Reactive oxygen species 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 defence 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.

Acclimatization
Also referred to as 'acclimation', a process by which plants adjust their metabolism, physiology and biochemistry to become accustomed to changes in their growth conditions or environment.

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 defence mechanisms and acclimatization. Dissecting and understanding how ROS orchestrate plant responses to stress will allow us to increase plant tolerance to stress and increase our ability to mitigate crop damage when crops are exposed to harsh environmental conditions[4].

The term 'reactive oxygen species' (ROS) describes a group of molecules derived from molecular oxygen (O_2). Whereas O_2 is generally non-reactive 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 with 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 ($^1O_2$), the hydroxyl radical ($HO^{\cdot}$) and various forms of organic and inorganic peroxides[5–9] (FIG. 1a and Supplementary Table 1). 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, and controls ROS signalling reactions and their outcomes (FIG. 1b).

Several hundred genes encode the different proteins and enzymes that regulate ROS metabolism and signalling in plants[6–8] (Supplementary Table 2). ROS are produced 'passively', by housekeeping enzymes or as by-products 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 homologues (RBOHs), which are the functional equivalents of mammalian NADPH oxidases (NOX proteins)[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[5–9] (Supplementary Table 2 and Supplementary Box 1). 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

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Aquaporins
(AQPs). Transmembrane water channel proteins that allow the diffusion of H_2O_2 from one side of the membrane to the other in a regulated manner.

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 C_3 plants.

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 root and leaf microbiomes. Different biotic and abiotic stresses can disrupt this homeostasis, uncouple metabolic pathways and lead to the accumulation of ROS in different cell compartments.

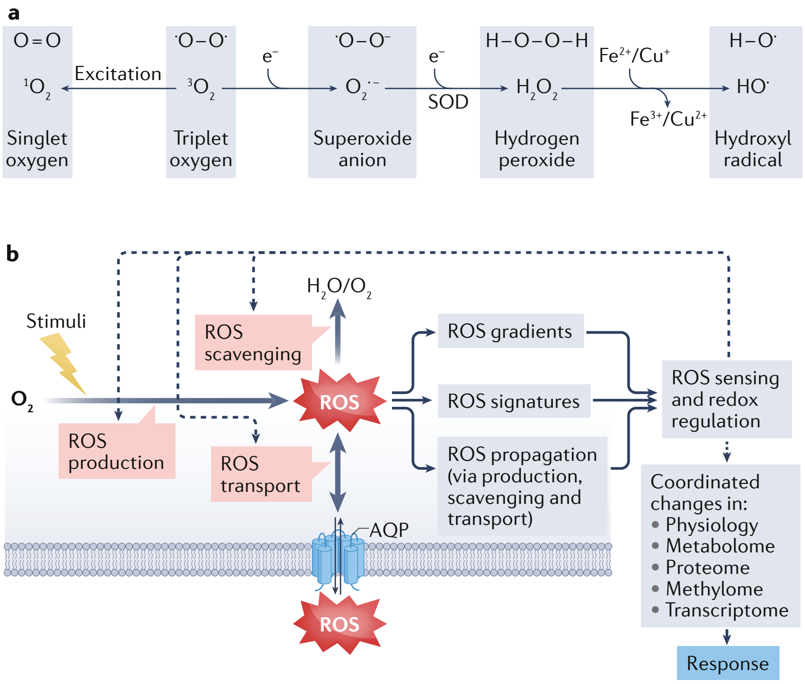


Fig. 1 | **Regulation of ROS metabolism and signalling in plants. a** | Formation of reactive oxygen species (ROS) by excitation or reduction of atmospheric oxygen. **b** | Cellular ROS concentrations are regulated by three distinct processes: ROS production, scavenging and transport. These processes 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), which 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; SOD, superoxide dismutase. Part **a** adapted with permission from REF.[7], Annual Reviews. Part **b** adapted with permission from REF.[208], Elsevier.

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 (for example, in C_3 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 channelled 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[28,29] (Supplementary Box 1).

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]. By 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 into 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 defence mechanisms (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[50,51] (Supplementary Box 2), 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 defence responses, it is first important to understand how ROS are sensed in cells.

ROS perception and redox regulation

Unlike for 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

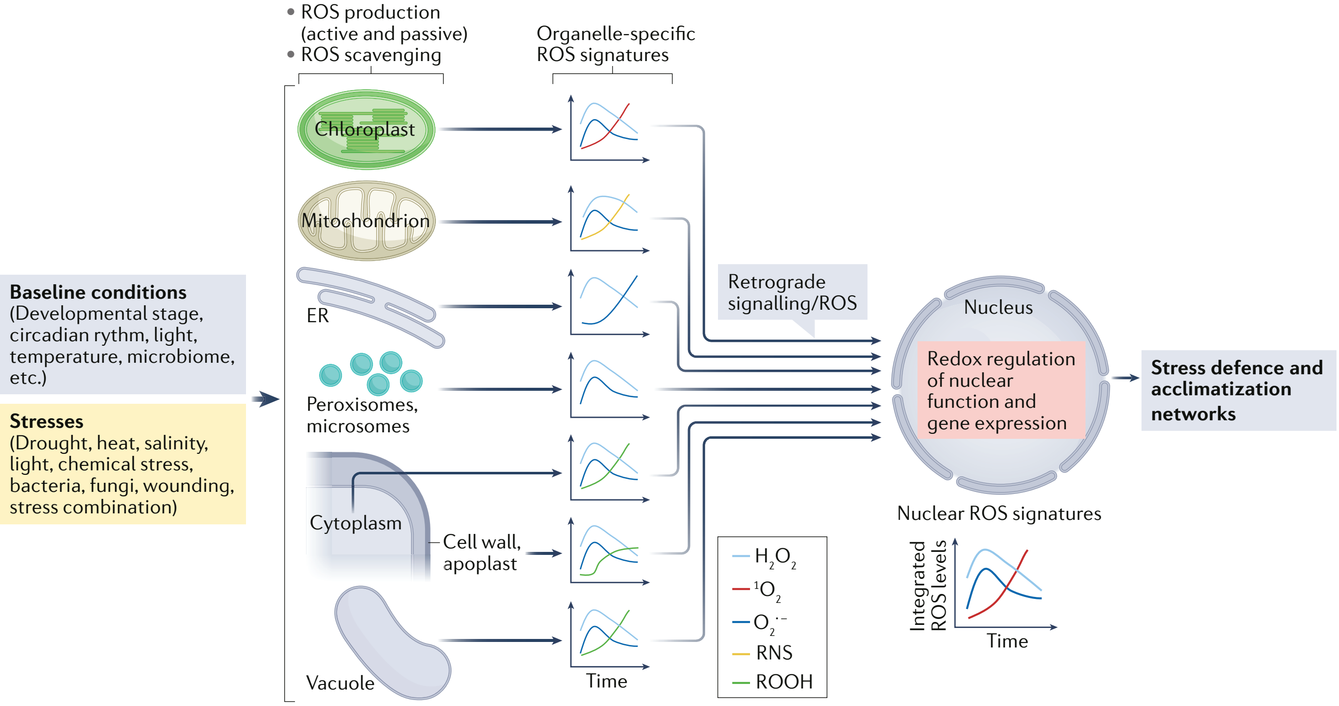


Fig. 2 | **Production and scavenging of ROS in different compartments in plants during stress.** The interplay between reactive oxygen species (ROS) production and scavenging in each cell compartment, including the cell wall and apoplast, during stress generates compartment-specific ROS signatures (hypothetical signatures are indicated on the right). These are integrated with other (non-ROS) retrograde signals that reach the nucleus, alter the nuclear ROS signature and trigger defence 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; RNS, reactive nitrogen species.

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 residues of many proteins are susceptible to nucleophilic attack by ROS. 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 the formation of which is 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 formation 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 intermolecular or intramolecular (that is, mixed disulfides), or with small molecules such as glutathione (GSH; that is, *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 of *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 methionine sulfoxides, which can be reduced back to methionine by methionine sulfoxide reductases[63]. If they are not reduced back, methionine sulfoxide can be further converted into methionine sulfone.

Recent studies have shown that most 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 reverse 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 to form the original thiol by glutaredoxins (GRXs), whereas protein disulfides are mostly reduced back by thioredoxins (TRXs)[64–67] (FIG. 3a). 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

C_3 plants
A large group of plants in which the initial product of the assimilation of CO_2 through photosynthesis is 3-phosphoglycerate, which contains three carbon atoms.

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

Nucleophilic attack
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.

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 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 PrxR 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, makes some GPXs ideal candidates for these signalling functions. For example, 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 *Arabidopsis thaliana*, a dual role of scavenging and signalling was proposed for GPXL3, as loss-of-function *gpxl3* mutants displayed higher sensitivity to H_2O_2 treatments and, in vitro, GPXL3 suppressed the activity of type 2C serine/threonine protein phosphatase 2A (PP2A)[71] (FIG. 3b).

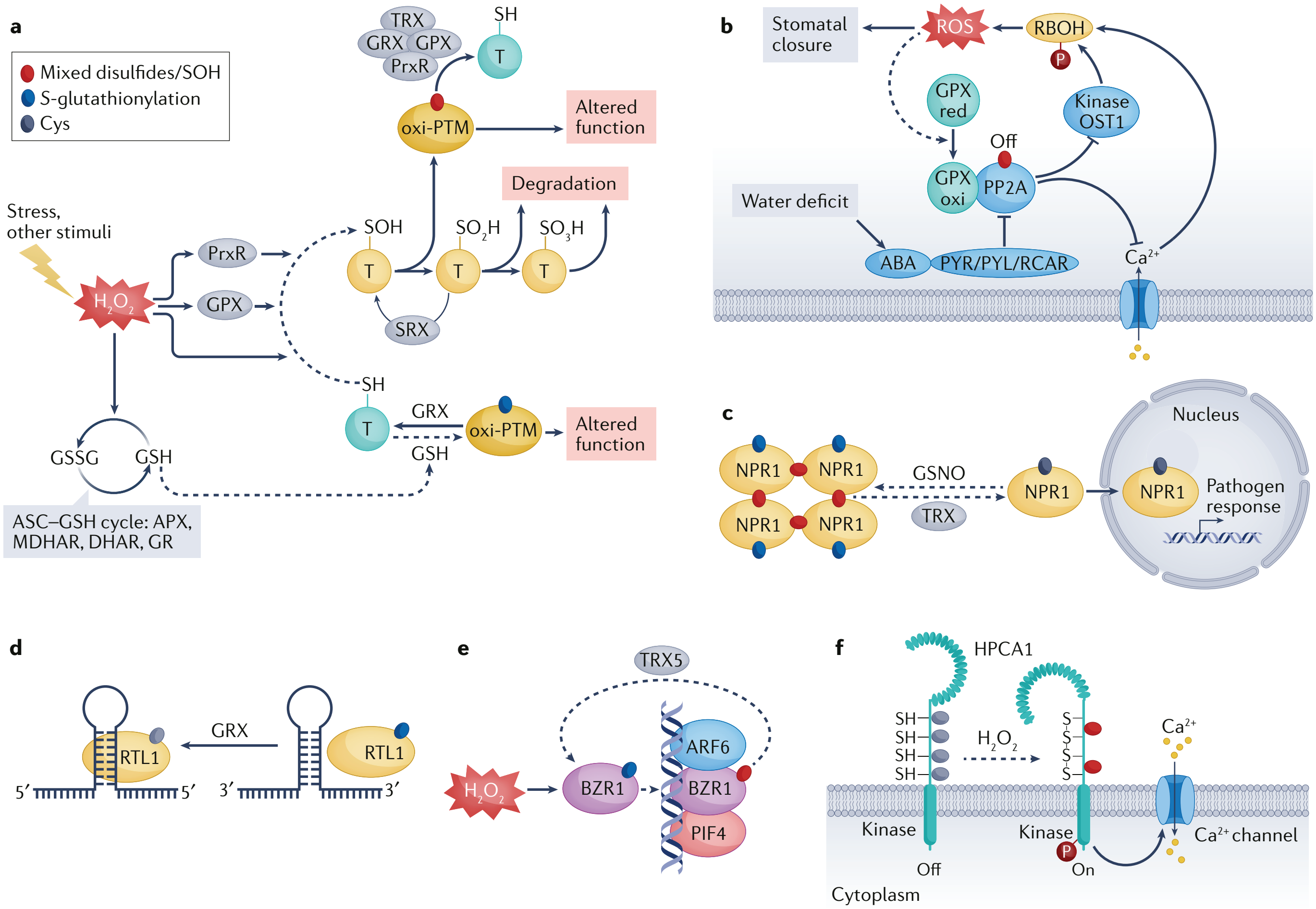


Fig. 3 | **Mechanisms of ROS and redox sensing in plants. a** | H_2O_2 alters protein structure and function through oxidation of cysteine (Cys) thiols (directly or through the function of glutathione peroxidases (GPXs) or peroxiredoxins (PrxRs)). H_2O_2 also affects the ratio between oxidized and reduced glutathione (GSH) (directly or through the function of the ascorbate (ASC)–GSH cycle), further altering protein structure and function through *S*-glutathionylation. These oxidative post-transcriptional modifications (oxi-PTMs) can be reversed through the function of glutaredoxins (GRXs), PrxRs and thioredoxins (TRXs), allowing reactive oxygen species (ROS) such as H_2O_2 to activate or suppress different cellular functions in a reversible fashion. **b** | Regulation of protein phosphatase 2A (PP2A) function by protein oxidation, used to control stomatal aperture closing by abscisic acid (ABA) in response to water deficit stress. **c** | Regulation of translocation of the transcription factor NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) into the nucleus by Cys oxidation and *S*-glutathionylation, used to control gene expression in response to pathogens. **d** | Regulation of small interfering RNA binding by the plant protein RNASE III-LIKE 1 (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 BRASSINAZOLE-RESISTANT 1 (BZR1), used to control brassinosteroid responses to many different abiotic stresses, including heat and drought. **f** | Regulation of the ROS/redox receptor HYDROGEN-PEROXIDE-INDUCED CALCIUM INCREASES 1 (HPCA1) by protein oxidation during responses to pathogen infection. Dashed arrows indicate regulation by redox changes. APX, ascorbate peroxidase; ARF6, AUXIN RESPONSE FACTOR 6; DHAR, DEHYDROASCORBATE REDUCTASE; GR, GLUTATHIONE REDUCTASE; GSNO, *S*-nitrosoglutathione; GSSG, glutathione disulfide; MDHAR, monodehydroascorbate reductase; OST1, OPEN STOMATA 1; oxi, oxidized; P, phosphate; PIF4, PHYTOCHROME-INTERACTING FACTOR 4; PYR/PYL/RCAR, pyrabactin resistance/PYR-like/regulatory components of abscisic acid receptors; RBOH, respiratory burst oxidase homologue; red, reduced; SRX, sulfiredoxin; T, target.

Type 2C serine/threonine protein phosphatase 2A
(PP2A). A family of phosphatases that generally function as negative regulators of different stress responses in plants and are inhibited by reactive oxygen species-induced redox reactions.

Dicer proteins
Endoribonucleases that cleave double-stranded RNA and pre-microRNAs into short double-stranded RNA fragments such as small interfering RNA and microRNA.

Brassinosteroid
Member of a class of polyhydroxysteroids that function as plant hormones involved in many developmental processes and responses to stress.

Leucine-rich repeat receptor kinase
A large protein family in plants composed of a leucine-rich repeat-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.

GSH and the ascorbate–GSH cycle. The Foyer–Asada–Halliwell pathway (also known as the ascorbate (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 defence against excessive H_2O_2 accumulation, changes in the oxidation state of the GSH pool (that is, changes in the ratio of GSH to glutathione disulfide (GSSG)), 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-driven and redox-driven oxi-PTMs regulate many metabolic reactions in plant cells (for example, 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 tyrosine phosphatase (PTP), class 2 serine/threonine protein phosphatase (PP2A or PP2C) (FIG. 3b), the catabolic phosphatase SAL1 and the phosphatase STARCH-EXCESS 4 (SEX4)[56,77–79], which are involved in hormone, metabolic and retrograde signalling. By contrast, ROS-induced oxi-PTMs activate mitogen-activated protein kinase (MAPK) cascades such as the MEKK1–MM1/MM2–MPK4/MPK6 cascade, and serine/threonine kinases, which are required for the full activation of MPK3 and MPK6 (REFS.[80–82]), that play key roles in the induction of pathogen and stress responses.

Additional examples of important oxi-PTM targets during stress include transcriptional regulators such as NON-EXPRESSOR 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], which are involved in pathogen, heat, cold and retrograde signalling, respectively. Although histones are not typically subjected to oxi-PTMs, chromatin and histone modifiers such as the methyltransferase PROTEIN ARGININE METHYLTRANSFERASE 5 (PRMT5) and the Dicer proteins DCL3 and DCL4 and RNASE III-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, which is 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[91] (FIG. 3e). 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-localized Ca^{2+} channels[92], which 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], which is required for the interaction of parasitic plants with their hosts.

A recent study identified QUIESCIN SULFHYDRYL OXIDASE 1 (QSOX1) as a redox sensor that inactivates *S*-NITROSOGLUTATHIONE REDUCTASE (GSNOR), which leads to increased levels of *S*-nitrosoglutathione, 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 cryogenic electron microscopy analysis of the plant glutamate receptor-like channel GLR3.4, which plays a key role in Ca^{2+} signalling, revealed that GSH regulates GLR3.4 channel activity by binding to Cys205 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 next.

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 in 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 have the greatest role in ROS signalling at the apoplast (FIG. 4a).

RBOHs are highly regulated transmembrane proteins that use cytosolic NADPH to generate $O_2^{\bullet-}$ in the apoplast (converted to H_2O_2 spontaneously or by superoxide dismutases)[6–8]. They are thought to reside at the plasma membrane in nanodomains together with several ancillary proteins involved in their regulation[96–98].

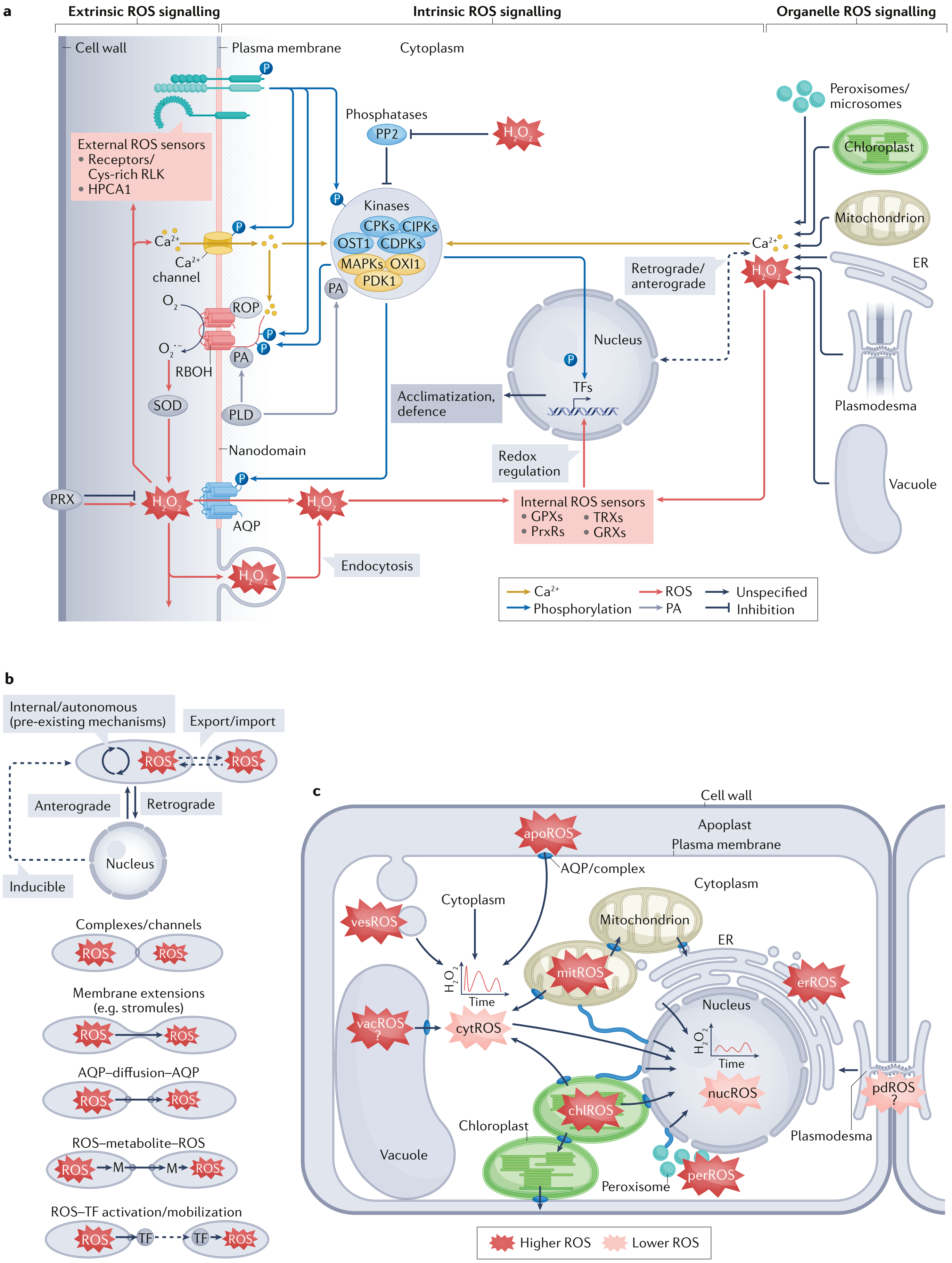

a
Extrinsic ROS signalling
Intrinsic ROS signalling
Organelle ROS signalling
Cell wall
Plasma membrane
Cytoplasm
P
External ROS sensors
• Receptors/ Cys-rich RLK
• HPCA1
Phosphatases
PP2
H_2O_2
Kinases
CPKs
CIPKs
OST1
CDPKs
MAPKs
OXI1
PDK1
PA
Ca^{2+}
Ca^{2+} channel
O_2
$O_2^{\cdot-}$
ROP
RBOH
PA
SOD
PLD
Nanodomain
PRX
AQP
Endocytosis
Peroxisomes/ microsomes
Chloroplast
Mitochondrion
ER
Plasmodesma
Vacuole
Retrograde/ anterograde
Nucleus
TFs
Acclimatization, defence
Redox regulation
Internal ROS sensors
• GPXs
• TRXs
• PrxRs
• GRXs
Ca^{2+}
ROS
Unspecified
Phosphorylation
PA
Inhibition
b
Internal/autonomous (pre-existing mechanisms)
Export/import
ROS
ROS
Anterograde
Retrograde
Nucleus
Inducible
Complexes/channels
ROS
ROS
Membrane extensions (e.g. stromules)
ROS
ROS
AQP–diffusion–AQP
ROS
ROS
ROS–metabolite–ROS
ROS
M
M
ROS
ROS–TF activation/mobilization
ROS
TF
TF
ROS
c
Cell wall
Apoplast
Plasma membrane
apoROS
AQP/complex
Cytoplasm
Cytoplasm
vesROS
Mitochondrion
ER
H_2O_2
Time
mitROS
erROS
Nucleus
vacROS ?
cytROS
H_2O_2
Time
nucROS
pdROS ?
chlROS
Chloroplast
Plasmodesma
Vacuole
Peroxisome
perROS
Higher ROS
Lower ROS

Fig. 4 | **Integration of ROS signals in plant cells. a** | Reactive oxygen species (ROS) signalling in plants can be divided into extrinsic, intrinsic and organelle-localized pathways. These are integrated through the function of respiratory burst oxidase homologues (RBOHs), aquaporins (AQPs), various Ca^{2+} channels, receptors and various kinases and phosphatases that link ROS signalling with calcium, phosphorylation, phosphatidic acid (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 to or 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 at 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 stimulus-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 defence mechanisms. Dashed arrows indicate retrograde signalling. Question marks indicate that ROS levels are not known yet. apoROS, apoplastic reactive oxygen species; CDPK, Ca^{2+}-dependent protein kinase; chlROS, chloroplastic reactive oxygen species; CIPK, calcineurin B-like-interacting protein kinase; CPK, Ca^{2+}-dependent protein kinase; cytROS, cytosolic reactive oxygen species; ER, endoplasmic reticulum; erROS, endoplasmic reticulum-associated reactive oxygen species; GPX, glutathione peroxidase; GRX, glutaredoxin; HPCA1, HYDROGEN PEROXIDE-INDUCED CALCIUM INCREASES 1; MAPK, mitogen-activated protein kinase; mitROS, mitochondrial reactive oxygen species; nucROS, nuclear reactive oxygen species; OST1, OPEN STOMATA 1; OXI1, OXIDATIVE SIGNAL-INDUCIBLE 1; P, phosphate; PA, phosphatidic acid; pdROS, plasmodesmatal reactive oxygen species; perROS, peroxisomal reactive oxygen species; PRX, peroxidase; PrxR, peroxiredoxin; PDK1, 3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE 1; PLD, phospholipase D; PP2, protein phosphatase 2; RLK, receptor-like kinase; ROP, Rho of plants; SOD, superoxide dismutase; TF, transcription factor; TRX, thioredoxin; vacROS, vacuolar reactive oxygen species; vesROS, vesicular reactive oxygen species.

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

ROS production by RBOHs can be regulated by the binding of Ca^{2+} to EF-hand domains in their cytosolic amino-terminal region, phosphorylation/dephosphorylation of their cytosolic amino or carboxy 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 ubiquitylation, 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[30,114–116] (FIG. 4a). Cell wall-bound peroxidases 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[118] (Supplementary Table 2).

ROS that accumulate in the apoplast can directly or indirectly (potentially through redox-transducing proteins), react with different receptors (for example, 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 (REFS. [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 post-translational modifications 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[127,128] (FIG. 4a). In addition, the cytosol contains many different signalling hubs, such as MAPK cascades, calcium-dependent protein kinases, calcineurin B-like-interacting protein kinases, ROP/RAC small GTPases, different phosphatases (PP2A, PP2C and PTPs)[129] and different redox sensing networks (for example, PrxRs, GRXs and TRXs), which integrate different ROS signals with other signalling molecules, such as Ca^{2+} and different hormones (FIG. 4a).

As AQPs found at the plasma membrane 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 defence 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-dependent and redox-dependent activation of many transcriptional regulators that control plant stress responses, such as NPR1, HSFA and ANAC transcription factors, occurs in the cytosol before these proteins enter the nucleus[46,133–136].

Compared with ROS regulation in the cytosol, regulation of ROS and redox levels in the nucleus is poorly understood. The plant nucleus contains several ROS and redox-regulating proteins, such as GRXs, TRXs, PrxRs and GPXs, as well as GSH[137,138] (Supplementary Table 2). 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 proposed by 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 ROS-producing mechanisms that regulate ROS signalling within each organelle and participate in organelle-to-organelle and organelle-to-nucleus communication[6–8,72,142] (Supplementary Table 2). 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 (for example, stromules, peroxules and matrixules), and organelle-to-organelle protein complexes that form membrane contact sites and may contain AQPs[39] (FIG. 4b). Examples of these mechanisms include stress-response ROS signalling mediated by subpopulations of chloroplasts found close to the nucleus, and the formation of stromules that mediate ROS signals between chloroplasts and the nucleus in response to pathogens, excess light, H_2O_2 or salicylic acid[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[46,56,83,134,150] (FIG. 4b). 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 therefore 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 and the vacuole (FIG. 2 and Supplementary Table 2). The endoplasmic reticulum and the vacuole are thought to have highly oxidized environments, and plasmodesmata were 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 in 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. 5). 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 as a response to an isolated event occurring in a particular compartment. Moreover, because ROS can accumulate to high levels in some compartments and remain at high levels for a long time without causing toxic effects — for example, levels of ROS produced in the apoplast of *Arabidopsis* by RBOHD remained high for 3–6 h following a 10-min excess light stress treatment)[152], some cell compartments could serve as a reservoir of ROS. Similarly to calcium being stored in certain compartments, such as the endoplasmic reticulum 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 AQPs (FIG. 4c).

Regulation of plant defence and acclimatization by ROS

Changes in ROS levels in different cell compartments and integration of such signals during stress activate defence 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 channels that detect heat stress; receptor-like kinases and ROP proteins that detect osmotic changes; and photoreceptors such as PHYTOCHROME B (PHYB) and cryptochrome 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[152–159] (FIG. 5a).

The physical proximity of some of these receptors to RBOHs (for example, when they reside in the same nanodomains at the plasma membrane or when chloroplasts are near the plasma membrane 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+}-dependent and/or phosphorylation-dependent activation of RBOHs

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 hydroxy group, involved in the response of plants to different biotic and abiotic stresses.

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

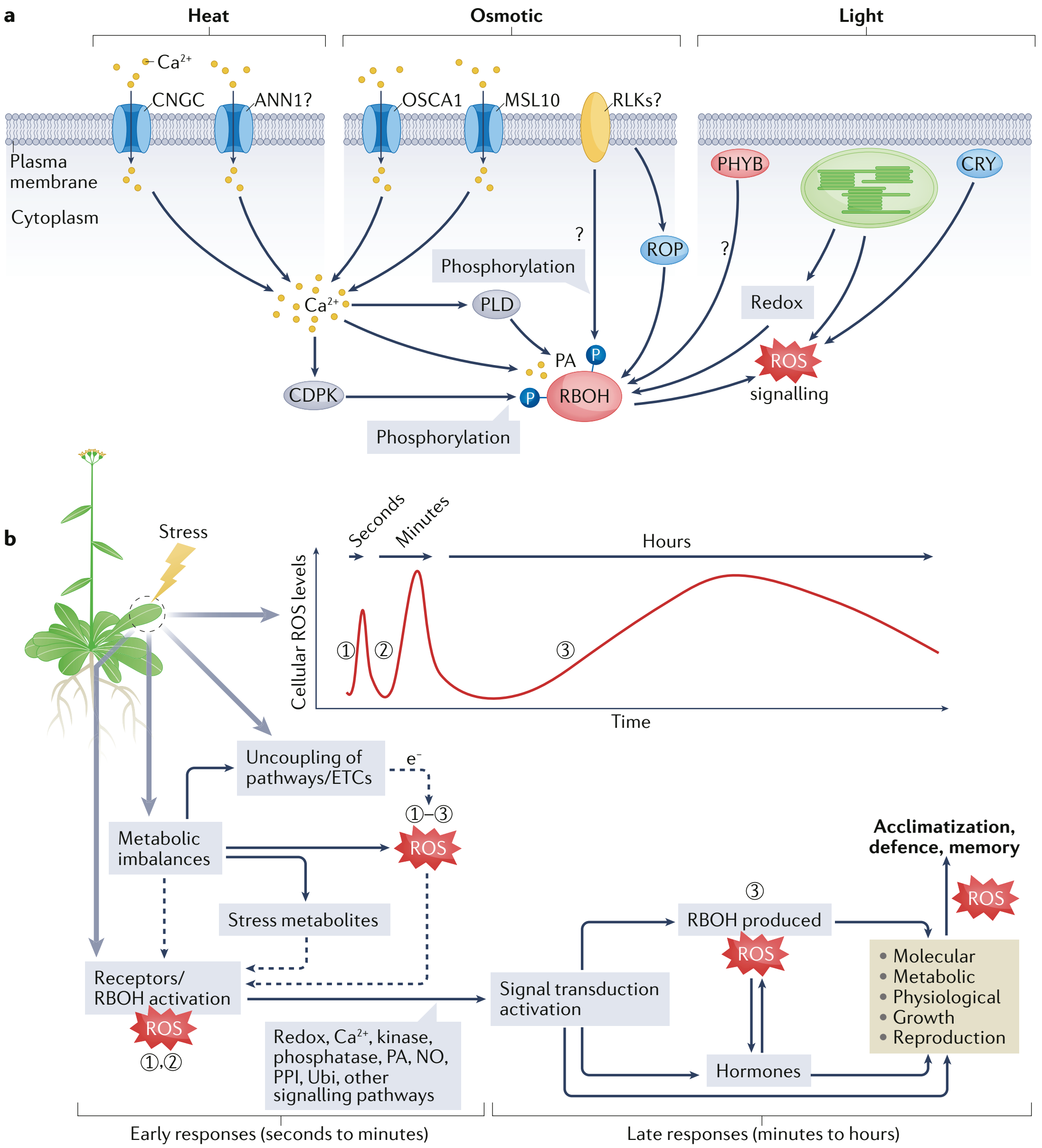
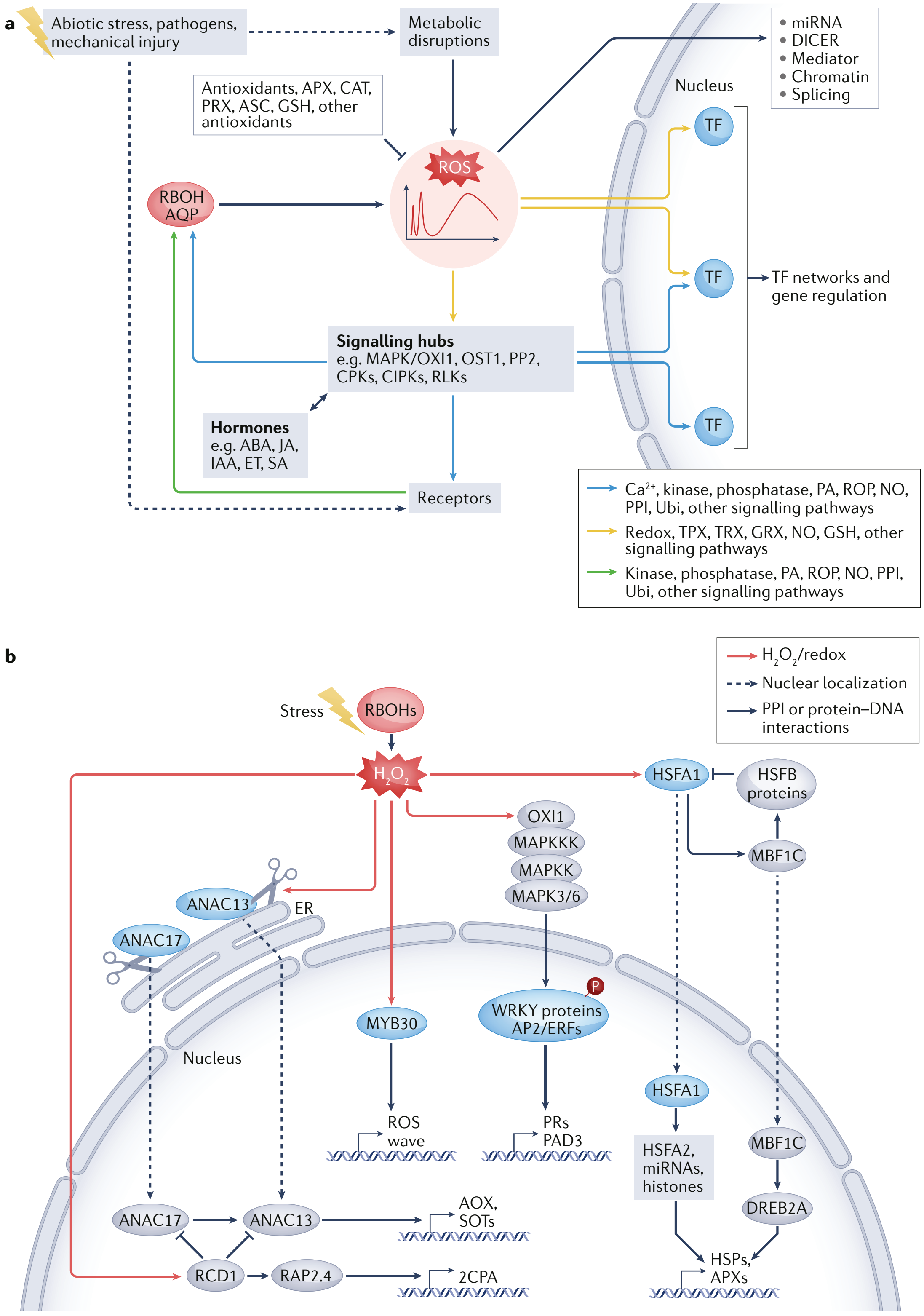


Fig. 5 | **ROS in early and late responses of plants to stress. a** | Different plant sensors for changes in environmental conditions such as temperature, light intensity/quality and osmotic potential are directly linked to reactive oxygen species (ROS) signalling through the respiratory burst oxidase homologue (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 defence, and induce stress memory. Dashed arrows indicate ROS and other stress metabolites used for early stress signalling. Question marks indicate possible links. ANN1, ANNEXIN 1; CDPK, Ca^{2+}-dependent protein kinase; CNGC, cyclic nucleotide-gated channel; CRY, cryptochrome; 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 interaction; RLK, receptor-like kinase; ROP, Rho of plants; Ubi, ubiquitylation.

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 diffuse to the cytosol through AQPs[6–8], and this 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: (1) nucleus-associated chloroplasts that mediate chloroplast-to-nucleus signalling and (2) 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[152] (FIG. 5a).

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

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 networks through different signalling hubs involving Ca^{2+}, phosphorylation, phytohormone function and many other signal transduction reactions. Reactive oxygen species (ROS) alter many components of these signalling hubs through oxidative post-translational modifications and directly trigger ROS-dependent and redox-dependent transcription factor networks. ROS also regulate transcription by modifying proteins involved in mRNA splicing, microRNA (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 monitors and/or memory signals. The dashed arrows indicate early responses to stress. **b** | H_2O_2 can trigger the mobilization and activation of TFs such as heat shock transcription factor A (HSFA) proteins or *Arabidopsis* NAC domain-containing proteins (ANAC) from the cytosol or endoplasmic reticulum, respectively, to the nucleus, trigger the activation of mitogen-activated protein kinase (MAPK) cascades that phosphorylate and activate transcription factors such as WRKY proteins and AP2/ethylene-response factors (ERFs) and/or directly impact the binding of transcription factors such as MYB30 to DNA. These regulatory functions of ROS are controlled by H_2O_2-derived oxidative post-transcriptional modifications and directly link transcription to H_2O_2 levels in cells during responses to biotic and abiotic stresses. 2CPA, two-cysteine peroxiredoxin A; ABA, abscisic acid; AOX, alternative oxidase; APX, ascorbate peroxidase; AQP, aquaporin; ASC, ascorbate; CAT, catalase; CIPK, calcineurin B-like-interacting protein kinase; CPK, Ca^{2+}-dependent protein kinase; DREB2A, dehydration-responsive element-binding protein 2A; HSP, heat shock protein; ET, ethylene; GRX, glutaredoxin; GSH, glutathione; IAA, indole-3-acetic acid; JA, jasmonic acid; MBF1C, MULTIPROTEIN BRIDGING FACTOR 1C; miRNA, microRNA; NO, nitric oxide; OST1, OPEN STOMATA 1; OXI1, OXIDATIVE SIGNAL-INDUCIBLE 1; P, phosphate; PA, phosphatidic acid; PAD3, PHYTOALEXIN DEFICIENT 3; PP2, protein phosphatase 2; PPI, protein-protein interaction; PR, pathogen-related protein; PRX, peroxidase; RAP2.4, RELATED TO APETALA 2.4; RBOH, respiratory burst oxidase homologue; RCD1, RADICAL-INDUCED CELL DEATH 1; RLK, receptor-like kinase; ROP, Rho of plants; SA, salicylic acid; SOT, sulfotransferase; TF, transcription factor; TPX, thiol peroxidase; TRX, thioredoxin; Ubi, ubiquitylation.

slower responses that activate many different defence 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[163] (FIG. 5b). This new view of plant responses to stress suggests that different stresses are rapidly sensed by stress-specific receptors (FIG. 6a) 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 the levels of hormones, for example newly synthesized jasmonic acid, or release of abscisic acid and salicylic acid from conjugated forms[23,167–170].

The activation of acclimatization and defence networks resulting from these early signalling events further alters ROS signatures, increasing plant resilience to stress[11–21,163,169,171] (FIG. 5b). 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).

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

ROS roles during exposure to multiple stresses. In nature, plants are often exposed to different stresses simultaneously; for example, a combination of drought, high light levels 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/MPK6 and MPK4 cascades play antagonistic roles in the triggering of defence 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 (for example, heat) occurs on the background of a particular developmental stage (for example, 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: (1) stress-derived or ROS-derived changes in phosphorylation, Ca^{2+}-binding, sumoylation and/or other signal transduction mechanisms that alter transcription factor function and (2) 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 AQPs (FIGS. 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 microRNAs, as well as modulate mRNA splicing[184–186] (FIG. 6a). 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, microRNA function or interactions with the Mediator complex for their expression (for example, 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/ANAC017/ANAC 089, which are involved in responses to biotic and abiotic stresses, from the cytosol or the outer membranes of the endoplasmic reticulum to the nucleus following their activation[87,133–136,169] (FIG. 6b). The translocation of these transcriptional regulators into the nucleus then triggers gene expression networks and enhances plant resilience to stress. A recent study that used a supervised learning approach to generate an ROS-response integrated gene regulatory network, using DNA motifs, open chromatin regions, transcription factor-binding sites 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, which 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[188] (FIG. 6b).

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 next.

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[134,169] (FIG. 6b). Moreover, BZIP28 is activated and translocated from the endoplasmic reticulum to the nucleus[189]. HSFA and MBF1C cooperate in the transcriptional activation of many heat shock proteins and other transcription factors, such as dehydration-responsive element-binding factors, and are both required for the acquisition of thermotolerance[181] (FIG. 6b). BZIP28 cooperates with BZIP60 to transcriptionally activate the unfolded protein response 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) 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, which 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 Cys82 and Cys216 (REFS[83,84]) (FIG. 6c). Salicylic acid triggers a reduction of these bonds that is mediated by thioredoxins (TRX3 and TRX5) and results in monomerization of NPR1 (REF.[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 protein-encoding genes and transcription factors, such as WRKY[133]. Interestingly, the plant hormone jasmonic acid antagonizes this process by promoting the S nitrosylation of NPR1 on Cys156, causing its oligomerization[192] (FIG. 6c). NPR1 is also involved in the response to other abiotic stresses (for example, salinity)[193], and could be 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, ZAT12, 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 endoplasmic reticulum to the cytosol and the negative regulator RCD1[46] (FIG. 6b). 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 occurs (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]. In the next section we 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 $O_2^{\bullet -}$ 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 neighbouring cells, between different organelles or along membranes (FIG. 7a). This new concept in ROS signalling stems from

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

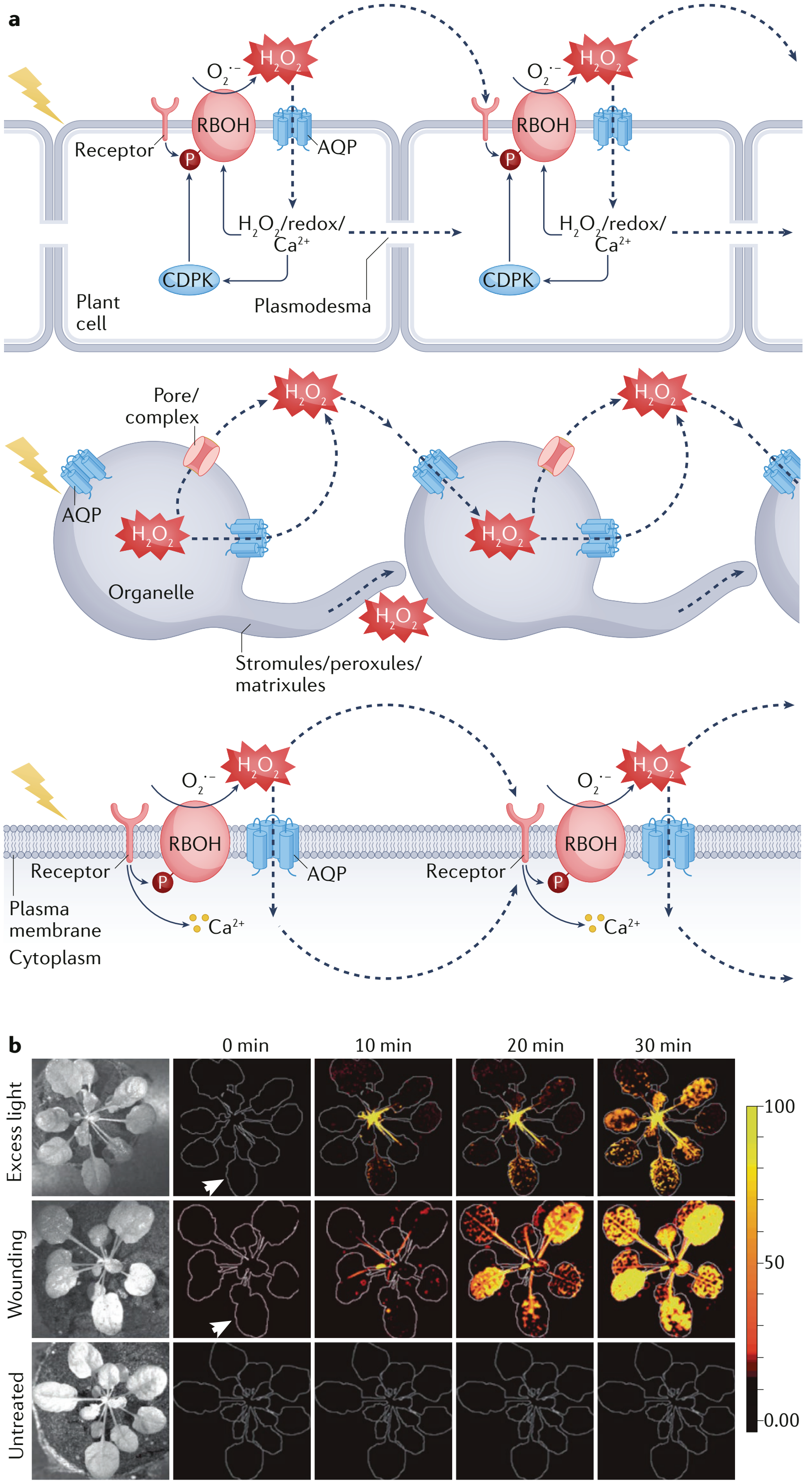


Fig. 7 | **Propagation of ROS signals within and between cells. a** | Because reactive oxygen species (ROS) are rapidly scavenged in cells, they cannot diffuse over long distances in different biological systems. Instead, the state of their 'production, scavenging and transport' can propagate as an 'on–off ROS accumulation' or 'altered ROS' state between cells (top), between 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, between 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. The colour scale bar in part **b** indicates signal intensity. AQP, aquaporin; CDPK, Ca^{2+}-dependent protein kinase; P, phosphate; RBOH, respiratory burst oxidase homologue.

studies that reported cell-to-cell (in plants)[116,151,163,197–200] and mitochondrion-to-mitochondrion (in mammalian cells)[201–203] signalling pathways that involve an 'ROS-induced-ROS production' response by each cell or organelle that communicate with another cell or organelle, and could in principle be extended to membrane nanodomain-to-nanodomain ROS signalling (FIG. 7a). Although mitochondrion-to-mitochondrion 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 neighbouring 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[116,151,169,197,200,204] (FIG. 7a). This process, termed the 'ROS wave', is autopropagating 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 autopropagating 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 autopropagating 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 started 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 AQPs or other transporters. Furthermore, our perspective on how ROS are produced in cells during stress should be re-evaluated. Whether the finding that most 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 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 autopropagating 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 will it be possible to fully understand the functions of ROS in plants in response to stress.

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