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Redox signaling: an evolution from free radicals to aging

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Abstract

Redox biology has evolved from studies of the pathology that involves oxidants to an understanding of how our oxidants participate in normal as well as aberrant signal transduction. Although the concept that signal transduction involved changes in the redox state dates from the 1930s, the modern history of redox biology began with the discovery of superoxide dismutase by McCord and Fridovich. The initial focus was on free radicals and damage of macromolecules, which remains an important topic. But, over time it was realized that hydroperoxides, especially H_2O_2 produced by NADPH oxidases, and electrophiles derived from lipid peroxidation or metabolism, played essential roles in physiologically relevant signaling. The mechanisms through which H_2O_2 and other electrophiles signal became an important area of study that provided insight into how these reactive molecules were involved in major signaling pathways and regulation of transcription factors. Thus, the field of redox signaling that is the overlap of signal transduction with redox biology was established. Alterations in redox signaling are observed in aging, but we also now know that redox signaling is essential in physiological homeostasis and that sustained deviation from redox homeostasis results in disease. This is a review of the history of redox biology from a personal perspective of nearly fifty years working in this field that hopefully provides some insights for the reader.

Keywords

oxidative stress; redox signaling; superoxide; hydrogen peroxide; NADPH oxidase; AP-1; Nrf2; aging; homeostasis; antioxidant; air pollution; thiolate; glutathione

Introduction

From the time of Joseph Priestly and the other co-discoverers of oxygen in the eighteenth century, the potential toxicity of this essential gas has been known. But, understanding of the mechanism did not advance significantly until the 1950s when Rebecca Gerschman and coworkers noted that oxygen poisoning resembled the damage from X-rays and proposed that free radicals were involved [1]. Shortly after, Denham Harman proposed that aging could also involve free radicals based upon radiation chemistry [2]. The modern history of

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redox biology however, began with the discovery by Joe McCord and Irwin Fridovich of superoxide dismutase [3].

I was fortunate to become a postdoctoral fellow in the Fridovich laboratory at a time when the field, then known as free radical biology, was just beginning to expand. It is a very good thing to be in the right place at the right time. It is also good to be inspired by brilliant minds. My graduate mentor, Philip Feigelson, my postdoctoral mentor, Irwin Fridovich, and the person who gave me the opportunity to begin my independent career, Aron Fisher, are the scientists who most influenced my career.

Albert Szent-Gyorgyi, the Nobel Prize winning discoverer of vitamin C [4], proposed the involvement of free radicals in biological systems well before the discovery of superoxide dismutase [5]. Szent-Gyorgyi often quoted the statement, attributed to both Schopenhauer and Johnathan Swift in various forms, that, "Discovery consists of seeing what everybody has seen and thinking what nobody has thought." That this statement itself evolved provides an example of what underlies most of the breakthroughs in science. We all depend upon what came before, and make advances by seeing things in different ways with the assistance of technologies that our predecessors did not have. Likewise, my contributions have depended on the work of many others in the field of redox biology, some of whom I have had the privilege to work with directly. They are listed at the end of this review. The lesson learned from the words of Szent-Gyorgyi that influenced my career is to keep your eyes and mind open.

Superoxide as a product or intermediate in enzymatic reactions – free radical biology begins Irwin Fridovich suggested that xanthine oxidase produced superoxide as an intermediate in the oxidation of sulfite [6], eight years before he and his student, Joe McCord discovered superoxide dismutase (SOD) [3]. In 1968, as a graduate student investigating the catalytic mechanism of tryptophan oxygenase with Philip Feigelson at Columbia University, we proposed that the enzyme formed a ferric heme-superoxide intermediate that did not release superoxide from the enzyme [7]. This began my interest in free radicals and I fell under the influence of the old work of Szent-Gyorgyi. About that time, Frank Brady, who had done his graduate work at Duke University with Philip Handler and K.V. Rajagopalan in the laboratory next to the Fridovich lab, joined the Feigelson laboratory. Frank introduced us to the concept of using superoxide dismutase as a tool to study biological oxidations [8]. He also introduced me to Irwin Fridovich. Frank passed away at a relatively early age, but he had a great positive and lasting influence on those who knew him.

Philip Feigelson *Irwin Fridovich*

The Spring of 1968, my second semester in graduate school, was tumultuous. Early in the semester, a riot broke out and the police occupied the Columbia University campus where we attended most of our classes. In April, Martin Luther King was assassinated and riots broke out in several cities. During the summer of 1968, Robert Kennedy was assassinated. Not long after, a riot broke out outside the Democratic convention in Chicago. For a liberal New Yorker, it was a time that becoming radical could have happened easily. Fortunately, free radical biology was the clear choice over radical politics.

Superoxide dismutase

In 1969, Joe McCord and Irwin Fridovich published their classic paper on the discovery that erythrocuprein had SOD activity [3]. This discovery and the burst of publications from the Fridovich laboratory over the next few years (summarized in [9][10], provided a foundation for the field. A postdoctoral fellowship from the National Institutes of Health allowed me to work with Irwin and resulted in four publications [11][12][13][14]. These papers included determining the active site histidines in SOD1, the rate constants for different SODs, and the role of zinc in SOD 1. The findings regarding zinc in structural stability of SOD1 were cited fourteen years later in a ground-breaking paper suggesting a role for abnormal zinc binding in SOD1 that causes the enzyme to gain a nitration catalysis function and thereby participate in the pathology of amyotrophic lateral sclerosis [15]. The environment in the Fridovich laboratory was wonderful. It taught me that one should always try to take advantage of being in a place where new development is occurring.

Mitochondrial superoxide production

The budget consequences of the Vietnam War had a devastating economic impact on science. Under the circumstances, the best position offered was a Research Associate position in Kansas City. Without dwelling on how, at the age of twenty-six I nearly left academia, thanks to James Kennedy a position at the Veterans Administration Hospital, which led to a Research Assistant Professor appointment at the University of Kansas Medical Center developed. While at the VA, I began work with James on pyrimidine biosynthesis, which unexpectedly led to one of my most fortunate and important findings.

In the early 1970s, Alberto Boveris, Nozumo Oshino, and Britton Chance described the production of H_2O_2 by mitochondria [16]. In 1974, Loschen, Azzi, Richter and Flohe, discovered that succinate oxidation in mitochondria produced $O_2^{\cdot-}$ and suggested that it was the source of the H_2O_2 [17]. At almost the same time, we discovered that the oxidation of dihydroorotate, which is the one step in pyrimidine biosynthesis that occurs in mitochondria, also produced $O_2^{\cdot-}$ [18]. But, the more important discovery in that study was that the reaction producing $O_2^{\cdot-}$ was thermodynamically unfavorable and actually pulled forward by SOD. We did not know what the exact reaction was that produced $O_2^{\cdot-}$, but soon after a debate began about cytochrome b versus ubiquinone as the source. Work by Enrique Cadenas and Alberto Boveris [19] strongly indicated that ubisemiquinone oxidation was the source of $O_2^{\cdot-}$. Christine Winterbourn showed that the reaction of semiquinones with oxygen to produce $O_2^{\cdot-}$ was thermodynamically unfavorable and that SOD could pull the reaction forward [20]. This work explained our previous finding that SOD promoted the production of $O_2^{\cdot-}$ in which $Q^{\cdot-} + O_2 \rightleftharpoons Q + O_2^{\cdot-}$ is displaced to the right by SOD in the reaction $2O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$. As later pointed out by Forman and Azzi, this results in mitochondrial $O_2^{\cdot-}$ concentration being negligible [21]. Only a reaction with a rate constant competitive with the near diffusion limited rate of SOD, such as the reaction of $\cdot NO$ with $O_2^{\cdot-}$ to produce peroxynitrite [22], would be likely to involve $O_2^{\cdot-}$ directly.

Alberto Boveris

Although work on mitochondrial H_2O_2 and $O_2^{\cdot-}$ production continued, and has had a resurgence in the last few years [23], my work in that area ended when the Biochemistry Study Section of the National Institutes of Health told me that, “Superoxide production by mitochondria is an uninteresting artefact.” Moving on, I always have wondered whether my project would have been funded if the “artefact” was more interesting to those reviewers.

Pulmonary oxygen and paraquat toxicity

As mentioned at the beginning of this article, the toxicity of oxygen has been known since its discovery when Lavoisier noted in 1783 that mice exposed to pure oxygen had damage to their lungs [24]. Probably the clearest example in terms of human experience is the toxicity of oxygen therapy for premature infants, which notably can cause lifelong consequences for lung function and blindness. But, for a century and a half, the underlying cause of oxygen toxicity remained unknown. While free radicals were proposed by Gerschman based on the resemblance to injury from X-rays [1], there was little direct evidence. Interest in the field increased during World War II when it was noticed that divers breathing pure oxygen had seizures. In 1945, Haugaard and coworkers however, demonstrated the loss of activity of enzymes after hyperbaric oxygen exposure [25].

Rats exposed to 100% oxygen at 1 atmosphere die within 3 days. Rats exposed for 5 days to 80% oxygen adapt and become able to survive indefinitely in 100% oxygen. In the 1970s, Crapo and Tierney [26], using this model of adaptation to hyperoxia, demonstrated that increased SOD activity in the lung correlated with adaptation to hyperoxia. About the same time, studies were being made of the toxicity of the herbicide, paraquat, which caused lethal lung damage through generation of $O_2^{\cdot-}$ continuously [27]. In 1978, I was recruited to the University of Pennsylvania by Aron Fisher, who introduced me to pulmonary physiology and toxicology. Investigating how different cells in the lungs of adapting rats changed in the activities of SOD1 and SOD2 along with other enzymes that contributed to antioxidant defense, we found that the cells that appeared to be most responsive in terms of increasing these activities were the granular type II epithelial cells [28]. With Tom Aldrich, we also explored how paraquat accumulates specifically into those cells making type II epithelial cells the target of the toxicity [29]. It was interesting to observe that the cells that adapted best to hyperoxia were the specific target of paraquat.

Aron B. Fisher

Phagocytic superoxide production

The signaling pathway and function we chose to focus on was the respiratory burst of alveolar macrophages. Bernie Babior discovered in 1973 that phagocytes generated $O_2^{\cdot-}$ [30]. We began our studies looking at how paraquat might cause loss of the ability of macrophages to produce $O_2^{\cdot-}$ when stimulated. Through serendipity and the careful notebook keeping of June Nelson, we found that in the absence of glucose, paraquat depleted the substrate, NADPH, for the phagocyte oxidase and only later became toxic to the cells in the presence of glucose [31]. But, paraquat did not deplete NADH. Babior learned about our results and said that there was an ongoing debate about whether NADPH or NADH was used by the phagocyte oxidase and that our work on toxicity contributed to resolution of this debate in favor of NADPH. Sometimes one gets very lucky.

Bernard M. Babior

In the 1980s free radical biological research was largely focused on mechanisms of cell injury and death. When I moved into my first fully independent position, we laboratory began to study the mechanisms whereby oxidants cause inhibition of signaling, as we observed changes in macrophage functions in exposure to hyperoxia and low exogenous concentrations of hydroperoxides both in vitro and in vivo without cell death [32,33]. This was important because we get sick more often than we die! Furthermore, if one is going to use in vitro models, it is essential needed to avoid using “the thermonuclear attack model of toxicology” in which cells die from doses of added hydroperoxides that has no resemblance to actual physiological or even pathological conditions.

Glutathione biosynthesis

Glutathione (GSH), γ -L-glutamyl-L-cysteinylglycine, is the reducing substrate for the glutathione peroxidases and peroxiredoxin 6 in removal of hydroperoxides, and for the glutathione S-transferases that help remove toxicants as well as participate in leukotriene C4 synthesis. GSH is synthesized and degraded in a cycle that was described by Alton Meister [34]. Helmut Sies, who coined the term oxidative stress [35], suggested that GSH oxidation to the disulfide (GSSG) and formation of mixed protein-glutathione disulfides was the essential determinant of that stress. Research in our laboratory on altered cell function led us into studies of glutathione redox cycling, primarily by Mark Sutherland, that were cited recently [36] in an issue of Archives of Biochemistry and Biophysics dedicated to Helmut Sies upon his retirement as Editor in Chief of the journal.

Leopold Floh Fulvio Ursini Helmut Sies

An increase in total GSH plus GSSG (GSH is almost all in the GSH form in unstressed cells) occurred in many situations where cells or animals were exposed to sublethal oxidative stress. We decided to pursue the question of how this occurred. Using redox cycling quinones, menadione that could be conjugated to GSH and another, 1,2-dimethoxy-naphthoquinone that could only redox cycle, Ming Shi, Li Tian, Rui-Ming, and Amir Kugelman demonstrated that both catalytic and modulatory subunits of the first enzyme in de novo GSH biosynthesis, glutamate cysteine ligase (GCL, also known as γ -glutamylcysteine synthetase) could be transcriptionally induced by mild oxidative stress [37–39]. We also showed that γ -glutamyltranspeptidase, which is involved in GSH recycling was also transcriptionally inducible by quinones [40].

The studies of GSH biosynthesis began at the Institute for Toxicology at the University of Southern California. Paul Hochstein, Alex Sevanian, Kelvin Davies, Enrique Cadenas, Joseph Landolph and others in this group that focused on free radical biology provided an outstanding environment for our work. I also was fortunate to become the Head of the Cell Biology Group at Children's Hospital of Los Angeles, where we collaborated with Thomas Coates and Martine Torres among others. The latter became my main collaborator for many years until her retirement. Martine, an expert in signal transduction, influenced much of my thinking on the roles of oxidants in signaling. Retaining relationships over decades has been of great value to my career. With Tom Coates, we are currently looking at how sickle cell disease alters redox homeostasis in blood, and as described below, we currently collaborate with Kelvin Davies on studies of aging.

Alex Sevanian Kelvin Davies Enrique Cadenas Martine Torres

Redox signaling

In the 1930s Warburg and Szent-Gyorgyi proposed that redox reactions regulate cell function, but tools for studying this were absent as was the understanding that $O_2^{\cdot -}$ and H_2O_2 were generated in cells. In the 1970s a few studies showed that H_2O_2 could mimic insulin [41,42], but the mechanisms were unknown. Beginning in the late 1980s studies showed that exogenous H_2O_2 could modulate multiple signaling pathways, but specific targets were not clear with a few notable exceptions. Gopalakrishna and Anderson showed that classical protein kinase C activity could be elevated by H_2O_2 in the absence of calcium

[43], while Lands and others showed that a lag in the activation of cyclooxygenase [44] and 5-lipoxygenase [45] was abolished by low concentrations of H_2O_2 . Lands coined the term “peroxide tone” and suggested that the production of eicosanoids was regulated by the endogenous level of H_2O_2 in cells.

But, almost all the early work on the activation of cell signaling by H_2O_2 was done through addition of H_2O_2 to cells. This included our own work on the respiratory burst of alveolar macrophages [46]. In those studies, we had switched from looking at how hydroperoxides inhibited the respiratory burst without killing the cells to investigating the mechanism underlying an serendipitous discovery that low concentrations of hydroperoxides increased subsequent stimulation of the respiratory burst [47], which we eventually found was due to an elevation of intracellular calcium [48] and activation of phosphatidylcholine specific phospholipase C (PC-PLC) [49]. This work was carried out by two graduate students, Judith Murphy and Carolyn Hoyal, and Julio Giron-Calle, a postdoc.

One of the most interesting and influential studies of signaling caused by the addition of exogenous H_2O_2 was the activation of the NF- κ B transcription factor [50]. Realizing that our experimental model cells, macrophages, generated H_2O_2 upon stimulation, my colleague Martine Torres and I wondered if the amount of H_2O_2 generated would be sufficient to activate NF- κ B in these cells. It was [51]. Working in collaboration with Rayadu Gopalakhrisna, Nalini Kaul from our laboratory found that the activation of NF- κ B by endogenously produced H_2O_2 was due to the activation of protein kinase C [52]. We also showed that increased tyrosine phosphorylation of many proteins and activation of the ERK pathway by stimulants of the respiratory burst in macrophages was H_2O_2 dependent [53]. At the time we discovered this, it was widely believed that the only cells that produced H_2O_2 in response to stimulation were phagocytes. Thus, despite our being able to publish this work and even get funded by the NIH to study it, the activation of signaling by endogenously generated H_2O_2 was considered an oddity of macrophages and other phagocytes. That changed dramatically in 1999 when David Lambeth and coworkers discovered that other cells had a related NADPH oxidase [54] and then that there were seven mammalian NADPH oxidases labeled as NOXs and DuOXs [55] distributed in every cell type. With that discovery, the field of redox signaling now clearly had identified a major source in all cell types of H_2O_2 produced upon stimulation. While mitochondria, other organelles and some oxidoreductases generate H_2O_2 , it remains to be determined whether anything other than the NOX and DuOX proteins are truly involved in stimulated signaling. The work on redox signaling resulted in one of the first reviews [56] and two edited books on the topic [57,58]. Going back to the suggestion of Lands regarding peroxide tone [59], it seems the lipoxygenases and cyclooxygenases have the best chance of fitting that description by providing lipid hydroperoxides in response to stimulation. That hydroperoxides rather than other so-called reactive oxygen species are the mediators of signaling has been addressed in detail in recent reviews [60,61].

*1996 USC lab –top from left Rui-Ming Liu
Li Tian, Huanfang Zhou, HJ Forman, Tim
Robison, Chang-Jun Yue. Bottom from left –
Carolyn Hoyal, Nalini Kaul, and Jinah
Choi.*

In 1999, we moved to the University of Alabama at Birmingham where I became the Chair of the Department of Environmental Health Sciences and joined the Center for Free Radical Biology headed by Bruce Freeman and Victor Darley-USmar. It was fortunate that almost all of the graduate students and postdocs in my laboratory at USC moved to UAB. Both USC and UAB were great environments in which we interacted with some of the best investigators in the free radical biology field.

At UAB, we continued to investigate how H_2O_2 generated by cells acts as a signaling molecule, particularly on the activation of the AP-1 transcription factor [62]. Ichijo and colleagues demonstrated that oxidation of thioredoxin (Trx) by addition of H_2O_2 to cells activated ASK1, a kinase that is upstream of both p38MAPK and JNK [63]. Honglei Liu, a postdoc in our laboratory, showed that the activation of H_2O_2 production in macrophages led to activation of the JNK pathway through the Trx mediated activation of ASK1 [64]. Subsequently, Im and coworkers showed that the oxidation of Trx in this system is through the activity of peroxiredoxin [65].

Mechanisms of signaling by electrophiles

The non-enzymatic oxidation of polyunsaturated lipids leads to the production of a large variety of products. Hermann Esterbauer was the discoverer of 4-hydroxy-2-nonenal (HNE), one of the more abundant and biologically active products [66,67]. Along with Dianzani, Comporti, Poli, Slater, and Esterbauer showed that HNE appeared to be responsible for pathology of several diseases [68–71]. Poli then demonstrated that low concentrations of HNE could act as stimulants of signaling [72–74]. We first learned about HNE when we began to examine the mechanism through which exposure to the atmospheric pollutant, nitrogen dioxide, caused loss of macrophage function. Working with Michael J. Thomas, Tim Robison and I showed that NO_2 exposure of macrophages resulted in the formation of many aldehydes, and that HNE was the most abundant [75]. Then Rui-Ming Liu, Dale

Dickinson, Karen Iles, and Hongqiao Zhang showed that HNE could induce GSH biosynthesis through transcriptional elevation of GCL and GGT gene expression [76,77]. Expression of GCL genes appeared to be under the control of the AP-1 transcription factor [78].

Giuseppe Poli

2001 UAB lab –back row from left Hongqiao Zhang, Charles Venglarik, Dale Dickinson, David Krzywanski, Amanda Wigley, Julio Giron-Calle. Front row from left- Nabuo Watanabe, Karen Iles, Xue Zhang, Rui-Ming Liu, and Henry Jay Forman

It was becoming clear that while generation of H_2O_2 could induce GCL, other electrophiles were actually stronger inducers. Nrf2 is the transcription factor that appears to be a master rheostat in transcriptional regulation of a large number of enzymes involved in protection of cells from stress. The regulatory element, first called the antioxidant response element (ARE) was discovered by Cecil Pickett [79]. Shortly after, a more accurate name for these group of related sequences, the electrophile response element (EpRE) was supplied by

Violet Daniel [80]. Around the time we began to look at HNE-induced GCL expression, Tim Mulcahy demonstrated that the two subunits of GCL were regulated by Nrf2 in response to electrophiles [81,82]. So, we examined whether the electrophiles, HNE, curcumin, and 15-deoxy- 12,14-prostaglandin J2, the latter in collaboration with Anna-Liisa Levonen and Victor Darley-USmar, induced GCL gene expression through Nrf2 [83–86]. What was clear from our studies and those of others was that the GCL genes are dually regulated by both AP-1 and Nrf2 [84].

In 2003, we moved to the new University of California campus that was being built in Merced, a small city in the agricultural heartland of the Central Valley of California. Once again, several members of the UAB laboratory made the move to UC Merced. There, surrounded by cows, we continued studies on how both hydroperoxides and HNE activated the ERK, JNK pathways.

Two new postdocs, Alessandra Rinna and Smadar Levy joined the lab along with a graduate student, Chris Mahaffey. Smadar discovered that c-Myc played a role in regulation of Nrf2 by apparently binding to Nrf2 in the Nrf2-EpRE complex and thereby inhibiting transcription of several phase II genes. Moreover, c-Myc also appeared to cause degradation of the nuclear pool of Nrf2 [87]. Chris showed that HNE could induce multidrug resistance protein 3 through activation of Nrf2 in human bronchial cells and non-small cell lung carcinoma cell lines, where many of the latter have defective Keap1 that causes constitutively high MRP3 expression [88][89].

2004 UCM lab –from left Alessandra Rinna, Christopher Mahaffey, Gayatri Premasekharan, Smadar Levy, Jinah Choi, Henry Jay Forman, and Hongqiao Zhang.

Alessandra demonstrated that activation of H₂O₂ production caused transient inactivation of protein tyrosine phosphatase 1B (PTP1B) through glutathionylation stimulated by the endogenous generation of H₂O₂ [90]. PTP1B is the enzyme that prohibits activation of the ERK pathway by dephosphorylating Raf1. Another of her studies demonstrated that HNE could activate the JNK pathway through inhibition of the protein tyrosine phosphatase, SHP-1 [91].

Studies on the pathophysiological role of HNE have continued in a collaboration with Giuseppe Valacchi [92,93]. We first met on the tennis courts of the University of California at Davis when Giuseppe was a postdoc in Carroll Cross's laboratory.

Air pollution

Collaborations with people at UC Davies led to development of a joint training program with UC Merced in air pollution. During this time, I was appointed by the Governor of California to the Governing Board of the San Joaquin Valley Air Pollution Control District. Meeting a movie star and the niece of a president, Arnold Schwarzenegger and Maria Shriver was nice, but the appointment was a unique opportunity to see how research could be helpful in formulating policy that affects the health of the population.

Over many decades, there have been a great many studies on the effect of airborne particulates and manufactured nanoparticles on health [94,95]. In terms of free radical biology, some of the most notable work has been done by Andre Nel and coworkers [96]. Studies by Andrij Holian on silica induced toxicity interested us as well [97]. At UC Merced, Honglei Liu and Hongqiao Zhang began our studies of the mechanism through which respirable silica activates the production of pro-inflammatory cytokines. Their studies implicated activation of PC-PLC in the signaling [98]. Gayatri Premasekharan, a graduate student working in materials science with Valerie Leppert, took this work further by demonstrating an essential role for surface iron and the role of lipid raft disruption in the mechanism [99].

Aging

In 1999, Kelvin Davies asked me if I was interested in returning to the University of Southern California and offered to share space in his laboratory in the Leonard Davis School of Gerontology. For the following six years, my laboratory was at USC, while all of my teaching responsibilities remained at UC Merced. This was an undergraduate organic chemistry and a graduate level course in signal transduction.

Hongqiao Zhang and Honglei Liu, who moved from UAB to UC Merced also moved with the laboratory to USC. A very positive part of coming back to USC was the opportunity to collaborate with Kelvin along with Caleb (Tuck) Finch, leaders in aging research. USC is also the home of the Southern California Environmental Health Sciences Center, where my lab can interact with many experts in air pollution, including Costas Sioutas and Frank Gilliland. In 2015, I became a Distinguished Emeritus Professor of Biochemistry at UC Merced and the next day went to USC to my current position, which involves almost full time research.

At USC, we began a collaboration with Davies and Finch to try to understand the mechanisms that cause increased susceptibility to the deleterious consequences of air pollution in aging. We suspected that this involved a decrease in responsiveness of Nrf2 signaling that had been reported by Tory Hagen and my former postdoc and colleague, Rui-Ming Liu [100]. Our studies demonstrated that the age-related loss in Nrf2-regulated transcription due to chronic exposure to air pollution was observed not only in lungs, but in the cerebellum and liver as well [101]. The results also suggested that an increase during aging in c-Myc and Bach 1, another inhibitor of Nrf2 transcription, may be responsible for the loss of inducibility of Nrf2-regulated genes [101]. This work is currently being pursued

in our laboratory. We continue also to collaborate with colleagues at UC Merced, particularly Valerie Leppert and Peggy O'Day, who are analyzing the surface chemistry of air pollution nanoparticles and synthesizing model nanoparticles to allow for a more uniform material for the mechanistic studies we pursue at USC. Our work on signaling in which H_2O_2 production by cells is involved is illustrated in Figure 1.

Redox signaling chemistry and redox homeostasis

In 1990, Fulvio Ursini and Matilde Maiorino invited me to come to the University of Padova for a month long visit. The purpose was to begin a collaboration on the effect of hydroperoxides on signaling. Over the past 26 years, the collaboration has evolved, as did the field, toward attempting to understand how hydroperoxides work as signaling molecules, understanding how antioxidants work, and how redox signaling contributes to homeostasis. Too many people have contributed significantly to these areas to mention all of them here. But, the work of many of those contributors are cited in our recent articles.

In two reviews [60,61], we have argued that among the so-called reactive oxygen species, only H_2O_2 and other hydroperoxides fulfill the requirements for functioning as a second messenger. The important points we made in these reviews were based on understanding of kinetic constraints that are frequently ignored. The targets for hydroperoxide signaling are protein cysteine in their thiolate form ($-\text{S}^-$). But, non-enzymatic oxidation of thiolates by H_2O_2 is, in most cases, too slow to be involved in signaling. Indeed, the rate of non-enzymatic oxidation of GS^- , which is 10^5 times as great as the protein thiolate. Thus, the intermediate formation of a sulfenic acid through oxidation of a protein thiolate must involve enzymatic catalysis of thiol oxidation.

In another review with Kelvin Davies [102], we described how the kinetics of antioxidant defenses requires that, with the exception of vitamin E scavenging of hydroperoxyl radicals, rules out a role for scavenging of oxidants including free radicals by non-enzymatic mechanisms. Instead, enzymes with fast rate constants remove superoxide and hydroperoxides. This then prevents formation of hydroxyl radical, which no enzyme or small molecule can do efficiently as $\cdot\text{OH}$ reacts with all molecules with rate constants near the limit of diffusion. The likely reason why many dietary compounds, including isocyanates and flavonols that have been called antioxidants work is because they or their metabolites can alkylate Keap1. By activating Nrf2, transcription of many antioxidant enzymes as well as the enzymes that provide the substrates for the antioxidant enzymes are elevated. This maintains a nucleophilic tone in cells. This brings the discussion back to Albert Szent-Gyorgyi. In 1936, he proposed that the dietary phytochemicals, flavonols, should be called vitamin P foreshadowing again an important component of redox biology [103]. Indeed, as with other vitamins, it is difficult to demonstrate any positive effect for health with high doses. It remains to be seen whether an insufficiency of electrophiles in the diet (if that can actually be achieved without general malnutrition) would reveal that they are indeed vitamins.

Our most recent publication [104] concerns the maintenance of redox homeostasis, which involves signaling by electrophiles including HNE along with hydroperoxide signaling to

maintain nucleophilic tone. The principle arguments we made, besides reiterating what we described in the prior reviews, are: that redox signaling acts as a rheostat in a continuous dynamic process of oxidant production balanced by reduction rather than an on-off switch; redox signaling is determined by reaction kinetics and does not rely on reaching thermodynamically defined ratios; redox signaling involves reactions of specific electrophiles with specific protein thiolates, primarily through enzyme catalyzed reactions; and that challenges to redox homeostasis generally stay within the bounds of a physiological defined range of electrophiles and nucleophiles, but that a condition of prolonged and/or enhanced exposure to environmental stressors in either the oxidized (pro-inflammatory) or reductive direction results in a new steady state that is abnormal. Thus, while adaptation may preserve function, it results a shift away from physiological redox homeostasis.

Key publications in the field

Table 1 is a biased list of key publications in the field. These are some of the very many key citations that influenced me. Citations on nitric oxide have been included in this table although not described in the text. This is because the author worked in this field only as a collaborator, and had the pleasure of Lou Ignarro as a frequent visitor during a period of several years when a group of LA radicals met in his home for monthly chalk talks. Studies on H₂S and other important areas are not cited. While that work certainly influenced my thinking, I did not want to have thousands of references here. My Mendeley database has over 10,000 entries, most of which could have been included. But, I warned you that this was biased! The table ends a decade ago because history has taught me that one needs the perspective of time to see what is the most significant work.

Summary of lessons learned

Keep your eyes and mind open.

Take advantage of being in a place where new development is taking place.

Study mechanisms rather than phenomena.

Use experimental designs that mimic reality rather than produce spectacular results.

Thermodynamics predict what can happen eventually, but kinetics tell us what is happening.

Retain good relationships with your colleagues when you move. It isn't true in science that you can't go home again.

Acknowledgments

As stated at the beginning, my contributions to the field of redox biology would not have occurred without the help of a great number of people. My primary mentors were Philip Feigelson, Irwin Fridovich, and Aron B. Fisher.

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Highlights

Free radical biology began with Szent-Gyorgyi and became modern with Fridovich.

First focus was damage and oxidative stress and sources, then alteration of signaling.

H₂O₂ is a second messenger used by peroxidases to oxidize signal protein thiolates.

Redox signaling pathways include tyrosine protein kinases and transcription factors.

Redox homeostasis involves Nrf2 activity, while aging causes decreased Nrf2 activation.

Figure 1. Studies on H₂O₂ signaling from the Forman laboratory

The studies are for the endogenous production of H₂O₂. H₂O₂ produced by Nox2 can activate Src kinases [105] and cause inactivation of PTP1B through glutathionylation [90]. Together the activation of a Src kinase and inactivation of PTP1B cause ERK activation [53]. H₂O₂ produced by Nox2 is also used to oxidize thioredoxin, which allows activation of the ASK1 to JNK pathway. Stimulation of Nox2 also leads to H₂O₂-dependent NF-κB activation [51]. H₂O₂ produced by redox cycling of quinones activates Nrf2 and Phase 2 gene expression [37]. Silica activates Nox2 generation of H₂O₂ that is then used in the Fenton reaction to initiate localized lipid peroxidation that results in lipid raft disruption, activation of PC-PLC, and NF-κB-dependent cytokine production.

Table 1

Key papers in the history of redox biology - a biased view.

Year	Key finding	Reference
1954	Gerschman and coworkers notes oxygen poisoning resembles X-ray damage and propose free radical involvement	[1]
1956	Harman proposes the free radical theory of aging	[2]
1958	Szent-Gyorgyi proposed involvement of free radicals in biological systems	[5]
1967	Esterbauer describes the production of 4-hydroxy-2-nonenal (HNE)	[66]
1969	McCord and Fridovich discover superoxide dismutase	[3]
1970	Meister describes the γ -glutamyl cycle in glutathione biosynthesis	[34]
1972	Boveris Oshino, and Chance discover production of H_2O_2 by mitochondria	[16]
1973	Babior and coworkers demonstrate O_2 production by phagocytes	[30]
1974	Two labs demonstrate O_2 production by mitochondrial respiratory chain	[17,18]
1974	Crapo and Tierney demonstrate adaptation to hyperoxia in rats correlates with increased superoxide dismutase	[26]
1976	Cadenas and Boveris show that ubisemiquinone oxidation is the source of mitochondrial $O_2^{\cdot-}$	[19]
1977	Murad demonstrates nitric oxide induction of cyclic GMP	[106]
1979	Ignarro demonstrates muscle relaxation by nitro compounds including NO	[107]
1981	Furchgott describes endothelial derived relaxing factor (EDRF)	[108]
1983	Lands demonstrates peroxide tone regulates cyclooxygenase	[44]
1984-1985	Comporti, Dianzani, Poli, Slater, and Esterbauer demonstrate pathology due to HNE	[66,67] [70]
1985	Sies defines oxidative stress	[35]
1987	Poli demonstrates that HNE acts as a stimulant of signaling	[72]
1989	Gopalakrishna and Anderson demonstrate H_2O_2 activation of classical protein kinase C	[43]
1991	Schreck and coworkers demonstrate NF- κ B activation by addition of exogenous H_2O_2 to lymphocytes	[50]
1991	Rushmore and Pickett discover the antioxidant response element (aka EpRE)	[79]
1994	Forman and coworkers demonstrate that quinones induce glutamate cysteine ligase (GCL)	[38]
1995	Mulcahy and coworkers show that EpRE regulates	[81]
1996	Kaul and Forman demonstrate activation of NF κ B by endogenously generated H_2O_2 in macrophages	[51]
1996	Venugopal and Jaiswal identify Nrf2 as regulator of EpRE	[109]
1998	Denu and Tanner demonstrate reversible inactivation of PTP1B by H_2O_2	[110]
1999	Lambeth and coworkers discover the NOX DuOX family of enzymes is found in almost all cells	[55]
1999	Chock demonstrates glutathionylation of PTP1B	[111]
2001	Kensler, Yamamoto and coworkers demonstrate link of Nrf2 deficiency to carcinogenesis	[112]
2002	Talalay, Yamamoto, and coworkers demonstrate Keap1 modification by electrophiles	[113]
2003	Nel and coworkers demonstrate a role for oxidants in signaling by air pollution particle exacerbation of asthma	[114]
2003	Yamamoto, Hayes and coworkers demonstrate Keap1 facilitates Nrf2 degradation	[115]

Year	Key finding	Reference
2004	Levonen, Darley-USmar and coworkers show HNE conjugates to Keap1	[116]
2004	Hagen and coworkers demonstrate loss of Nrf2 signaling in aging	[100]
2006	Forman and coworkers demonstrate selective and reversible PTP1B glutathionylation during signaling	[90]

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