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ROS are universal cell-to-cell stress signals

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Abstract

The interplay between reactive oxygen species (ROS) and the redox state of cells is deeply rooted in the biology of almost all organisms, regulating development, growth, and responses to the environment. Recent studies revealed that the ROS levels and redox state of one cell can be transmitted, as an information 'state' or 'currency', to other cells and spread by cell-to-cell communication within an entire community of cells or an organism. Here, we discuss the different pathways that mediate cell-to-cell signaling in plants, their hierarchy, and the different mechanisms that transmit ROS/redox signaling between different cells. We further hypothesize that ROS/redox signaling between different organisms could play a key role within the 'one world' principle, impacting human health and our future.

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Abiotic stress, Cell-to-cell, Climate change, 'One world', Reactive oxygen species (ROS), Redox, Systemic signaling.

Introduction

Cell-to-cell signaling within a tissue (*i.e.*, local) and between tissues or across the entire organism (*i.e.*, systemic) is fundamental for the development, growth, and reproduction of multicellular organisms. Local and systemic cell-to-cell signaling pathways are also important for unicellular organisms living in a microbiome, crust, or community, as well as for interactions between microorganisms and multicellular organisms such as plants and animals [1–4]. Transmitting chemical, electrical, and physical signals between cells coordinates many processes within an organism/community of cells and is essential for stress responses and acclimation to changes in environmental conditions [1–7]. Here, we focus on systemic cell-to-cell signaling in plants, the reactive oxygen species (ROS) wave [8], and ROS/redox signaling within and between different organisms [6]. We define ROS as activated forms of oxygen, including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($HO^{\cdot}$), and various forms of organic and inorganic peroxides, and redox/redox state as the ratio between the reduced and oxidized forms of protein/peptide cysteines as well as other redox-dependent molecules in the cell [5–8].

Systemic cell-to-cell signaling in plants: Hydraulic, electric, calcium, ROS, redox, volatile, and chemical communication

Almost any change in environmental conditions, and/or the activation/suppression of developmental or metabolic programs sensed by, or occurring in, a single plant cell is communicated to other cells immediately adjacent to it, within the same tissue or organ, and in many cases systemically to other tissues/organs/cells and/or the entire organism [1–14]. This type of cell-to-cell communication is mediated through plasmodesmata (PD) [15], the apoplastic space of cells [16], through the vascular system of plants [17–21], and/or by volatiles [22]. There are many examples of cell-to-cell signals, including different electric waves that propagate by changes in membrane potential between cells *e.g.*, [18,19,21,23–25], hydraulic waves that propagate through changes in water pressure along vascular bundles *e.g.*, [10,20,26], calcium and ROS/redox waves that propagate from cell to cell through PDs and/or the apoplast *e.g.*, [9,12,16,27–33], volatiles that are transmitted through internal air spaces within plant tissues, and/or between tissues/leaves [22], and hormones, peptides, proteins, and other metabolites, such as the amino acid glutamic acid, that are transmitted between cells through PDs/apoplast, between cells within the vascular system *e.g.,* [34], and/or released from cells into the apoplast *e.g.,* [20].

Recent studies shed new light on many of the components that mediate systemic cell-to-cell signaling in plants, including how the release of different compounds into the xylem triggers electric waves upon injury [18–21], how long-distance turgor pressure changes (hydraulic waves) induce local activation of plant glutamate receptor-like channels [20], how volatiles communicate calcium signals between different plant tissues [22], how electric waves trigger calcium channels [25,35], how changes in pH driven by proton pumps regulate calcium channels [24,36], and how the sensing of H_2O_2 in the apoplast triggers calcium signaling [16]. In addition, new studies conducted on roots, using a split-root design, revealed multiple systemic responses at the root level *e.g.,* [37,38], as well as between roots and shoots *e.g.,* [34,39], and at least one study addressed the question of what will happen if two different stresses impact two different leaves at the same time and trigger opposing systemic responses? [40]. These findings add to our growing understanding of systemic cell-to-cell signaling in plants and demonstrate that the sensing of wounding, pathogen infection, and/or abiotic stress by a group of cells located on one tissue of the plant induces multiple acclimation responses in the affected tissue, as well as triggers several different systemic signals that travel at different speeds through the plant (Figure 1) [5–41].

The fastest systemic signals measured to date are electric and hydraulic waves that travel at several to tens of centimeters per minute [19–21]. These are followed by the calcium and ROS waves that are interlinked and travel at rates of 0.1–0.5 cm per minute [15–17], and the redox wave that follows the ROS wave and triggers acclimation responses in all cells it reaches [27]. Along the way, the different waves 'excite' or 'activate' all cells they transverse, cause in them transcriptomic and metabolic changes, trigger the release from conjugates/biosynthesis of different hormones like jasmonic acid (JA), induce a state of acclimation/defense to the initial treatment, and carry with them other, yet unknown, signals/compounds that determine specificity/context in systemic signaling (Figures 1, 2)[5–42].

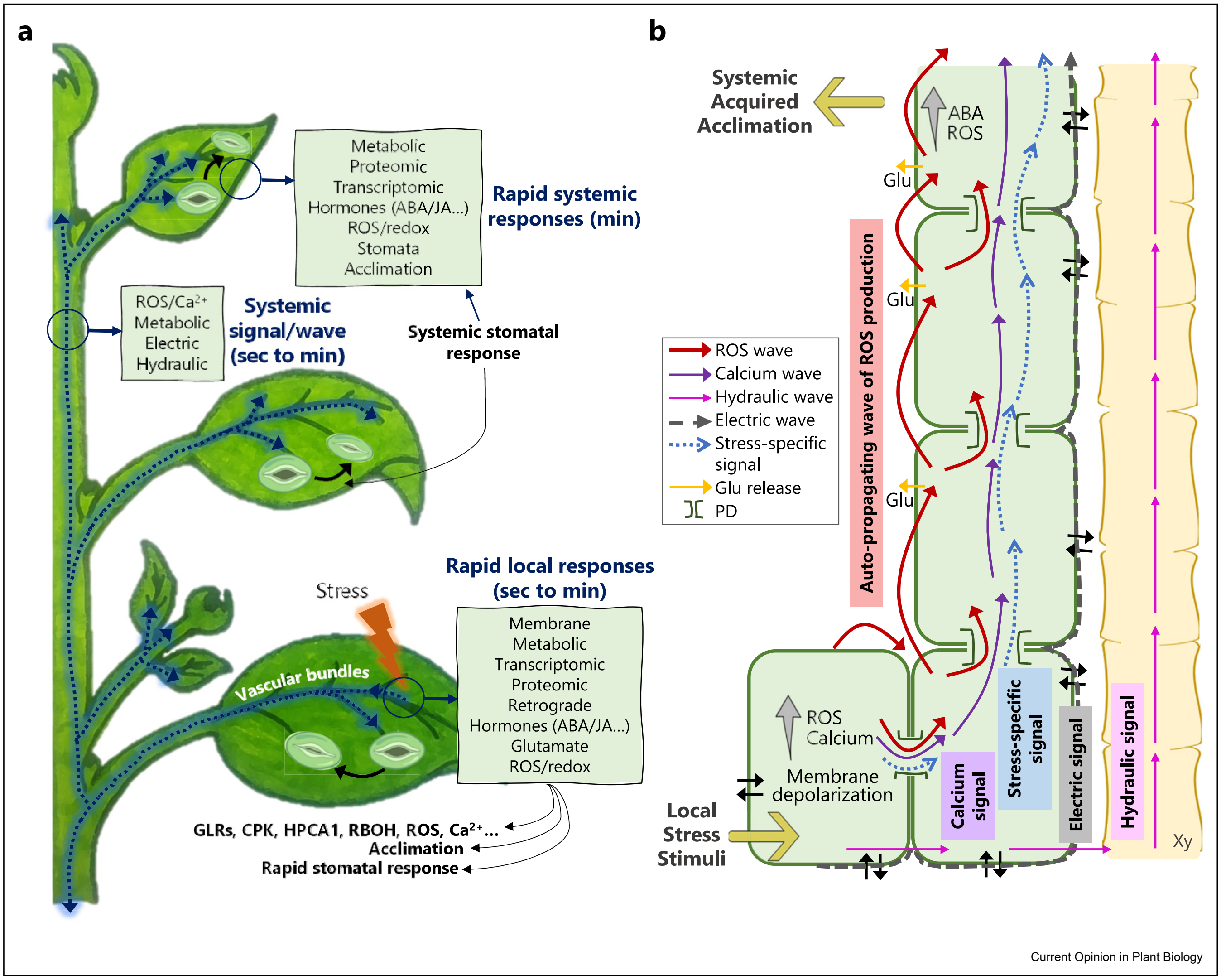
Due to the rapid rates of systemic signals propagation, it is generally believed that they are not mediated by the diffusion of different compounds from the local to the systemic tissues. At least one recent study suggested however that glutamic acid that is released from cells at the signal initiation site diffuses over long distances in plants [33]. Recent opposing evidence suggested, nonetheless, that, instead of diffusion, the hydraulic wave causes the release of glutamic acid from different cells along its path [20]. In addition to the activation of different molecular and metabolic responses in tissues along the path of the signal, the different signals are thought to cause physiological responses, such as stomatal aperture changes, that enhance plant acclimation *e.g.,* [40,42]. The different systemic signals described above coordinate therefore the response of the entire plant to an initial stress stimulus that affects only one tissue of the plant (Figure 1). For example, a heat treatment applied to only one leaf of an *Arabidopsis thaliana* plant caused the activation of molecular acclimation mechanisms to heat and stomatal opening in this leaf, as well as in all other leaves the systemic signal reached [40,42]. In contrast, the application of excess light stress to a single leaf triggered the activation of molecular acclimation mechanisms to excess light stress and stomatal closing in this leaf, as well as in all other leaves the systemic signal reached [40,42]. While both local treatments triggered the ROS, calcium, and electric wave responses [10,40], they resulted, nonetheless, in different molecular, physiological, and acclimation responses in the systemic leaves [40], highlighting the existence of additional systemic signals that provide specificity/context to the systemic response. These should be identified in future studies, as they are highly important for our understanding of basic processes in plant biology. Together, the advances described above have helped in decoding the hierarchy of systemic signals in plants, suggesting that the hydraulic and electric waves 'excite/activate' cells and prime them for the calcium and ROS waves that are interlinked and cause changes in redox and calcium levels in cells along the path of the signal, triggering in turn acclimation and defense mechanism (Figure 2) [12,15,16,19–21,23–25,32,35,36,42]. Below we will focus on the ROS wave that was recently discovered to have a universal function as a cell-to-cell stress signal.

The ROS wave mechanism and evolution: A universal cell signaling system

Reactive oxygen species, and especially H_2O_2, are important regulators of protein structure, stability, and function [6,43–45]. Although the potential chemical toxicity of ROS is undeniable, the control of ROS production, scavenging, and transport, as well as the chelation of free metals, and repair of oxidative damage to proteins (*i.e.,* The 5 principles of living with ROS), mitigate ROS toxicity and allow compatibility with cellular function (Figure 3a) [45].

The ROS wave, discovered in 2009 [8], is an auto-propagating cell-to-cell signaling process in which each cell along the path of the signal is prompted to produce ROS. An initiating cell would therefore start to produce ROS that is sensed by neighboring cells and cause them to actively produce ROS as well, triggering a cascade of

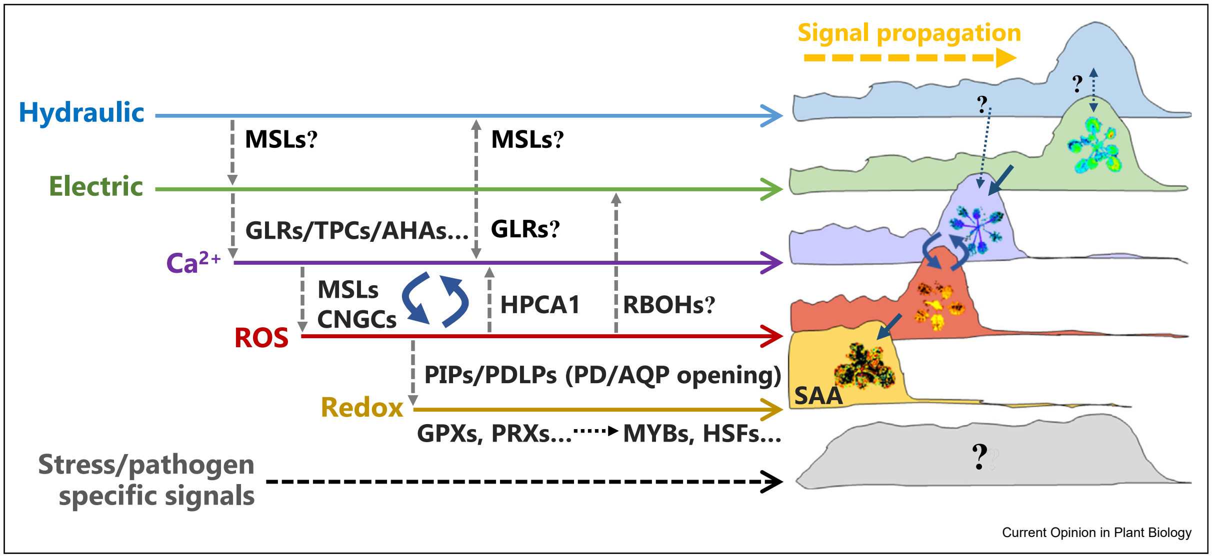
Figure 1



Rapid systemic signaling and the principle of cell-to-cell communication. **a.** A local application of stress to a single leaf is shown to trigger local responses in this leaf within seconds to minutes, induce local acclimation pathways, and initiate systemic signaling. The systemic signals (electric, hydraulic, ROS/Ca^{2+}, redox, and other chemical signals), then spread within seconds to minutes to other parts of the plant (parts that did not sense the stress) and trigger in them systemic responses and acclimation pathways. The physiological (*e.g.,* stomatal responses), molecular, and metabolic responses, occurring in the stressed leaf are therefore communicated to all other parts of the plant and coordinate the response of the entire plant to the initial stress that triggered systemic signaling. **b.** Different cells along the path of systemic signaling (a) are shown to transfer the different systemic signals from one to another generating a cell-to-cell signaling chain or path. As the different systemic signals propagate from cell-to-cell at different speeds, some of them (*e.g.,* electric and hydraulic) prime the cells along the path of the signal, and allow others (*e.g.,* ROS/Ca^{2+}/Glu release and redox) to propagate through them. Please see text for more detail and Figure 2 for the proposed hierarchy of the different systemic signals. Abbreviations: ABA, abscisic acid; CPK, calcium-dependent kinase; GLRs, glutamate receptor-like; Glu, glutamate; HPCA1, H_2O_2-induced Ca^{2+} increases 1; JA, jasmonic acid; Min, minutes; PD, plasmodesmata; RBOH, respiratory burst oxidase homolog; ROS, reactive oxygen species; Sec, seconds; Xy, xylem. Adapted from [53,54].

cell-to-cell 'enhanced ROS production' state *e.g.,* [5–8,45]. Although ROS do not diffuse systemically *per se* in this signaling mechanism, they are nevertheless required for the ROS wave to propagate, as external application of ROS scavengers, inhibitors of NADPH oxidases (NOXs), and/or grafting experiments with NOX mutants, applied centimeters away from the initiation site, block it [5–8,15–17]. Recent studies revealed some of the key mechanisms that regulate the ROS wave (Figure 3b). For example, it was found that phytochrome B, functioning in the cytosol, is required for its activation by different stimuli [46]. In addition, the plasma membrane (PM) receptor HPCA1 (HYDROGEN-PEROXIDE-INDUCED Ca^{2+} INCREASES 1), and the calcium channel MSL3 (MECHANOSENSITIVE CHANNEL OF SMALL

Figure 2

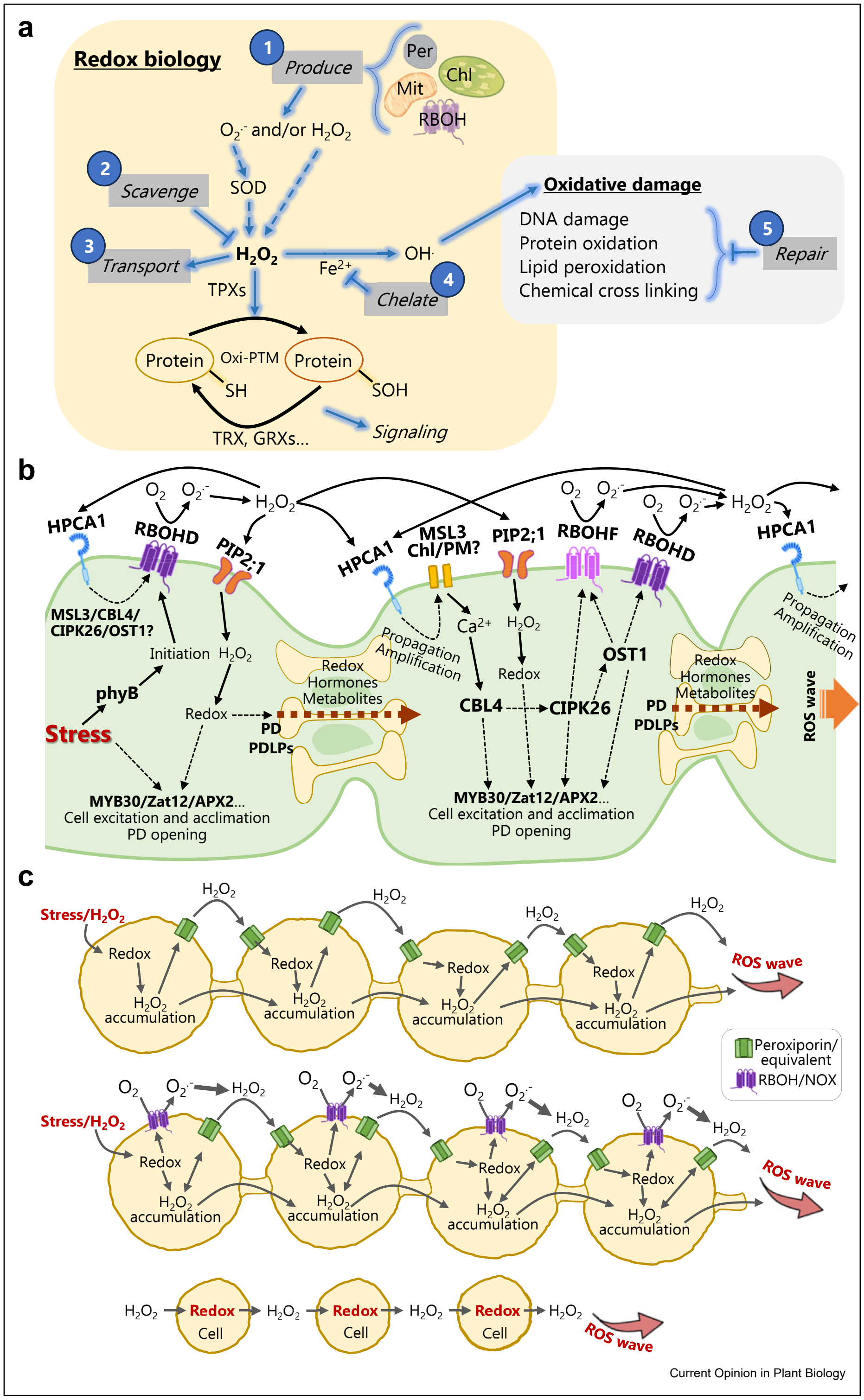


The hierarchy between different systemic signals. The hydraulic and electric waves are shown to be the fastest and to 'excite' or 'prime' cells along their path to transmit the ROS and calcium waves. This is mediated through different mechanosensory channels, pumps, and other sensors/channels. The activation of the calcium wave by the electric and/or hydraulic waves is then shown to trigger the ROS wave, and the two waves, that are mostly dependent on each other (*e.g.,* through the function of HPCA1/MSL3 and other channels) then propagate through the different tissues of the plant via a cell-to-cell process. At each cell the ROS wave arrives, it triggers the redox wave that activates different acclimation and defense mechanisms via different redox-dependent TFs. Similarly, at each cell the calcium wave arrives it triggers acclimation and defense mechanisms via calcium signaling. As the ROS and calcium waves are interlinked, both redox and calcium signaling reactions are coordinated in the different cells along the path of the signal. The different waves are also priming cells to mediate other, yet unknown, signals that provide specificity to systemic signaling. Please see text for more details. Abbreviations: AHA, autoinhibited plasma membrane H^+-ATPase; AQP, aquaporin/peroxiporin; CNGC, cyclic nucleotide-gated channel; GLR, glutamate receptor-like; GPX, glutathione peroxidase; HPCA1, H_2O_2-induced Ca^{2+} increases 1; HSF, heat shock transcription factor; MSL, small conductance mechanosensitive ion channel-like; MYB, myeloblastosis viral oncogene homolog; PD, plasmodesmata; PDLP, plasmodesmata localized protein; PIP, plasma membrane intrinsic protein; PRX, peroxiredoxin; RBOH, respiratory burst oxidase homolog; ROS, reactive oxygen species; SAA, systemic acquired acclimation; TF, transcription factor; TPC, two-pore channel. Adapted from [42].

CONDUCTANCE-LIKE 3), were found to be required for its propagation, potentially linking the calcium and ROS waves (Figures 2, 3b) [16]. In addition, PD localized proteins (PDLP1 and 5) were found to be required for its propagation, and ROS produced by NOXs (RESPIRATORY BURST OXIDASE HOMOLOG D; RBOHD) were found to transiently open PD pores in a process that required PDLP5 [15]. The redox changes caused in cells actively involved in this process activate in turn redox-dependent transcription factors (TFs) such as MYB30 that control and activate acclimation mechanisms (Figure 3b) [27,47]. The ROS and calcium waves, linked by HPCA1/MSL3, therefore induce in cells along the path of the ROS wave acclimation/defense mechanisms, serving as the 'implementors/executioners' of systemic signaling.

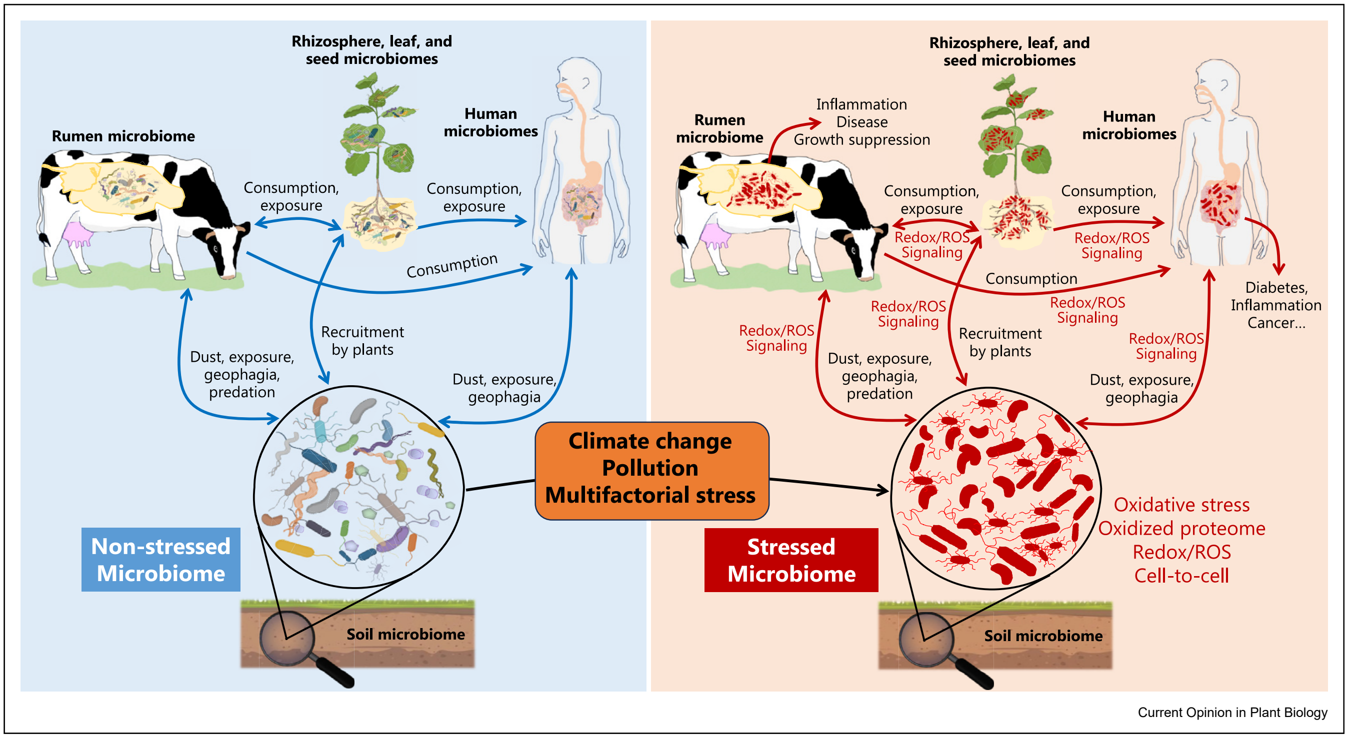
Recent studies demonstrated that the ROS wave also functions in lawns/communities (growing on the surface of agar plates) of microorganisms such as *Chlamydomonas reinhardtii* and *Dictyostelium discoideum*, monolayers of mammalian cells (human epithelial breast cancer and rat cardiomyocytes cells grown in culture), and even entire isolated hearts from female or male mice [7,48]. The ROS wave in these organisms/organs was initiated by a local stress/wounding treatment, inhibited by catalase, NOX, or in mammalian cells gap junction inhibitors, and was absent in a *Chlamydomonas* NOX mutant [7,48]. In addition, proteomics studies in *Chlamydomonas* and mammalian cells revealed that it is accompanied by changes in the abundance of hundreds of proteins in cells along its path [7,48]. The ROS wave in *Chlamydomonas* was also blocked by the presence of a stretch of NOX mutant cells, or NOX inhibitors, placed on the agar plate several centimeters away from the initiation site, demonstrating that, like the ROS wave in plants, it is an active/auto-catalytic process [7]. The studies described above led to a hypothesis that the ROS wave evolved as an early cell-to-cell signaling process that linked/coordinated the redox state of different cells within a crust/microbiome/community and enabled them to survive changes in environmental conditions — an early form of 'multicellularity' in a community of microorganisms (Figure 3c) [7]. In this process, the initiating cell would change its redox state upon ROS sensing, trigger ROS production, and secrete (through

Figure 3



Keeping ROS at the redox biology range, current understanding of the ROS wave, and an ancient origin for cell-to-cell ROS communication. **a.** Five different processes (control of ROS production, scavenging, and transport, chelation of free metals, and repair of oxidative damage to proteins) are highlighted as important to keep ROS from damaging cells. These allow ROS such as H_2O_2 to function as important cell-to-cell signals. **b.** The different molecular mechanisms mediating the ROS wave. Stress is shown to trigger ROS production at the initiating cell via RBOHD and phyB function, and H_2O_2 produced at the apoplast is sensed by the initiating cell and all cells around it via HPCA1 and aquaporin/peroxiporin-facilitated diffusion into cells. H_2O_2 that enters cells and calcium signals triggered in each cell (by HPCA1/MSL3, or equivalent) then trigger further production of ROS and mediate the ROS wave between cells in a process that requires plasmodesmata and apoplast connection between cells. **c.** The principle of ROS-redox-ROS as the 'engine'

Figure 4



Cell-to-cell ROS and redox signaling within the 'one world one health' concept. The ROS levels and redox states of different microorganisms within our environment/ecosystem can directly impact our health and longevity. The altered ROS levels and redox states of different soil microorganisms, within our ecological and agricultural ecosystems, could for example impact the ROS levels and redox states of the cells and microbiomes of plants and animal we consume, altering the ROS levels and redox states of our own microbiomes (via consumption or ingestion of dust particles, *i.e.,* geophagia), thereby impacting our overall physical and mental health. This can occur via cell-to-cell ROS/redox signaling, the consumption of oxidized proteomes, and/or the ingestion of dust particles (as well as other more complex processes that connect different organisms within an ecosystem and are not shown in the figure). As ROS (H_2O_2) and redox are universal cell-to-cell stress signals, and directly impact multiple processes in different cells, they serve as universal stress signals within the one world one health concept. Due to the growing impact of global warming, climate change, and different anthropogenic pollutants on our environment and the enhanced stress levels they induce in different microbiomes, plants, and animals, the relationship(s) between stress, ROS/redox signaling, and communication within and between different organisms needs to be further studied. Abbreviations: ROS, reactive oxygen species. Adapted from [50].

its PM and/or facilitated via ancient forms of peroxiporins/equivalents) ROS to neighboring cells that will repeat this process generating a chain of cell-to-cell signaling pathway (Figure 3c, top panel). With the evolution of NOXs, the production of ROS was 'moved/relocated' to the extracellular side of the plasma membrane/apoplast compartment of plant cells (Figure 3c, middle panel), but the principle of 'ROS sensing leading to redox changes leading to ROS production', by a chain of cells, remained the same (Figure 3c, bottom panel) [7]. It is of interest to note that some NOXs, such as NOX4, function in the mitochondria and nucleus of mammalian cells, further suggesting the possibility of concomitant signaling within and between

of cell-to-cell ROS signaling. The ROS wave is proposed to have evolved as an ancient cell-to-cell signaling mechanism allowing cells to communicate an 'alert' signal/state between them. Changes in redox in the initiating cell are shown to trigger ROS production within (top), or within and outside (after RBOHs/NOXs evolved; middle) it, and the ROS produced by this cell are shown to alter the redox state of other cells around it causing all neighboring cells to trigger ROS production and transmit the altered redox state from one cell to the other via a chain of propagating cell-to-cell H_2O_2/redox communication events (bottom). This ROS/redox signaling process has now been shown to occur in communities of microorganisms, plants, and animal cells/tissues. Please see text for more details. Abbreviations: APX2, ascorbate peroxidase 2; HPCA1, H_2O_2-induced Ca^{2+} increases 1; CBL4, calcineurin B-like calcium sensor 4; Chl, chloroplast; CIPK26, CBL4-interacting protein kinase 26; GRX, glutaredoxins; MSL3, mechanosensitive ion channel like 3; MYB30, myeloblastosis domain protein 30; Mit, mitochondria; NOX, NADPH oxidase; OST1, open stomata 1; Oxi-PTM, oxidative posttranslational modification; PD, plasmodesmata; PDLP, plasmodesmata localized protein; Per, peroxisome; PhyB, phytochrome B; PIP2; 1, plasma membrane intrinsic protein 2; 1; PM, plasma membrane; RBOHD, respiratory burst oxidase homolog D; RBOHF, respiratory burst oxidase homolog F; ROS, reactive oxygen species; SOD, superoxide dismutase; TPX, thiol peroxidases; TRX, thioredoxin; ZAT12, zinc finger of *Arabidopsis thaliana* 12. Adapted from [5,7,45].

different subcellular compartments [43–45]. As H_2O_2, is relatively stable and non-toxic [6,43–45], it could have served as one of the first stress hormones to appear during evolution on Earth.

ROS as universal cell-to-cell signaling molecules within the 'one world' concept

The concept of ROS/H_2O_2 as universal cell-to-cell stress signals (in microorganisms, plants, and mammalian cells) and the fundamental link between ROS, redox, and biological function (*i.e.*, ROS causing redox changes that cause changes in protein structure, stability, and function) [6,7,43–45], coupled with recent studies showing that the ROS wave can be transferred between plants [49], and many studies showing that ROS play an important role in plant and animal interactions with microorganism, place ROS at a central crossroads within the 'one world, one health' principle (Figure 4) [50]. The 'one world' concept is based on the growing understanding that humans, animals, plants, microbiomes, and the environment are inseparably linked and impact each other constantly. The stress levels (and thus the ROS levels and redox state) of different organisms within an ecosystem could therefore be transmitted from one to another through cell-to-cell ROS/redox signaling [6]. As plants, soil microbiomes, animals, and other ecosystem components are increasingly subjected to stresses, stress combinations, and multifactorial stress combinations, caused by global warming and climate change [51,52], their enhanced stress level could directly impact the microbiomes of humans (though consumption, and/or ingestion of dust particles, for example) causing ailments/conditions such as diabetes, inflammation, and cancer. This could be caused by the transmission of altered ROS levels, redox states, and multiple other signals altered/triggered by ROS/redox, from the different plants, soil microbiomes, and animals we consume, to our microbiomes that impact our health, using a shared signaling axis, that of ROS and redox (*e.g.*, following consumption/ingestion of 'high ROS' producing microorganisms with highly oxidized proteomes). In future studies, it would be interesting to follow, using different protein oxidation markers, how a state of stress, sensed by for example plants or soil microbiomes within an ecosystem or an agricultural field, impacts animals and humans. As redox and H_2O_2 are so deeply rooted in the biology of almost all organisms and ecosystems (*e.g.*, the redox code) [6,43–45], deciphering and understanding how ROS/H_2O_2 and redox signals are exchanged between different cells and organisms is at the foundation of biological sciences and could play a key role in our ability to face the challenges of our future on this constantly changing planet [51,52].

Author contributions

M.A.P.V., Y.F., S.I.Z., C.H.F., and R.M. wrote the manuscript.

Declaration of Generative AI and AI-assisted technologies in the writing process

None.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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