Hydrogen peroxide and central redox theory for aerobic life A tribute to Helmut Sies: Scout, trailblazer and Redox Pioneer

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Abstract

When Rafael Radi and I wrote about Helmut Sies for the Redox Pioneer series, I was disappointed that the Editor restricted us to the use of "Pioneer" in the title. My view is that Helmut was always ahead of the pioneers: He was a scout discovering paths for exploration and a trailblazer developing strategies and methods for discovery. I have known him for nearly 40 years and greatly enjoyed his collegiality as well as brilliance in scientific scholarship. He made monumental contributions to 20th century physiological chemistry beginning with his first measurement of H_2O_2 in rat liver. While continuous H_2O_2 production is dogma today, the concept of H_2O_2 production in mammalian tissues was largely buried for half a century. He continued this leadership in research on oxidative stress, GSH, selenium, and singlet oxygen, during the timeframe when physiological chemistry and biochemistry transitioned to contemporary 21st century systems biology. His impact has been extensive in medical and health sciences, especially in nutrition, aging, toxicology and cancer. I briefly summarize my interactions with Helmut, stressing our work together on the redox code, a set of principles to link mitochondrial respiration, bioenergetics, H_2O_2 metabolism, redox signaling and redox proteomics into central redox theory.

Keywords

Oxidative stress; Hydrogen peroxide; Redox biology; Oxidation-reduction; Bioenergetics

1. Intercellular versus intracellular O_2 gradients

My PhD research in the early 1970's was on the biochemistry of hypoxia with Howard S. Mason in Portland, Oregon; by the time I graduated I was introduced to the leading redox biochemistry research of the day, including recent research on superoxide dismutase by McCord and Fridovich, chemiosmotic coupling by Mitchell, and catalase function in liver by Sies and Chance. Arguments abounded at that time about whether cytochrome P-450 and cytochrome P448 were really two enzymes or only one. This was before molecular biology; enzymology was king. Methods were relatively primitive for protein purification, computers were very limited (1K of memory cost $1000), and advanced spectroscopic methods were rapidly being development. The major discovery of my dissertation research was that under

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hypoxic conditions, the mitochondrial O_2 consumption in hepatocytes was sufficient to establish intracellular concentration gradients of O_2 to mitochondria while O_2 dependence of cytochrome P450 in the endoplasmic reticulum experienced only a minimal O_2 gradient [1]. This research directly linked me to Helmut Sies, who was also studying tissue O_2 gradients at the time.

And this connection continues to current times. At the recent Oxygen Club of California Meeting in Valencia, Spain, Salvador Moncada delivered a splendid keynote address in which he described his work with a newly developed device which he described as an "oxystat". As I walked by after his presentation, I overheard Helmut cordially telling Salvador that his group had developed an "oxystat" a few decades earlier [2]. I could not resist teasing Helmut that he was late with his development, that I had described an oxystat earlier as part of my PhD research [3]. Helmut seized the moment and commented that we should certainly obtain a photo of this historic oxystat trio (Fig. 1).

Upon receipt of my PhD, I went to Cornell University in Ithaca to study enzymology of stearoyl-CoA desaturase with Jim Gaylor. The nature of this enzyme was only poorly characterized at this point in time, and Jim was interested in gaining an understanding of a protein fraction that stimulated desaturase activity. Generation of hydrogen peroxide upon addition of NADPH to microsomes was known from Lars Ernster's research, and Jim was interested in whether the auxiliary protein was functioning to change the stoichiometry of the desaturase from an H_2O_2-producing to a non--H_2O_2--producing activity. My first stoichiometry studies on the desaturase activity showed that the enzyme produced no H_2O_2. But because I had no positive control, i.e., an enzyme which definitely produced H_2O_2, I did not have a satisfactory answer. So I proceeded to develop a different H_2O_2 production assay, and finally a third H_2O_2 production assay, before I finally concluded that H_2O_2 was not produced by stearoyl desaturase [4]. In the process of these "failed" experiments, I became an authority on measurement of H_2O_2 production. When I moved to Stockholm the following year to continue my studies with Sten Orrenius, I was already an expert in measurement of H_2O_2 production.

The time at Cornell was doubly serendipitous. During this period of time, Britton Chance invited me to the University of Pennsylvania to work with him on respiration-dependent O_2 gradients in hepatocytes (which I published later independently from my own laboratory; [5]). This gave me hands on experience with the methods that Helmut Sies had used in his pioneering studies on H_2O_2 production in perfused rat liver [6]. In the process of my studies with Britt, I obtained independent confirmation of O_2 gradients in hepatocytes. More importantly, Efraim Racker, a Cornell biochemist noted for his pioneering work on the mitochondrial ATPase (Complex V, ATP synthase), was hosting Helmut Sies as a visitor to the Department. Efraim knew that I had worked with Britt and felt that this would be useful for me to meet Helmut.

As I recall, Helmut and I had a good discussion. After I explained my PhD research and conclusions, however, he told me that he had already shown this and he would send a copy of his paper. As imaginable, I was totally deflated. But then when I received his paper [7], I found that even though he referred to intracellular indicators in his title, he had only

concluded that there were "intercellular" O_2 gradients in liver. Thus, my detection of "intracellular" O_2 gradients, was the first publication showing heterogeneity of O_2 concentration within cells [1]. I followed this with studies showing O_2 gradients in cardiac myocytes [8] and proximal kidney cells [9], ATP concentration gradients in hepatocytes [10], pH gradients in proximal tubule cells [9], and H_2O_2 gradients in hepatocytes [11]. These provided the foundation for the concept that kinetic limitations are central to the redox organizational structure as we recently included in the redox code (described below; [12]).

Years later, when I recalled this first meeting to Helmut, he had no recollection. As I guess this meeting should have been: he was much more impressive to me than I was to him. At least he remembered me from the Glutathione meeting in Schloss Reisensburg the following year (see: [13]).

2. H_2O_2 production in hepatocytes

I had the remarkable good fortune to join Sten Orrenius in 1977, just after he had purchased an Aminco-Chance dual beam spectrophotometer. I arrived with experience using Britt's homemade prototype instrument and also had my experience measuring H_2O_2 during studies of the stoichiometry of the desaturase. Don Reed was visiting on sabbatical at the time and had recently developed a sensitive HPLC method for GSH and GSSG [14]. Within a very short period of time, we were able to show that certain cytochrome P450 substrates stimulated H_2O_2 production in hepatocytes [15] as measured by both the spectrophotometric method that Helmut had pioneered [6] and by stimulation of GSSG efflux, a process also described by Helmut [16].

I pursued research on H_2O_2 metabolism when I set up my own laboratory at Emory. These studies included collaboration with Sten showing that H_2O_2 metabolism is spatially resolved, with low rates of H_2O_2 production in peroxisomes being metabolized in peroxisomes and low rates of H_2O_2 production in the endoplasmic reticulum being metabolized by GSH peroxidase rather than peroxisomal catalase [11]. Years later, Michel Toledano concluded that specificity in H_2O_2 signaling is obtained by localized H_2O_2 generation and metabolism [17], apparently without realizing that local generation and metabolism of H_2O_2 was established decades earlier [11]. From our series of studies on intracellular gradients, we also derived an equation to predict when intracellular gradients will occur [18]. Application of this to redox signaling shows conditions under which substantial local H_2O_2 gradients exist for redox signaling (Fig 2).

My controlled titration studies [19] provided an independent confirmation of Helmut's finding that intracellular H_2O_2 concentration is in the low nanomolar range. More recent modeling studies of H_2O_2 metabolism also support this interpretation [20]. Thus, the modern view of H_2O_2 generation and metabolism is largely developed from Helmut's pioneering research published in 1970. Available evidence indicates that H_2O_2 is a ubiquitous metabolite under aerobic conditions. As we developed in the redox code, this steady-state concentration of H_2O_2 provides a context to maintain redox organization and function.

3. Functions of GSH

Helmut had already been studying GSH for a few years [21] before I began to appreciate its importance [15]. I was well aware of Helmut's research on subcellular compartmentation of NADH/NAD and NADPH/NADP systems in liver [22]. He established the steady-state values for these couples in mitochondria, cytoplasm and nuclei, and these data provided a foundation for research in my laboratory to subsequently determine steady-state redox potentials for GSH/GSSG and thioredoxin couples within the different compartments [23–26].

My introduction to GSH research came from the nutritional story of the selenium requirement for GSH peroxidases [27]; my introduction to Helmut's research came from his studies on GSH and GSSG release from liver [28]. Helmut had made important contributions on Se deficiency effects on GSH peroxidase and cellular GSSG release from liver in studies with Ray Burk [29]. For my research, his series of prescient experiments showing the disequilibrium between the NADPH/NADP couple and the GSH/GSSG couple in liver [21] were probably most important. In a conversation that I had with Sir Hans Krebs in the late 1970's, it was apparent that Krebs understood the fundamental importance of Helmut's research. Krebs described regulation of the pentose phosphate pathway by GSSG as a mechanism to stimulate NADPH supply to maintain GSH [30].

Dan Ziegler, a noted biochemist known for his characterization of the flavin amine oxidase [31], told me that he thought that Helmut's interpretation was completely wrong. He felt that all GSSG and protein disulfide measurements were artifacts and that if everything were done strictly anaerobically, everything would be reduced [32]. However, I had measured pyruvate in hepatocytes even under strictly anaerobic conditions [1]. The results showed that even with anoxia, the redox potentials of NADH/NAD do not swing wildly to negative potentials but only achieve values approaching the NADPH range. I believe that this is a consequence of the presence of many other "oxidants" in physiologic systems, including carbonyls, quinones, disulfides and other electron-accepting functional groups that serve to protect against reductive stress.

When we obtained results in the 1990's clearly showing that the GSH/GSSG and cysteine/cystine couples were not in equilibrium in human plasma [33], I initially assumed that it was an artifact. Larry Lash had tabulated data on plasma thiols and disulfides [34], which supported our analytical measurements. Based upon the earlier redox studies of the NADH/NAD and NADPH/NADP systems [35] [22], we recognized that redox systems can appear out of equilibrium for multiple reasons. In cells, the couples could be differentially distributed among compartments, or binding proteins could prevent interaction of one or more components. Plasma does not have membrane barriers, however, and our equilibrium dialysis experiments with plasma provided insufficient non-covalent binding to account for the differences. But it was really Hiram Gilbert's emphatic support for Helmut's results and interpretation [36] that made me recognize kinetic limitation between thiol/disulfide systems as one of the fundamental principles of aerobic life [37,38].

Although we only published our finding of disequilibrium between GSH/GSSG and Cys/CySS system in plasma in 2000 [33], by 2002 we had evidence for differences as a function of age in humans [39], for cysteine/cystine redox control cell growth [40], and for differences between GSH/GSSG and Trx (reduced/oxidized) redox systems during proliferation and differentiation of CaCo2 cells [41]. We went on to characterize compartmentalization of the thiol/disulfide systems [23] in the same way Helmut had done for the NAD and NADP systems [22].

4. Refining the definition of oxidative stress

Helmut is perhaps best known for his definition of oxidative stress [13,42]. I was in Stockholm in 1981 on one of several "mini-sabbaticals" studying with Sten Orrenius, and I remember well Sten and Peter Moldéus exuberantly discussing the Kappus and Sies paper on redox cycling [43]. From this paper, which clearly presents oxidative reactions in an abnormal context of lipid peroxidation, the nature of chemically induced oxidative stress is effectively defined as a disruption in normal function leading to extensive oxidation of membrane lipids. In his extension of the concept more globally to macromolecular damage in the 1985 definition of oxidative stress [42], Helmut brought together many disparate fields of radiation biology, cancer, aging, nutrition, etc [13].

As large-scale, double blind trials of radical-scavenging antioxidants accumulated to show little or no health benefit [44–52], arguments abounded concerning possible problems with study design, choice of antioxidants and accessibility of antioxidants to critical targets. While I felt that all of those could contribute, my interpretation from the kinetic limitations in electron transfer between thiol/disulfide systems was that there was a general misconception about the nature of redox systems. In our 2004 paper describing cysteine/cystine as a hub in the redox organizational structure [37], we introduced the concept that thiol/disulfide redox circuits can be described in analogy to the well-defined mitochondrial electron transfer pathways. My 2006 paper, "Redefining Oxidative Stress" [53] discussed disruption of these redox circuits as a replacement to Helmut's definition. In my conversations with Helmut, he said that he would prefer the term "refined" to "redefined" and pointed out that my definition overlooked the very real evidence for DNA damage from radiation and hepatic failure from carbon tetrachloride or halothane intoxication. Thus, the following year we introduced a consensus definition (if one can consider agreement between two individuals as "consensus") as "An imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage." [54].

5. The redox code

Studies by Ward Kirlin in the 1990's showed that the calculated steady-state GSH/GSSG redox potential in HT-29 cells changed as a function of differentiation and after exposure to benzylisothiocyanate, an activator of the antioxidant response element (ARE) [55]. We recognized the potential importance of this in terms of control of cell function; if proteins responded rapidly enough to this change in potential, then changes in this steady state could control cell functions [55]. However, the data available at that time were insufficient to

conclude that this actually happens. Considerable data showed that protein oxidation occurs following addition of oxidants, but limited data provided evidence for oxidation under normal physiologic conditions. As a consequence of Ward's finding, I began efforts to determine whether changes in protein oxidation occur naturally during the life cycle of cells. Jiyang Cai and Bert Watson developed an assay for thioredoxin-1 [56] based upon earlier studies of bacterial thioredoxin by Arne Holmgren [57], and Yvonne Nkabyo showed that unlike the oxidation of GSH/GSSG, thioredoxin-1 did not become oxidized during spontaneous differentiation of CaCo2 cells in culture [41].

The first disease-associated signaling pathway response of cells to an imposed change in redox potential was obtained by Young-Mi Go in 2005 [58]. This study did not identify the redox-responsive protein but showed downstream activation of pro-inflammatory cytokines in mouse aortic endothelial cells in response to varied extracellular cysteine/cystine redox potential. This motivated our work with Jan Pohl to develop the redox ICAT method to measure protein thiol oxidation in cells under usual physiologic conditions [59]. The results of these studies confirmed data of others [60–62] showing that cysteine residues of many proteins are partially oxidized under usual cell growth conditions [63]. Importantly, the finding that the percentage oxidation of cysteines map to functional pathways for the proteins rather than to subcellular distribution of the protein [63], provided impetus to consider the character of the redox proteome [64] within central redox theory, a subject receiving little attention since Helmut's detailed review in 1982 [22].

I had been thinking about this for several years but could not reconcile all of the information. So in late 2010, I approached Helmut to inquire whether he would be willing to help me with this. He agreed, probably not remembering that I am not as efficient as he is. The writing of "The Redox Code" [12] took approximately five years for completion; most of the delay was due to my limitations, not Helmut's.

Starting with research from Bücher and Klingenberg in the 1950's [65] and Krebs in the 1960's [66], summarized and expanded by Helmut in his 1982 review [22], a first redox principle was evident: Reversible electron accepting and donating properties of nicotinamide in NAD and NADP provide organization of metabolism, operating at near-equilibrium [12]. Substrate oxidations are linked to reduction of NAD^+ and $NADP^+$, which in turn are linked to ATP production, catabolism and anabolism, respectively. Helmut and I discussed whether a related principle was needed to connect 1-electron transfer (radical) processes with 2-electron transfer (non-radical) processes, such as occur with flavoproteins, semi-quinones and ascorbate. We ultimately decided, however, that this should be left to future updates to elaborate the code.

Our most extensive discussions concerned the second principle, that metabolism is linked to protein structure through kinetically controlled redox switches in the proteome, which determine tertiary structure, macromolecular interactions and trafficking, activity and function. The evolution of my thinking, from defined thiol redox circuits [37,38] to relatively non-defined redox networks [67–69], greatly complicated definition of this principle. I attempted to detail variations in abundance of proteins and reactivity of sulfur switches, but ultimately Helmut prevailed with his clarity of thought. We agreed that

considerable research will be needed to define the orders of magnitude variations to understand the specificity and/or network structure; the central principle that we recognized was that sulfur switches define the connectivity of the bioenergetics and metabolic systems to the macromolecular structures [12]. A corollary to this principle is that other redox-dependent, high-energy metabolites, e.g., acetyl-CoA, S-adenosylmethionine, NAD^+, etc., elaborate a broader connectivity of the bioenergetics and metabolic systems to the macromolecular structures and functions through acetylation/deacetylation, methylation/demethylation, ADP-ribosylation and other epiproteomic switches.

The third principle exemplified the synergy from our collaboration. Helmut wanted to incorporate innovative imaging methods of Hung et al. [70] and the exciting findings of Love et al. [71] and Knoefer et al. [72] showing that oxidative processes occur during development and wound repair. I wanted to incorporate the concept that linked, opposing reactions involving NADPH and H_2O_2 are used to maintain stable, non-equilibrium conditions [73] in cellular regulation. Our conversations on these points led to the recognition of a third principle, that activation/deactivation cycles of redox metabolism, especially involving H_2O_2, support spatio-temporal processes in differentiation and life cycles of cells and organisms [12].

This principle ultimately links back to Helmut's original measurement of H_2O_2 generation in mammalian tissues [6]. The central depiction of H_2O_2 as a universal pool of oxidant remains only a conceptual framework because there is no way to calibrate the steady-state concentration in most biologic systems. My interpretation is that upon evolution of O_2 in the Earth's atmosphere, the rate of chemical oxidation of thiols contributed to maintenance of an H_2O_2 concentration in the low nanomolar range and that H_2O_2 generation by mitochondria and NADPH oxidases evolved as systems to maintain a steady oxidative tone for redox organization. This provides a type of capacitor in which the potential difference between the NADPH/NADP couple and the H_2O_2/water couple is available to control the structural dynamics through reversible switches. The waves of H_2O_2 generation and elimination operate within this 3-dimensional structure. One must recognize however, that the number of discrete examples of activation/deactivation cycles is limited. Despite this, the third principle provides a foundation for new experimental paradigms to test spatiotemporal sequencing and related mechanisms in disease [12] and aging [74].

A fourth principle emerged from our discussions of the relevance of the principles of the redox code to the genetic code, histone code and epigenetic code. Christopher Wild had defined the concept of the exposome [75] as the cumulative lifelong exposures of an individual, which complements an individual's genome in accounting for disease. The fourth principle can be considered in analogy to this genome-exposome complement: the genome defines an individual, while the redox code, along with histone and epigenetic codes, define how an individual interacts with its environment. Young-Mi Go and I had proposed that the interaction of the genome and the exposome occurs through a redox interface [76]. The fourth principle fits naturally with this concept, namely, that redox networks form an adaptive system to respond to environment, from microcompartments through subcellular systems to the levels of cell and tissue organization. This adaptive redox network structure is

required to maintain health in a changing environment and, if functionally impaired, contributes to disease and organism failure [12].

6. Summary and perspective

Through these brief comments, I have emphasized how Helmut Sies has had considerable impact on redox biology, especially through his fundamental research on oxidative stress and redox biochemistry. I have illustrated how my own research has been enriched both by standing upon his shoulders and also by directly bearing the load of the intensive thought to refine the definition of oxidative stress and elaborate central redox theory. His lifelong career in application of quantitative methods anchored the first principle of the redox code. His demonstration that the GSH/GSSG redox couple is not in equilibrium with the NADPH/NADP couple provided a foundation for contemporary understanding of kinetic control in the redox proteome. His measurements showing that H_2O_2 is present at low nanomolar concentrations in mammalian cells provided a context for understanding the general feature that cysteines within proteins exist in partially oxidized states and that cyclical activation/deactivation cycles of oxidation serve to control and direct complex processes in cellular differentiation and organ development. Thus, an understanding of the redox code is a direct derivative of his pioneering research and underscores the critical and long-term impact of his research in redox biology and medicine.

Acknowledgments

References

1. Jones DP, Mason HS. Gradients of O2 concentration in hepatocytes. J Biol Chem. 1978; 253:4874–4880. [PubMed: 209020]

2. de Groot H, Noll T, Sies H. Oxygen dependence and subcellular partitioning of hepatic menadione-mediated oxygen uptake. Studies with isolated hepatocytes, mitochondria, and microsomes from rat liver in an oxystat system. Arch Biochem Biophys. 1985; 243:556–562. [PubMed: 2417562]

3. Jones DP, Mason HS. Apparatus for automatically maintaining cell suspension at low, constant oxygen concentrations: the oxystat. Anal Biochem. 1978; 90:155–166. [PubMed: 727460]

4. Jones DP, Gaylor JL. Regulation of microsomal stearoyl-coenzyme a desaturase. Purification of a non-substrate-binding protein that stimulates activity. Biochem J. 1979; 183:405–415. [PubMed: 43136]

5. Jones DP, Kennedy FG. Intracellular oxygen supply during hypoxia. Am J Physiol. 1982; 243:C247–C253. [PubMed: 7137335]

6. Sies H, Chance B. The steady state level of catalase compound I in isolated hemoglobin-free perfused rat liver. FEBS Lett. 1970; 11:172–176. [PubMed: 11945479]

7. Sies H. Cytochrome oxidase and urate oxidase as intracellular O2 indicators in studies of O2 gradients during hypoxia in liver. Adv Exp Med Biol. 1977; 94:561–566. [PubMed: 207168]

8. Jones DP, Kennedy FG. Intracellular O2 gradients in cardiac myocytes. Lack of a role for myoglobin in facilitation of intracellular O2 diffusion. Biochem Biophys Res Commun. 1982; 105:419–424. [PubMed: 6284154]

9. Aw TY, Wilson E, Hagen TM, Jones DP. Determinants of mitochondrial O2 dependence in kidney. Am J Physiol. 1987; 253:F440–F447. [PubMed: 2820242]

10. Aw TY, Jones DP. ATP concentration gradients in cytosol of liver cells during hypoxia. Am J Physiol. 1985; 249:C385–C392. [PubMed: 2998197]

11. Jones DP, Eklow L, Thor H, Orrenius S. Metabolism of hydrogen peroxide in isolated hepatocytes: relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H2O2. Arch Biochem Biophys. 1981; 210:505–516. [PubMed: 7305340]

12. Jones DP, Sies H. The Redox Code. Antioxid Redox Signal. 2015

13. Jones DP, Radi R. Redox pioneer: professor helmut sies. Antioxid Redox Signal. 2014; 21:2459–2468. [PubMed: 25178739]

14. Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW, Potter DW. High-performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide, and related thiols and disulfides. Anal Biochem. 1980; 106:55–62. [PubMed: 7416469]

15. Jones DP, Thor H, Andersson B, Orrenius S. Detoxification reactions in isolated hepatocytes. Role of glutathione peroxidase, catalase, and formaldehyde dehydrogenase in reactions relating to N-demethylation by the cytochrome P-450 system. J Biol Chem. 1978; 253:6031–6037. [PubMed: 567217]

16. Akerboom TP, Bilzer M, Sies H. The relationship of biliary glutathione disulfide efflux and intracellular glutathione disulfide content in perfused rat liver. J Biol Chem. 1982; 257:4248–4252. [PubMed: 7068633]

17. D'Autreaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol. 2007; 8:813–824. [PubMed: 17848967]

18. Jones DP, Aw TY, Sillau AH. Defining the resistance to oxygen transfer in tissue hypoxia. Experientia. 1990; 46:1180–1185. [PubMed: 2253719]

19. Jones DP. Intracellular catalase function: analysis of the catalatic activity by product formation in isolated liver cells. Arch Biochem Biophys. 1982; 214:806–814. [PubMed: 6284037]

20. Adimora NJ, Jones DP, Kemp ML. A model of redox kinetics implicates the thiol proteome in cellular hydrogen peroxide responses. Antioxid Redox Signal. 2010; 13:731–743. [PubMed: 20121341]

21. Sies H, Summer KH. Hydroperoxide-metabolizing systems in rat liver. Eur J Biochem. 1975; 57:503–512. [PubMed: 1175655]

22. Sies, H. Metabolic Compartmentation. Sies, H., editor. Academic Press; London; New York: 1982. p. 205-231.

23. Go YM, Jones DP. Redox compartmentalization in eukaryotic cells. Biochim Biophys Acta. 2008; 1780:1273–1290. [PubMed: 18267127]

24. Halvey PJ, Hansen JM, Johnson JM, Go YM, Samali A, Jones DP. Selective oxidative stress in cell nuclei by nuclear-targeted D-amino acid oxidase. Antioxid Redox Signal. 2007; 9:807–816. [PubMed: 17508907]

25. Hansen JM, Zhang H, Jones DP. Differential oxidation of thioredoxin-1, thioredoxin-2, and glutathione by metal ions. Free Radic Biol Med. 2006; 40:138–145. [PubMed: 16337887]

26. Watson WH, Jones DP. Oxidation of nuclear thioredoxin during oxidative stress. FEBS Lett. 2003; 543:144–147. [PubMed: 12753922]

27. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973; 179:588–590. [PubMed: 4686466]

28. Sies H, Koch OR, Martino E, Boveris A. Increased biliary glutathione disulfide release in chronically ethanol-treated rats. FEBS Lett. 1979; 103:287–290. [PubMed: 467672]

29. Sies H, Bartoli GM, Burk RF, Waydhas C. Glutathione efflux from perfused rat liver after phenobarbital treatment, during drug oxidations, and in selenium deficiency. Eur J Biochem. 1978; 89:113–118. [PubMed: 699901]

30. Eggleston LV, Krebs HA. Regulation of the pentose phosphate cycle. Biochem J. 1974; 138:425–435. [PubMed: 4154743]

31. Poulsen LL, Ziegler DM. The liver microsomal FAD-containing mono-oxygenase. Spectral characterization and kinetic studies. J Biol Chem. 1979; 254:6449–6455. [PubMed: 36396]

32. Ziegler DM. Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation. Annu Rev Biochem. 1985; 54:305–329. [PubMed: 2862840]

33. Jones DP, Carlson JL, Mody VC, Cai J, Lynn MJ, Sternberg P. Redox state of glutathione in human plasma. Free Radic Biol Med. 2000; 28:625–635. [PubMed: 10719244]

34. Lash LH, Jones DP. Distribution of oxidized and reduced forms of glutathione and cysteine in rat plasma. Arch Biochem Biophys. 1985; 240:583–592. [PubMed: 4026295]

35. Chance B, Cohen P, Jobsis F, Schoener B. Intracellular oxidation-reduction states in vivo. Science. 1962; 137:499–508. [PubMed: 13878016]

36. Gilbert HF. Molecular and cellular aspects of thiol-disulfide exchange. Adv Enzymol Relat Areas Mol Biol. 1990; 63:69–172. [PubMed: 2407068]

37. Jones DP, Go YM, Anderson CL, Ziegler TR, Kinkade JM Jr, Kirlin WG. Cysteine/cystine couple is a newly recognized node in the circuitry for biologic redox signaling and control. Faseb J. 2004; 18:1246–1248. [PubMed: 15180957]

38. Jones DP. Disruption of mitochondrial redox circuitry in oxidative stress. Chem Biol Interact. 2006; 163:38–53. [PubMed: 16970935]

39. Jones DP, Mody VC Jr, Carlson JL, Lynn MJ, Sternberg P Jr. Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses. Free Radic Biol Med. 2002; 33:1290–1300. [PubMed: 12398937]

40. Jonas CR, Ziegler TR, Gu LH, Jones DP. Extracellular thiol/disulfide redox state affects proliferation rate in a human colon carcinoma (Caco2) cell line. Free Radic Biol Med. 2002; 33:1499–1506. [PubMed: 12446207]

41. Nkabyo YS, Ziegler TR, Gu LH, Watson WH, Jones DP. Glutathione and thioredoxin redox during differentiation in human colon epithelial (Caco-2) cells. Am J Physiol Gastrointest Liver Physiol. 2002; 283:G1352–G1359. [PubMed: 12433666]

42. Sies, H. Oxidative Stress, Orlando. Sies, H., editor. Academic Press; London: 1985. p. 1-8.

43. Kappus H, Sies H. Toxic drug effects associated with oxygen metabolism: redox cycling and lipid peroxidation. Experientia. 1981; 37:1233–1241. [PubMed: 7035210]

44. Brion LP, Bell EF, Raghuveer TS. Vitamin E supplementation for prevention of morbidity and mortality in preterm infants. Cochrane Database Syst Rev. 2003:CD003665.

45. Fishbane S, Durham JH, Marzo K, Rudnick M. N-acetylcysteine in the prevention of radiocontrast-induced nephropathy. J Am Soc Nephrol. 2004; 15:251–260. [PubMed: 14747371]

46. Goodman GE, Thornquist MD, Balmes J, Cullen MR, Meyskens FL Jr, Omenn GS, Valanis B, Williams JH Jr. The Beta-Carotene and retinol efficacy Trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping beta-carotene and retinol supplements. J Natl Cancer Inst. 2004; 96:1743–1750. [PubMed: 15572756]

47. Lonn E, Bosch J, Yusuf S, Sheridan P, Pogue J, Arnold JM, Ross C, Arnold A, Sleight P, Probstfield J, Dagenais GR. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. Jama. 2005; 293:1338–1347. [PubMed: 15769967]

48. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol. 2005; 25:29–38. [PubMed: 15539615]

49. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL Jr, Valanis B, Williams JH Jr, Barnhart S, Cherniack MG, Brodkin CA, Hammar S. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and retinol efficacy Trial. J Natl Cancer Inst. 1996; 88:1550–1559. [PubMed: 8901853]

50. Scott JA, King GL. Oxidative stress and antioxidant treatment in diabetes. Ann N Y Acad Sci. 2004; 1031:204–213. [PubMed: 15753146]

51. Tylicki L, Rutkowski B, Horl WH. Antioxidants: a possible role in kidney protection. Kidney Blood Press Res. 2003; 26:303–314. [PubMed: 14610334]

52. Williams KJ, Fisher EA. Oxidation, lipoproteins, and atherosclerosis: which is wrong, the antioxidants or the theory? Curr Opin Clin Nutr Metab Care. 2005; 8:139–146. [PubMed: 15716791]

53. Jones DP. Redefining oxidative stress. Antioxid Redox Signal. 2006; 8:1865–1879. [PubMed: 16987039]

54. Sies, H.; Jones, DP. Oxidative Stress. Elsevier; San Diego: 2007.

55. Kirlin WG, Cai J, Thompson SA, Diaz D, Kavanagh TJ, Jones DP. Glutathione redox potential in response to differentiation and enzyme inducers. Free Radic Biol Med. 1999; 27:1208–1218. [PubMed: 10641713]

56. Watson WH, Pohl J, Montfort WR, Stuchlik O, Reed MS, Powis G, Jones DP. Redox potential of human thioredoxin 1 and identification of a second dithiol/disulfide motif. J Biol Chem. 2003; 278:33408–33415. [PubMed: 12816947]

57. Holmgren A, Fagerstedt M. The in vivo distribution of oxidized and reduced thioredoxin in Escherichia coli. J Biol Chem. 1982; 257:6926–6930. [PubMed: 7045097]

58. Go YM, Jones DP. Intracellular proatherogenic events and cell adhesion modulated by extracellular thiol/disulfide redox state. Circulation. 2005; 111:2973–2980. [PubMed: 15927968]

59. Go YM, Pohl J, Jones DP. Quantification of redox conditions in the nucleus. Methods Mol Biol. 2009; 464:303–317. [PubMed: 18951192]

60. Le Moan N, Clement G, Le Maout S, Tacnet F, Toledano MB. The Saccharomyces cerevisiae proteome of oxidized protein thiols: contrasted functions for the thioredoxin and glutathione pathways. J Biol Chem. 2006; 281:10420–10430. [PubMed: 16418165]

61. Leichert LI, Gehrke F, Gudiseva HV, Blackwell T, Ilbert M, Walker AK, Strahler JR, Andrews PC, Jakob U. Quantifying changes in the thiol redox proteome upon oxidative stress in vivo. Proc Natl Acad Sci U S A. 2008; 105:8197–8202. [PubMed: 18287020]

62. Sethuraman M, McComb ME, Huang H, Huang S, Heibeck T, Costello CE, Cohen RA. Isotope-coded affinity tag (ICAT) approach to redox proteomics: identification and quantitation of oxidant-sensitive cysteine thiols in complex protein mixtures. J Proteome Res. 2004; 3:1228–1233. [PubMed: 15595732]

63. Go YM, Duong DM, Peng J, Jones DP. Protein cysteines map to functional networks according to steady-state level of oxidation. J Proteom Bioinform. 2011; 4:196–209.

64. Go YM, Jones DP. The redox proteome. J Biol Chem. 2013; 288:26512–26520. [PubMed: 23861437]

65. Bücher T, Klingenberg M. Wege des Wasserstoffs in der lebendigen Organisation (Pathways of hydrogen in the living organisation). Angew Chem. 1958; 70:552–570.

66. Williamson DH, Lund P, Krebs HA. The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochem J. 1967; 103:514–527. [PubMed: 4291787]

67. Jones DP. Redox sensing: orthogonal control in cell cycle and apoptosis signalling. J Intern Med. 2010; 268:432–448. [PubMed: 20964735]

68. Jones DP, Go YM. Mapping the cysteine proteome: analysis of redox-sensing thiols. Curr Opin Chem Biol. 2011; 15:103–112. [PubMed: 21216657]

69. Go YM, Jones DP. Thiol/disulfide redox states in signaling and sensing. Crit Rev Biochem Mol Biol. 2013; 48:173–181. [PubMed: 23356510]

70. Hung YP, Albeck JG, Tantama M, Yellen G. Imaging cytosolic NADH-NAD(+) redox state with a genetically encoded fluorescent biosensor. Cell Metab. 2011; 14:545–554. [PubMed: 21982714]

71. Love NR, Chen Y, Ishibashi S, Kritsiligkou P, Lea R, Koh Y, Gallop JL, Dorey K, Amaya E. Amputation-induced reactive oxygen species are required for successful Xenopus tadpole tail regeneration. Nat Cell Biol. 2013; 15:222–228. [PubMed: 23314862]

72. Knoefler D, Thamsen M, Koniczek M, Niemuth NJ, Diederich AK, Jakob U. Quantitative in vivo redox sensors uncover oxidative stress as an early event in life. Mol Cell. 2012; 47:767–776. [PubMed: 22819323]

73. Jones DP. Redox potential of GSH/GSSG couple: assay and biological significance. Methods Enzymol. 2002; 348:93–112. [PubMed: 11885298]

74. Jones DP. Redox theory of aging. Redox Biol. 2015; 5:71–79. [PubMed: 25863726]

75. Wild CP. Complementing the genome with an exposome: the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev. 2005; 14:1847–1850. [PubMed: 16103423]

76. Go YM, Jones DP. Redox biology: interface of the exposome with the proteome, epigenome and genome. Redox Biol. 2014; 2:358–360. [PubMed: 24563853]

77. Jones, DP.; Aw, TY. Use of Enzymes and Transport Systems as in Situ Probes of Metabolite and Ion Gradients. Eiley-Liss; New York: 1989.

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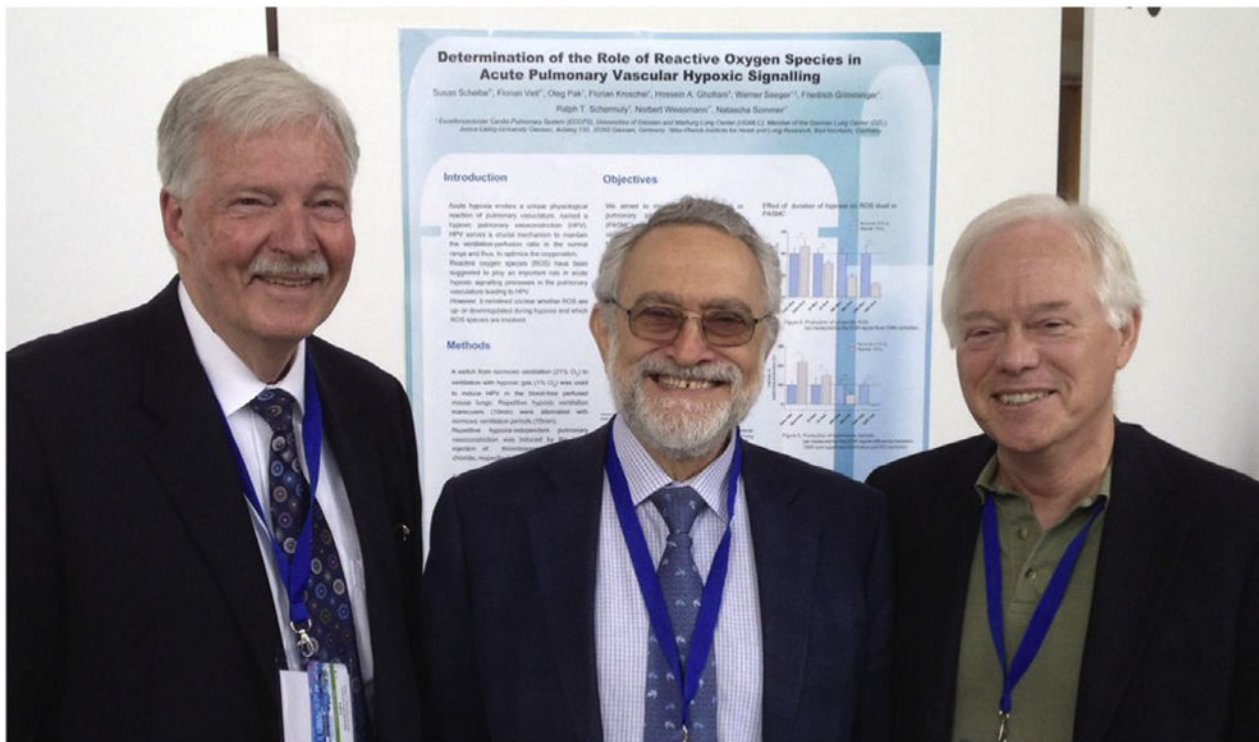Author Manuscript

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Fig. 1. Legendary Oxystat Trio
Author with Professor Helmut Sies (Left) and Professor Salvador Moncada (Center)
following Professor Moncada's keynote presentation at the 2015 Oxygen Club of California
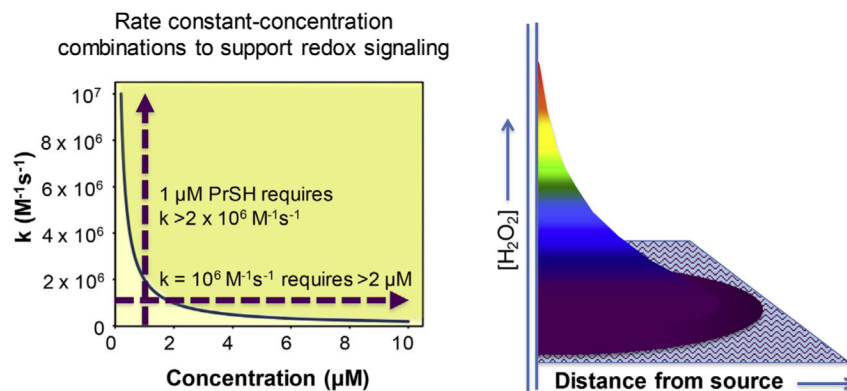meeting in Valencia, Spain.

Fig. 2. A limited range of rate constant-concentration combinations can support localized redox signaling

Based upon empirical data showing that solute heterogeneity (C/C) occurs in volumes with turnover >2 s^{-1} [18,77], local gradients occur for a system with $v = $ k[PrSH][H$_2$O$_2$] only when rate constant (k) x protein concentration (PrSH) are in the darker yellow region of the left panel; for details of calculation, see Figure 4 of [64]. Such conditions may occur with activation of H$_2$O$_2$ generation by NADPH oxidases in association with signaling complexes. A schematic depiction of the diffusion/consumption gradient is shown on the right, where the magnitude of the gradient is largely determined by the site-specific H$_2$O$_2$ production rate.