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INVITED REVIEW

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The redox code of plants

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Abstract

Central metabolism is organised through high-flux, Nicotinamide Adenine Dinucleotide (NAD$^+$/NADH) and NADP$^+$/NADPH systems operating at near equilibrium. As oxygen is indispensable for aerobic organisms, these systems are also linked to the levels of reactive oxygen species, such as H_2O_2, and through H_2O_2 to the regulation of macromolecular structures and activities, via kinetically controlled sulphur switches in the redox proteome. Dynamic changes in H_2O_2 production, scavenging and transport, associated with development, growth and responses to the environment are, therefore, linked to the redox state of the cell and regulate cellular function. These basic principles form the 'redox code' of cells and were first defined by D. P. Jones and H. Sies in 2015. Here, we apply these principles to plants in which recent studies have shown that they can also explain cell-to-cell and even plant-to-plant signalling processes. The redox code is, therefore, an integral part of biological systems and can be used to explain multiple processes in plants at the subcellular, cellular, tissue, whole organism and perhaps even community and ecosystem levels. As the environmental conditions on our planet are worsening due to global warming, climate change and increased pollution levels, new studies are needed applying the redox code of plants to these changes.

KEYWORDS

cell, development, metabolism, network, reactive oxygen species (ROS), stress

1 | INTRODUCTION

Biochemical and metabolic reactions exchange energy with each other in many forms. Among the more ancient 'energy coins' used for such purpose are Nicotinamide Adenine Dinucleotide (NAD$^+$/NADH), and its phosphorylated derivative NADP$^+$/NADPH molecules, that couple many different reactions in cells. These important co-factors exchange different oxidation (i.e., loss of hydrogen or loss of electron) and reduction (i.e., gain of hydrogen or gain of electrons) states between and/or within different pathways in cells (e.g., glycolysis, the pentose phosphate pathway, respiration …) and form one of the basic principles for 'coupled cellular metabolism' that defines life as we know it. *Bioenergetics, catabolism and anabolism are, therefore, organised through high-flux, NAD$^+$/NADH and NADP$^+$/NADPH systems operating at near equilibrium with central metabolism*, and this process constitutes the first principle of the cellular redox code, as described below (The term 'near equilibrium' is used here to describe reactions in which there is essentially zero free energy available from the electron(s) transferred/exchanged because the reaction is maintained so close to an equilibrium distribution between reactants and products; The term 'code' is used here to describe a set of principles or rules; Jones & Sies, 2015). In the presence of oxygen, that is, under aerobic conditions, that significantly enhance energetic metabolism (but also sometimes under semi- or full-anaerobic conditions), several other players are introduced into biological systems, and some of them take the form of reactive oxygen species (ROS), such as H_2O_2. The formation of ROS under aerobic conditions is unavoidable as many proteins and pathways in cells (e.g., respiration and photosynthesis) can reduce or excite atmospheric oxygen, forming ROS (for a more comprehensive overview of ROS formation, reactivity, transport and detoxification, the reader is directed to the many reviews addressing this subject: e.g., Foyer & Hanke, 2022; Mittler, 2017; Mittler et al., 2022; Sies & Jones, 2020; Sies et al., 2022; Smirnoff & Arnaud, 2019; Waszczak et al., 2018). H_2O_2 can inhibit or accelerate different metabolic reactions, either by modifying protein structure and function directly or through the activation/suppression of transcriptional/posttranscriptional mechanisms (Foyer & Hanke, 2022; Mittler, 2017; Mittler et al., 2022; Sies & Jones, 2020; Sies et al., 2022; Smirnoff & Arnaud, 2019; Waszczak et al., 2018). The gradual switch from a strictly anaerobic metabolism to aerobic/anaerobic metabolism that began as oxygen-evolving photosynthesis evolved on Earth, more than 2.5 billion years ago (Gutteridge & Halliwell, 2018; Inupakutika et al., 2016; Jabłońska & Tawfik, 2021), forced, therefore, an adjustment of central metabolism, from a strictly 'energy-redox' driven coupled flow of reactions, to an 'energy-redox-H_2O_2' driven coupled flow of reactions (Figure 1a). In this review, we describe how this change altered metabolism and signalling processes in cells, generating a 'redox state-H_2O_2 level network', termed the 'redox code' (Jones & Sies, 2015), that coordinates growth, development, differentiation and responses to the environment in different biological systems. Our review focuses on H_2O_2 as a central ROS in cell signalling and regulation in plants; readers must be aware, however, that superoxide anion radical ($O_2^{\cdot-}$) is also important in plant cells, and that some analytic methods may not discriminate between $O_2^{\cdot-}$, H_2O_2, other ROS and other nonoxygen-related oxidants (Sies et al., 2022). In addition to highlighting the many different biological roles of the redox code in plants, we discuss in this review the role of the redox state-H_2O_2 level network in cell-to-cell, systemic and plant-to-plant communication, as well as address the role of redox regulation in plant responses to multiple environmental stress conditions caused by global warming and climate change.

2 | THE RELATIONSHIP BETWEEN H_2O_2 AND REDOX

As described above, the flow of energy through many metabolic reactions can generate ROS, such as H_2O_2 (metabolic H_2O_2). Among other molecules in the cell, H_2O_2 can react with many proteins, by, for example, targeting their exposed cysteine (Cys) residues. These reactions, that are sometimes mediated through the function of different peroxidases (e.g., glutathione peroxidases [GPXs] or peroxiredoxins), cause the oxidation of Cys residues and alter protein structure and function (Foyer et al., 2018; Noctor et al., 2018; Young et al., 2019; Zhou et al., 2023). Among the many proteins potentially subjected to such oxidative posttranslational modifications (i.e., oxi-PTM) are different transcription factors, receptors, channels and metabolic enzymes (>200 000 Cys are estimated to be involved in such redox networks; Jones & Sies, 2015). The fluctuations in the levels of metabolic H_2O_2, that reflect changes in metabolism and the

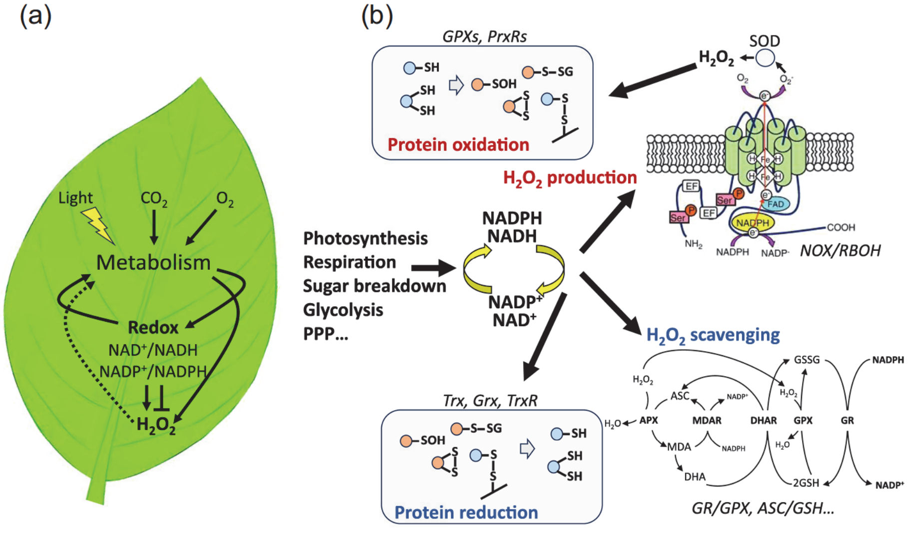

FIGURE 1 The basic interactions underlying the relationships between redox state and H_2O_2 levels. (a) Metabolic reactions use NAD^+/NADH and $NADP^+$/NADPH as redox coins. They are further accompanied by the formation of H_2O_2. H_2O_2 and the redox state are linked and affect the overall metabolism of the plant. (b) Metabolic reactions alter the levels of NAD^+/NADH and $NADP^+$/NADPH that are directly linked to (i) H_2O_2 production via NOX/RBOH/SOD enzymes; (ii) H_2O_2 scavenging via multiple pathways such as the ascorbate-glutathione pathway and (iii) the regulation of the proteome redox state through different enzymes such as Trxs, Grxs and TrxR. The levels of H_2O_2, generated by NOX/RBOH/SOD, using NADPH, further regulate the proteome redox status through the function of GPXs and/or PrxRs. See text for more details. APX, ascorbate peroxidase; ASC, ascorbate; DHA, dehydroascorbate; DHAR, DHA reductase; GPX, glutathione peroxidase; GR, glutathione reductase; Grx, glutaredoxins; GSH, glutathione; GSSG, glutathione disulphide; MDA, monodehydroascorbate; MDAR, MDA reductase; NAD, nicotinamide adenine dinucleotide; NOX, NADPH oxidase; PrxR, peroxiredoxin; RBOH, respiratory burst oxidase homolog; ROS, reactive oxygen species; SOD, superoxide dismutase; TPX, thiol peroxidase; Trx, thioredoxin. [Color figure can be viewed at wileyonlinelibrary.com]

redox status of the cell (reflected in the ratios of NAD^+/NADH and $NADP^+$/NADPH), can, therefore, alter or regulate many cellular processes via H_2O_2-derived oxi-PTM reactions involving key regulatory proteins (Figure 1b). In addition to H_2O_2 being generated via metabolic reactions (metabolic H_2O_2), cells actively produce superoxide $O_2^{\cdot -}$ and H_2O_2 for signalling purposes (signalling H_2O_2) via enzymes such as NADPH oxidases (NOXs; called in plants Respiratory Burst Oxidase Homologs; RBOHs) and superoxide dismutates (SODs; Figures 1b, 2). These enzymes represent a prime example of how an energy coin such as $NADP^+$/NADPH can be converted into H_2O_2. The state of $NADP^+$/NADPH (that is associated by different cellular reactions to the state of NAD^+/NADH) is, therefore, directly linked to H_2O_2 production via the highly regulated function of NOXs/RBOHs coupled with SODs (Mittler et al., 2022; Torres et al., 2002), as well as to oxi-PTM reactions and protein function (Figure 1b).

To prevent cellular damage associated with overaccumulation of ROS, $O_2^{\cdot -}$ and H_2O_2 are continuously being scavenged by multiple cellular pathways and enzymes, such as SODs, the ascorbate-glutathione (ASC-GSH) pathway, the water–water cycle and catalase (Decros et al., 2023; Foyer & Hanke, 2022; Sies & Jones, 2020; Mittler, 2017; Mittler et al., 2022; Sies et al., 2022; Smirnoff & Arnaud, 2019; Waszczak et al., 2018). Many of these pathways, for example, the ASC-GSH pathway (also known as the Foyer-Asada-Halliwell pathway) and GPXs, require NADH and NADPH to function. The metabolic and redox state (i.e., the ratio between the reduced and oxidised forms of Cys and other redox-dependent molecules in the cell) of cells and the ratios of NAD^+/NADH and $NADP^+$/NADPH, are, therefore, also linked to the levels of $O_2^{\cdot -}$ and H_2O_2 through the many different $O_2^{\cdot -}$ and H_2O_2 scavenging pathways of the cell (Figures 1b, 2). In addition to the direct link between the redox state of the cell and the levels of $O_2^{\cdot -}$ and H_2O_2 (via $O_2^{\cdot -}$ and H_2O_2 production and scavenging that require NADPH/NADH, described above; Figures 1b, 2), there is also a direct link between the ratios of NAD^+/NADH and $NADP^+$/NADPH in the cell and the reduced/oxidised state of the proteome. Countering the oxidising effects of H_2O_2 on the proteome (e.g., oxidation of Cys residues; Figure 1b) are, therefore, multiple enzymes that repair or reverse protein oxidation. These include thioredoxins (Trxs), glutaredoxins and/or Trx reductases (TrxR) that can revert the oxidation state of a Cys residue using reducing power in the form of NADPH or NADH (Figures 1b, 2; Jones & Sies, 2015; Mittler et al., 2022; Sies & Jones, 2020; Sies et al., 2022).

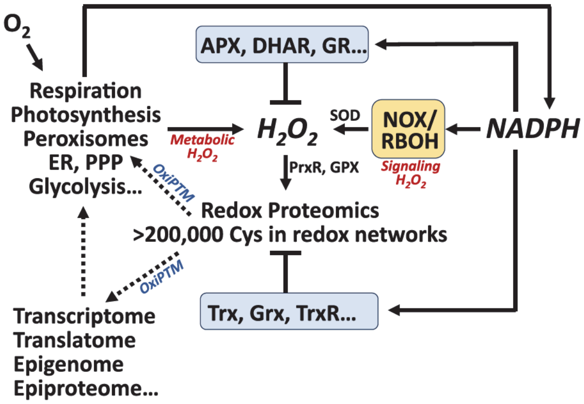

FIGURE 2 The interactions between H_2O_2 and redox state control cellular functions. Metabolic reactions such as respiration and photosynthesis are shown to produce metabolic H_2O_2 and to affect the levels of NADPH. NADPH levels, in turn, are shown to affect the levels of signalling H_2O_2 through the function of NOX/RBOH/SOD, as well as to affect H_2O_2 scavenging (through the function of enzymes such as DHAR and GR; part of the ASC-GSH pathway), and the redox state of the proteome (protein reduction through the function of Trxs, Grxs and TrxR). The levels of H_2O_2, controlled by the interplay between H_2O_2 scavenging and production are shown to affect the proteome redox status (protein oxidation) through PrxR and GPX. Changes in the proteome redox status, regulated by the opposing processes of protein oxidation and reduction, are shown to control metabolism and the transcriptome, translatome, epigenome and epiproteome of the plant via oxi-PTM reactions. See text for more details. APX, ascorbate peroxidase; ASC, ascorbate; Cys, cysteine; DHAR, dehydroascorbate reductase; ER, endoplasmic reticulum; GPX, glutathione peroxidase; GR, glutathione reductase; Grx, glutaredoxins; GSH, glutathione; NAD, nicotinamide adenine dinucleotide; NOX, NADPH oxidase; Oxi-PTM, oxidative posttranslational modification; PPP, pentose phosphate pathway; PrxR, peroxiredoxin; RBOH, respiratory burst oxidase homolog; ROS, reactive oxygen species; SOD, superoxide dismutase; Trx, thioredoxin; TrxR, Trx reductase. [Color figure can be viewed at wileyonlinelibrary.com]

The redox state of the cell, determined by its metabolic activity and signalling reactions, can, therefore, affect the levels of H_2O_2 (via $O_2^{\cdot -}$ and H_2O_2 production and scavenging), that in turn affect the redox state of the cell by affecting protein structure and function, and vice versa, in a complex relationship that is determined by the energetic state of the cell, its developmental and differentiation programmes, proliferation rate and interaction(s) with the environment (Figures 1, 2). As the evolution of aerobic organisms occurred in the presence of ROS, this complex and intertwined relationship became a key feature of aerobic life and cellular function. In addition to the direct links between H_2O_2, redox and the oxi-PTM state of the proteome, described above, cells contain many redox-active small molecules that function as buffers or sensors for the redox-ROS state of the cell (redox coins). Ascorbic acid and GSH function as such buffers/sensors in plants as they can react with H_2O_2, and detoxify it (directly or enzymatically via ascorbate peroxidases (APXs) and GPXs, respectively); at the cost of their oxidation. In counter reactions, oxidised ASC and GSH can, however, be reduced back using NADPH/NADH via enzymes such as monodehydro- and dehydroascorbate reductases, and glutathione reductase (Figure 1b), respectively. By functioning as 'couplers' for H_2O_2 levels and redox state, these molecules can buffer rapid changes in H_2O_2/redox in cells, as well as function as sensors for their levels (Considine & Foyer, 2014; Decros et al., 2023; Mittler et al., 2022; Smirnoff & Arnaud, 2019; Figures 1, 2).

The complex relationships between the redox state and H_2O_2 levels of the cell, outlined in a simplified manner in Figure 1b, are, therefore, extended to include the opposing effects of H_2O_2 and redox on the proteome, depicted in Figure 2, and define the second principle of the redox code: *Macromolecular structure and activities are linked to bioenergetic systems through kinetically controlled sulphur switches in the redox proteome* (Jones & Sies, 2015).

3 | SUBCELLULAR H_2O_2 LEVELS AND REDOX REGULATION

The interactions between metabolism, energy, redox state and H_2O_2 levels, outlined in Figures 1 and 2, occur in almost all subcellular compartments of the cell (Figure 3). These interactions define and regulate each compartment and are directly linked to the specialised metabolism that occurs within it (Diaz-Vivancos et al., 2015; Mittler et al., 2022; Noctor & Foyer, 2016; Sevilla et al., 2023; Smirnoff & Arnaud, 2019). For example, due to their specialised metabolism, compartments such as the chloroplast, apoplast, ER and peroxisomes could have a higher oxidation state compared to the cytosol and nuclei (Figure 3). Such differences between the redox potential and H_2O_2 content of each subcellular compartment are further altered based on the metabolic state of the cell, its developmental programme and its interaction(s) with the environment. For example, in plants (that depend on light for their metabolism), the redox state and H_2O_2 levels of the chloroplast and mitochondria (the two powerhouses of plant cells), might be significantly different between day and night. Differences in the redox state and H_2O_2 levels can also occur during responses to bacterial pathogens that elicit a high rate of $O_2^{\cdot-}$ and H_2O_2 production in the apoplast and chloroplast. Although each compartment has its own characteristic redox state and H_2O_2 levels, in recent years, it became apparent that in plants different compartments are connected to each other, for example, through membrane extensions such as stromules, peroxules and matrixules (Figure 3; Mittler et al., 2022; Noctor & Foyer, 2016; Smirnoff & Arnaud, 2019). The dynamics, scope and regulation of the redox state and H_2O_2 exchanges occurring through these extensions are a research area in need of further development. In addition, some compartments can exchange H_2O_2 with others via aquaporins/peroxiporins (Figure 3; Rodrigues et al., 2017), and of course, NADH, NADPH and/or other redox/energy 'coins' via different shuttles. These pores, channels and shuttles are highly regulated and together with the rates of H_2O_2 production and scavenging, and the redox state changes in each compartment, determine the overall redox state-H_2O_2 balance of each compartment. A coupled and integrative network of redox state and H_2O_2 levels in each compartment, therefore, determines the overall 'landscape' of H_2O_2 and redox state in the cell and defines its function (Mittler et al., 2022). This network/landscape is continuously and dynamically altered based on the cell metabolic function, external conditions (e.g., changes in light intensity, temperature, humidity …), developmental and differentiation programmes, and interactions with the environment (e.g., pathogens, stress …). Because H_2O_2 levels and the redox state control the proteome of each organelle and subcellular compartment, they are also linked to many other cellular and signalling processes that occur between different compartments of the cell (e.g., retrograde and anterograde signalling), and could regulate these based on the overall redox state-H_2O_2 levels/landscape of the cell (Mittler et al., 2022).

The different interactions between the redox state and H_2O_2 levels that occur in each subcellular compartment and are dynamically altered in response to different stimuli, as well as linked and

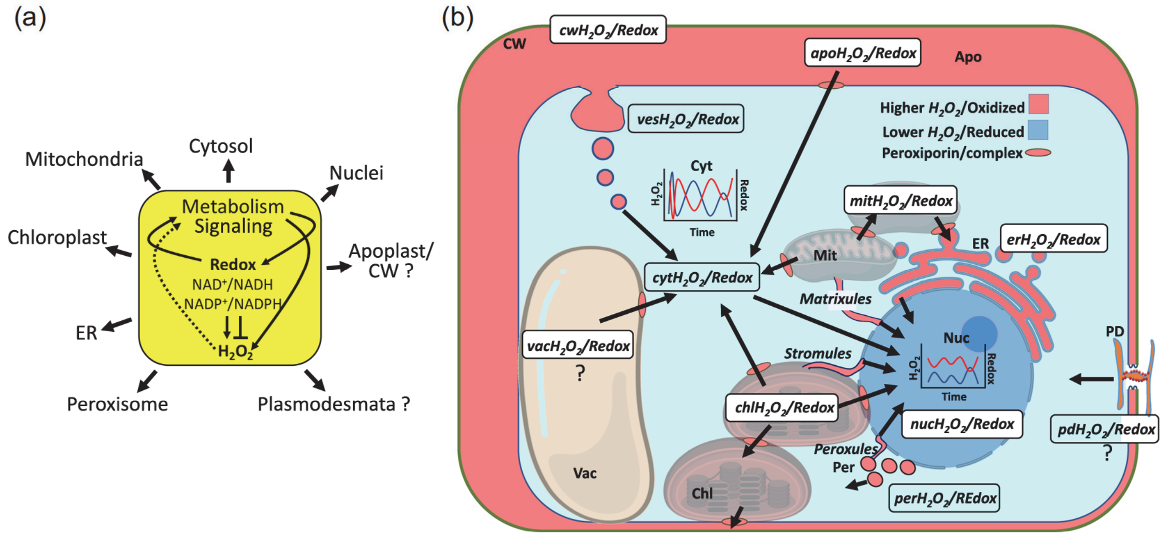

FIGURE 3 Subcellular localisation of H_2O_2 levels-redox state interactions. (a) The interactions between redox state and H_2O_2 levels that control metabolism and other functions in cells occur in almost all subcellular compartments. (b) The H_2O_2 levels-redox state interactions at the different subcellular compartments are linked with each other generating a 'landscape' of redox state and H_2O_2 levels. See text for more details. Apo, apoplast; Chl, chloroplast; CW, cell wall; Cyt, cytosol; ER, endoplasmic reticulum; H_2O_2/Redox, H_2O_2 levels/redox state; Mit, mitochondria; Nuc, nuclei; PD, plasmodesmata; Per, peroxisome; Vac, vacuole; Ves, vesicle. [Color figure can be viewed at wileyonlinelibrary.com]

coordinated with each other within cells, define the third principle of the redox code: *Activation/deactivation cycles of H_2O_2 production linked to NAD and NADP systems support redox signalling and spatiotemporal sequencing for cellular function* (Jones & Sies, 2015).

4 | REDOX STATE AND H_2O_2 LEVELS IN CELL-TO-CELL COMMUNICATION

As each cell within each tissue of the plant, and the different tissues of the plants, are connected to each other via symplastic (e.g., plasmodesmata; PD) and/or apoplastic routes, the state of redox and H_2O_2 levels within each cell can affect or alter the levels of H_2O_2 and redox state of the different cells adjacent to it; and in principle the redox state and H_2O_2 levels of the entire plant (Figure 4). This concept gained support from recent studies demonstrating the cell-to-cell spread of different signals including H_2O_2, redox state, calcium, membrane potential and hydraulic pressure waves over long distances in plants (Fichman et al., 2019, 2021; Fichman & Mittler, 2021a, 2021b; Gao et al., 2023; Grenzi et al., 2023; Hu et al., 2022; Miller et al., 2009; Mousavi et al., 2013; Nguyen et al., 2018; Toyota et al., 2018). For example, stimulating or stressing a group of cells in one leaf of an *Arabidopsis thaliana* plant, with a localised high light stress or wounding treatments, resulted in the spread of an 'enhanced H_2O_2 production and accumulation state' from cell-to-cell until it reached all aboveground parts of the plant. Likewise, signals that were initiated in roots were shown to spread to the upper parts of the plant in a systemic H_2O_2-dependent manner. The active cell-to-cell transfer of enhanced H_2O_2 levels was also recently shown to occur in monolayers of mammalian cells and isolated hearts (Fichman, Rowland, et al., 2023).

At least two different routes can be used to communicate redox state and H_2O_2 signals between two adjacent plant cells. These include PD pores and the ER-associated membrane structures that span them, and the apoplastic space that two adjacent cells share (Figure 4a). Recent studies show that both routes are needed to communicate the high H_2O_2 production and accumulation signal between cells. One study showed that PD-localised proteins are essential for the cell-to-cell transfer of the H_2O_2 signal, as well as for the H_2O_2-mediated transient opening of PD during this process

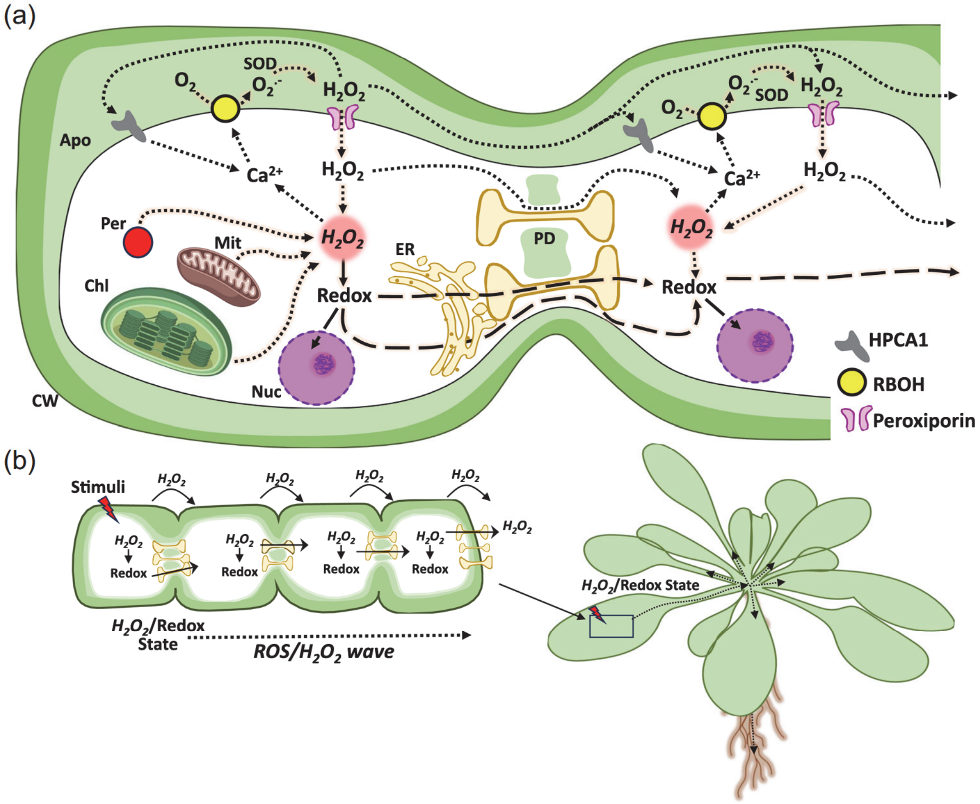

FIGURE 4 Redox state and H_2O_2 levels in cell-to-cell communication. (a) A simplified model for the H_2O_2 levels-redox state interactions between two adjacent cells. H_2O_2 can be exchanged between two adjacent cells via the apoplast and/or PD, while redox state can be exchanged through the membranes that span the PD and/or the PD pore. (b) The H_2O_2 levels and redox state of one cell can be transferred from cell-to-cell via an actively propagating process termed the 'ROS/H_2O_2 wave'. This process requires symplastic and apoplastic functions and can mediate a ROS/H_2O_2/redox state signal from a small group of cells located at a single tissue (e.g., a leaf), to the entire plant. See text for more details. Apo, apoplast; Chl, chloroplast; CW, cell wall; ER, endoplasmic reticulum; H_2O_2/Redox, H_2O_2 levels-redox state; HPCA1, H_2O_2-induced Ca^{2+} increases 1; Mit, mitochondria; Nuc, nuclei; PD, plasmodesmata; Per, peroxisome; RBOH, respiratory burst oxidase homolog; ROS, reactive oxygen species; SOD, superoxide dismutase. [Color figure can be viewed at wileyonlinelibrary.com]

(Fichman et al., 2021). Another study showed that the perception of an apoplastic H_2O_2 signal by the receptor H_2O_2-induced Ca^{2+} increases 1 (HPCA1) was required for this process as well (Fichman et al., 2022). In addition, the function of aquaporins/peroxiporins was shown to be required for the mobilisation of H_2O_2 and redox state signals between cells (Fichman et al., 2021). Taken together, the accumulation of H_2O_2 and redox state changes that occur in one cell can alter the H_2O_2 level-redox state of an adjacent cell via PD pores and/or the membranes that span them, as well as a combination of NOX/RBOH/SOD, coupled with the apoplast to cytosol H_2O_2 transfer function of aquaporins/peroxiporins, and the function of apoplastic exposed receptors that control calcium levels in the cytosol (Figure 4a). This process can be further amplified, by NOX/RBOH activation, and propagated from cell-to-cell until it reaches all parts of the plant (termed the 'ROS/H_2O_2 wave'; Figure 4b; Fichman et al., 2019; Fichman, Rowland, et al., 2023; Miller et al., 2009). Another possible route for the transfer of redox state and H_2O_2 levels between different parts of the plant could be the vascular bundles (i.e., phloem, xylem and bundle sheath cells; Zandalinas, Fichman, & Mittler, 2020; Zandalinas & Mittler, 2021). However, further research is needed to address this possibility, for example, by expressing roGFP2/ro-GFP2-Orp1 proteins (or equivalent) in these tissues and following the dynamic changes in ROS and redox states in them in live plants in response to different stimuli.

The coordination and/or coupling of redox state and H_2O_2 levels between different organelles (Figure 3) and/or between different cells (Figure 4), and the changes that occur in the different cellular/subcellular redox state-H_2O_2 level networks in response to different stimuli (see below), define the fourth principle of the redox code: *Redox/ROS networks from microcompartments to cells, tissues and the entire organism, coordinate adaptive responses to the environment with development, differentiation and growth* (Jones & Sies, 2015).

5 | FROM ORGANELLES TO CELLS, TISSUES AND PLANTS: A DYNAMIC REDOX STATE-H_2O_2 LEVEL NETWORK

The levels of H_2O_2 and the redox state in each subcellular compartment (Figure 3) respond in a dynamic manner to different stimuli (Figure 5a). Recent studies revealed that these changes can occur within seconds to minutes and are associated with rapid changes in the transcriptomic, proteomic and metabolomic landscapes of the plant cell (Choudhury et al., 2018; Kollist et al., 2019; Suzuki et al., 2015). As the levels of H_2O_2 and redox state are linked between the different subcellular compartments of plant cells (Figure 3), and changes in H_2O_2 levels and redox state in cells alter the oxi-PTM state of many proteins (Figures 1, 2), altering

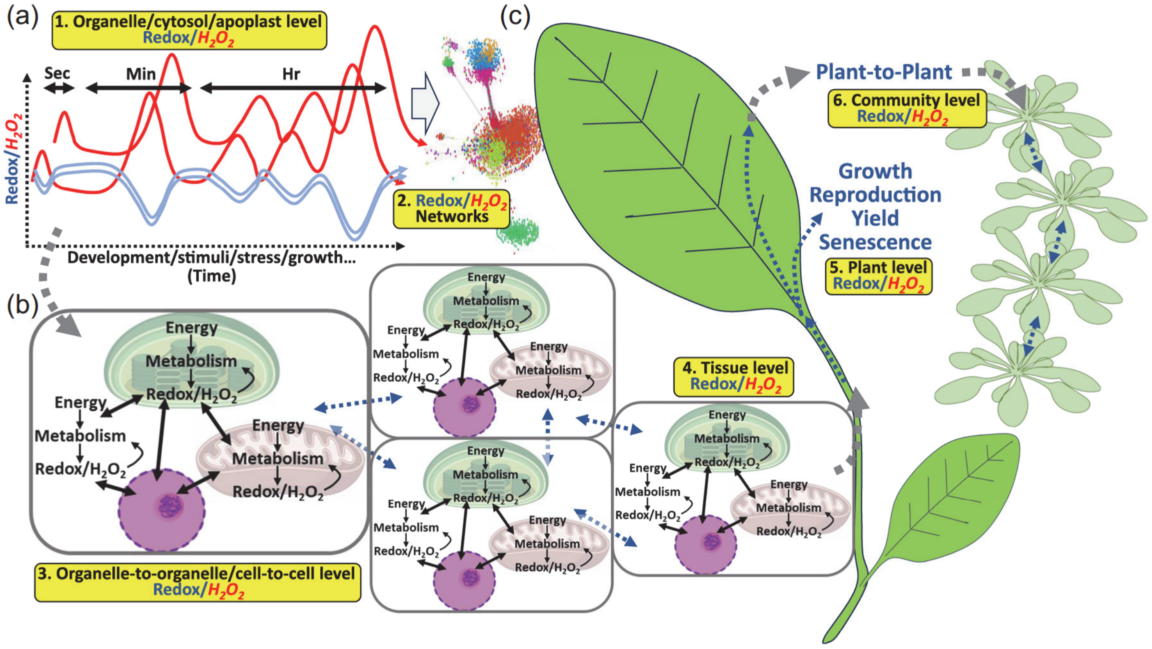

FIGURE 5 The dynamic and integrative nature of the redox code. (a) A hypothetical line graph showing the changes in H_2O_2 levels/redox state in the different organelles of the cell in response to different developmental programmes, external conditions and other stimuli. These dynamic changes are shown to alter the H_2O_2 levels-redox state networks of the cell. (b) The changes in H_2O_2 levels-redox state in each organelle are shown to interact within each cell, and each cell is shown to interact with its adjacent cells, coordinating the H_2O_2 levels-redox state of the different cells within the entire tissue. (c) The H_2O_2 levels-redox state of different tissues within the plant are coordinated with each other controlling and orchestrating the plant overall growth, reproduction, yield and senescence. The H_2O_2 levels-redox state of the entire plant is also shown to affect neighbouring plants and potentially coordinate the overall H_2O_2 levels and redox states of an entire plant community. See text for more details. H_2O_2/Redox, H_2O_2 levels-redox state; Hr, hour; Min, minute; ROS, reactive oxygen species; Sec, second. [Color figure can be viewed at wileyonlinelibrary.com]

transcription, translation and the allosteric behaviour of different enzymes, the H_2O_2 level and redox state networks of the cell are directly linked to the regulation of plant responses to different stimuli, developmental programmes and the plant environment (Figure 5a).

As the different cells of the plant are linked with each other (Figure 4), changes in the redox state and/or H_2O_2 levels in one cell (or even in one compartment of one cell) can initiate a cascade of H_2O_2 levels-redox states reactions that spread from the initiating cell to neighbouring cells, and eventually the entire tissue (Figure 5b; Mittler et al., 2022). These dynamic changes in H_2O_2 levels and redox states of cells can further spread from cell-to-cell, within minutes, to the entire plant and alter and/or coordinate its growth, development, differentiation and responses to the environment, potentially even affecting reproductive tissues and yield (Figure 5c). Changes in the redox state and H_2O_2 levels of a group of cells subjected to an external stimulus (e.g., wounding or high light stress) were shown to spread to the entire plant via the actively propagating function of the ROS/H_2O_2 wave that is dependent on NOX/RBOH activation in each cell along the path of the signal (Fichman et al., 2019; Miller et al., 2009). In addition, physiological responses such as stomatal aperture changes, enhanced acclimation and changes in the transcriptome and metabolome were shown to occur in remote plant tissues in response to a local stress (applied to a single leaf) that triggered the ROS/H_2O_2/redox state wave (Choudhury et al., 2018; Devireddy et al., 2018; Zandalinas, Fichman, Devireddy, et al., 2020; Zandalinas et al., 2019). These changes were suppressed or altered in mutants deficient in NOX/RBOH, demonstrating that they depend on the function of the ROS/H_2O_2 wave. The dynamic changes in the redox status and H_2O_2 levels of plant cells, that are dependent on interactions between the plant environment and its developmental stage, are, therefore, coordinated via organelle-to-organelle and cell-to-cell H_2O_2 levels and redox state signalling reactions, driving plant metabolism, growth and reproduction, and supporting the overall survival of plants (Figure 5; The fourth principle of the redox code, as described above).

Recent studies also showed that changes in ROS/H_2O_2 levels in one plant can alter the ROS/H_2O_2 status of an adjacent plant, as long as the two plants were physically touching each other and conditions were sufficiently humid (Szechyńska-Hebda et al., 2022), or that the two plants were connected via a parasitic plant (Fichman, Kaluwella Mudalige, et al., 2023). This plant-to-plant H_2O_2 communication pathway was further shown to coordinate physiological responses in a community of plants and proposed to support the survival of the entire community to different stresses (Szechyńska-Hebda et al., 2022). Changes in the redox state-H_2O_2 levels in one plant can, therefore, alter the physiology and metabolism of another plant that is in close physical proximity to it (Figure 5c), and a community of such plants communicating with each other (via plant-to-plant H_2O_2/redox state signalling) could coordinate their overall responses to different signals, such as insect or pathogen attack that initially impacted a single plant in the community (Szechyńska-Hebda et al., 2022; Figure 5c).

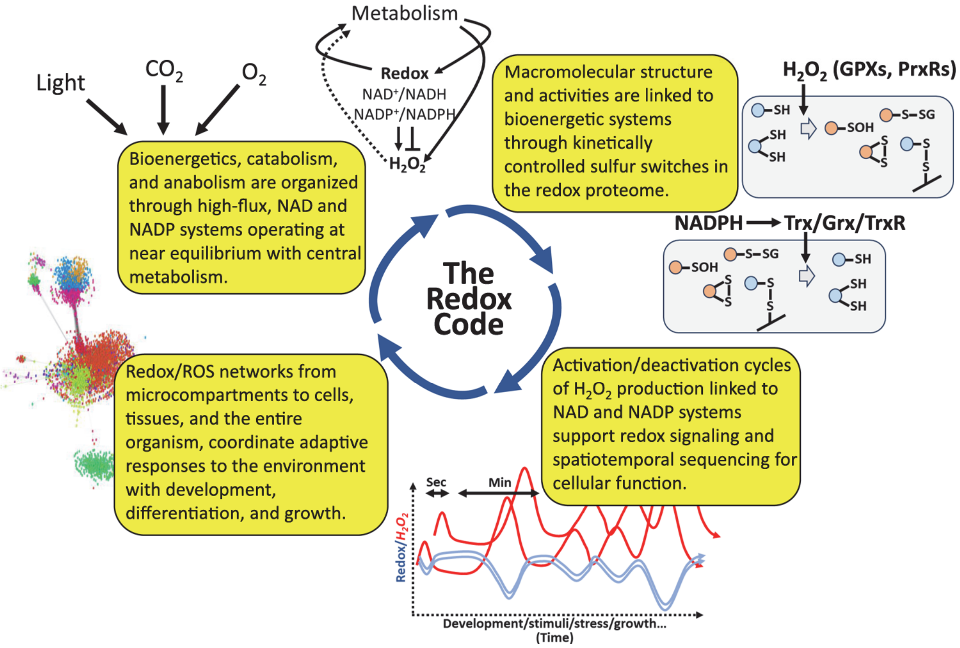

FIGURE 6 The redox code. The different tiers of the redox code are shown to interact with each other beginning with the ratios of NAD^+/NADH and $NADP^+$/NADPH that are dependent on metabolism, through the effects of H_2O_2 levels/redox state on the proteome and the dynamic coordination of subcellular, cellular, tissue and whole organism functions. See text for more details. GPX, glutathione peroxidase; Grx, glutaredoxins; NAD, nicotinamide adenine dinucleotide; PrxR, peroxiredoxin; ROS, reactive oxygen species; Trx, thioredoxin; TrxR, Trx reductase. *Source*: Adapted from Jones and Sies (2015). [Color figure can be viewed at wileyonlinelibrary.com]

6 | PUTTING IT ALL TOGETHER: THE REDOX CODE OF PLANTS

The basic processes at the foundation of almost all biological systems, that is, bioenergetics, catabolism and anabolism, are organised through high-flux, NAD^+/NADH and $NADP^+$/NADPH systems operating at near equilibrium with central metabolism. As oxygen is indispensable for aerobic organisms, they are also linked to the levels of H_2O_2 and through H_2O_2 to the regulation of macromolecular structure and activities; through kinetically controlled sulphur switches in the redox proteome (Figures 1, 2). The activation/deactivation cycles of H_2O_2 production, associated with development, growth and responses to the environment in many organisms are further linked to NAD^+/NADH and $NADP^+$/NADPH systems and support redox signalling and spatiotemporal sequencing for cellular function (Figures 3–5). The dynamic redox state-H_2O_2 level networks established from subcellular compartments to cells, tissues and the entire organism, coordinate adaptive responses to the environment with development, differentiation and growth (Figure 5). These basic principles of the redox code were first defined in a landmark paper by D. P. Jones and H. Sies (2015) and are here applied to plants in which studies have now shown that they can explain organelle-to-organelle, cell-to-cell and even plant-to-plant signalling processes (Mittler et al., 2022; Szechyńska-Hebda et al., 2022; Fichman, Kaluwella Mudalige, et al., 2023; Fichman, Rowland, et al., 2023). The redox code (Figures 5, 6; Jones & Sies, 2015) is, therefore, an integral part of biological systems as we know them, spanning microbes, plants and animals, and can be used to explain multiple processes at the subcellular, cellular, tissue, whole organism and perhaps even community and ecosystem levels. As the environmental conditions on our planet are worsening due to global warming, climate, change and increased levels of industrial/agricultural pollution, causing conditions of stress combinations and even multifactorial stress combinations (Masson-Delmotte et al., 2021; Zandalinas et al., 2021; Rillig et al., 2019), more studies addressing how the redox code applies to these changes in multiple organisms are needed. These studies are especially critical as new research identified H_2O_2 level and redox state systems as underlying the response of plants to stress/multifactorial stress combinations (Sinha et al., 2023; Peláez-Vico et al., 2023; Zandalinas et al., 2021), associated with many of the global change factors described above.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in https://pubmed.ncbi.nlm.nih.gov/ at https://pubmed.ncbi.nlm.nih.gov/.

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