Trends in Plant Science

CellPress

Opinion

ROS Are Good

Ron Mittler[1,*]

Reactive oxygen species (ROS) are thought to play a dual role in plant biology. They are required for many important signaling reactions, but are also toxic byproducts of aerobic metabolism. Recent studies revealed that ROS are necessary for the progression of several basic biological processes including cellular proliferation and differentiation. Moreover, cell death–that was previously thought to be the outcome of ROS directly killing cells by oxidation, in other words via oxidative stress–is now considered to be the result of ROS triggering a physiological or programmed pathway for cell death. This Opinion focuses on the possibility that ROS are beneficial to plants, supporting cellular proliferation, physiological function, and viability, and that maintaining a basal level of ROS in cells is essential for life.

Introduction to ROS

Reactive oxygen species (ROS; e.g., $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, $^1O_2$) are partially reduced or excited forms of atmospheric oxygen (Figure 1A) [1]. They function in cells as signaling molecules, but are also thought of as the unavoidable toxic byproducts of aerobic metabolism [2–5]. ROS most likely appeared on Earth together with the first atmospheric oxygen molecules about 2.4–3.8 billion years ago, and have been a constant companion of aerobic life ever since (Figure 1B) [1,6–10]. Taking into account the highly-reducing conditions and the high levels of soluble (reduced) iron in the primordial oceans, it is possible that most atmospheric oxygen initially produced by biological systems on Earth was almost immediately converted into ROS or some other form of oxygen-derived intermediate or radical (Figure 1B) [8,10]. The finding that the ROS scavenging enzyme superoxide dismutase (SOD) is found in all kingdoms of life, and has evolved even before the differentiation of eubacteria from archaea, strongly supports this possibility [9]. The evolution of aerobic life on Earth therefore occurred in the presence of ROS, and this fact should be kept in mind when we consider the roles ROS currently play in different biological systems. As signaling molecules, ROS are highly versatile owing to their diverse properties that include different levels of reactivity, sites of production, and potential to cross biological membranes (Figure 1C) [1–6]. They were most likely first used by cells as signaling molecules to sense unsafe levels of atmospheric oxygen, or to monitor different metabolic reactions, but have since evolved to regulate almost all aspects of life in plants, animals, and most eukaryotic organisms [6]. In higher plants, for example, ROS were found to regulate development, differentiation, redox levels, stress signaling, interactions with other organisms, systemic responses, and cell death [2–6,11]. As toxic byproducts of aerobic metabolism, ROS are primarily formed in chloroplasts, mitochondria, and peroxisomes, but also at any other cellular compartment that includes proteins or molecule with a sufficiently high redox potential to excite or donate an electron to atmospheric oxygen. They are then removed or detoxified by an array of antioxidative enzymes and antioxidants (Figure 1C) [11]. This process of ROS production as a byproduct of aerobic metabolism, coupled with ROS removal by cellular antioxidative mechanisms, occurs constantly in cells to prevent some of the potential toxic effects of ROS that could include DNA, RNA, protein, and membrane oxidation and damage (collectively referred to as **oxidative stress** (see Glossary; Figure 1C) [1–6,11]. The many antioxidative systems of the cell therefore keep ROS at a basal non-toxic level, and any deviation from this balance could be used for ROS signaling reactions [11].

Trends

ROS function in cells as signaling molecules, but are also thought of as the unavoidable toxic byproducts of aerobic metabolism.

Some organisms display tolerance to extreme ROS levels, highlighting the possibility that ROS might not be as toxic as previously thought.

Some cell death processes originally thought to result from the direct toxicity of ROS (i.e., oxidative stress) were recently shown to be part of a programmed/physiological cell death pathway.

Recent studies suggest that a basal level of ROS is necessary for basic biological processes such as cellular proliferation and differentiation.

ROS are predominantly beneficial to cells, supporting basic cellular processes and viability, and oxidative stress is only an outcome of a deliberate activation of a physiological cell death pathway.

Maintaining a basal level of ROS in cells is essential for life.

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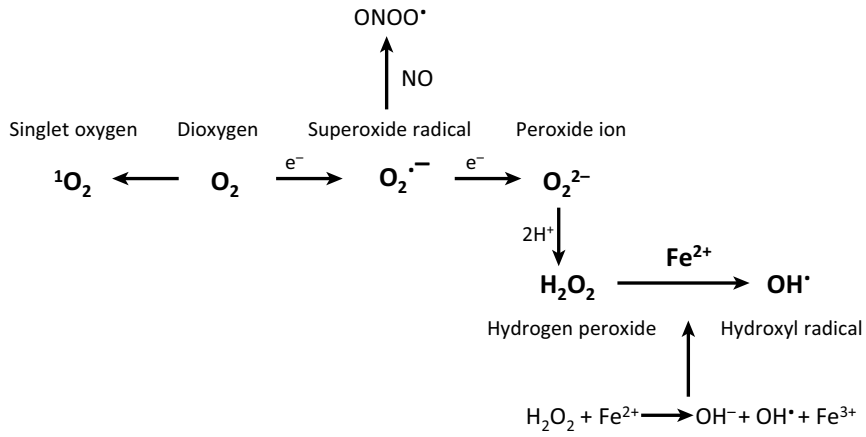
(A)



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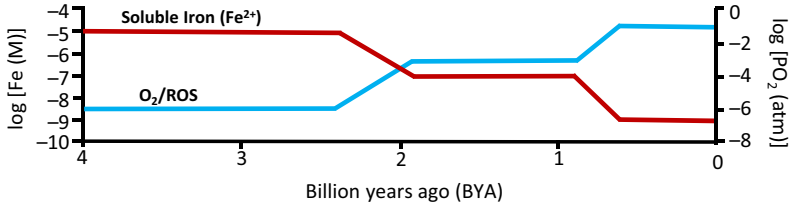

(C)

ROS	$t_{1/2}$	Migration distance	Mode of action	Production site	Scavenging systems
Superoxide ($O^{\bullet-}_2$)	1–4 µs	30 nm	Reacts with Fe–S proteins Dismutates to H_2O_2	Apoplast (RBOHs), chloroplasts, mitochondria, peroxisomes, electron transfer chains	SOD, flavonoids, ascorbate…
Hydroxyl radical (OH•)	1 ns	1 nm	Extremely reactive with all biomolecules including DNA, RNA, lipids, and proteins	Iron and H_2O_2 (Fenton reaction)	Flavonoids, proline, sugars, ascorbate,…
Hydrogen peroxide (H_2O_2)	>1 ms	>1 µm	Reacts with proteins by attacking cysteine and methionine residues. Reacts with heme proteins. Reacts with DNA.	Peroxisomes, chloroplasts, mitochondria, cytosol, apoplast	APX, CAT, GPX, PER, PRX, ascorbate, glutathione,…
Singlet oxygen ($^1O_2$)	1–4 µs	30 nm	Oxidizes lipids, proteins (Trp, His, Tyr, Met, and Cys residues), and G residues of DNA	Membranes, chloroplasts, nuclei	Carotenoids and α-tocopherol

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Figure 1. Properties and Reactivity of ROS. (A) Formation of different ROS and reactive nitrogen species from atmospheric oxygen. Atmospheric oxygen (dioxygen; O_2) is shown to undergo excitation or reduction to form singlet oxygen ($^1O_2$) or superoxide radical ($O_2^{\bullet-}$), respectively. Superoxide is shown to dismutate to form hydrogen peroxide (H_2O_2), and hydrogen peroxide is shown to interact with Fe^{2+} and to form hydroxyl radicals ($OH^\bullet$) via the Fenton reaction. (B) The appearance of atmospheric oxygen coincided with iron oxidation and precipitation in the primordial oceans [8,10]. The appearance of ROS is hypothesized to have taken place at the same time as atmospheric oxygen. It should be noted that, although the great oxidation event is thought to have occurred about 2.4 billion years ago (BYA), the first appearance of oxygen-evolving photosynthetic organisms on Earth is thought to have occurred as early as 3.6–3.8 BYA [71,72]. (C) Properties ($t_{1/2}$, migration distance), reactivity (mode of action), formation (typical production systems), and scavenging (typical scavenging systems) of ROS in plant and animal cells [1–6]. Abbreviations: APX, ascorbate peroxidase; CAT, catalase; GPX, glutathione peroxidase; PER, peroxidase; PRX, peroxiredoxin; RBOH, respiratory burst oxidase homolog; SOD, superoxide dismutase.

Glossary

Fenton reaction: the reaction of H_2O_2 (or dismutated $O_2^{\bullet-}$) with Fe^{2+} to form the highly-reactive hydroxyl radical ($OH^\bullet$).

Ferroptosis: a programmed or physiological cell death process that kills the cell by inducing oxidative stress, mainly in the form of lipid peroxidation. The activation of ferroptosis is highly regulated, much like that of apoptosis.

Oxidative stress: the result of ROS accumulation in cells to levels that exceed the capacity of the ROS scavenging and damage repair systems of the cell. The overaccumulated ROS can react with different cellular components to cause oxidative cellular injury and cell death. The presence of labile iron (Fe^{2+}) in the cell considerably increases the risk of oxidative stress occurring as a result of the Fenton reaction.

Redox biology: a broad term used to describe the regulation of gene expression, metabolic pathways, and signal transduction mechanisms by redox reactions mediated through the oxidation and reduction of cysteine (Cys) residues of proteins that alter their structure and function. The overall redox state and the regulation of redox reactions in cells is directly linked to ROS levels because ROS such as H_2O_2 can oxidize Cys residues at physiological pH.

Regulated necrosis: a term used to describe an array of different types of physiological or programmed cell death processes that are not apoptosis. Much like apoptosis, these forms of programmed cell death are controlled by a genetic program, are highly regulated, can be triggered by ROS, and are different from classical necrosis that is externally induced. Ferroptosis is often referred to as one of the regulated necrosis pathways of the cell.

ROS network: the collection of genes and proteins that control ROS production, perception, and scavenging in cells.

ROS signaling is mediated by a highly regulated process of ROS accumulation at specific cellular compartments. This may be mediated, for example, by plasma membrane-bound NADPH-oxidases (respiratory burst oxidase homolog, RBOH; termed NOX in animals), enzymes that produce ROS at the apoplast [12–15]. This family of enzymes may also be found in the vacuole, endoplasmic reticulum (ER), nuclei, or mitochondria, and are highly regulated via calcium and different phosphorylation/dephosphorylation reactions [12,15]. In addition to RBOHs, ROS signaling was found to be mediated by peroxidases at the apoplast, as well as by the accumulation of ROS in different cellular compartments including the chloroplast, mitochondria, peroxisomes, and nuclei [1–6,11]. A fine balance therefore exists between ROS production for signaling, the baseline of metabolically produced ROS, the rate of ROS diffusion and reactivity, and ROS removal and ROS perception in the different cellular compartment of the plant (the **ROS network**) [11], and the integration of these different ROS-dependent reactions/signals determines the overall response of the cell to a particular stimulus. It is important to remember in this context that ROS accompanied aerobic organisms throughout their entire evolutionary path, and that every step of this evolution and increased organismal complexity required a solution for ROS at each compartment and at each stage of the life cycle. Owing to their beneficial but also toxic nature, ROS have traditionally been referred to as the double-edged sword of life [1,6,9–11]. This article focuses on the possibility that ROS are predominantly beneficial to cells, supporting cellular processes and viability, and that oxidative stress is only an outcome of a deliberate activation of a cell death pathway such as **ferroptosis** or **regulated necrosis** [16–18]. For excellent recent reviews on ROS metabolism, signaling, and detoxification in plants the reader is referred to [2–5].

How Toxic Are ROS?

The main targets of ROS during oxidative stress are thought to be DNA, RNA, proteins, and lipids [1]. Different ROS have different degrees of reactivity toward these cellular components (Figure 1), and the availability of free iron in the form of Fe^{2+} is considered paramount for ROS toxicity due to the role of iron in the **Fenton reaction** that drives the formation of hydroxyl radicals (Figure 1A) [1–6]. Despite the presumed toxicity of ROS to biological systems, some organisms were found to tolerate extreme levels of ROS, bringing the degree of cellular sensitivity to oxidative stress into question. One such example is the Gram-positive, red-pigmented bacterium *Deinococcus radiodurans* that is highly tolerant to ROS generating agents, ionizing radiation, and treatments with 100 mM H_2O_2 [19]. This type of resistance to oxidative stress is not unique to *D. radiodurans*, and can also be found in *Bacteroides fragilis* and in other members of the *Deinococcus* family such as *D. gobiensis* [19–21]. How can these organisms survive such high levels of ROS? From the many studies on the *Deinococcus* family it seems that several different factors contribute to the resistance of this bacterium to ROS. These include reducing the risk of ROS injury by biochemical and cellular adaptations, reducing endogenous ROS production, actively scavenging ROS, and having a heightened ability to remove, replace, or repair damaged DNA, nucleotides, and proteins. A key example of an adaptation that reduces the risk of ROS injury in *D. radiodurans* is a reduced cellular content of iron and iron–sulfur (Fe–S) proteins, as well as sequestering the majority of cellular iron in the area outside the cytosol in the septum between dividing cells [19]. This could be a key adaptation that prevents the formation of hydroxyl radicals (Figure 1A). An additional significant adaptation is a high content of Mn^{2+} that is found in complex with amino acids, peptides, nucleotides, and carbohydrates. These manganese complexes are efficient scavengers of $O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$. In addition, Mn^{2+} can replace iron in some proteins, preventing iron toxicity upon oxidative stress [19]. The adaptations described above are backed by an extremely efficient ability of *D. radiodurans* to remove, replace, and/or repair oxidized proteins and broken/nicked/oxidized DNA [19,21]. A combination of safeguarding iron levels with unique scavenging capabilities and robust repair mechanisms can therefore enable cells to mitigate the toxic effects of ROS, demonstrating that ROS toxicity, even at extreme levels, can be avoided in a biological system.

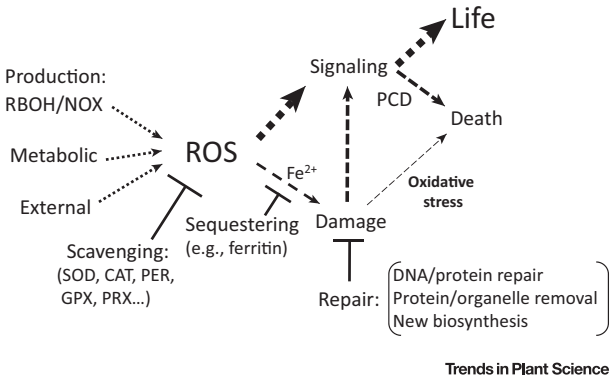

Figure 2. A Combination of ROS Scavenging and Damage Repair/Removal Pathways Cooperate in Cells To Prevent Cell Death by Oxidative Stress. The accumulation of ROS in cells, mediated by NADPH oxidases (RBOH/NOX), metabolic pathways (Metabolic), or external (External) sources, is attenuated by cellular ROS scavenging mechanisms (Scavenging). If ROS reacts with Fe^{2+} to form hydroxyl radicals and causes cellular damage, an array of repair, removal, and replacement pathways repair this damage (Repair) and prevent the accumulation of oxidized cellular components. Cellular damage can also be prevented by keeping the level of labile iron low (Sequestering). Cell death by oxidative stress can therefore be prevented. However, cell death can occur as a result of the activation of physiological pathways (physiological cell death; PCD), and this process can be triggered by ROS/redox reactions or by cellular surveillance systems that monitor the level of cellular damage. Abbreviations: CAT, catalase; GPX, glutathione peroxidase; NOX, NADPH oxidase; PER, peroxidase; PRX, peroxiredoxin; RBOH, respiratory burst oxidase homolog; SOD, superoxide dismutase.

How can we extrapolate from the example of *D. radiodurans* to plant and animal cells? One important addition to the previous model of balancing ROS production with ROS scavenging, as a means to prevent ROS toxicity [11], is the emerging importance of mechanisms for the repair and removal of damaged DNA and proteins (Figure 2). Thus, ROS production that could be metabolic, signaling (e.g., RBOH/NOX activation). or external (e.g., ROS produced by a neighboring cell or a pathogen) in its origin, is balanced by ROS scavenging mechanisms [e.g., SOD, catalase (CAT), and peroxidase (PER)]. Cellular ROS levels are then used for signaling or, if ROS interacts with labile iron, cause cellular damage through the Fenton reaction (Figures 1A,2). Cellular damage caused by hydroxyl radicals is in turn mitigated by DNA/protein repair mechanisms, and/or by replacement of damaged cellular components via removal (degradation/autophagy) and new biosynthesis (Figure 2). If too much damaged cellular components accumulate, oxidative stress ensues and death can occur (Figure 2). By contrast, damaged cellular components, or ROS, can act as signals to trigger death via programmed or physiological cell death (PCD; Figure 2). The question of what kills the cell is perhaps the most important one because it will distinguish between death by oxidative stress (that could be avoided by the normal ROS scavenging, iron sequestering, and repair mechanisms of the cell) and physiological cell death that is a consequence of ROS signaling. Because plant and animal cells have high ROS scavenging capacity, and the examples described above provide evidence that ROS, even at very high levels, cannot kill cells, it is becoming more and more plausible that ROS-induced cell death is mediated through a programmed genetic pathway (e.g., ferroptosis or regulated necrosis) [16–18] and is not a result of the cell simply succumbing to an overwhelming ROS injury (Figure 2). Perhaps the emerging concept of **redox biology** and the large body of literature that backs it can provide more clarification to this question.

Redox Biology: The Good Side of ROS

The term redox biology refers to ROS acting as signaling molecules to regulate and maintain normal physiological functions mainly via interacting with cysteine (Cys) residues of proteins [22–24]. H_2O_2 interacts, for example, with Cys thiolate anions ($Cys–S^-$) at physiological pH and oxidizes them to their sulfenic form (Cys–SOH), causing structural changes within the target protein and altering its function (Figure 3). These redox-derived changes in protein function can

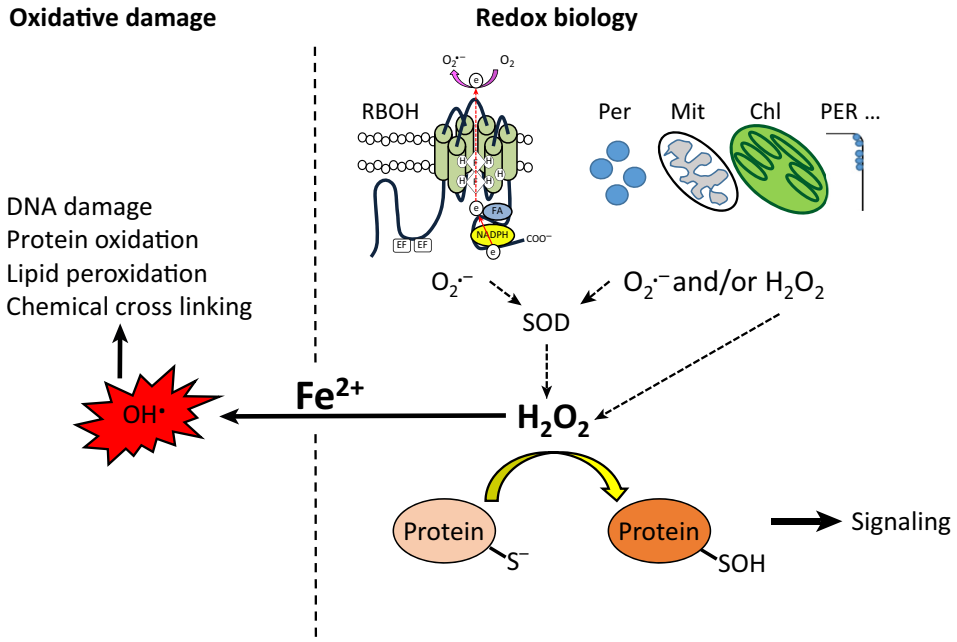

Figure 3. ROS and Redox Biology. ROS produced by respiratory burst oxidase homologs (RBOHs), peroxisomes (Per), mitochondria (Mit), chloroplasts (Chl), and cell wall bound peroxidases (PER) result in the accumulation of H_2O_2 that mediates the oxidation of cysteine residues on proteins, affecting their structure and function and triggering/regulating cellular signaling pathways (Signaling). However, the presence of labile iron in the form of Fe^{2+} can tip the cellular balance of ROS/redox reactions and cause oxidative stress via the formation of hydroxyl radicals. Maintaining the cellular pool of labile iron as low as possible is therefore crucial for redox biology and for the regulation of metabolism and other cellular functions by ROS.

affect transcription, phosphorylation, and other important signaling events, and/or alter metabolic fluxes and reactions in the cell by altering enzymatic properties [22–24]. In addition, many redox relays exist in cells, and these can transduce and/or amplify an initial ROS-derived redox event [24]. A key role for ROS in promoting cell proliferation via redox reactions was, for example, identified in cancer cells [22,25–29]. To continue to proliferate, cancer cells (as well as several different types of stem cells, as outlined below) maintain a higher than normal level of ROS that drives redox-signaling reactions in favor of enhanced proliferation via pathways involving hypoxia-inducible factors (HIFs), phosphoinositide 3-kinase (PI3K), nuclear factor κ light-chain-enhancer of activated B cells (NF-κB), and mitogen-activated protein kinases (MAPKs). Cancer cells keep ROS levels, therefore, at a moderately high tumorigenic level, above a low cytostatic level, but below levels that will be cytotoxic. This is achieved by a fine balance between ROS production via the mitochondria and NADPH oxidases, ROS scavenging via SODs, glutathione peroxidases (GPXs), peroxiredoxins (PRXs), and other antioxidants such as glutathione (GSH), and removal of damaged proteins and other cellular components via the proteasome and autophagy [29]. Another example of a crucial role for ROS-driven redox signaling is in the innate immune response. Activation of surveillance receptors was found to increase ROS production that is required for the release of the proinflammatory cytokines interleukin 1β (IL-1β), tumor necrosis factor ∝ (TNF∝), and interferon β (IFN-β), that in turn are required for orchestrating an appropriate immune response [30–34]. Low ROS levels therefore prevent immune-response activation and lead to immunosuppression, whereas high ROS levels cause autoimmunity through increasing the release of proinflammatory cytokines [30–34]. ROS-mediated redox signaling was also found to play a key role in the emerging field of stem cell research. Thus, the generation of low levels of ROS by NOX/mitochondria was found to be

necessary for the activation of proliferative pathways, supporting stem cell renewal and differentiation, whereas the accumulation of high levels of ROS activates signaling pathways that limit self-renewal in a pathway that involves the DNA-damage checkpoint kinase ataxia-telangiectasia mutated (ATM) [35–41]. In addition, several different types of stem cells were found to reside in a highly-oxidizing environment [39–41]. Of course, the specific response of any given cell type to ROS levels depends on its identity [35–41].

The examples provided above point to a key beneficial function for ROS in supporting normal physiological and metabolic functions, as well as in promoting cellular proliferation and differentiation. Of course these beneficial functions of ROS could be hijacked, for example by cancer cells that tilt the ROS balance in their favor and maintain a higher basal level of ROS that promotes cellular proliferation [22,25–29,42]. Another example is provided by necrotrophic plant pathogens that use ROS to induce plant necrosis and feed on the dead plant tissue [4,5]. The essential roles ROS play in cellular proliferation, immune response, cellular differentiation, development, circadian rhythms, and cell death regulation (these are only a few selected examples) highlight a new view of ROS as being beneficial for life. This should not come as a surprise because ROS were present throughout the evolution of aerobic life and have most likely become essential to normal processes in most multicellular organisms. What therefore determines if ROS are good or bad, and how is this balance maintained in cells? As outlined in Figure 3, redox biology results from ROS such as H_2O_2 modifying the structure and function of target proteins and affecting signaling. Provided that the concentrations of ROS are maintained within the normal range in cells (Figure 2), this process can continue unperturbed and normal cellular processes can carry on. Moreover, the high capacity and high redundancy of ROS scavenging systems in plants and other organisms ensure that ROS levels will not exceed, or drop below, the normal cellular range of ROS. This balance can of course be perturbed by the presence of high levels of labile iron (Fe^{2+}) in cells that would tip the cell from the redox biology range into oxidative stress owing to the enhanced production of hydroxyl radicals via the Fenton reaction (Figure 3) [1,42–44]. Recent studies have indeed shown that proliferating cancer cells are susceptible to perturbations in their iron homeostasis [44]. Maintaining levels of cellular iron under control is therefore paramount for keeping ROS within the redox biology range.

Plants and Redox Biology

Plants have almost all of the components that mediate ROS homeostasis and redox signaling in animals [2–6,11,45–50]. These include NOX-like proteins (RBOHs), SODs, CATs, PRXs, GPXs, iron uptake/storage regulating mechanisms, and a network of thio- and glutaredoxins. Moreover, attempts to disrupt ROS scavenging or production in plants have typically resulted in affecting ROS-mediated redox signaling, rather than causing cell death via oxidative stress (e.g., [51]). These endeavors have also resulted in the formation of smaller plants that were deficient in growth and reproduction, but were still alive. Of course many differences exist between plant and animal cells in ROS production and scavenging. These include the high production of ROS in chloroplasts and peroxisomes during photosynthesis and photorespiration, the presence of the vacuole with its mostly unknown ROS-redox capabilities, the presence of the cell wall/apoplast with its multiple ROS production and scavenging peroxidases and oxidases, and the presence of plant-specific ROS scavenging enzymes such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHR). Regardless of these differences, the high capacity of plants to detoxify ROS may suggest that ROS could have a beneficial effect on plants, comparable to some of the examples described above for animals. Further research is needed to address these possibilities, and particularly with respect to cellular proliferation and differentiation. The high complexity of the RBOH family in plants and its diverse roles in the regulation of plant immunity, acclimation to abiotic stress, growth, and development, and the regulation of physiological responses such as stomatal aperture [12], is of course highly supportive of this possibility. In addition, priming by ROS

application was shown to improve plant performance and growth [52], and ROS were found to be important for the regulation of many beneficial processes in plants (e.g., [53–64]). The contributions of ROS to plant and animal physiology, development, and differentiation underscore the possibility that the beneficial effects of ROS in different organisms outweigh the risks ROS pose to biological systems, focusing again on the question: are ROS good or bad?

ROS Are Good!

Several different studies in plants and animals are supporting a view that ROS are essential for promoting normal cellular processes, as opposed to having a toxic effect on life. Even cell death that was previously thought to result from oxidative damage is now considered as the result of ROS triggering a physiological pathway for cell death [16–18], rather than directly killing cells (Figure 2). Moreover, the findings that decreasing the ROS levels of cells to below a particular threshold could result in suppressed cellular proliferation, as well as negatively affect differentiation and immunity [22,25–41], strongly argue that a basal level of ROS is essential for normal life. Maintaining a basal level of ROS which is above a cytostatic level, but below cytotoxic, therefore enables proper redox biology reactions and the regulation of numerous processes essential for life (Figures 3 and 4A). The dependency of life on ROS could also be viewed as a bell curve-shaped response with an optimum that depends on the environmental conditions, developmental stage, cell identity, and other factors that affect the whole plant (Figure 4B). Thus, ROS levels that are too low or too high impair plant growth and development, whereas maintaining ROS levels within the right range promotes plant health. Of course, alterations in ROS levels that are part of the normal function of the plant should not exceed the threshold boundary between redox biology and cytotoxic or cytostatic levels. The limitations on the amplitude of ROS signals, in other words not to exceed or drop below cytotoxic or cytostatic levels, respectively, might have been one of the driving forces behind the evolution of apoplastic ROS signaling. Responses to pathogens or rapid systemic signaling in the form of the ROS wave result in RBOH activation

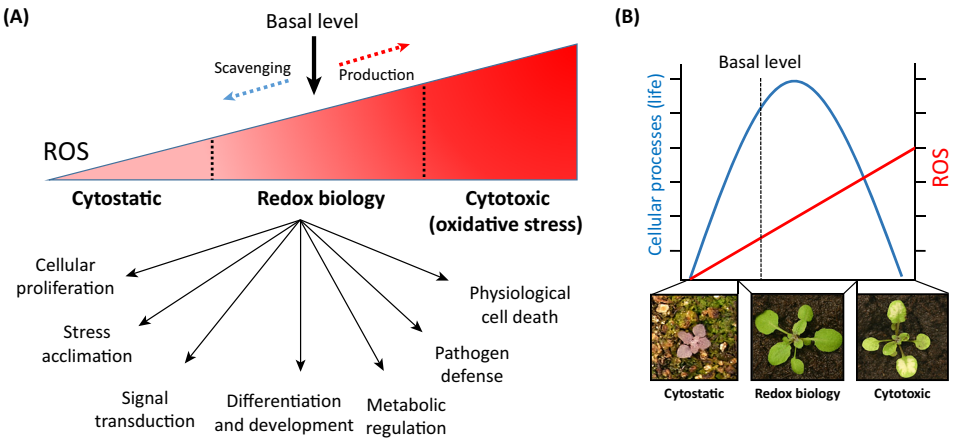

Figure 4. Maintaining a Basal Level of ROS in Cells is Essential for Proper Cellular Function.

For a Figure360 author presentation of Figure 4, see the figure online at http://dx.doi.org/10.1016/j.tplants.2016.08.002#mmc1.

(A) The effect of different levels of ROS on the regulation of different cellular processes. ROS and redox signaling is shown to occur within a defined window of ROS concentrations and to control cellular processes. ROS levels that are too low are thought to be cytostatic for cells, whereas ROS levels that are too high are cytotoxic. A basal level of ROS is therefore required for proper ROS and redox signaling in cells, and this level is maintained by the balance between ROS production and ROS scavenging. (B) The dependency of cellular functions and viability on ROS concentrations. A bell-shaped curve is hypothesized to represent the dependency of maintaining proper cellular processes on increasing ROS concentrations. Normal plant metabolism requires therefore an optimum range of ROS levels that enable the plant to achieve its maximal growth and developmental potential.

and the accumulation of high levels of ROS in the apoplast [59,62,64–66]. Because cells may be able to control the rate of H_2O_2 diffusion through the plasma membrane [67], and/or have a high cytosolic buffering capacity for ROS [6,11,51], the high apoplastic levels of ROS are filtered down or attenuated before they reach the nuclei such that they will be in the range of redox biology, and not the oxidative stress range. At least a subgroup of RBOH/NOX proteins might have therefore evolved to produce ROS exclusively at the external side of the plasma membrane–primarily to allow ROS signaling at the apoplast and away from the nuclei [11–15]. As indicated above, the possibility that ROS are good for cells should not come as a surprise. ROS were present throughout the evolutionary path of aerobic organisms and have most likely become essential for the maintenance of key biological processes that support life. From the standpoint of signaling, and taking into the account the hypothesis that ROS were used as early signals to detect oxygen levels, it should also not come as a surprise that ROS are required for cellular proliferation in aerobic organisms. For an aerobic organism, if oxygen is present, life can occur, potentially explaining the positive effects ROS have on cellular proliferation, differentiation, and aging [22,35,39–41,68–70]. In conclusion, if you are an aerobic organism, ROS are good, but too much, or too little, of a good thing can be bad for you. . .

Outstanding Questions

Are there regulated necrosis and/or ferroptosis pathways in plants? Plants rely on iron storage for the prevention of ROS toxicity and have some of the proteins involved in ferroptosis in animals, for example, GPXs.

Are ROS required for cell division and cellular proliferation in plants?

How essential are RBOHs for the maintenance of basic cellular processes in plants? The RBOH family in *Arabidopsis* contains 10 genes; what would happen if all of them would be inactivated?

How programmed is oxidative stress in plants? Can we identify mutants that do not die in the presence of high ROS concentrations? Some reports, for example with *flu/Executer* mutants, support this possibility.

Should we consider developing transgenic crops with a higher basal cellular level of ROS (that is of course not cytotoxic)? Would they grow faster and be more tolerant to pathogens and abiotic stresses? Should we activate the higher ROS level-inducing transgene only at specific developmental stages, or in response to specific environmental conditions?

Acknowledgments

This work was supported by funding from the National Science Foundation (IOS-1353886, IOS-0639964, IOS-0743954, IOS-1557787, MCB-1613462) and the University of North Texas, College of Arts and Sciences. The author would like to thank Mr Amith R. Devireddy for his help with formatting the references and assembling the Table shown in Figure 1C. The funders had no role in the design, data collection, analysis, decision to publish, or preparation of the manuscript.

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