

Oxidative Stress in Applied Basic Research
and Clinical Practice

Stephen M. Roberts
James P. Kehrer
Lars-Oliver Klotz *Editors*

Studies on Experimental Toxicology and Pharmacology

 Humana Press

Oxidative Stress in Applied Basic Research and Clinical Practice

Editor-in-Chief
Donald Armstrong

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Donald Armstrong
Editor-in-Chief

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Part I
Toxicological Mechanisms
and Evaluation of Oxidative Stress

Chapter 1

A Historical Perspective on Oxidative Stress and Intracellular Redox Control

Ethiene Castellucci Estevam, Muhammad Jawad Nasim, Lisa Faulstich, Marina Hakenesch, Torsten Burkholz, and Claus Jacob

1.1 Introduction

Most of us are familiar with the rather curious, popular expression that “History repeats itself”. Whilst this may well apply to certain economic cycles and swings in popular political opinion, it seems rather alien to most natural scientists who believe in Science as a unidirectional, ever evolving process. At closer inspection, however, we notice that there are also certain cycles in Science, and research into themes such as Oxidative Stress, intracellular redox processes and related preventive and therapeutic interventions in the form of “antioxidants” form no exception. The history of that particular field of research, which also represents the theme of this book, has witnessed many pioneering studies, leading to the true heydays of research, only to turn into a certain decline to be followed by yet another cycle of rise and fall. Ultimately, the term **Renaissance** not only applies to Italian culture, but also to research, and oxidative stress research even features its very own “Cysteine Chapel” [1, 2].

Indeed, just a couple of months ago, a rather enthusiastic piece has appeared in the journal *Biological Chemistry* entitled “Redox Biology on the rise”, apparently ushering in an entirely new era of Redox Biology [3]. Such rather graphic titles do, of course, raise some suspicion. Has Redox Biology only just begun to rise, as one may assume, and if so, where has it been before? What happened to the various previous redox related discoveries in Biology, such as oxidative phosphorylation, the various redox enzymes, free radical oxidative stress theories and glutathione measurements? Or how about everyday products, including the glucose oxidase-based glucose sensor used by diabetics and, on a more trivial note, the antioxidants

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we cherish as part of our daily food? Are these discoveries not “redox” or perhaps not “biology”, or just not “on the rise”? Or is the new Redox Biology just old wine in new barrels and the whole matter a cunning PR stunt?

As always, there is no direct answer to such a question, yet a look into the recent history may at least provide some ideas. As part of this chapter, we will therefore take a rather unusual, historical turn and in doing so, direct our attention to the various developments the field of Redox Biology and Biological Redox Chemistry has seen during the last couple of decades. Here, we will find a range of key discoveries which have subsequently led to – or at least influenced – basic concepts in this field which we still value and use today. In order to understand such historic processes fully, we will necessarily have to wear two hats, one of a redox chemist or biologist, and one of a historian of science.

Along the way, we will then dwell on key discoveries, such as the presence of free radicals in the body, or oxidative signaling, we will witness the appearance of new analytical methods, such as techniques for the detection of reactive oxygen species (ROS), and will see the rise, evolution and even demise of certain concepts (Fig. 1.1).

1.2 The Search for the Grandmaster

Our journey will start with a simple yet revealing question often asked by scientists and lawyers alike: “Who invented it?” A more naïve tactic to find a quick answer to this question, unfortunately rather popular those days, may involve the use of one of the many (scientific) search engines to see when “oxidative stress” first appeared on the scene, i.e. in the title of a listed publication.

Here, Web of Science delivers us a very early publication on “oxidative stress” by the Norwegian Svein Ore, published in 1955 in *Acta Chemica Scandinavica*, which unfortunately is on “oxidative stress relaxation of natural rubber vulcanized with di-*tertiary*-butyl peroxide” [4]. It is not worth following this lead any further, as comparing this kind of “oxidative stress” with our modern *medical* or *biological* concept of “oxidative stress” would be like comparing apples with horse apples.

Nonetheless, this rather surprising appearance of an homonym provides us with an early warning, namely that certain concepts may be rooted in different disciplines where they may have different meanings altogether, yet could be mutually stimulating once their details are understood correctly.

A more specific, refined search for the first appearance(s) of the expression “oxidative stress” in a biological context then brings us to the 1970s and to a range of publications. Here, we meet groups such as the one of Ernest Beutler (1928–2008), which consider links between glutathione, oxidative stress and wider aspects of metabolism [5]. Yet these studies deal primarily with individual, often rather limited aspects of oxidative stress research, and do not aim at any more general unifying concepts. Ernest Beutler, despite his considerable contribution to haematology and hence also to Redox Biology, is therefore not the one and only ‘inventor’ either.¹

¹Curiously, whilst Ernest Beutler never won the Nobel Prize for his groundbreaking work in the field of haematology, his son, Bruce Alan Beutler (*b.* 1957) shared the 2011 Nobel Prize in Physiology or Medicine for “discoveries concerning the activation of innate immunity”.

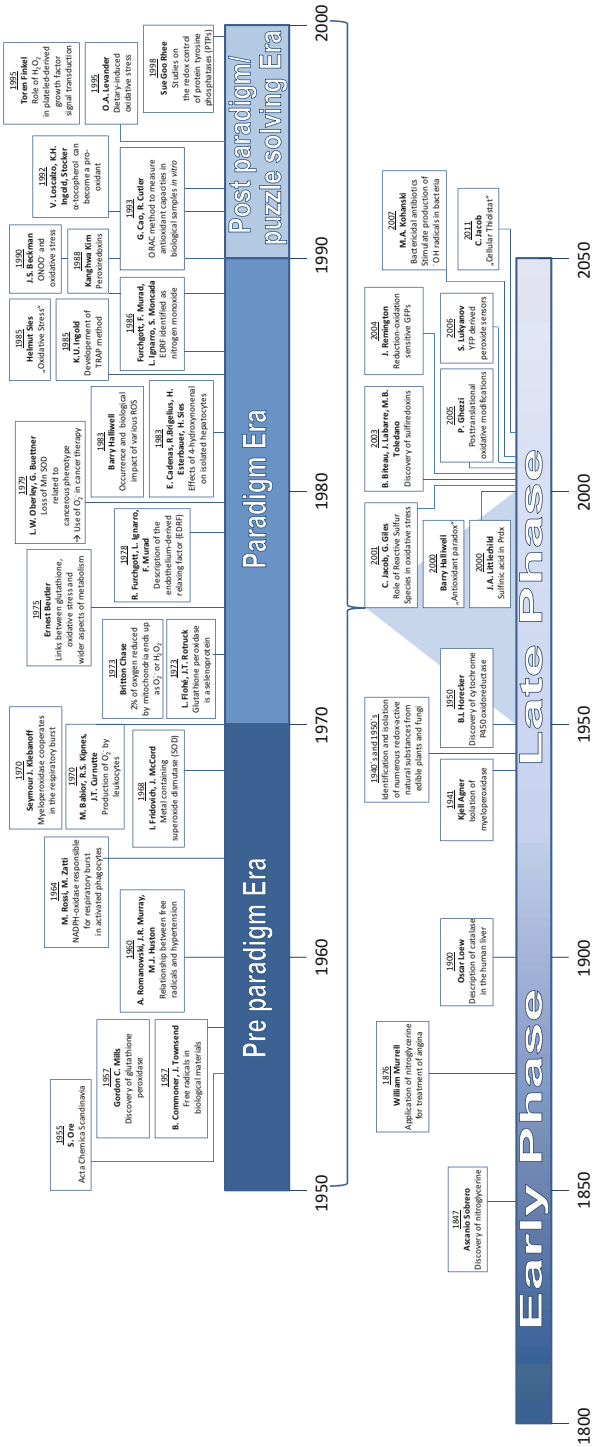


Fig. 1.1 Timeline of major findings in oxidative stress research. See text for details

Hence the simple attempt to illuminate the history of oxidative stress research and Redox Biology by using search engines tempts us to get lost in the mist of time. Here, another strategy is needed, this time based on contemporary witnesses. Indeed, when posing the question of the founding fathers and mothers of our field of research to more senior colleagues, the latter may contemplate for a moment and then point towards some of the early publications in the field of oxidative stress research they had come across at a time when we were a lot younger and also had a lot more hair, and which were “hot as a two-barrel shotgun”, as Bert Vallee tended to put it.

Following this lead, we soon identify such a landmark paper entitled “Free radicals in biological materials”. Authored by Barry Commoner and Jonathan Townsend and published in 1957 in *Nature*, this piece describes the widespread presence of (redox active) radical species in organisms [6]. Yet are Commoner and Townsend the founders of oxidative stress research, its true ‘inventors’? After all, their work on free radicals predates the concepts of Ernest Beutler by over a decade, although, admittedly, it comes *after* the use of the homonym “oxidative stress” by Svein Ore.

Or should we rather consider Britton Chase and colleagues as inventors, who in 1973 showed that about 2 % of oxygen reduced by mitochondria ends up as superoxide radicals or hydrogen peroxide (and not just water)? Or does this honour, after all, go to Helmut Sies, who in 1985 really coined the expression of “oxidative stress” in the book appropriately entitled “Oxidative Stress”? [7, 8]. At this point “oxidative stress” is no longer just a term with a new, modern meaning, but also a programme, a programme for an entire field of research (see below).

After the rather futile search for a key ‘inventor’ or ‘event’ of “oxidative stress” in the laboratory, literature or field, we must admit that the hunt for the one inventor, the grandmaster of our field of research, in the end is an illusion. It is nourished by the common belief that such fields are initiated by one or a few outstanding scientists who subsequently may – or may not – receive the Nobel Prize. As natural scientists, we must therefore depart from yet another of our most cherished ideas. We must face the hard reality that humans do not have a penis bone and that such fields of research do not have just one or a handful of enthusiastic inventors.

We must rather embrace the notion that such concepts have emerged from a range of different strands of investigation, which initially have perhaps been running in parallel in different disciplines for a long time, unaware of each other and with their very own language, terminology and experimental basis. Only much later, such seemingly separate strands of investigation may have come together to develop a common set of ideas and hypotheses which have then slowly crystallized into unified, interdisciplinary theories and perhaps even laid the basis for a true Kuhnian Paradigm (see below).

1.3 The Pre-paradigm Era

As part of our search for the more subtle strands of investigation, which may be seen as the tributaries to the concept of oxidative stress and redox signaling even without mentioning such concepts or terms explicitly, we have to go back further in History,

and to take a path which soon parts into several trails winding through different disciplines. These individual trails seem to lead us to several events during the first two-thirds of the twentieth Century. At this time, Redox Biology has not been “on the rise” but has already been “all around us”. For researchers investigating redox processes relevant to Biology, such as Otto Warburg, Oscar Loew and Chester Cavallito, the redox theme at the time held considerable promise, yet they were (pre-)occupied with their own research within their own disciplines. In retrospect, we can identify research into what we would nowadays call Redox Biology thriving at that time in various disciplines. This kind of research stretched from the exploration of cellular energy metabolism (e.g. fermentation, oxidative phosphorylation), human host defence (e.g. in form of ROS generating macrophages) and the appearance of free radicals during physical exercise to the presence of glutathione in animal cells, and the rather curious circumstance that the human body contains enzymes able to decompose ROS.

The antioxidant enzyme catalase, for instance, was described rather comprehensively by Oscar Loew (1844–1941) in 1900 and studied extensively afterwards. Considering that this enzyme occurs in the human liver and redox decomposes H_2O_2 , the thought of a particular role of H_2O_2 in human cells would not have been far-fetched, even during these early days of biochemistry. Nonetheless, it still took another couple of decades for researchers to realize that redox events associated with such ROS play an integral and important role in human biochemistry. These decades witnessed the identification of apparently damaging, oxidizing species in the human body (e.g. by Barry Commoner and Jonathan Townsend, 1957), as well as the discovery of additional “antioxidant” enzymes able to remove such reactive species, such as glutathione peroxidase (Gordon C. Mills, 1957) – now known to be a selenoenzyme – and the various metal-containing superoxide dismutases (SOD) by Irwin Fridovich and Joe McCord in 1968 [9–11].

Here it is interesting to highlight the discovery of cytochrome P450 oxidoreductase, originally described by Horecker in 1950 as cytochrome *c* reductase and finally recognized by Martin Klingenberg and David Garfinkel in 1958, later being exhaustively investigated by Jud Coon in the 1960s [12–15]. Many studies concerning P450 oxidoreductase could be cited, but we will highlight the description of the microsomal ethanol-oxidizing system (MEOS) by Lieber and DeCarli in 1968 [16–18].

In 1964 Filippo Rossi and Mario Zatti proposed that a Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase was responsible for the respiratory burst that occurred in activated phagocytes and in 1970 Seymour J. Klebanoff demonstrated that myeloperoxidase (isolated for the first time in the early 1940s by Kjell Agner) contributes to this burst [19–21].

At around the same time, and in an entirely different context, numerous redox active natural products with a pronounced biological activity were identified in and isolated from various plants, including edible ones, and, together with their synthetic analogues, were subsequently studied extensively regarding their biological activity. During this period, we find thriving research programmes into the chemistry and biology of various antioxidant vitamins, catechins, flavons, anthocyanidins, coumarins, aurones, tannins, resveratrol and related polyphenols, to mention just a few [22]. From the perspective of modern-day Redox Biology, the discovery of numerous biologically active Organic Sulfur Compounds (OSCs) in plants, fungi

and lower organisms is particularly noteworthy, as those compounds – later on – will play a major role as selective, yet also effective redox modulators. Many of these natural substances, such as allicin from garlic, were isolated and characterized in the 1940s and 1950s, i.e. well before their true impact as nutraceuticals became apparent [23]. Indeed, these early studies on redox active natural products have provided the basis – and substances – for emerging new fields of antioxidant and nutritional research, such as the ones dealing with “chemoprevention”, “functional foods”, “nutraceuticals” and “nutri-epigenetics”.

The various discoveries during the first six decades of the twentieth Century have provided a wealth of new insights in the field of redox chemistry and biology, but also new (natural) redox active substances and innovative new analytical methods to follow redox events, which together have paved the way for more comprehensive, systematic concepts.

1.4 The 1980s and the Emergence of More General Concepts

During the 1970s, the individual, mostly independent strands of investigation slowly began to interact and the first holistic concepts entered the scene, heralding a new era of concepts and paradigms.

Our first look to the 1970s will start with the publication by Bernard M. Babior, Ruby S. Kipnes and John T. Curnutte of the paper “The production by leukocytes of superoxide, a potential bactericidal agent”, which proposed that superoxide participates in bacterial killing and this could be associated with numerous alternative oxygen dependent mechanisms [24].

During the same decade, nitrogen monoxide (nitric oxide, *NO) became in evidence although its history *de facto* began already much earlier, in 1847, with the discovery of nitroglycerine by Ascanio Sobrero and its application for angina treatment by William Murrell in 1876. It was only in 1978, however, that endothelium-derived relaxing factor (EDRF) was described by Robert Furchgott and colleagues, and in 1986 identified as nitrogen monoxide (nitric oxide, *NO) by Furchgott, Ferid Murad, Louis Ignarro and Salvador Moncada² [25, 26]. After this, many other roles of nitric oxide in biological systems were studied, such as its function in activation, recruitment and aggregation of platelets at Joseph Loscalzo’s laboratory in 1989 [27–29].

After the discovery of nitric oxide as EDRF, the role of oxidative stress in the pathophysiology of hypertension and atherosclerosis became more evident, although the fact that the relationship between free radicals and hypertension had been suggested already in 1960 by Romanowski, Murray and Huston in a paper entitled “Effects of hydrogen peroxide on normal and hypertensive rats” [30]. In this context, the mode of action of nitroglycerin as an indirect donor of *NO, was finally understood

²Furchgott, Murad and Ignarro, but not Moncada, subsequently shared the 1998 Nobel Prize in Physiology or Medicine for this discovery.

[31–33]. Moreover, cytokine-activated macrophages produce high levels of $\cdot\text{NO}$ which leads to the destruction of targeted cells, such as tumor cells and bacteria [34].

At around the same time, in 1978, Dillard and his colleagues published their study on the “Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation” reaffirming the whole topic of physical stress, redox events, oxidation of biomolecules and subsequent physiological impediments [35]. Interestingly, this and related publications entering the scene during the 1970s possess a new quality: They reflect an emerging holistic approach, which is able to integrate basic (inorganic, radical) chemistry and biomolecule-chemistry with biochemical events and physiological changes. This new approach has many authors, and can also be found in the rather stimulating publication by well-known figures in the field of oxidative stress research, such as Enrique Cadenas, Regina Brigelius, Hermann Esterbauer and Helmut Sies, who in 1983 published together on the “Effects of 4-hydroxynonenal on isolated hepatocytes” [36]. Or we may consider the groundbreaking work of Barry Halliwell and his colleagues on the nature, occurrence and biological impact of various ROS [37]. The 1970s also witnessed the beginning of the studies of Leopold Flohé about the different functions of Glutathione Peroxidase (GPx) [38].

At this point we can see that research began to point to the various physiological roles of oxidative stress and the change in the concept that oxygen, nitrogen and other reactive species were the “bad guys”.

At the beginning of the 1980s, the time was therefore ripe for the emergence of new, more general paradigms in oxidative stress and redox signaling research – and the following decade may therefore be seen as the heydays in this field. In 1985, Helmut Sies published his book accurately entitled “Oxidative Stress” and in 1986 the manuscript “Biochemistry of Oxidative Stress” in *Angewandte Chemie*, pointing out that “This field of research provides new perspectives in biochemical pharmacology, toxicology, radiation biochemistry as well as pathophysiology” [7, 39].

Historically, these publications may well be considered as key events in oxidative stress research. Whilst oxidative stress and Redox Biology research itself were not *invented* at this point, and the authors mentioned above were the proverbial “dwarfs standing on the shoulders of giants”, these (and related) publications provided a *common basis for this field across disciplines*. This epistemological difference between inventing or discovering a phenomenon on one side, and inventing a new paradigm on the other, is important. The mid 1980s are the time when this highly interdisciplinary area of research began to consolidate as a separate field, with its own concepts, techniques, experimental methods, mutually accepted tools and, above all, language. As part of this Gestalt Switch, previous discoveries were integrated into the new concepts, revisited or reinterpreted under the new paradigm.³ It was also the time

³ Unfortunately, the role of language and terminology in science is often belittled, but their power should not be underestimated. The concept of phlogiston was dead once Lavoisier introduced his own language referring to oxidation and entirely removing the “P-word” from his vocabulary and journals, almost like Stalin had the image of Trotzky removed from official photographs. Or as some philosophers would say: “If you have no word for it, it does not exist.” While expressions

when researchers from vastly different disciplines, such as Wim Koppenol from inorganic chemistry, and Helmut Sies from biochemistry and physiological chemistry joined forces to provide the expertise for such a highly multidisciplinary adventure.

To supply new tools to this great adventure, Ingold and colleagues in 1985 proposed a quantitative method to measure the total secondary antioxidant content of a biological fluid known as TRAP method and later, in 1993, Guohua Cao and Richard Cutler developed the ORAC method (Oxygen Radical Absorbance Capacity) to assess antioxidant capacities in biological samples *in vitro* [40, 41].

When turning to the 1990s, the paradigms embracing “oxidative stress” and redox signaling in biology were therefore firmly established, analytical methods for the detection and quantification of ROS in biological samples were provided and the role of antioxidants as the “good guys” could be studied. There was also mounting evidence from clinical trials and epidemiological studies pointing towards a specific role of antioxidants in human health, which was subsequently used to discuss apparently healthy foods and opened up the field of nutraceuticals.

1.5 Puzzle Solving

In the words of Thomas Kuhn, the subsequent decades may therefore be characterized as the time when scientists were performing their “puzzle solving activities”, i.e. certain post-revolutionary mopping up exercises under the new paradigm(s) of Redox Biology. Here, we find various strands of investigation slowly meandering through their own fields, from bioinorganic and analytical chemistry (e.g. colleagues like Wim Koppenol) all the way to nutrition (e.g. Helmut Sies, Norbert Latruffe), medicine, cosmetics and even to Agriculture (e.g. Alan Slusarenko).

In the field of Redox Biology, we may highlight the rather pivotal research on redox control of the transcription factor NF- κ B (by H₂O₂) by Patrick Baeuerle and colleagues in the early 1990s [42–44]. These studies were reflected by the work by Toren Finkel and colleagues on the role of H₂O₂ in (platelet-derived growth factor) signal transduction, published from 1995 onwards [45–47]. Such early discoveries of redox controlled cell signaling events were followed by numerous studies on the redox control of key cellular signaling pathways, for instance by the landmark studies on the redox control of protein tyrosine phosphatases (PTPs) by Sue Goo Rhee and his colleagues [48, 49].

At this point we should mention peroxiredoxins (Prdx, discovered in 1988 by Kanghwa Kim and colleagues from the group of Sue Goo Rhee) and sulfiredoxins (first described by Benoit Biteau, Jean Labarre and Michael B. Toledano in 2003) and their role in signal transduction as well as regulation of post-translational glutathiolation that has been recognized as a means of redox-modulation of enzyme activities [50–54].

such as “oxidative stress” and “free radicals” ultimately ushered in a new era of research, other entities, such as the “caged radical” also appeared on the scene for a while to stimulate research but subsequently escaped from their cages into the mist of time.

1.6 Clouds on the Horizon

Towards the end of the 1990s, the field of oxidative stress research began to move on from ROS and antioxidant research to some of the more hidden redox regulatory events. At this point, the old paradigm defined in 1985, that “oxidative stress” is “a disturbance in the prooxidant/antioxidant balance in favor of the prooxidants, leading to potential damage” [7] was facing its first serious anomalies, clearly demanding a certain refinement.

The traditional view that ROS are entirely ‘bad’ and damage cells, whilst antioxidants, such as vitamin C or vitamin E are ‘good’ because they protect cells, came under pressure. First of all, it turned out that antioxidants were not nearly as efficient in preventing or even curing human ailments as early nutritional studies had suggested. In fact, it now seemed that an excessive use of antioxidants such as vitamin E or C may even cause damage (see below). Secondly, there was mounting evidence of widespread cellular redox signaling which involved beneficial, and not just detrimental, oxidative events and pathways. Indeed, it became more and more apparent that a wide range of cellular signaling events rely on the presence of oxidants, such as H_2O_2 , and would not function in their absence. Some beneficial adaptive processes, for instance during physical exercise, even require a build-up of ROS in order to be successful, and fail miserably if antioxidants are used to neutralize such ‘good’ ROS.

Vincent Bowry, Keith U. Ingold and Roland Stocker (1992), for instance, studied the relationship between ascorbic acid and alpha-tocopherol demonstrating that, depending on some conditions, an antioxidant such as alpha-tocopherol becomes a pro-oxidant [55]. Indeed, in the field of nutrition, Levander and colleagues studying infection and oxidative stress created the term “dietary-induced oxidative stress” in 1995 [56].

At around the same time, Halliwell studied nutrition and oxidative stress searching for optimization strategies of nutritional intake of antioxidants, since the large doses of dietary antioxidants didn’t show any preventive or therapeutic effect, and because of this he introduced the “antioxidant paradox” concept [57–59]. Here, the notion of equilibrium is highlighted: “antioxidant defenses act as a balanced and coordinated system and each relies on the action of the others”.

This whole conundrum of pro- and antioxidants therefore required an amendment of the previous concepts. At this point, the traditional, crude view of oxidative stress as an entirely damaging event gave way to a more differentiated view on cellular redox signaling. Whilst in 1985, “oxidative stress” was defined by Helmut Sies as “a disturbance in the prooxidant/antioxidant balance in favor of the prooxidants, leading to potential damage” [7], 20 years later, the focus on “damage” had been expanded to a more refined view also paying tribute to “signaling” and “control”. “Oxidative stress” was now defined 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” [60, 61].

Historically speaking, these changes represent an evolution of the original paradigm in response to certain anomalies discovered during the puzzle solving period, rather than a scientific ‘revolution’ with a change of paradigm. Here, the focus of

Redox Biochemistry and Biology has shifted from the damaging effects of free radicals on proteins, membranes and DNA to oxidative modifications involved in cellular signaling. Such oxidative signaling events do not necessarily result in ‘negative’ actions, such as uncontrolled proliferation or apoptosis, but may also lead to more ‘positive’ outcomes, such as adaptation, or intrinsic antioxidant responses.

As part of the next two sections, we will therefore briefly consider how such reinterpretations and amendments of the original paradigm(s) have stimulated research into the field of intracellular redox control and also led to a certain renaissance of the antioxidants.

1.7 The Journey to the Cysteine Chapel

The historical developments discussed above may probably be illustrated best when considering the evolution which has taken place during the last 15 years in the field of sulfur-based redox systems and processes. Sulfur-based redox systems have been around for a long time, but research into their darker side only gathered steam during the first decade of the Twenty-first Century. Before then, redox active sulfur was mostly – but not always – considered as a cellular antioxidant in the form of a thiol which could be oxidized to a disulfide. The 1990s were the time, however, when it became obvious that redox active sulfur provides a considerably more diverse and, in any case, extraordinarily facet-rich biological redox chemistry.

Sulfur is a true redox chameleon able to occur in biology in over ten different formal oxidation states ranging from -2 in hydrogen sulfide (H_2S) to $+6$ in sulfate (SO_4^{2-}), and counting fractional states, such as -1.5 in the disulfide radical anion RSSR^- . This extraordinary flexibility in oxidation states subsequently translates into numerous different chemical appearances, which range from the better known thiols (RSH) and disulfides (RSSR') to lesser known chemical species, such as thiyl radicals (RS^\bullet), sulfenic acids (RSOH), sulfinic acids (RS(O)OH), sulfonic acids ($\text{RS(O)}_2\text{OH}$), thiosulfonates ($\text{RS(O)SR}'$), thiosulfonates ($\text{RS(O)}_2\text{SR}'$), polysulfanes ($\text{RS}_x\text{R}'$, $x \geq 3$ and $\text{R}, \text{R}' \neq \text{H}$) and partially reduced per- and hydropolysulfanes (RSSH and RS_xH , $x \geq 3$ and $\text{R} \neq \text{H}$, respectively). At the same time, these sulfur species are able to participate in a vast variety of redox processes ranging from one- and two-electron transfer to radical reactions, hydride and oxygen transfer and the omnipresent nucleophilic ‘exchange’ reactions (e.g. thiol/disulfide exchange).

Not surprisingly, therefore, the last 15 years have witnessed a dramatic increase in the studies addressing the chemistry, biochemistry and biological activity of such Reactive Sulfur Species (RSS). There have also been numerous attempts to use this truly unique ‘chemistry’ in the fields of medicine, pharmacy and agriculture. Based on extensive prior work on redox active sulfur in biology and medicine, the concept of “Reactive Sulfur Species” (RSS) as a unique class of redox active stressors and regulatory elements emerged in 2001 [62]. This concept represented a further amendment or extension of the original paradigm of oxidative stress and was soon followed by a number of discoveries, which can be divided

into three different, mutually not exclusive groups: (a) RSS as oxidative stressors, (b) RSS as a particular group of natural products, similar but not identical to organic sulfur compounds (OSCs), and (c) RSS as posttranslational cysteine modifications found in many proteins and enzymes. The last decade has witnessed considerable progress in all three of these areas.

In the field of oxidative stress research, a number of chemical sulfur species have been identified which are highly reactive and able to cause – sometimes severe – levels of oxidative stress in cells. Such a stress may be useful, for instance in the fight against cancer cells and microbes. Indeed, many rather unusual sulfur compounds found in nature can be described as RSS and are currently under consideration as potential drugs, including thiosulfates, polysulfanes, 1,2-dithiols, 1,2-dithiole-3-thiones and various isothiocyanates. Such compounds often exhibit considerable cytotoxic activities and have been discussed in the context of chemoprevention, antibacterial activity, antifungal activity, anticancer activity and activity against scleroderma [63–69]. Since many of these agents occur naturally, often even in edible plants (for instance in garlic, onions, mustard, asparagus) or in ‘semi-natural’ preparations more or less safe for human consumption (e.g. 1,2-dithiole-3-thiones in Haarlem Oil), such RSS feature highly in the arena of nutraceuticals and nutri-epigenetics.

Many of these substances also react fast, efficiently and selectively with cysteine residues in proteins and enzymes and hence exert pronounced, yet well-characterized effects on cells. These ‘effects’ often include widespread modifications of cysteine residues, a surge in ROS, cell cycle arrest and induction of apoptosis. Whilst the exact chains of events are still not fully understood, it seems that oxidative cysteine modifications play a major role. Here, the hunt for such unusual cysteine modifications is now on, and ‘cysteine hunters’ such as Kate S. Carroll, Philip Eaton, Jakob R. Winther and Joris Messens have identified quite a number of different RSS in proteins and enzymes during the last 10 years. Such modifications seem to occur in cells during normal cellular metabolism or are induced by internal or external (oxidative) stress. In order to emphasize the importance of such RSS, colleagues like Kate Carroll have even coined a new terminology using expressions such as the “cellular sulfenome” [70, 71]. The latter is referring to the widespread formation of sulfenic acids in proteins and enzymes.

While these investigations have focused primarily on stress situations, other groups, such as the ones of Jenny A. Littlechild, Leslie B. Poole or Michel B. Toledano have begun to search for the presence of unusual sulfur species in catalytic cycles of enzymes, such as the peroxiredoxins (Prdx) [72–74]. The Prdx enzymes catalytically remove H_2O_2 from the cell and also serve as a sensor for oxidative stress. Their normal catalytic cycle involves a thiol, sulfenic acid and disulfide, whilst overoxidation results in the formation of an unusual sulfenic acid, which shuts down the enzyme and opens the ‘floodgate’ for H_2O_2 to cause damage to the cell and to ultimately lead to apoptosis. The sulfenic acid formed in Prdx may be reduced back to the sulfenic acid (and thiol) in the presence of the redox protein sulfiredoxin (Srx), a process which probably involves the formation of a sulfenic acid phosphoryl ester and a thiosulfinate as reactive intermediates. As a result, the

catalytic and regulatory cycle of Prdx in concert with Srx includes six different sulfur modifications, of which four (i.e. sulfenic acid, sulfinic acid, sulfinic acid phosphoryl ester, thiosulfinate) clearly represent more unusual forms of RSS.

At the same time, other researchers, such as Pietro Ghezzi and his colleagues, began to shed some light on the events associated with the formation of such post-translational modifications, which obviously do not go unnoticed in the cell. Indeed, many of these oxidative modifications result in a loss of protein function or enzyme inhibition, and hence trigger wider cellular signaling [75, 76].

Indeed, once cell biologists became aware of these developments, the quest for the “cellular redoxome” was truly on [77, 78]. The evaluation of the various chains of oxidative events inside living cells first relied on “intracellular diagnostics” based on a combination of redox sensitive dyes to visualize intracellular redox changes, analytical techniques (such as Western Blots) to monitor oxidative changes to proteins and functional / activity assays to assess oxidatively induced changes in protein function and enzyme activity. These methods were soon joined by sophisticated protein-based ‘redox sensors’, such as the green fluorescent protein (GFP)-based sensors – “reduction-oxidation-sensitive GFPs” (roGFPs), developed by James Remington and colleagues – or yellow fluorescent protein (YFP)-derived peroxide sensors such as HyPer, developed by Sergey Lukyanov and colleagues [79, 80].

At some stage, the different strands of investigation, which include (a) the effective yet selective modification of cellular thiols by RSS and ROS, (b) the discovery of the resulting, widespread and often reversible cysteine modifications in proteins and enzymes, and (c) the pronounced effects such modifications exert on cellular processes, have merged in the concept of the “cellular thiolstat” first proposed in 2011 [81, 82]. This concept postulates that certain – but not all – intracellular cysteine proteins and enzymes form targets for redox modulation and hence serve as a sophisticated sensing and regulatory network which ultimately controls cell proliferation, differentiation and apoptosis. As redox processes involving cysteine residues are fast, effective and often reversible, the thiolstat enables the cell to respond to internal or external redox changes in an efficient, measured, appropriate and reversible manner.

Epistemologically, the concept of the “cellular thiolstat” represents another important cornerstone of our modern perception of oxidative stress and cellular redox control. Similar to the other concepts sheltered under the general paradigm, it is not an absolute dogma but rather designed to explain certain experimental findings, and to stimulate further research in this field, for instance by providing the necessary problems, leads and directions [83].

1.8 The Rise, Demise and Renaissance of the Antioxidant

A similar development has also taken place in the context of the “antioxidant”. As in the field of sulfur biochemistry, changes in the perception of “oxidative stress” in general have resulted in a wealth of knock-on effects, which has also led to a redefinition of the antioxidant concept and a re-evaluation of functional foods. As mentioned before, the 1980s saw the emergence of the traditional concept of “oxidative stress”,