Editorial

Viewing oxidative stress through the lens of oxidative signalling rather than damage

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Concepts of the roles of reactive oxygen species (ROS) in plants and animals have shifted in recent years from focusing on oxidative damage effects to the current view of ROS as universal signalling metabolites. Rather than having two opposing activities, i.e. damage and signalling, the emerging concept is that all types of oxidative modification/damage are involved in signalling, not least in the induction of repair processes. Examining the multifaceted roles of ROS as crucial cellular signals, we highlight as an example the loss of photosystem II function called photoinhibition, where photoprotection has classically been conflated with oxidative damage.

Introduction

Redox signalling is an essential component of cellular energy homeostasis and responses to the environment in animals and plants, redox-sensitive proteins functioning as sensors that trigger repair mechanisms and regulate cell division, growth and defence processes. Despite a growing acceptance in the animal and plant literature that ROS accumulation and programmed cell death are not the enemy but rather hallmarks of survival [1,2], old paradigms die hard. The concept that ROS mediate their principal effects by causing indiscriminate irreversible inactivation of proteins and/or loss of function of other cellular components (i.e. damage) became strongly anchored within the literature with the advent of initiatives to confer general stress tolerance on plants by overexpression of antioxidative enzymes. This notion remains surprisingly persistent to this day, probably because it is extremely simple. According to the ‘damage’ paradigm, overproduction of ROS in conditions such as excess light availability induces a general loss of cellular functions through processes such as photoinhibition, lipid peroxidation and protein oxidation, the accumulation of damage leading ultimately to death. Not before time, this simple paradigm is finally being laid to rest. Evidence that is often cited in apparent support of the ‘reduction good/oxidation bad’ paradigm is that oxidation leads to loss of enzyme activity. However, such effects have often only been demonstrated in vitro, sometimes using oxidant concentrations that are not biologically relevant. Furthermore, the literature contains physiologically relevant counterexamples, such as oxidative activation of chloroplast glucose-6-phosphate dehydrogenase [3,4] and protein kinase signalling cascades [5]. Crucially, the biological relevance of oxidative changes must be understood within the context of cellular functions: loss of activity of a given protein may activate a function at the cellular level.

ROS at the heart of intracellular and cell-to-cell signalling

ROS play numerous important roles in plant development and environmental responses. ROS functions in plants are tightly intertwined with signalling pathways through phytohormones. It has long been apparent from studies of plant responses to the gaseous pollutant ozone that ROS interact with stress hormones such as ethylene, salicylic acid and jasmonic acid [6]. More recently, it has been
established that ROS and related molecules such as thiols interact with auxins, gibberellins and cytokinins to control plant growth and development [7–9]. ROS-related redox processes probably intervene at multiple levels of signalling for any given phytohormone. For example, thiol-dependent steps are probably involved in both synthesis and signalling of salicylic acid [10]. In this regard, ROS can act either as a life or a death signal, dependent on the molecular and cellular context in which ROS accumulate. The outcomes of such signalling depend on many parameters, principally the chemical nature of the ROS form produced (i.e. superoxide, hydrogen peroxide or singlet oxygen) and the nature of the interacting partner (protein thiol, metabolite, lipid or DNA molecule), as well as cell identity, but all types of oxidative modification (reversible or irreversible) can be viewed as part of the redox signalling matrix because they induce regulatory, repair or death responses. Together with cellular oxygen tension, ROS control many crucial aspects of animal and plant biology, not least cell proliferation, stem cell homeostasis and differentiation lineage commitment [11–14].

Publications continue to appear that are based on the premise that low doses of ROS play beneficial roles in signalling, while higher doses have detrimental effects. Cell death is often cited as an example of the latter, supposedly undesirable effects, even though this process is central to renewal, immunity and defence responses. In multicellular organisms, the elimination of certain cells is a beneficial process, whether it is in control of uncontrolled cell division in mammalian cells or in the hypersensitive response in the case of plants resisting pathogen attack. Even at the local (cellular) level, there is abundant evidence that cell death in plants does not only occur generally through damage that overwhelms the cell’s defences, but rather through genetically programmed pathways, with signalled processes that are controlled by specific genes and that may involve the programmed withdrawal of antioxidative systems. The importance of specific ‘executor’ genes was first reported in plants accumulating singlet oxygen [15]. Genetic control over ROS-induced cell death is equally apparent from studies of the effects of H₂O₂ in catalase-deficient plants, using both reverse and forward genetics [16–18].

**ROS and photosynthesis**

Chloroplasts were one of the very first sources of superoxide and H₂O₂ to be described in plants [19,20]. Electron flow to oxygen rather than NADP⁺ relieves reductive pressure within the electron transport chain and balances ATP:NADPH ratios by allowing proton pumping without net reductant generation (Figure 1). Moreover, like ROS formed at other subcellular locations, thylakoid-generated ROS may play crucial roles as signal transducers (Figure 1). ROS generated during light capture and electron transport are situated at the interface between the environment and the molecular machinery of photosynthesis, thereby providing the cell with crucial information on current status [21,22].

As well as superoxide and H₂O₂ production by the photosynthetic electron transport chain, energy transfer within the photosystems leads to generation of singlet oxygen, a ROS that is formed by excitation energy transfer from triplet chlorophyll to O₂ [23,24]. The high reactivity of membrane proteins and lipids to singlet oxygen makes these prime targets for signalling from photosystem II (PSII) to the nucleus. While oxidative modification of PSII structural and repair proteins and lipids is unavoidable [25,26], considerable uncertainty remains concerning the extent to which such processes impair PSII function within a physiological context.

**Photoinhibition and regulation of PSII**

The management of light interception and energy conversion is one of the most fundamental concepts in understanding the regulation of photosynthesis. The chloroplast is faced with the problem of balancing light harvesting with the generation of ATP and NADPH, in appropriate ratios, at rates that match the demands of metabolism [27]. The fast turnover of these pools in the light leaves little room for imbalances in rates of energy production and consumption, explaining the evolution of a plethora of stabilising mechanisms that come into play, as required, to ensure smooth running of the system over the wide range of irradiances that occur in the natural and field environments. Such mechanisms include pH-triggered non-photochemical chlorophyll fluorescence quenching (NPQ) at PSII and the direct transfer of energy and electrons to oxygen leading to the production of reactive oxygen species (ROS) such as singlet oxygen, superoxide and H₂O₂ (Figure 1), all of which play important roles in the regulation of photosynthesis [27].

An extensive body of literature has accumulated over the last 30 years on phenomena that are included under the term ‘photoinhibition’. Considerable confusion persists because this term includes both photodamage and down-regulation of PSII function. Even though increasing evidence suggests that photodamage is not generally the dominant component, photoinhibition tends still to be equated with ‘damage’. For this reason, in the following critique, photoinhibition is used to denote this long-standing notion of ‘photodamage’.
Excess sunlight saturates PSII, causing build-up of excess excitation energy in its antenna. This unused energy is potentially dangerous because it can lead to the inactivation of the reaction centres (RCIs), resulting in a sustained decrease in the quantum efficiency of PSII and the subsequent electron transport rate, a phenomenon termed photoinhibition [28–30]. Indeed, the photosynthetic pigments of oxygen-evolving PSII should be potentially vulnerable to photoinhibition since the RCII possesses a very strong oxidative potential of ∼1.17 V that is required to oxidise water. Under conditions where electron donation to P680 is less efficient than its photo-oxidation, an increase in the P680+ lifetime will occur. This powerful oxidant may oxidise the nearest pigments and amino acids, causing their degradation and a subsequent degradation of the key RCII D1 protein [29]. In other circumstances, when the acceptor side is less efficient, a radical pair will be formed. The recombination of this pair will lead to the formation of a P680 triplet state that was proposed to interact with atmospheric triplet oxygen, causing formation of highly reactive singlet oxygen, which in turn can lead to the degradation of the key RCII component, D1 protein [30–33]. Hence, initially photoinhibition was thought to lead to a decreased number of active RCIs.

Mechanisms to deal with high light exposure are required to minimise the build-up of potentially photodamaging excess energy in PSII. An imbalance between ATP generation and utilisation, caused, for example, by a failure of metabolism to keep pace with the thylakoid reactions, will cause protons to rapidly accumulate in the intrathylakoid space (Figure 1). This leads to decreased PSII light-harvesting efficiency through a process...
called NPQ that protects RCII from the damage via prompt dissipation of excess energy as heat [34]. Apart from being triggered by the proton gradient (ΔpH), NPQ is strongly enhanced by the xanthophyll cycle activity that leads to conversion of violaxanthin into zeaxanthin. This reaction is also dependent on the ΔpH, albeit on a somewhat slower timescale than onset of either ΔpH or, in many cases, the pH-dependent NPQ. Importantly, PSII quantum efficiency can be decreased by both photodamage to RCII and NPQ (see below). Until recently, the only criterion that was used to separate photodamage from the protective reduction in the PSII yield (otherwise called down-regulation) was the timescale of the recovery of these processes in the dark. Indeed, while the repair from the damage to RCII takes hours, the down-regulation via NPQ was believed to take only minutes to recover [33]. The evidence for the former was taken from biochemical data (D1 protein repair) [34], whereas the evidence for the latter was taken from the so-called pulse amplitude-modulated chlorophyll fluorescence analysis, the interpretation of which remains a source of controversy [35].

**Interpreting chlorophyll fluorescence**

Chlorophyll fluorescence has been used for several decades for prompt and non-destructive assessment of PSII efficiency in a variety of photosynthetic organisms [34,36]. A typical fluorescence-quenching experiment is depicted in Figure 2. The active (open) PSII RCII is efficient quenchers of antenna chlorophyll fluorescence ($F_o$) excited by a very weak light (measuring light). Application of a saturating light pulse (10 000 μmol m$^{-2}$ s$^{-1}$) for 1 s closes all RCII, bringing the fluorescence to the $F_m$ level. Now, the quantum efficiency of PSII can be expressed as $\Phi_{PSII} = (F_v)/F_m$, where $F_v = F_m - F_o$. Application of continuous illumination for 5 min causes a gradual decline not only in $F_v$, a steady-state fluorescence level, but also in $F_m$, which becomes $F_m'$. The decline in $F_m$ is called non-photochemical quenching (NPQ) and is often expressed as $\text{NPQ} = (F_m - F_m')/F_m$. NPQ decreases the yield of PSII under continuous illumination that can now be expressed as

$$\Phi_{PSII} = \frac{F'_v}{F'_m} = qP \times \frac{(F_v/F_m)}{[1 + (1 - F_v/F_m) \times \text{NPQ}]}$$

(1)

**Figure 2. A typical pulse amplitude-modulated chlorophyll fluorescence induction measurement.**

The modulated low intensity measuring light of $\sim 1$ μmol $m^{-2}$ s$^{-1}$ is used to excite chlorophylls of the PSII antenna fluorescence ($F_o$ level). In these conditions, fluorescence is highly quenched by working RCs (RCII). Application of a saturating light (10 000 μmol m$^{-2}$ s$^{-1}$) for 1 s causes the closure of all reaction centres for the measuring light so that they stop quenching of the antenna fluorescence and the level rises to $F_m$. After $\sim 1$ min, a continuous illumination is applied (actinic light) of an intensity of $\sim 800$ μmol m$^{-2}$ s$^{-1}$. This causes gradual quenching of $F_m$ to the $F_m'$ level. This quenching is triggered by the proton gradient and called NPQ. After $\sim 5$ min, the actinic light is turned off and NPQ begins to recover. The recovery that is not complete within 5 min of darkness is termed qI.
where \( qP \) is photochemical quenching \([qP = (F_m - F_o)/(F_m - F_s)]\). Hence, the yield of PSII is a function of both NPQ and \( qP \). In the dark, following moderate levels of illumination, \( qP = 1 \) and NPQ recovers gradually but not completely (Figure 2). The slowly reversible NPQ component is called \( qI \) [equal to \((F_m - F_{m}''')/(F_m'''')\)] and was first proposed to reflect the photodamage to RCII [36,37] diminishing the PSII yield in the dark as can be seen from the formula (1). However, later it was discovered, mainly by the groups of Adams and Demming-Adams, that a large part of \( qI \) does not reflect the damage to RCIIIs but relates to the synthesis of zeaxanthin, and promotion of the slowly reversible NPQ components [38,39] at high light and low temperature conditions [40–43]. Hence, the PSII yield in the classical fluorescence measurements was established to be dependent on both the RCII photodamage and the sustained NPQ. To quantify the true effect of photodamage upon \( \Phi_{PSII} \), a new method has been developed [44,45]. It uses a gradually increasing actinic light illumination and the periodic measurements of \( \Phi_{PSII} \) \([F_m - F_o]/(F_m - F_s)\] and compares them with the analytically derived \( \Phi_{PSII} \) using the formula (1). The measured and calculated values of \( \Phi_{PSII} \) match each other very well at somewhat low actinic light intensities, but gradually fall apart at somewhat higher light. This disparity can be easily corrected if the \( qP \) values in the dark were <1. Indeed, these values have been obtained using the comparison between measured and calculated values of \( F_o \) [46]. Therefore, the \( qP \) in the dark \( (qP_d) \) has been proposed as a true indicator of photoinhibition reflecting the percentage of the inactivated RCIIIs [46]. The use of this parameter demonstrated that a prolonged exposure to the saturating light intensities (1500–2000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) only caused inhibition to at most 25% of PSII RCIIIs of established mature plants [45,47]. Hence, photoinhibition may not be a common phenomenon in nature. If the D1 repair process is ongoing and recovery without damage is facilitated by the protective component of NPQ, the loss of the PSII yield due to photoinhibition will greatly decrease [45,47]. Therefore, the sustained decline in \( F_o/F_m \) should be interpreted as predominantly related to PSII down-regulation via sustained components of protective NPQ and not as damage resulting from photoinhibition, and the \( qP_d \) parameter should be used to quantify this change [44,45,47,48].

Conclusions and perspectives

The Manichean notion that sets evil ROS on one side and benevolent antioxidants on the other is impossible to defend. Different oxidants may antagonise each other, and antioxidants such as glutathione may play an integral part not only in controlling ROS but also in transmitting oxidative signals [10,49]. Enzymes that play important antioxidative roles can also promote ROS production or ROS-dependent processes [50,51]. While plant cell functions operate at rather negative redox potentials in the soluble phase [21,22], the paradigm that oxidation is bad, while reduction is good, is too simplistic given the complexity of redox interactions [52]. This is particularly true in photosynthesis, which is driven by large redox and energy gradients, and which is the major source of ROS in plant cells. ROS production, signalling and removal associated with photosynthesis provide flexibility and control in the management of high light stress. Grasping the implications of this paradigm shift is key to addressing global issues such as food security and the production of crops in a sustainable manner for a growing world population. This challenge has led to an upsurge of interest in improving photosynthesis through the manipulation of processes that alter the light use efficiency of photosynthesis [48]. Current initiatives such as the introduction of characteristics of \( C_4 \) photosynthesis into important \( C_3 \) species such as rice and, more generally, improving the capture of light energy and its conversion into biomass [45,53] might benefit from an enlightened appreciation of the beneficial roles of ROS as a central integrator of functions at the cellular and whole-plant level.

Abbreviations

PSII, photosystem II; RCIIIs, reaction centres; ROS, reactive oxygen species.

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Competing Interests

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32 De Las Rivas, J., Shipton, C.A., Ponticos, M. and Barber, J. (1993) Acceptor side mechanism of photoinduced proteolysis of the D1 protein in photosystem-II reaction centers. Biochemistry 32, 6944–6950 doi:10.1021/bi00078a019
33 Aro, E.-M., Virgin, I. and Andersson, B. (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. Biochim. Biophys. Acta, Bioenergetics 1143, 113–134 doi:10.1016/0005-2728(93)90134-2
34 Demmig-Adams, B., Garab, G., Adams, III, W.W. and Govindjee (eds) (2014) Nonphotochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria, Advances in Photosynthesis and Respiration, vol. 40, Springer Netherlands
35 Ruban, A.V. (2016) Nonphotochemical chlorophyll fluorscence quenching: mechanism and effectiveness in protecting plants from photodamage. Plant Physiol. 170, 1903–1916 doi:10.1104/pp.15.01935
36 Baker, N.R. and Horton, P. (1987) Physiological factors associated with fluorescence quenching during photoinhibition. In Topics in Photosynthesis, Photoinhibition (Amtzzen, C.J., Kyle, D.J. and Osmond, C.B., eds.), vol. 9, pp. 145–168, Elsevier, Amsterdam
37 Krause, G.H. and Weis, E. (1991) Chlorophyll fluorescence measurements: the basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 313–349 doi:10.1146/annurev.pp.42.060191.001525
38 Demmig-Adams, B. and Adams, III, W.W. (1992) Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43, 599–626 doi:10.1146/annurev.pp.43.060192.003123
39 Jahns, P. and Holzwarth, A.R. (2012) The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim. Biophys. Acta 1817, 182–193 doi:10.1016/j.bbapap.2011.04.012
40 Adams, III, W.W., Demmig-Adams, B., Verhoeven, A.S. and Barker, D.H. (1995) ‘Photoinhibition’ during winter stress: involvement of sustained xanthophyll cycle-dependent energy dissipation. Funct. Plant Physiol. 22, 261–276 doi:10.1071/PP9950261
41 Adams, III, W.W., Zarter, C.R., Mueh, K.E. and Demmig-Adams, B. (2008) Energy dissipation and photoinhibition: a continuum of photoprotection. In Photoprotection, Photoinhibition, Gene Regulation, and Environment (Demmig-Adams, B., Adams, III, W.W. and Mattoo A.K., eds.), pp. 49–64, Springer, The Netherlands
42 Demmig-Adams, B., Cohu, C.M., Muller, O. and Adams, III, W.W. (2012) Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. Photosynth. Res. 113, 75–88 doi:10.1007/s11120-012-9761-6
43 Adams, III, W.W., Muller, O., Cohu, C.M. and Demmig-Adams, B. (2013) May photoinhibition be a consequence, rather than a cause, of limited plant productivity? Photosynth. Res. 117, 31–44 doi:10.1007/s11120-013-9849-7
44 Ruban, A.V. and Murchie, E.H. (2012) Assessing the photoprotective effectiveness of non-photochemical chlorophyll fluorescence quenching; a new approach. Biochim. Biophys. Acta, Bioenergetics 1817, 977–982 doi:10.1016/j.bbabio.2012.03.026
45 Ruban, A.V. and Beljo, E. (2014) The relationship between maximum tolerated light intensity and non-photochemical chlorophyll fluorescence quenching: chloroplast gains and losses. Philos. Trans. Roy. Soc. Lond. B, Biol. Sci. 369, 20130222 doi:10.1098/rstb.2013.0222
46 Oxborough, K. and Baker, N.R. (1997) Resolving chlorophyll a fluorescence of photosynthetic activity into chemical components — calculation of qP and Fv/Fm without measuring Fo. Photosynth. Res. 54, 135–142 doi:10.1023/A:1005936823310
47 Ware, M.A., Beljo, E. and Ruban, A.V. (2015) Photoprotective capacity of nonphotochemical quenching in plants acclimated to different light intensities. Photosynth. Res. 126, 261–274 doi:10.1007/s11120-015-0102-4
48 Kromdijk, J., Glowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Nyogi, K.K. et al. (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Science 354, 857–861 doi:10.1126/science.aab8878
49 Creissen, G., Firmin, J., Fryer, M., Kular, B., Leyland, M., Reynolds, H. et al. (1999) Elevated glutathione biosynthetic capacity in the chloroplasts of transgenic tobacco paradoxically causes increased oxidative stress. Plant Cell 11, 1277–1291 doi:10.1105/tpc.11.7.1277
50 Hackenberg, T., Juul, T., Auzina, A., Gwizdoz, S., Malepszy, A., Lehmann Nielsen, K. et al. (2013) Catalase and its regulator NO CATALASE ACTIVITY 1 (NCA1) promote autophagy-dependent cell death in Arabidopsis. Plant Cell 25, 4616–4626 doi:10.1105/tpc.113.117192
51 Johnston, E.J., Ryffel, E.L., Beynon, E., Lorenz, A., Chechik, V. and Bruce, N.C. (2015) Monodehydroascorbate reductase mediates TNT toxicity in plants. Science 349, 1072–1075 doi:10.1126/science.aaa3472
52 Noctor, G. (2015) Lighting the fuse on toxic TNT. An enzyme that helps control reactive oxidants sensitizes plants to TNT pollution. Science 349, 1052–1053 doi:10.1126/science.aaa3941
53 Kirk, S., Rizal, G. and Quick, W.P. (2013) Improvement of photosynthesis in rice (Oryza sativa L.) by inserting the C4 pathway. Rice 6, 28 doi:10.1186/1939-8433-6-28