

## Editorial

# Hydrogen Peroxide in Adaptation

Ivan Spasojević,<sup>1</sup> David R. Jones,<sup>2</sup> and Michael E. Andrades<sup>3</sup>

<sup>1</sup> Life Systems Department, Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, 11000 Belgrade, Serbia

<sup>2</sup> Paterson Institute for Cancer Research, University of Manchester, Wilmslow Road, Withington, Manchester M20 4BX, UK

<sup>3</sup> Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-903 Porto Alegre, RS, Brazil

Correspondence should be addressed to Ivan Spasojević, redoxsci@gmail.com

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The perception of the roles of H<sub>2</sub>O<sub>2</sub> in living systems has come a long way, transcending from H<sub>2</sub>O<sub>2</sub> being considered as (i) exclusively damaging; (ii) a necessary evil “unwanted but an inevitable product of aerobic metabolism”; (iii) important for specific biological processes that involve ROS aggressiveness, such as the battle of the innate immune system with pathogens; and (iv) signalling species [1–6]. The list does not end here. A number of studies have illustrated that at concentrations in the high physiological range, H<sub>2</sub>O<sub>2</sub> induces more permanent, modifying changes, adaptations, increasing the resistance of biological systems to the same stimulus (hormesis) or other stressors (cross-adaptation), or enabling the adaptation to altered ecology. The capability of H<sub>2</sub>O<sub>2</sub> to induce the synthesis of a large number of proteins and to provide cross-resistance implies that living systems may intentionally produce H<sub>2</sub>O<sub>2</sub> as a component of adaptation in response to different fluctuations and perturbations shifting the system away from homeostasis [7–9]. Some data even implicate an important role of H<sub>2</sub>O<sub>2</sub> in interspecies communication and in the development of multicellularity [10].

Examples of signalling roles of H<sub>2</sub>O<sub>2</sub> emerge at progressing pace, although the field of redox research is yet to take a fully organised and systematised profile. To date it has been shown that H<sub>2</sub>O<sub>2</sub> participates in signalling pathways responsible for the regulation of cell differentiation, proliferation, migration, survival and apoptosis, and mitochondrial relocation and of various biological processes, such as vascularisation, angiogenesis, vascular tone control, maintenance of glucose level, oxygen tension regulation, calcium metabolism regulation, immune system control, and wound healing [3–6]. Redox signalling was first described

in *prokaryotes*. An excellent example of an H<sub>2</sub>O<sub>2</sub> sensor is the transcription factor OxyR which is activated by H<sub>2</sub>O<sub>2</sub> oxidation of one specific cysteine (thiol) residue. The thiol residue is modified to –SOH, –S–SG, or –S–S– (with another cysteine that emerges onto the protein surface due to conformation changes). Activated OxyR targets multiple genes involved in peroxide removal and the regulation of iron metabolism (the latter is aimed at preventing the Fenton reaction). An example of H<sub>2</sub>O<sub>2</sub> signalling in bacteria which is involved in the regulation of processes other than redox control is the *Staphylococcus aureus* virulence factor MgrA, the oxidation of which results in increased resistance to antibiotics. A system composed of the enzyme Orp1 and the transcription factor Yap1 is yeast’s analog of OxyR. A specific –SH residue in Orp1 is oxidised by H<sub>2</sub>O<sub>2</sub> to –SOH, further reacting with a cysteine on Yap1 to form a disulfide bridge. The consequent change in conformation leads to nuclear accumulation of Yap1 which promotes the expression of a number of redox enzymes. A similar system is found in another fungus, *Schizosaccharomyces pombe*, but with an additional feature that Pap1 (the Yap1 homologue) is not activated if H<sub>2</sub>O<sub>2</sub> is present at concentrations exceeding 1 mM. In this case, the critical cysteine residue in the activator Tpx1 (the Orp1 analogue) is oxidised to –SO<sub>2</sub>H resulting in Tpx1’s inability to oxidise Pap1 and to provoke the formation of a specific intramolecular disulphide bond in Pap1 [1–3]. Cells and tissues in mammals and other complex organisms are much more relaxed with respect to an external prooxidative environment as they have specialised organs to deal with the hazards. As a consequence, they generally do not need rapid redox responses and are in position to develop redox signalling pathways that are involved in the regulation

of processes other than feedback activation of antioxidative defence.

The basic principles of  $H_2O_2$  signalling in mammals have been described [3–6]. They are as follows. (i) The inactivation of phosphatases. The oxidation of a specific thiol residue to  $-SOH$  prevents it from accepting phosphate. In some cases a disulphide bond is formed thus preventing further oxidation to  $-SO_2H$  or  $-SO_3H$  and irreversible inactivation. (ii) The activation or inhibition of kinases. ASK1 is activated by  $H_2O_2$ -mediated oxidation of two cysteine residues to  $-SOH$  which leads to intermolecular disulphide bond formation and multimerisation. In vascular smooth muscle cells, Src kinase is activated by  $H_2O_2$ , which results in cofilin and myosin light-chain kinase inactivation and consequent F-actin stabilisation and promotion of actin-myosin interactions, thus enabling migration. (iii) The activation/derepression of transcription factors. NF- $\kappa$ B, p53, HIF-1 $\alpha$ , and AP-1 have redox sensitive thiol groups in DNA-binding domains. In addition, it has been proposed that  $H_2O_2$ -provoked oxidation of specific thiol residues in DJ-1 and the Nrf2-inhibitor KEAP1 results in derepression of Nrf2-regulated transcription of a set of enzymes involved in antixenobiotic and cytoprotective response. NF- $\kappa$ B is activated by thiol oxidation, while I $\kappa$ B kinase can be inhibited by  $H_2O_2$ , which together makes a biphasic redox-sensing mechanism. (iv) The activation of ion channels such as ATP-sensitive  $K^+$  channels or TRPA1. (v) The modification of activity of a number of other proteins. Very recent findings show that  $H_2O_2$  is essential for keeping the immune system under control. The oxidation of T-cell surface thiol switches by  $H_2O_2$  results in suppressed activity and proliferation of T cells. In order to activate T cells, dendritic cells release glutathione which is then cleaved to cysteine to reduce  $-SOH$  groups back to  $-SH$ , thus turning the redox switch-on [11]. It is important to note that  $H_2O_2$  signalling pathways are intertwined with the effects of other signalling species, such as NO and CO.

On the other hand, excessive production of  $H_2O_2$  or some other ROS, and a consequential supraphysiological level of oxidation (i.e., oxidative stress), has been related almost to all human diseases that one may think of [12]. Pertinent to this, the isolation of “natural” or the creation of synthetic antioxidants has become a very lucrative activity, which may explain the “explosion” of antioxidative research in the past four decades. However, promising *in vitro* data have not been translated into success in human clinical trials. If we put aside the fact that many researchers in the field neglect important issues such as (i) which ROS is/are overproduced in the particular condition they are trying to treat? (ii) At which site(s) are ROS produced? (iii) Are ROS important for pathophysiology, or do they merely represent byproducts (in other words, a cause or consequence)? (iv) What are the metabolic properties and targets of the proposed antioxidants (e.g., ascorbate seems to exert some beneficial effects in cancer and sepsis treatment via its prooxidative interplay with iron, but not due to its antioxidative effects)?; the key problem is that the importance of redox signalling has not been appreciated [13]. Such careless, almost concept-free approach is giving antioxidants

a bad name and erases the belief of the importance of redox processes in (patho)physiology in the general (scientific) population, thus compromising the entire redox field. This frustrates the experts and represents the subject of concern for authorities. Barry Halliwell and coworkers have made some extreme examples to point out where this carelessness may end up. They have shown that human urine and feces possess reasonable antioxidative capacities [14]. Although those are “natural products,” there seems to be no interest on the market yet. On the other hand, the European Food Safety Authority recently presented negative scientific opinions on a staggering number of health claims that various compounds and products, some of which are widely accepted “antioxidants,” exert antioxidative/beneficial effects in humans [13]. The explanation for *in vivo* ineffectiveness of many *in vitro* antioxidants hides in the fact that redox signalling is too precious for the cells and tissues to allow exogenous meddling. In addition, the intrinsic enzymatic antioxidative system is more efficient than any supplementary antioxidants (of course, if there is no vitamin deficiency), so cells see the later more as a threat to normal redox signalling and less as a help [13, 14]. In order to maintain a flexible redox poise living systems have developed refractory mechanisms [15]. A good illustration of the activity of refractory mechanisms represents the fact that one may consume huge amounts of ascorbate or vitamin E, but the level of these in the blood will not rise above a specific level [15]. When exposed to an excess of exogenous antioxidants, the cells are even prepared to suppress the intrinsic enzymatic antioxidative defence in order to preserve redox homeostasis [16]. From the evolutionary point of view, the refractory system had to be developed or otherwise signalling pathways involved in the regulation of crucial biological processes would have been diet sensitive (e.g., excessive consumption of fruits and vegetables would result in proreductive conditions and the obstruction of redox signalling pathways). Do these facts altogether imply that there is no future for antioxidative therapy? Of course not! We still can adjust the redox milieu with more sophisticated approaches such as the modulation of enzyme and transcription factor activities, or we may offer compounds that have antioxidative effects but are not recognised by cells as antioxidants *per se* (examples being pyruvate, fructose, fructose 1,6-(bis)phosphate, oxaloacetate, fumarate, and metal chelators), to help the organism to fight intracellular oxidative stress against its own “will.”

$H_2O_2$  is important for the development and differentiation of multicellular communities of unicellular yeast and bacteria and for their adaptation to the ever-changing environment [17]. In yeast,  $H_2O_2$  at moderate concentrations increases longevity. More importantly,  $H_2O_2$  has been proposed to regulate the initial steps of ammonia production which synchronises the development of colonies. Finally,  $H_2O_2$  is involved in the programmed cell death that develops in the centre of a colony. The dead cells in the colony centre are likely to release nutrients that are then used by the younger prosperous cells at the edge of the colony to survive and colonise other localities [17]. It has been shown that many bacterial species express  $H_2O_2$ -producing enzymes L-amino acid oxidase, lysine oxidase, and pyruvate

oxidase [18]. In developing colonies, bacteria seem to be programmed to produce  $H_2O_2$  which cleaves DNA resulting in genetic rearrangements via double-strand DNA break repair and the release of extracellular DNA fragments that are incorporated into the chromosome by competent cells. In this way, bacteria acquire genetic flexibility and advantageous gene variations which enable them to adapt to altered environments during initial phases of the development of biofilms [19]. In multispecies oral biofilms, streptococci use  $H_2O_2$  (at concentrations above 1 mM) during active growth, as a mean of biochemical warfare against competitors, particularly in times of low carbohydrate (food) availability. However, some species recognise streptococcal-produced  $H_2O_2$  as a signal to upregulate the resistance to host innate immune system responses, which also uses  $H_2O_2$  [20]. It is noteworthy that the two host-derived peroxidases in the human oral cavity, salivary peroxidase and myeloperoxidase, use streptococcal-generated  $H_2O_2$  to produce an antimicrobial substance, hypothiocyanite [21]. So in summary what we have here looks like a love/hate redox triangle. It has been documented in complex organisms that exposure to mild oxidative stress initiated by ROS or low level radiation increases resistance to later challenges with higher concentrations or doses of the initial stressor [22]. For example, nematodes exposed to low level oxidative stress cross-adapt, developing increased resistance to other stressors, such as heavy metals [23]. The key players in the adaptive response of nematodes seem to be antioxidative defence, heat-shock proteins and metal binding proteins, which are regulated by a branched gene pattern. It has been reported that nematodes activate intrinsic ROS production when exposed to environmental stress, such glucose deficiency [24]. This implies that oxidative stress/other stressor cross-adaptation may be based upon the role of  $H_2O_2$  signalling in adaptive processes in general. Some insects seem to use  $H_2O_2$  in a rather creative manner in order to survive subzero temperatures. It has been shown recently that the Arctic springtail (*Megaphorura arctica*) suppresses catalase activity and increases  $H_2O_2$  production when exposed to freezing temperatures [25]. Combined with cryoprotective dehydration, this results in the accumulation of  $H_2O_2$  in body fluids to surprisingly high level (15 to 20%, v/v). If we take into account that the mixture of  $H_2O$  and  $H_2O_2$  represents a eutectic system, where the freezing point of 20% (v/v)  $H_2O_2$  solution is at  $-15^\circ\text{C}$  [26], it is clear how arctic insects may use  $H_2O_2$  to prevent deadly freezing in a harsh environment. These findings could also represent the basis for new concepts in cryopreservation research. In mammals, the increased production of  $H_2O_2$  in cardiomyocytes is actively involved in hypoxia-induced preconditioning to ischemia [27]. The preconditioning protects ischemic cardiomyocytes through upregulation of antioxidative defence and by  $H_2O_2$ -provoked opening of mitochondrial  $K^+$ -ATP channels. The importance of  $H_2O_2$  in the development of increased resistance to ischemia by intermittent hypoxia preconditioning is implicated by the fact that treatment with the antioxidant N-acetylcysteine in the preconditioning phase may completely prevent the development of cardioprotection [27]. It has been suggested

that  $H_2O_2$  plays a key role in the endothelial adaptation to exercise by stimulating an upregulation of endothelial NO synthase [28]. Finally,  $H_2O_2$  has been reported to provoke hormesis in mitochondria of pancreatic  $\beta$  cells [29]. An important role in this process seems to be played by the increased expression of mitochondrial uncoupling protein UCP2. Even more,  $H_2O_2$  promotes the secretion of insulin from pancreatic  $\beta$  cells, which generally exhibit low levels of antioxidative defence and are therefore highly redox-sensitive [29]. Complimentary to the cellular level where mitochondria play a dual role ( $H_2O_2$ -producing/energy-converting organelles), there may be a link between the redox and energy status of the living system as a whole.

In this special issue we present you seven (excellent, in our honest opinion) papers providing various examples of adaptive and signalling roles of  $H_2O_2$ . We would like to invite you to find others in the literature, and there will be, for sure, more to come.

Ivan Spasojević  
David R. Jones  
Michael E. Andrade

## References

- [1] S. G. Rhee, " $H_2O_2$ , a necessary evil for cell signaling," *Science*, vol. 312, no. 5782, pp. 1882–1883, 2006.
- [2] B. D'Autréaux and M. B. Toledano, "ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 10, pp. 813–824, 2007.
- [3] C. E. Paulsen and K. S. Carroll, "Orchestrating redox signaling networks through regulatory cysteine switches," *ACS Chemical Biology*, vol. 5, no. 1, pp. 47–62, 2010.
- [4] H. J. Forman, M. Maiorino, and F. Ursini, "Signaling functions of reactive oxygen species," *Biochemistry*, vol. 49, no. 5, pp. 835–842, 2010.
- [5] E. Veal and A. Day, "Hydrogen peroxide as a signaling molecule," *Antioxidants and Redox Signaling*, vol. 15, no. 1, pp. 147–151, 2011.
- [6] Y. Wang, J. Yang, and J. Yi, "Redox sensing by proteins: oxidative modifications on cysteines and the consequent events," *Antioxidants and Redox Signaling*, vol. 16, no. 7, pp. 648–657, 2012.
- [7] M. J. Faulkner and J. D. Helmann, "Peroxide stress elicits adaptive changes in bacterial metal ion homeostasis," *Antioxidants and Redox Signaling*, vol. 15, no. 1, pp. 175–189, 2011.
- [8] W. Chadwick, Y. Zhou, S. S. Park et al., "Minimal peroxide exposure of neuronal cells induces multifaceted adaptive responses," *PLoS ONE*, vol. 5, no. 12, Article ID e14352, 2010.
- [9] V. I. Lushchak, "Adaptive response to oxidative stress: bacteria, fungi, plants and animals," *Comparative Biochemistry and Physiology*, vol. 153, no. 2, pp. 175–190, 2011.
- [10] H. Lalucque and P. Silar, "NADPH oxidase: an enzyme for multicellularity?" *Trends in Microbiology*, vol. 11, no. 1, pp. 9–12, 2003.
- [11] Z. Yan, S. K. Garg, and R. Banerjee, "Regulatory T cells interfere with glutathione metabolism in dendritic cells and T cells," *Journal of Biological Chemistry*, vol. 285, no. 53, pp. 41525–41532, 2010.
- [12] I. Spasojević, "Free radicals and antioxidants at a glance using EPR spectroscopy," *Critical Reviews in Clinical Laboratory Sciences*, vol. 48, no. 3, pp. 114–142, 2011.

- [13] M. É. Andrades, A. Morina, S. Spasić, and I. Spasojević, “Bench-to-bedside review: sepsis—from the redox point of view,” *Critical Care*, vol. 15, no. 5, Article ID 230, 2011.
- [14] B. Halliwell, “Free radicals and antioxidants: updating a personal view,” *Nutrition Reviews*, vol. 70, no. 5, pp. 257–265, 2012.
- [15] N. Lane, “A unifying view of ageing and disease: the double-agent theory,” *Journal of Theoretical Biology*, vol. 225, no. 4, pp. 531–540, 2003.
- [16] T. A. Kizhner, O. Shilovizki, and M. J. Werman, “Long-term fructose intake reduces oxidative defense and alters mitochondrial performance in mice,” *Nutrition Research*, vol. 27, no. 7, pp. 423–431, 2007.
- [17] L. Váchová and Z. Palková, “Physiological regulation of yeast cell death in multicellular colonies is triggered by ammonia,” *Journal of Cell Biology*, vol. 169, no. 5, pp. 711–717, 2005.
- [18] A. Mai-Prochnow, P. Lucas-Elio, S. Egan et al., “Hydrogen peroxide linked to lysine oxidase activity facilitates biofilm differentiation and dispersal in several gram-negative bacteria,” *Journal of Bacteriology*, vol. 190, no. 15, pp. 5493–5501, 2008.
- [19] B. R. Boles, M. Thoendel, and P. K. Singh, “Self-generated diversity produces “insurance effects” in biofilm communities,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 47, pp. 16630–16635, 2004.
- [20] M. M. Ramsey and M. Whiteley, “Polymicrobial interactions stimulate resistance to host innate immunity through metabolite perception,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 5, pp. 1578–1583, 2009.
- [21] A. Welk, P. Rudolph, J. Kreth, C. Schwahn, A. Kramer, and H. Below, “Microbicidal efficacy of thiocyanate hydrogen peroxide after adding lactoperoxidase under saliva loading in the quantitative suspension test,” *Archives of Oral Biology*, vol. 56, pp. 1576–1582, 2011.
- [22] S. I. S. Rattan, “Hormesis in aging,” *Ageing Research Reviews*, vol. 7, no. 1, pp. 63–78, 2008.
- [23] D. Wang, P. Liu, and X. Xing, “Pre-treatment with mild UV irradiation increases the resistance of nematode *Caenorhabditis elegans* to toxicity on locomotion behaviors from metal exposure,” *Environmental Toxicology and Pharmacology*, vol. 29, no. 3, pp. 213–222, 2010.
- [24] T. J. Schulz, K. Zarse, A. Voigt, N. Urban, M. Birringer, and M. Ristow, “Glucose restriction extends *caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress,” *Cell Metabolism*, vol. 6, no. 4, pp. 280–293, 2007.
- [25] G. Grubor-Lajšić, E. T. Petri, R. M. Worland et al., “Hydrogen peroxide and ecdysone in the cryoprotective desiccation strategy of *M. arctica*,” *Archives of Insect Biochemistry and Physiology*. In press.
- [26] J. M. Houtkoope and D. Schulze-Makuch, “A possible biogenic origin for hydrogen peroxide on Mars: the Viking results reinterpreted,” *International Journal of Astrobiology*, vol. 6, no. 2, pp. 147–152, 2007.
- [27] H. Y. Zhang, B. C. McPherson, H. Liu, T. S. Baman, P. Rock, and Z. Yao, “H<sub>2</sub>O<sub>2</sub> opens mitochondrial KATP channels and inhibits GABA receptors via protein kinase C-ε in cardiomyocytes,” *American Journal of Physiology*, vol. 282, no. 4, pp. H1395–H1403, 2002.
- [28] N. Lauer, T. Suvorava, U. Rütther et al., “Critical involvement of hydrogen peroxide in exercise-induced up-regulation of endothelial NO synthase,” *Cardiovascular Research*, vol. 65, no. 1, pp. 254–262, 2005.
- [29] P. Maechler, N. Li, M. Casimir, L. Vetterli, F. Frigerio, and T. Brun, “Role of mitochondria in β-cell function and dysfunction,” *Advances in Experimental Medicine and Biology*, vol. 654, pp. 193–216, 2010.