peroxidases at the apoplast[30,191]. This burst is followed by an increase in the reduced state of the cytoplasm, the accumulation of the plant hormone salicylic acid[133], and the deposition of callose at the cell wall and plasmodesmata, that prevents pathogen spread[191]. The enhanced accumulation of ROS and salicylic acid following pathogen recognition triggers a redox-regulated transcriptional response mediated by NPR1. Under controlled growth conditions NPR1 is localized to the cytoplasm as an oligomer held by intermolecular Cys bonds involving Cys 82 and Cys 216 (FIG 3c)[83,84]. SA triggers a reduction of these bonds that is mediated by thioredoxin (TRX-h3/h5) and results in monomerization of NPR1[192]. Monomeric NPR1 is transported to the nucleus, where it interacts in a redox-dependent manner with TGA1, and activates the transcription of different PATHOGENESIS-RELATED (PR) protein-encoding genes, and other transcription factors such as WRKY[133]. Interestingly, the plant hormone jasmonic acid antagonizes this process by promoting the S-nitrosylation of NPR1 on Cys 156 causing its oligomerization (FIG 3c)[192]. NPR1 is also involved in the response to other abiotic stresses (*e.g.*, salinity)[193] and could represent an important integrator between daily changes in redox levels and plant responses to biotic and abiotic stresses[141].

Excess light stress. Excess light stress causes oxidation of the chloroplast, apoplast and cytosol regulating nuclear transcription through multiple redox-response transcription factors, including MYB30, ZAT10/12, RELATED TO APETALA 2 (RAP2) and different HSFs[127,188,194,195]. During light stress, the chloroplastic 3'-phosphoadenosine 5'-phosphate (PAP) phosphatase SAL1 undergoes redox-dependent oxidative inactivation. This leads to the accumulation of PAP, which serves as a retrograde signal to regulate gene expression in the nuclei[49,56]. Interestingly, PAP is associated with another retrograde signalling pathway

involving the mitochondria. In this pathway, PAP levels are affected by an interplay between the redox activated ANAC transcription factors that translocate from the ER to the cytosol and the negative regulator RCD1 (FIG 6b)[46]. ROS and retrograde signals are therefore interlinked and mediate many signal transduction responses to stress, and this integration could play an important role when pathogen infection or heat stress occur, for example, under conditions of excess light.

The transcriptional changes triggered in response to elevated ROS levels during stress (FIG 6) cause the enhanced accumulation of different antioxidants, osmoprotectants, molecular chaperones, pathogen-response proteins and many other enzymes and proteins that together enable the plant to resist the stress and survive[11–21,169,174,188,196]. Below we will discuss how ROS signals can propagate from their localized site of production to other cells and tissues of the plant and coordinate its systemic, whole-plant, responses to stress.

ROS and cell-to-cell signalling

ROS such as $O_2^{\cdot-}$ or H_2O_2 are rapidly scavenged in cells, so they cannot diffuse over long distances in different biological systems. Instead, an 'altered ROS state', such as 'excessive ROS accumulation', can propagate, for example, through the regulation of ROS production, scavenging and transport mechanisms, between neighboring cells, different organelles, or along membranes (FIG 7a). This new concept in ROS signalling stems from studies that reported cell-to-cell (in plants)[116,151,163,199–202] and mitochondria-to-mitochondria (in mammalian cells)[197,198,203] signalling pathways that involve a 'ROS-induced-ROS production' response by each cell or organelle that communicate with each other, and could in principle be extended to membrane nanodomain-to-nanodomain ROS signalling (FIG 7a). Although mitochondria-to-mitochondria ROS signalling has not been demonstrated in plants, plants have an extensive network of organelle ROS signalling (FIG 4c), which is in principle capable of supporting similar organelle-to-organelle ROS transport and signalling cascades.

As the rigid structure of plant cell walls keeps cells in close physical proximity, ROS levels and/or the redox status in one cell can affect neighboring cells via plasmodesmata, the cell wall and/or the apoplast. Recent studies have shown that the apoplast and plasmodesmata are involved in transducing RBOH-mediated cell-to-cell ROS and redox signals in plants (FIG 7a)[116,151,169,199,202,204]. This process, termed the 'ROS wave', is auto-propagating and capable of transferring stress-induced ROS and redox signals from cell-to-cell over long distances, sometimes spanning the entire length of the plant (FIG 7b). The main difference between ROS diffusion and an auto-propagating process, such as the ROS wave, is that it is not ROS *per se* that are mobilized between two different locations; instead, it is a state of 'ROS production, scavenging, and

transport' that becomes activated across cells and along tissues (FIGs 1, 7). This distinction is important because unlike many other signalling molecules in plants, ROS are likely to be scavenged during transport over long distances. However, an auto-propagating state of 'ROS production, scavenging and transport' can maintain a certain steady-state ROS level or signature at almost any cellular location along its path. Moreover, it was recently reported that two ROS waves originating from different tissues of the same plant can integrate two different stress-induced signals leading to a state of enhanced acclimatization of the entire plant[196]. This finding indicates that the intracellular networks of ROS signalling in plants can extend to become an intercellular cell-to-cell network that integrates ROS signals from different cells or tissues and coordinate whole plant physiological responses that involve different molecular and metabolic mechanisms[162–164,169,196,205,206].

Conclusions and perspectives

The study of ROS biology in plants initiated with a focus on ROS scavenging and production mechanisms in chloroplasts. This emphasis has changed into studying active ROS production, by for example RBOHs, and its regulation by different post-translational modifications. As ROS levels depend on the interplay between production, scavenging and transport (FIG 1), it will be important to determine the mechanisms that regulate ROS transport, for example by aquaporins or other transporters. Furthermore, our perspective on how ROS are produced in cells during stress should be re-evaluated. Whether the findings that the majority of ROS accumulation in plants during excess light stress is dependent on RBOHs[152,160], rather than originating from chloroplasts, extends to other stresses and plant species should be determined. Moreover, this finding highlights that ROS might not be as toxic to cells as initially thought[6]. Further research is also needed to determine how organelle ROS signalling is linked to the cytosol, nucleus and the apoplast, and how information in the form of ROS signatures is transmitted between these different compartments. The roles of the vacuole, plasmodesmata and endoplasmic reticulum, and the underlying mechanisms that connect ROS signalling between these organelles and the rest of the cell, remain to be determined. Better understanding of the mechanisms that mediate auto-propagating ROS signals in plants and their link to stress responses is also needed. Furthermore, it is unknown whether different channels can actively transport (pump) H_2O_2 against a potential gradient, and whether cells contain different chaperone molecules that can transport ROS, such as H_2O_2, from one location to another while protecting them from degradation. The identification of new ROS and redox sensors, redox relays and hubs, and the study of ROS-responsive transcriptional networks, will increase our understanding of how ROS signals are integrated in response to stress. However, to fully elucidate ROS cellular networks, it is necessary to accurately determine ROS levels in different compartments using genetically encoded ROS and redox sensors[25,28,29,36,38,40-45,207], as well

as to study ROS fluxes between different compartments and organelles. Only by obtaining an all-encompassing portrait of the stress-induced ROS signalling landscape of the cell and linking it to plant transcriptional, metabolic and proteomic networks, it will be possible to fully understand the functions of ROS in plants in response to stress.

Glossary

Acclimatization/Acclimation A process by which plants adjust their metabolism, physiology, and biochemistry to become accustomed to changes in their growth conditions or environment.

Aquaporin A transmembrane water channel protein that allows the diffusion of H_2O_2 from one side of the membrane to the other in a regulated manner.

Brassinosteroids A class of polyhydroxysteroids that function as plant hormones involved in many developmental processes and responses to stress.

Photosystems I and II Multiprotein complexes that reside on the thylakoid membranes inside chloroplasts and participate in the harvesting of light energy for the purpose of CO_2 fixation and sugar biosynthesis.

Photorespiration A biochemical pathway that results in the accumulation of H_2O_2 in peroxisomes, triggered when CO_2 concentrations are limited in C3 plants.

C3 plants A large group of plants in which the initial product of the assimilation of CO_2 through photosynthesis is 3-phosphoglycerate, which contains 3 carbon atoms.

Nucleophilic attach Attack of an electron-rich species (the nucleophile) on an electron-deficient species (the electrophile), forming a new bond between the nucleophile and the electrophile.

YAP1 A redox-regulated transcription factor that is essential for yeast survival under conditions of oxidative stress.

2C-type Ser/Thr protein phosphatase 2A A family of phosphatases that generally function as negative regulators of different stress responses in plants and are inhibited by ROS-induced redox reactions.

DICER proteins Endoribonucleases that cleave double-stranded RNA and pre-microRNAs into short double-stranded RNA fragments called small interfering RNA or microRNA.

Leucine-rich-repeat receptor kinases A large gene family in plants composed of a leucine-rich-repeat (LRR)-containing extracellular domain, a transmembrane domain, and an intracellular kinase domain, involved in developmental processes and stress responses.

Quinones A redox active class of cyclic organic compounds containing two carbonyl groups, involved in many electron transport reactions, and signalling processes.

EF hand A helix–loop–helix structural domain, with an E and F structural orientation of the two α-helices, found in many calcium-binding proteins.

Stromules, peroxules, and **matrixules** Dynamic tubular membrane structures extending from the surface of chloroplasts, peroxisomes and mitochondria, respectively, used for the transport of signals between different organelles and the nucleus.

Salicylic acid A phytohormone, characterized by an aromatic ring and a hydroxyl group, involved in the response of plant to different biotic and abiotic stresses.

Mediator complex An important component of the eukaryotic transcriptional machinery, linking different transcription factors with RNA polymerase II.

Unfolded protein response A cellular stress response pathway triggered by the presence of unfolded proteins inside the endoplasmic reticulum.

Plasmodesmata Small channels or pores that transverse the plant cell walls connecting the cytoplasm and plasma membrane of neighboring cells with each other, establishing metabolic and signalling bridges between cells.

Stomata Specialized pore structures found in the epidermal layer of plants and used for gas exchange with the atmosphere.

List of Supplementary Material

Supplementary Box 1. ROS production and scavenging pathways of plants.

Supplementary Box 2. Evolution of ROS metabolism in cells.

Table S1. Biochemical, physical, and molecular properties of different ROS and RNS in cells.

Table S2. Plant (*Arabidopsis thaliana*) proteins and small molecules regulating ROS levels in cells

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Competing interests

The authors declare no competing interests.

Acknowledgments

We apologize to the many authors whose important work could not be referred to. This work was supported by funding from the National Science Foundation (IOS-2110017, IOS-1353886, MCB-1936590, IOS-1932639), National Institute of Health (GM111364), Interdisciplinary Plant Group, University of Missouri, Research Foundation-Flanders (project G0D7914N), and The Excellence of Science Research project 30829584.

Author contributions

RM, SIZ, YF and FVB wrote the review, designed the figures, and approved the final version of the manuscript.

Figures

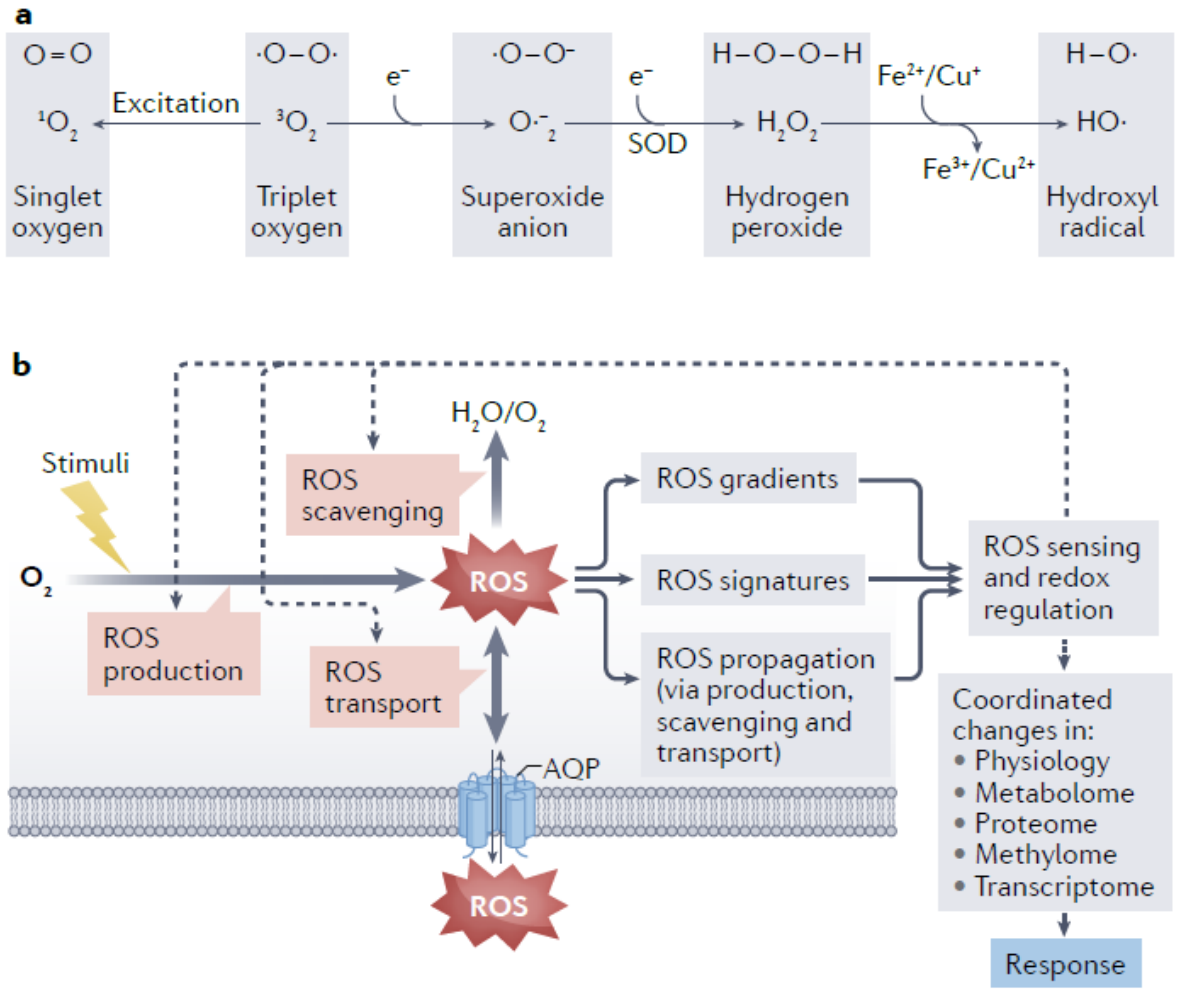

Fig. 1 | **Regulation of ROS metabolism and signalling in plants. a** | Formation of ROS by excitation or reduction of atmospheric oxygen. **b** | Cellular ROS concentrations are regulated by three distinct processes: ROS production, scavenging and transport. These processes determine the steady-state levels of ROS; they also generate different ROS signatures and gradients (characterized by different concentrations of the different types of ROS within organelles and cells), that function as signals. In response to external or internal stimuli, ROS levels change. ROS levels in cells are sensed and decoded through changes in the redox state of different proteins that lead to coordinated responses. In addition to their localized function within cells, ROS production, scavenging and transport can propagate, along membranes, between organelles or between cells, altering the steady-state levels of ROS in the entire plant. Dashed arrows indicate that ROS production, scavenging and transport can be regulated depending on the redox state of the cell. AQP, aquaporin; e, electron; ROS, reactive oxygen species; SOD, superoxide dismutase.

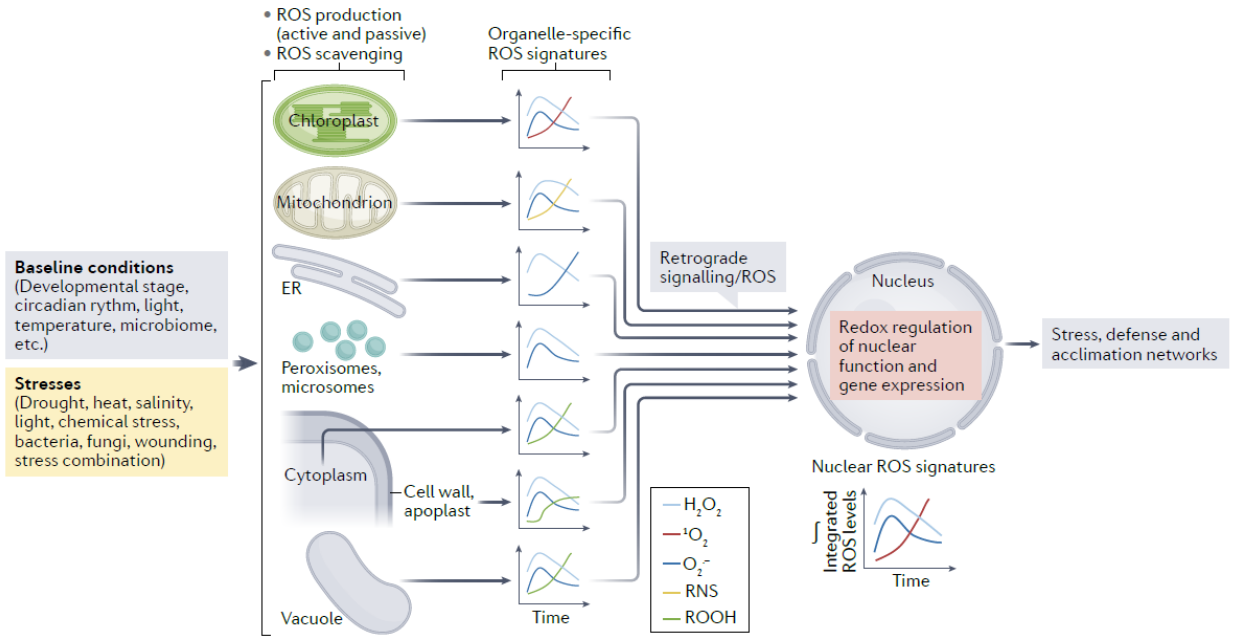

Fig. 2 | **Production and scavenging of ROS in different compartments in plants during stress.** The interplay between ROS production and scavenging in each cell compartment, including the cell wall and apoplast, during stress generates compartment-specific ROS signatures (hypothetical signatures are indicated on right). These are integrated with other (non-ROS) retrograde signals that reach the nucleus, alter the nuclear ROS signature and trigger defense and acclimatization responses. Organelle-to-organelle ROS communication is not depicted. A list of all ROS metabolism reactions and enzymes involved is included in Supplementary Table 2. ER, endoplasmic reticulum, ROOH, organic hydroperoxide; ROS, reactive oxygen species; RNS, reactive nitrogen species.

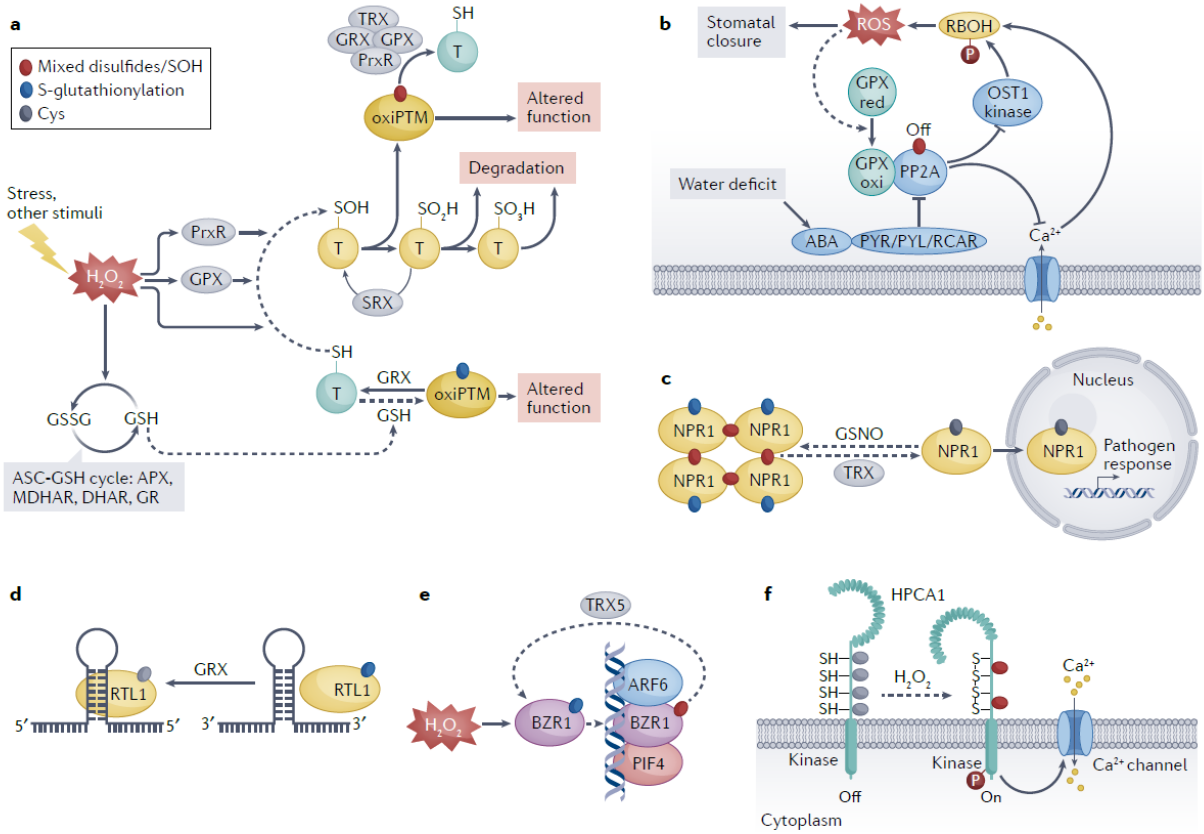

Fig. 3 | **Mechanisms of ROS and redox sensing in plants. a** | H_2O_2 alters protein structure and function through oxidation of Cys thiols (directly or through the function of GPXs or PrxRs). H_2O_2 also affects the ratio between oxidized and reduced GSH (directly or through the function of the ASC-GSH cycle), further altering protein structure and function through S-glutathionylation. These oxidative posttranscriptional modifications (oxi-PTMs) can be reversed through the function of GRXs, PrxR and TRXs allowing ROS such as H_2O_2 to activate or suppress different cellular functions in a reversible fashion. **b** | Regulation of PP2A (phosphatase) function by protein oxidation, used to control stomatal aperture closing by ABA in response to water deficit stress. **c** | Regulation of the transcription factor NPR1 translocation into the nucleus by Cys oxidation and S-glutathionylation, used to control gene expression in response to pathogens. **d** | Regulation of siRNA binding by the plant protein RTL1, used to control the function of the endoribonuclease complex DICER shown to be involved in responses to viral pathogens. **e** | Regulation of DNA binding by oxidation of the transcriptional switch BZR1, used to control brassinosteroid responses to many different abiotic stresses including heat and drought. **f** | Regulation of the ROS/redox receptor HPCA1 by protein oxidation during responses to pathogen infection. Dashed arrows indicate regulation by redox changes. ABA, abscisic acid; APX, ascorbate peroxidase; ARF6, auxin response factor 6; ASC, ascorbate; BZR1, brassinazole-resistant 1; CAT, catalase; Cys, cysteine; DHA, dehydroascorbate; GPX, glutathione peroxidases; GR, glutathione reductase; GRX, glutaredoxin; GSH, glutathione; GSNO, S-

nitrosoglutathione; GSSG, oxidized glutathione; HPCA1, hydrogen-peroxide-induced calcium increases 1; MDHAR, monodehydroascorbate reductase; NO, nitric oxide; NPR1, nonexpressor of pathogenesis-related genes 1; OST1, open stomata 1; oxi, oxidized; oxi-PTM, oxidative posttranscriptional modifications; P, phosphate; PIF4, phytochrome-interacting factor 4; PP2A, protein phosphatase 2A; PrxR; peroxiredoxin; PYR/PYL/RCAR, pyrabactin resistance/pyr-like/regulatory components of ABA receptors; red, reduced; RBOH, respiratory burst oxidase homolog; ROS, reactive oxygen species; RTL1, RNAse three like 1; SRX, sulfiredoxin; T, target; TRX, thioredoxins.

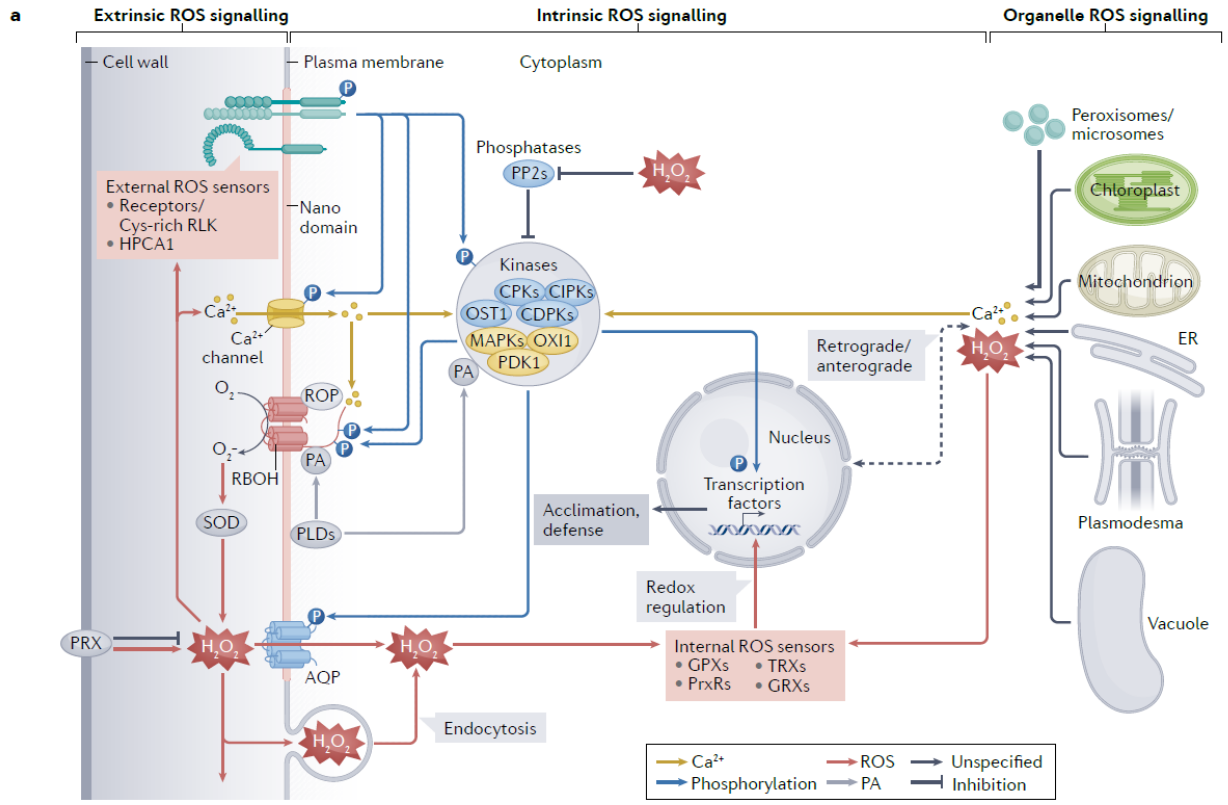

Fig. 4 | **Integration of ROS signals in plant cells. a** | ROS signalling in plants can be divided into extrinsic-, intrinsic- and organelle- localized pathways. These are integrated through the function of RBOHs, AQPs, various Ca^{2+} channels, receptors and various kinases and phosphatases that link ROS signalling with calcium, phosphorylation, PA and redox signalling, and trigger transcriptional responses to stress. **b** | The level of ROS in each organelle can be autonomously controlled through pre-existing organellar ROS production, sensing, scavenging and transport mechanisms, regulated by the nucleus through retrograde/anterograde signalling and newly synthesized (inducible) proteins, and/or modulated through ROS export/import from other organelles (Top). Organelles can impact the levels of ROS in each other or the nucleus through complexes, membrane extensions, diffusion, and/or metabolite/protein-derived signalling (Bottom). **c** | ROS can accumulate to high levels in different compartments of the cell and impact H_2O_2 levels in the cytosol and nuclei. Because different compartments are linked with each other and the transport of ROS between different compartments is regulated, different stresses can generate stimuli-specific 'maps' or 'landscapes' of ROS concentrations, across the different cellular compartments, that will alter H_2O_2 levels in the cytosol and nuclei and trigger stress-specific acclimatization and/or defense mechanisms. Dashed arrow indicates retrograde signalling. Question marks indicate that ROS levels are not known yet. AQP, aquaporin; apoROS, apoplastic ROS; CDPK, Ca^{2+}-dependent protein kinases; chlROS, chloroplastic ROS; CIPK, calcineurin B-like-interacting protein kinases; CPK, Ca^{2+}-dependent

protein kinases; cwROS, cell wall-associated ROS; cytROS, cytosolic ROS; ER, endoplasmic reticulum; erROS, endoplasmic reticulum-associated ROS; GPX, glutathione peroxidases; GRX, glutaredoxins; HPCA1, hydrogen-peroxide-induced Ca^{2+} increase 1; M, metabolite; MAPK, mitogen-activated protein kinase; mitROS, mitochondrial ROS; nROS, nuclear ROS; OST1, open stomata 1; OXI1, oxidative signal-inducible 1; P, phosphate; PA, phosphatidic acid; pdROS, plasmodesmatal ROS; perROS, peroxisomal ROS; PRX, peroxidases; PrxR, peroxiredoxin; PDK1, 3-phosphoinositide-dependent protein kinase1; PLD, Phospholipase D; PP2s, protein phosphatase 2; RBOH, respiratory burst oxidase homolog; RLK, receptor-like kinases; ROP, Rho of Plants; ROS, reactive oxygen species; SOD, superoxide dismutase; TRX, thioredoxins; vacROS, vacuolar ROS.

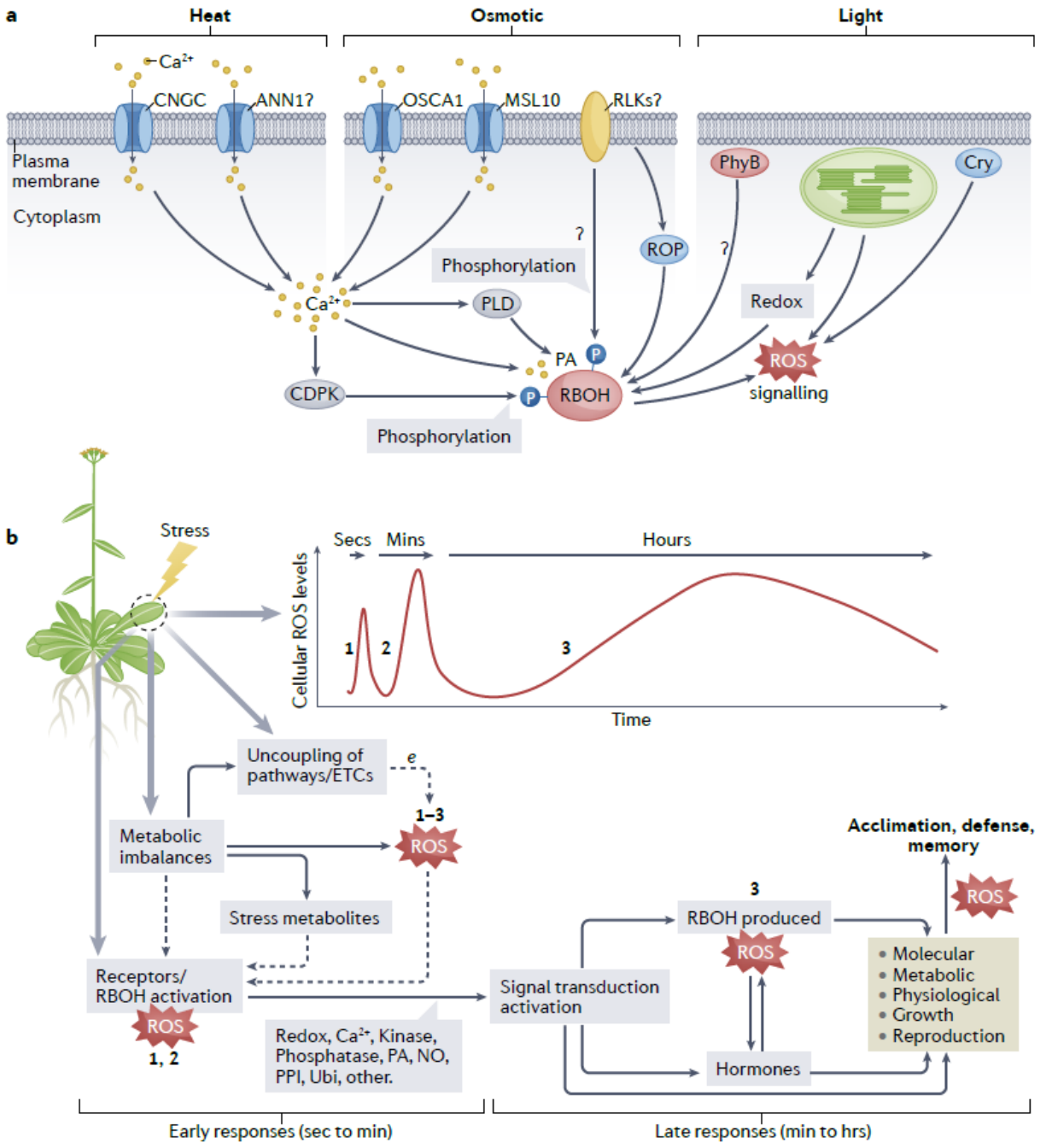

Fig. 5 | **ROS in early and late responses of plants to stress. a** | Different plant sensors for changes in environmental conditions such as temperature, light intensity/quality and osmotic potential are directly linked to ROS signalling through the RBOH signalling hub. These links allow ROS signalling to be triggered during early stages of stress sensing in plants. **b** | During early responses (seconds to minutes of stress initiation; stages 1 and 2), ROS produced 'actively' or 'passively' in cells are used to sense stress and trigger signal transduction mechanisms, while during late responses (minutes to hours of stress initiation; stage 3), ROS are used to regulate different networks and metabolic responses, balance plant

acclimatization and defense, and induce stress memory. Dashed arrows indicate ROS and other stress metabolites used for early stress signalling. Question marks indicate possible links. ANN1, annexin1; CDPK, Ca^{2+}-dependent protein kinases; CNGC, cyclic nucleotide gated channel; Cry, cryptochrome; e, electron; ETC, electron transport chain; MSL10, mechanosensitive channel of small conductance-like 10; NO, nitric oxide; OSCA1, Reduced hyperosmolality, induced Ca^{2+} increase 1; P, phosphate; PA, phosphatidic acid; PhyB, phytochrome B; PLD, phospholipase D; PPI, protein-protein interactions; RBOH, respiratory burst oxidase homolog; RLK, receptor-like kinases; ROP, Rho of Plants; ROS, reactive oxygen species; Ubi, ubiquitination.

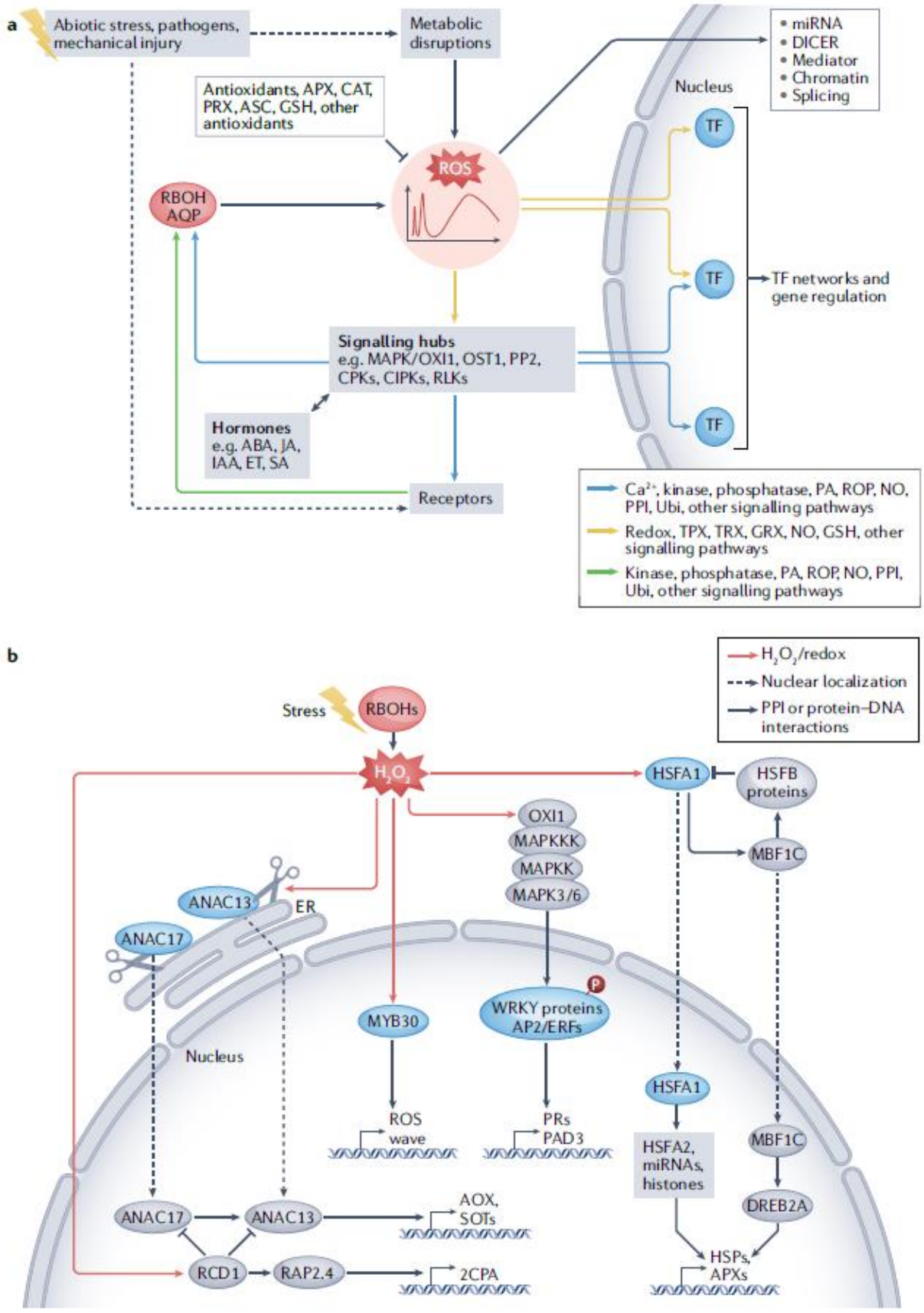
a
Abiotic stress, pathogens, mechanical injury
Metabolic disruptions
Antioxidants, APX, CAT, PRX, ASC, GSH, other antioxidants
ROS
RBOH AQP
Signalling hubs e.g. MAPK/OXI1, OST1, PP2, CPKs, CIPKs, RLKs
Hormones e.g. ABA, JA, IAA, ET, SA
Receptors
Nucleus
• miRNA
• DICER
• Mediator
• Chromatin
• Splicing
TF
TF
TF
TF networks and gene regulation
Ca2+, kinase, phosphatase, PA, ROP, NO, PPI, Ubi, other signalling pathways
Redox, TPX, TRX, GRX, NO, GSH, other signalling pathways
Kinase, phosphatase, PA, ROP, NO, PPI, Ubi, other signalling pathways
b
H2O2/redox
Nuclear localization
PPI or protein–DNA interactions
Stress
RBOHs
H2O2
HSFA1
HSFB proteins
MBF1C
OXI1
MAPKKK
MAPKK
MAPK3/6
ANAC13
ER
ANAC17
MYB30
WRKY proteins AP2/ERFs
P
Nucleus
ROS wave
PRs PAD3
HSFA1
HSFA2, miRNAs, histones
MBF1C
DREB2A
ANAC17
ANAC13
AOX, SOTs
RCD1
RAP2.4
2CPA
HSPs, APXs

Fig. 6 | **Integration of ROS signalling with stress-response networks in plants and transcriptional regulation by H_2O_2 during stress. a |** The sensing of stress triggers different transcription factor (TF) networks through different signalling hubs involving Ca^{2+}, phosphorylation, phytohormone function and many other signal transduction reactions. ROS alter many components of these signalling hubs through oxi-PTMs, as well as directly trigger ROS- and redox-dependent transcription factors networks. ROS also regulate transcription by modifying proteins involved in mRNA splicing, miRNA regulation, and the mediator complex. The integration of ROS with other stress-response signalling networks tunes these networks to the overall levels of ROS in cells that can serve as an initial alert, stress-level monitoring, and/or memory signals. **b |** H_2O_2 can trigger the mobilization and activation of transcription factors such as HSFAs or ANACs from the cytosol or ER, respectively, to the nucleus, trigger the activation of MAPK cascades that phosphorylate and activate transcription factors such as WRKYs and AP2/ERFs, and/or directly impact the binding of transcription factos such as MYB30 to DNA. These regulatory functions of ROS are controlled by H_2O_2-derived oxi-PTMs and directly link transcription to H_2O_2 levels in cells during responses to biotic and abiotic stresses. Dashed arrows in **a** indicate early responses to stress, and dashed arrows in **b** indicate nuclear translocation. 2CPA, 2-Cys peroxiredoxin A; ABA, abscisic acid; AOX, alternative oxidase; ANAC, Arabidopsis NAC; APX, ascorbate peroxidase; AQP, aquaporin; ASC, ascorbate; CAT, catalase; CDPK, Ca^{2+}-dependent protein kinases; CIPK, calcineurin B-like-interacting protein kinases; CPK, Ca^{2+}-dependent protein kinases; DREB, dehydration responsive element binding; ER, endoplasmic reticulum; ERF, ethylene-response factor; HSF, heat shock factor; HSP, heat shock protein; ET, ethylene; GRX, glutaredoxins; GSH, glutathione; IAA, indol-acetic acid; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; MBF1c, multiprotein bridging factor 1c; miRNA, micro RNA; NO, nitric oxide; Nuc, nucleus; OST1, open stomata 1; OXI1, oxidative signal-inducible1; oxi-PTM, oxidative post-translational modification; P, phosphate; PA, phosphatidic acid; PAD, phytoalexin deficient; PP2, protein phosphatase 2; PPI, protein-protein interactions; PR, pathogen-response; PRX, peroxidase; RAP2.4, related to APETALA 2.4; RBOH, respiratory burst oxidase homolog; RCD1, radical-Induced Cell Death 1; RLK, receptor-like kinases; ROP, Rho of Plants; ROS, reactive oxygen species; SA, salicylic acid; SOT, sulfotransferase; TF, transcription factor, TPX, thiol peroxidase; TRX, thioredoxins; Ubi, ubiquitination.

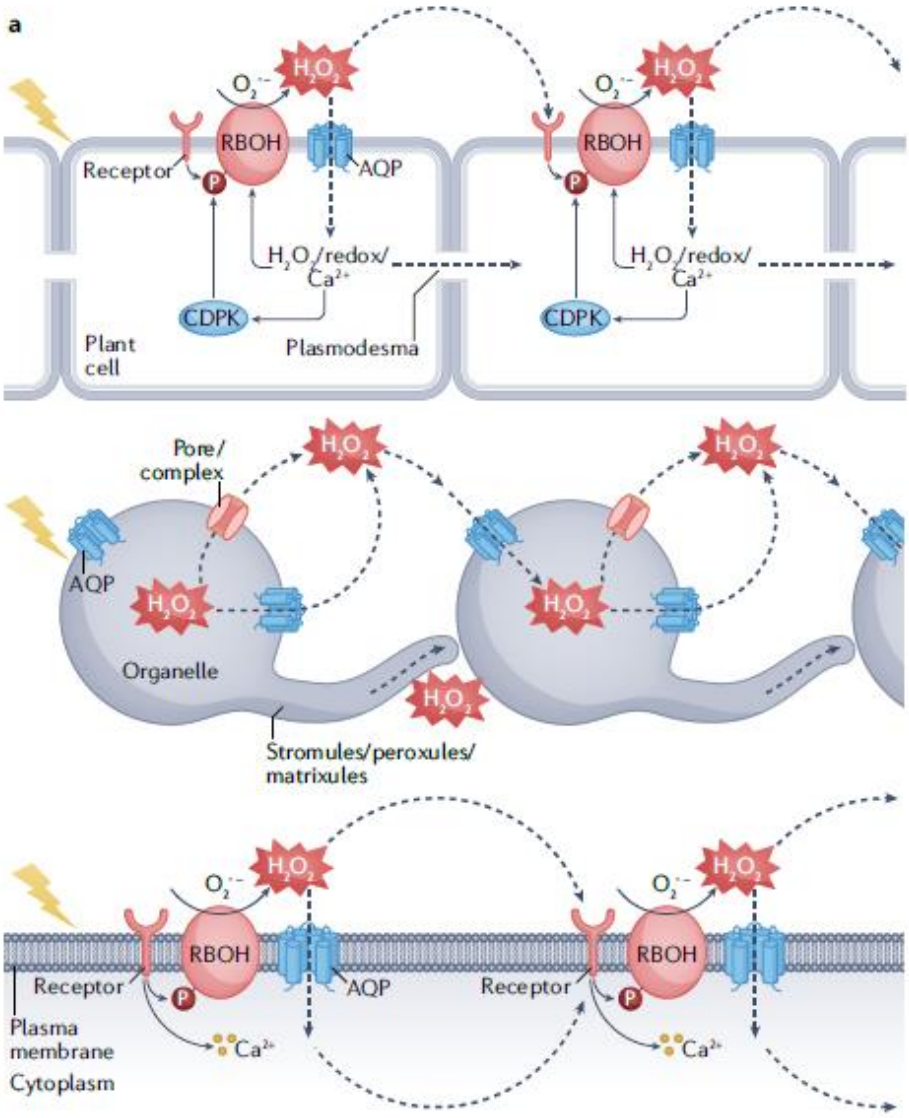
a
RBOH
Receptor
AQP
CDPK
Plant cell
Plasmodesma
Pore/ complex
Organelle
Stromules/peroxules/ matrixules
Plasma membrane
Cytoplasm
b
0 min
10 min
20 min
30 min
Excess light
Wounding
Untreated
100
50
0.00

Fig. 7 | **Propagation of ROS signals within and between cells. a** | Because ROS are rapidly scavenged in cells, they cannot diffuse over long distances in different biological systems. Instead, the state of their 'production, scavenging and transport' can propagate as an 'on/off ROS accumulation' or 'altered ROS' state, between cells (top), organelles (middle), or along membranes (bottom). This process, termed the 'ROS wave', is achieved by the coupling of ROS sensing, production and transport mechanisms between cells, organelles, or along membranes, and could involve calcium signalling and/or different protein phosphorylation networks. **b** | Time-lapse imaging of whole-plant ROS accumulation in *Arabidopsis thaliana* plants subjected to a localized stress such as wounding or excess light showing the spread of the ROS wave from the treated cells on one leaf (arrows) to the entire plants within minutes. Dashed arrows indicate the propagation of ROS. Colour scale bar in **b** indicates signal intensity. AQP, aquaporin; CDPK, Ca^{2+}-dependent protein kinases; P, phosphate; RBOH, respiratory burst oxidase homolog.