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Introduction

Claus Jacob and Paul G. Winyard

Redox events play a key role in biology. A wide range of biochemical processes, such as energy metabolism and host defense, critically depend on the smooth interplay of numerous cellular redox systems, which in turn involve a wealth of redox active organic molecules and inorganic ions and complexes. Many of these redox systems, such as the mitochondrial respiratory chain, have been known for decades and are well understood today. In most instances, the underlying redox events are tightly controlled by proteins and enzymes, which allows individual redox reactions to be studied and pieced together as part of a wider "redox puzzle". This area of "well behaved", almost unidirectional, straightforward and predictable redox chemistry forms a huge and important part of bioorganic and bioinorganic chemistry and biochemistry, which has long dominated the field of biological redox chemistry.

During the last two decades, however, it has become apparent that intracellular redox events do not always follow controlled and easy to understand linear reaction pathways. Research into oxidative stress, which has taken off since the 1980s, has demonstrated that numerous redox transformations inside and between cells follow highly complex transformation patterns which are quite difficult to study and to rationalize, especially when merely considering individual redox molecules, proteins or enzymes.

The effects hydrogen peroxide may have on a cell illustrate the inherent complexity of this kind of intracellular redox chemistry. First, there is no single or specific reaction partner for H_2O_2 inside the human cell. Numerous molecules may be affected simultaneously, such as cysteine proteins and enzymes, which may become oxidized "by design" or "at random". On the other hand, there are various enzymes dealing with H_2O_2, all of which may become involved with H_2O_2 at the same time. Furthermore, the effects of H_2O_2 may critically depend on its concentration. While lower concentrations may result in subtle redox signaling events or removal of H_2O_2 by enzymes such as glutathione peroxidase (GPx), peroxiredoxins (Prx) or catalase, higher concentrations may affect cellular components more widely, for instance by inhibiting cysteine enzymes and oxidizing metal/sulfur proteins. And finally, even if all chemical transformations triggered by H_2O_2 were fully understood, they would

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only provide the entry point for a plethora of biochemical signaling and response events involving small biomolecules, proteins, enzymes, lipids and genes leading to significant cellular changes, such as the expression of antioxidant molecules or the initiation of processes ultimately leading to cell death.

Many of these issues will be discussed in later chapters. Here, the peroxide example simply serves to illustrate that there is a dramatic difference between a well-controlled electron transport chain and a complex, yet mostly balanced redox system which is capable of responding to external or internal disturbances by employing an immense and diverse range of chemical and biochemical reactions. Needless to say, any investigation of such a complicated redox network is far from trivial.

As a result, redox signaling events in and between human cells have become an increasingly important area of biochemical research during the last decade. During this time, greatly improved and occasionally novel techniques have become available to track down, identify and characterize hitherto unknown redox modifications and associated processes in the cell. For instance, the use of the powerful technique of electron paramagnetic resonance (EPR) spectrometry, which is often aided by innovative spin traps, today allows studies of elusive sulfur radicals and other redox-active radicals *in vitro* and, more recently, *in vivo*. Similarly, synthetic chemistry has been able to produce a number of new, effective, yet highly selective labeling agents which allow scientists to trace "unusual" redox modifications, such as sulfenic acids, and redox events, such as iron release, inside cells. The availability of such "probes" has opened the door to several areas of redox biochemistry.

Furthermore, modern proteomic and gene mapping methods, which have burgeoned during the last decade, have turned out to be of immense value to redox research in cells. For instance, posttranslational modifications of proteins and enzymes can now be monitored with reasonable accuracy and correlated with (changes of) protein function or enzyme activity. At the same time, complex redox responses can be studied at the level of gene expression. Taken together, these modern chemical, analytical and biochemical techniques have the potential to provide a comprehensive picture of redox responses and signaling events which would not have been possible a decade ago.

Fifteen years ago, in 1992 the signaling molecule nitrogen monoxide, $^{\bullet}NO$, was chosen as "Molecule of the Year" by *Science* magazine. Since then research into biological redox processes has made significant progress. This molecule will be discussed in Chapter 12. Apart from the improvement of analytical techniques, one of the major driving forces behind this research has been the overarching importance of cellular redox events for human health and disease.

It has been known for many years that oxidative stress plays a major role in the progression of human illnesses, such as autoimmune and auto-inflammatory diseases, infectious diseases, diabetes, glaucoma and, perhaps most importantly, cancer. The exact relationship of reactive, oxidizing molecules and the ultimate formation or progression of a particular disease, is, of course, often complex, sometimes contradictory and still mostly poorly understood. This lack of knowledge has, on occasion, led some scientists to believe that matters such as oxidative stress in disease, antioxidants etc. mostly represent a red herring. It is increasingly becoming

Box 1.1: A Brief History of Oxidative Stress Research

This book presents a wealth of information regarding intracellular redox signaling and response processes. Although we are nowadays able to summarize the various cellular redox systems and their interdependencies on a few hundred pages, the discovery of these systems has taken many decades, and has often been filled with surprises, disappointments, and rather exciting scientific twists and turns. As part of this book, we are unfortunately not able to provide a comprehensive history of cellular redox signaling and control – this topic alone would fill a whole book – yet we would like to take the opportunity to mention some of the discoveries in the field. This presentation is necessarily incomplete.

The idea that redox processes play a role in human cells is probably as old as biochemistry itself. Redox processes are found not only in the context of signaling and regulation, but also as part of normal cell metabolism (e.g. energy generation), host defense and others. An early discovery in the field of intracellular redox processes was the redox enzyme catalase, which was described by Oscar Loew in 1900 and studied by him and others (e.g. D.W. May) at the beginning of the twentieth century. Coincidentally, this enzyme would later turn out to play a key role in oxidative stress and redox control. In 1957, a landmark paper entitled "Free radicals in biological materials" describing the widespread presence of (redox active) radical species in organisms was published by Barry Commoner and Jonathan Townsend in *Nature* (*Nature* 174, pp. 689–691, 1954). This discovery in many ways was ultimately rationalized in 1973 by Britton Chance and colleagues, who determined that about 2% of oxygen reduced by mitochondria ends up as superoxide radicals or hydrogen peroxide (and not just water, as one may have assumed). Although the 2% value is only a rough estimate – which has been confirmed on various occasions – this discovery identified one important source of reactive oxygen species (ROS) in the human cell, and explained the presence of various antioxidant enzymes there. In the 1960s and early 1970s, the term "oxidative stress" became widely used as a paradigm to describe the increase of oxidizing species – at that time mostly considered as being radicals – in the human cell (the term "antioxidant" was commonly used in the field of nutritional sciences from the 1940s onwards). The oxidative stress terminology was cemented further and expanded in the 1980s by "free radical" researchers, such as Helmut Sies.

In the meantime, various human antioxidant enzymes were discovered, lending further support to the notion that radicals do occur normally inside human cells and that these cells contain enzymes which are able to deal with such radicals accordingly. In 1957–1959, Gordon Mills reported the discovery and characterization of mammalian glutathione peroxidase (GPx). Several years later, in 1969, another antioxidant enzyme, superoxide dismutase (SOD), was described by Irwin Fridovich and Joe McCord. Other antioxidant enzymes, such as the peroxiredoxins (Prdx), were discovered in the 1970s and 1980s, often in various organisms and initially under different names.

Together, these developments provided a firm place for oxygen-based radicals (and peroxides) in human biochemistry, with well-defined formation and detoxification pathways. It became increasingly apparent, however, that the presence of such radicals in the human organism was not "all bad," but also seemed to have some significant benefits as well. Here, we need to mention two developments: On the one hand, the role of ROS in (human) host defense began to be elucidated during the 1950s and 1960s, with enzymes such as myeloperoxidase and – later – the various NADPH oxidases and dual oxidases being described. On the other hand, the 1980s and 1990s provided mounting evidence for the existence of cellular redox signaling molecules participating in extensive redox signaling networks.

One key discovery in this area involved the recognition of endothelium-derived relaxing factor (EDRF) by Robert Furchgott and colleagues in 1978, which was later (in 1986) identified as nitrogen monoxide (nitric oxide, $^{\bullet}NO$) by Furchgott, Ferid Murad, Louis Ignarro and Salvador Moncada (Furchgott, Murad and Ignarro subsequently shared the 1998 Nobel Prize in Physiology or Medicine for this discovery). In many ways, this discovery opened up the field of intracellular redox signaling based on reactive (redox) species.

Not surprisingly, the $^{\bullet}NO$ story was soon followed up by further findings of redox signaling based on ROS. Here we need to mention the key work on redox control of the transcription factor NF-κB (by H_2O_2) performed by Patrick Bäuerle and colleagues in the early 1990s and the work by Toren Finkel and colleagues on the role of H_2O_2 in (platelet-derived growth factor) signal transduction, published from 1995 onwards. Following these discoveries, there have been numerous studies on the redox control of key cellular signaling pathways. Here, we may mention the work by Barry Halliwell on free radicals in biology and the studies by Sue Goo Rhee and colleagues on the redox control of protein tyrosine phosphatases (PTPs).

More recent developments include the postulation of the concept of reactive sulfur species (RSS) in 2001. This was followed by the discovery of the key antioxidant protein sulfiredoxin by Michel Toledano and colleagues in 2003. Five years later, we are now in a position to look back at many of these discoveries and build on an increasing understanding of intracellular redox events, from chemical, biochemical, biological, medical and pharmacological perspectives.

apparent, however, that there are indeed significant links between redox events, such as oxidative stress, and numerous human illnesses. Once these processes are better understood, therapeutic interventions – some of which may well include forms of redox-regulatory drugs or antioxidants – are likely to become available for many of these disorders.

Interestingly, many of the aforementioned human illnesses, such as rheumatoid arthritis, various forms of cancer and diabetes, occur with advanced age. As described in Chapter 19, there seems to be a direct link between an aging organism, increases in

intracellular levels of oxidative stress, cell death, mutations and subsequently disease formation. Many aspects of this biochemical connection are still unexplored and little understood. A better understanding of redox events in aging is of great importance for many aging societies, such as the ones of Europe and North America. Research in this area is currently gathering considerable momentum, reflected by the increasing number of research institutes worldwide focusing on aging.

Most of these and related research centers thrive within a highly multidisciplinary research environment which embraces techniques from various scientific disciplines. Different branches of analytical and synthetic chemistry, for instance, are able to deliver probes and detection methods to monitor redox changes *in vitro* and *in vivo*; biochemical methods, including proteomics, gene expression and enzyme activity assays, are important to study more complex cellular responses and map out fairly intricate pathways; cell biology may allow insight into complex cellular events, including cell proliferation and apoptosis; animal experiments, perhaps combined with computer simulations, can be used to study the effects of redox changes on whole organisms; last but not least, medicine and drug design may enter the fray to apply research in order to prevent or even treat certain human disorders, as will become apparent in Chapter 19, where research into the dietary intake of amino acids is discussed briefly.

The cross-disciplinary approach in research in many ways reflects the multidisciplinary approach towards our understanding of cellular signaling and regulation. While each discipline provides individual pieces in our jigsaw image of redox events, only the final, emerging picture, embracing all of these pieces, allows us to fully comprehend the complicated interplay of cellular events we are really dealing with.

Such a cross-disciplinary approach clearly is not easy. As we have already seen, there are quite a few disciplines involved, and mastering an understanding in all of them is a massive, sometimes impossible task for individual researchers. The following chapters will therefore provide an introduction to some of the most important aspects of redox signaling and regulation in biology and medicine. Such an introduction is necessarily selective and incomplete. Nonetheless, by focusing on interesting and more recent developments, it may provide the reader with an understanding and basis for a more intensive consideration of the field.

In order to convey a more complete picture of the various aspects of redox signaling, control, response and regulation, the following chapters will consider various aspects, all the way from the relevant basic chemistry to the involvement of redox events in aging. It should be noted that the individual chapters are designed to "stand alone" in order to readily provide topic- or discipline-specific information, yet also systematically build on each other. The early chapters introduce basic aspects of redox systems and discuss the formation, properties and redox behavior of various relevant redox molecules and simple networks, while the later chapters consider more complex signaling and response arrangements which are operational within the human cell. This will culminate in a discussion of redox signaling and human health and disease (Chapter 18) and of redox changes associated with aging (Chapter 19).

Box 1.2: Oxidation, Reduction and Formal Oxidation States

There is often considerable confusion regarding the terms oxidation, reduction, pro- and antioxidants. Oxidation is the formal increase of the oxidation state of a particular atom in a compound or in the ionic state. For instance, the formation of Fe^{3+} from Fe^{2+} is an oxidation involving the removal of one electron from the reduced form of the iron ion and a change of oxidation state from $+2$ to $+3$. In contrast, reduction is a decrease of formal oxidation state. Importantly, oxidation and reduction processes may occur via (one or more) electron transfer processes, but do not have to. Formal oxidation states may also change as a result of "electron transfer-free" processes, such as radical reactions, atom transfers or substitution/exchange reactions. The substitution of hydroxide (HO^-) by hydride (H^-), for instance, frequently results in a formal decrease of oxidation state and hence should be considered as a reduction reaction, as seen in the reaction of sulfenic acid (RSOH) with H^- to produce a thiol (RSH). This particular hydride transfer chemistry plays a major role in some bacterial NADH (per-)oxidase enzymes, whereby sulfur is formally reduced from 0 to -2 (and H^- oxidized from -1 to $+1$).

Formal oxidation states can be positive and negative, and tend to cover a fairly wide range, for instance from -4 (carbon in CH_4) to $+7$ (manganese in MnO_4^-). More "extreme" oxidation states (i.e. beyond -4 or $+7$) are difficult to achieve. Oxidation states are formal numbers which can be assigned to any atom in an ion or molecule (although it is sometimes not very productive to do so). In essence, they serve to detect and describe redox reactions.

Chemists have various methods of assigning formal oxidation states. For instance, one may start with the charge of a compound and the known oxidation states of elements within this compound and then calculate the oxidation state in question. Here, hydrogen is usually $+1$ and oxygen -2, although exceptions exist (such as hydrides with hydrogen as -1 and peroxides with oxygen as -1). Only the element fluorine in compounds is always -1. This method allows us to calculate, for instance, that sulfur is $+6$ in sulfate SO_4^{2-}, since the overall charge is -2 and once all the other oxidation states are substracted, that is, 4×-2 (for oxygen) one arrives at $+6$ for sulfur ($+6$ is formally divided by 1 since there is only one sulfur atom).

Interestingly, this method fails for compounds that contain the same element in a different chemical environment, such as sulfur in thiosulfate $S_2O_3^{2-}$. In this case, the oxidation state(s) can be determined by writing down the (electronic) structure of the species, assigning the electrons based on electronegativity and then comparing the number of electrons left at a particular atom with the number of electrons for that particular atom in the neutral, elemental state. In this case, one sulfur atom in $S_2O_3^{2-}$ ends up with seven electrons (it had six before, hence formal oxidation state -1), while the other one, surrounded by the more electronegative oxygen atoms, has just one electron left (hence formal oxidation state $+5$). The traditional method would have simply given the average, that is, $+2$, ignoring the distinct differences in (redox) chemistry which exist between

the two sulfur atoms in this molecule (please note that mesomeric structures exist for $S_2O_3^{2-}$ which contain S in the formal oxidation states O and $+4$).

Three facts should be noted when discussing formal oxidation states: First, some textbooks also use Roman numerals to indicate oxidation states, such as Fe(III) for iron in Fe^{3+}. The use of this labeling system is awkward, however, when the oxidation states turn negative. Second, while oxidants and reductants are defined as electron acceptors and electron donors, respectively, Lewis acids and Lewis bases are defined as electron pair acceptors and donors, respectively. These two concepts must not be confused. Only in very rare instances are acid–base reactions also redox reactions, such as $H^+ + H^- \rightarrow H_2$. In contrast, the acid–base reaction $H^+ + NH_3 \rightarrow NH_4^+$ is not a redox reaction, since the formal oxidation states of H ($+1$) and in NH_3 (N is -3, H is $+1$) do not change. Third, the concepts of oxidants and biological pro-oxidants, as well as reductants and antioxidants must not be confused: not all oxidants behave as oxidizing species in biology, and not all reductants are antioxidants (e.g. Fe^{2+}, which reduces H_2O_2 to HO^- and $HO^\bullet$, with biological implications). On the other hand, there are chemical species which are themselves redox inactive yet trigger an antioxidative response (e.g. Zn^{2+} ions).

A multidisciplinary text such as this book often poses serious problems to its readers, especially when specialized, discipline-specific concepts or nomenclature are employed. In order to soften these matters somewhat, each chapter will provide one or more *Explanatory Boxes*. These are designed to provide a basic idea of the concepts, terminology etc. which are used in that chapter and deemed as being potentially problematic. Importantly, these boxes are written in a simple language – for instance to provide biochemists with an idea of some of the lesser known, yet relevant chemical concepts (and vice versa). Under no circumstances should such boxes be seen as expert, cutting edge or even comprehensive explanations.

In some rare instances, there may be a slight overlap of themes covered in individual chapters. This underlines the "stand alone" character of each chapter and also reflects the fact that it is sometimes necessary to briefly consider the wider context of redox signaling before turning toward a specific topic.

In Chapter 2, Thomas Hurd and Michael Murphy will begin with a brief overview of the various biological redox systems relevant for signaling and control. This chapter serves as a concise introduction to a range of important intracellular redox systems, including reactive oxygen species (ROS), protein thiols, peroxynitrite, $^\bullet NO$, nitrosothiols and lipid peroxidation products. Chemical and biochemical aspects of these redox systems will be discussed in more depth in the following chapters.

Lars-Oliver Klotz and Helmut Sies, for instance, will deal with the formation of cellular oxidants, such as ROS, in Chapter 3. These reactive species are of major importance for redox regulation, not only under conditions of oxidative stress. Their formation can follow various pathways, some of which are still little known or understood.

Once formed, reactive species and their cellular partners (or targets) exhibit a distinct chemical behavior as described in Chapter 4. Here, Claus Jacob, Mandy

Doering and Torsten Burkholz provide insight into some of the chemical properties of redox systems and their components, from their formation and parameters relevant for redox chemistry to reaction partners and possible reactions *in vitro* and *in vivo*. This chapter also introduces several key components of the chemical sensing and response reactions which are at the heart of the more extensive (bio-)chemical redox networks in the cell. The chemical and biochemical events emanating from this chemistry will be discussed in Chapters 5–7.

In Chapter 5, Pietro Ghezzi and Paolo Di Simplicio elaborate on the topic of *S*-glutathiolation. Recent research has indicated that glutathione (GSH) fulfills a range of interesting roles in redox signaling and responses, which transcend its established role as a cellular redox buffer and antioxidant. While the chemistry behind such GSH reactions has been considered in Chapter 4, Chapter 5 provides insight into the various consequences of protein glutathiolation and deglutathiolation, and the diverse factors affecting these processes.

Chapter 6 goes beyond the thiol/disulfide chemistry which is at the center of the thiolation and dethiolation processes. As part of this chapter, Jennifer Littlechild, Katalin Szabo and Paul Winyard consider the redox-modulation of protein activity in the context of the peroxiredoxin (Prx) enzymes. Together with the recently discovered protein sulfiredoxin (Srx), these incorporate a range of unusual sulfur modifications, such as a sulfenic and sulfinic acid and a transient thiosulfinate. This spectrum of sulfur redox states endows Prx enzymes with various biochemical functions, ranging from antioxidant enzyme and redox sensor to redox switch and chaperone.

After the discussion of the peroxiredoxins as key human redox enzymes, Chapter 7 is concerned with the wider implications of H_2O_2 and its effects on various biomolecules. As part of this chapter, Ewald Schröder and Philip Eaton consider a range of proteins modified by H_2O_2 and also discuss recently developed methods to detect posttranslational redox modifications of cysteine *in vitro* and in cell-based systems. This discussion also leads to related cellular signaling pathways, such as phosphorylation, which are affected by redox modifications, yet do not trigger exclusively redox events on their own.

The transmission of the redox signal into other cellular signaling pathways, for instance into phosphorylation/dephosphorylation, is discussed further in Chapter 8. In this chapter, Jeroen den Hertog describes the mechanism and physiological relevance of protein tyrosine phosphatase oxidation.

In a similar approach, Chapter 9, which is contributed by Adam Case and Frederick E. Domann, considers the effects of redox control on hypoxia-induced gene regulation. As in the previous chapter, a link is made between key redox events and subsequent signaling cascades, such as prolyl hydroxylase domain-containing proteins and p53, cascades which integrate redox events with various other "chemistries".

While Chapters 5–9 deal with established, protein-based signaling pathways, Chapter 10 takes a closer look at new aspects of redox signaling pathways based on lipids. As part of this chapter, Valerie B. O'Donnell introduces the formation, properties and signaling actions of eicosanoids, oxidized lipids which display a diverse array of bioactivities in virtually all organs and diseases.

Box 1.3: Radicals

The concept of radicals in chemistry dates back to the first half of the nineteenth century when radicals were seen as something like reactive fragments of (mostly) organic molecules. Nowadays, their definition is more exclusive: Radicals are chemical species that contain one or more unpaired electrons. In principle, this notion may apply to organic and inorganic molecules, including metals, metal ions and metal complexes. In practice, most of the discussion of radicals in biology and medicine is focussed on non-metal species, which exhibit their own, rather distinct "radical chemistry". The latter is characterized by the reactivity of the unpaired electron(s). For instance, radicals of the type $R^{\bullet}$ often abstract hydrogen atoms ($H^{\bullet}$) to form RH, remove electrons to form R^{-}, dimerize to form R-R, or react with other radicals $R'^{\bullet}$ to form R-R′. As part of this book, we will encounter a wide range of carbon-, nitrogen-, oxygen- and sulfur-centered radicals, among them triplet oxygen ($^3O_2$, a diradical with two unpaired electrons), the superoxide radical anion ($O_2^{\bullet -}$), nitric oxide ($^{\bullet}NO$) and the thiyl radical ($RS^{\bullet}$).

Radicals are frequently formed by (one) electron oxidation processes, $H^{\bullet}$ abstraction or simply by homolytic cleavage of a (weak) covalent bond. Apart from their reactivity, radicals are also electron spin "active" (the overall spin is not zero), and this often allows their detection and monitoring via electron paramagnetic resonance (EPR) spectrometry (or electron spin resonance; ESR). It is worth noting that this property of radicals is shared by many metal ions, in particular lanthanoides, which, perhaps not surprisingly, are used as "spin contrast agents" in medical diagnostic techniques such as magnetic resonance imaging (MRI).

With regard to radicals in biology, four important facts which run contrary to popular myth should be noted: first, not all radicals are charged (consider $^{\bullet}NO$); second, not all radicals are highly reactive (consider $O_2^{\bullet -}$); third, not all radicals are detrimental to human health (consider $^3O_2$); fourth, not all reactive species are radicals (consider H_2O_2).

This is followed by Chapter 11, which considers redox control in the context of transcription factors and gene expression. Gregory I. Giles describes how redox signals are transmitted to the level of genes, which are capable of redox responses by *de novo* expression of oxidative stress response proteins and enzymes. This chapter focuses on the antioxidant response element (ARE) and the Nrf2-Keap1 response, in particular, but also considers the wider role of genes in redox regulation.

After briefly covering some of the major redox signaling systems at the level of proteins, enzymes, lipids and genes, Chapters 12–14 consider specific redox signaling systems which have been at the forefront of research during the last couple of years. In Chapter 12, Dario Vitturi, David M. Kryzwanski, Edward M. Postlethwait and Rakesh P. Patel look at the role of $^{\bullet}NO$ in redox signaling. Extending the brief introduction of $^{\bullet}NO$ in the early chapters of this book, Chapter 12 provides a deeper insight into the formation and transformation pathways of this important signaling molecule, some of which are already well established while others are still under intense investigation.

Chapter 13 extents the discussion of gaseous (redox) transmitters. Here, Matthew Whiteman and Philip K. Moore consider the chemisty and biochemical impact of hydrogen sulfide (H_2S), a potent gasotransmitter whose role in human biology has only recently been discovered.

As part of a similar approach, Masuko Ushio-Fukai discusses aspects of NADPH oxidase- and dual oxidase-based signaling in Chapter 14. This chapter considers cellular aspects of signaling molecules, such as enzymatic formation and targets, and builds on the chemistry of such molecules that was introduced briefly in Chapters 2–4.

Chapter 15, in contrast, deviates from the mostly "bioorganic" signaling considered so far and takes a closer look at the bioinorganic chemistry of metal ion-based redox control. Andrew Pye, Yuktee Dogra, Jessica Tyrrell, Paul G. Winyard and Alison Curnow discuss redox active metal ions most commonly found in cellular signaling systems. This area of research is currently gathering steam, significantly fueled by the link between redox active metal ions and reactive species, but also by interesting therapeutic approaches based on metal ion control. This chapter completes the discussion of individual redox systems.

Chapters 16 and 17 move on to consider the wider impact of redox signaling and regulation. As part of Chapter 16, Christofanon Silvia, Dicato Mario, Ghibelli Lina and Marc Diederich describe various aspects of the rather complicated relationship between oxidative stress and apoptosis and provide a brief historical overview of this exciting area of research.

Edith Charlier, Jacques Piette and Geoffrey Gloire then take a closer look at redox control of apoptosis in the context of immune cells, an issue with particular relevance in inflammatory and infectious diseases. After the general introduction to the field in the previous chapter, the authors turn to immune-specific regulation, such as induction of apoptosis by exogenous and endogenous ROS, immune receptor stimulation and matters surrounding spontaneous versus bacterial-induced neutrophil apoptosis.

With a link between oxidative stress, cell survival and cell death firmly established, Chapters 18 and 19 provide the final perspective on redox regulation in biology and medicine by considering events at the level of whole organisms. In many ways, these chapters represent the synthesis and culmination of the previous chemical, mechanistic, biochemical and cell-related discussions.

Chapter 18, which is written by Katalin E. Szabo, Nicholas J. Gutowski, Janet E. Holley, Jennifer A. Littlechild and Paul G. Winyard, considers the numerous emerging links between redox signaling on the one hand and human health and disease on the other. This chapter also includes an introduction to the most recent developments in research dealing with inflammatory and neurodegenerative diseases and cancer.

The final chapter looks at oxidative stress from the perspective of the aging organism. By considering various chemical, biochemical and medicinal processes in an aging organism, Alberto Sanz, Gustavo Barja, Reinald Pamplona and Christiaan Leeuwenburgh show how a deeper understanding of processes at the level of an organism can be in part be explained by events taking place at lower levels of complexity. This chapter embraces a critical discussion of the mitochondrial free radical theory of aging, considers changes at the level of DNA and

Box 1.4: Human Illnesses Associated with a Disturbed Redox Balance

Research conducted over the last two decades has demonstrated that cellular redox regulation and control is essential for human health. In the following chapters, we will encounter a range of redox signaling molecules which help to maintain the well-being of mammalian cells, such as $^{\bullet}NO$ and H_2S. We will also encounter H_2O_2 and HOCl, which not only act as signaling but also as host defense molecules. It has become apparent that disturbed redox balances, and in particular oxidative stress, play a major role in cancer, neurodegenerative, cardiovascular, inflammatory, autoimmune and infectious diseases, especially in rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, glaucoma and diabetes. Some of these illnesses will be discussed as part of Chapter 18. Among the disorders linked to oxidative stress are the most common causes of death in the developed world, that is, cardiovascular diseases and cancer. At the same time, aging organisms seem to lose some of their redox control mechanisms. This matter will be examined in Chapter 19 and again, poses a serious concern for modern society in terms of the aging human population in many developed countries.

The causal relationships emerging between oxidative stress and these diseases are complex. Although oxidative stress may be found in many human illnesses, this does not necessarily imply that oxidative stress is also the ultimate cause of these disorders. Far from it: in most cases, it may be just a side-effect of a genetic mutation (e.g. in cancer cells), a malfunctioning immune system (e.g. in rheumatoid arthritis) or a viral/bacterial invasion. Nonetheless, there are also certain diseases, such as cancer, where oxidative stress may play a role as the initiating culprit, for example, by causing oxidative mutations to DNA.

In any case, once oxidative stress appears as part of a certain disorder, it is likely to have damaging effects on cells, tissues and organs. It is therefore important to reduce oxidative stress in these diseases if at all possible. Although this may not ultimately cure the disease, it may help to alleviate some of the damage associated with it or slow progression of the disease.

Although disturbed intracellular redox balances are usually associated with oxidative stress, there are also several human illnesses which exhibit cells with an abnormally reducing redox environment. Such reductive stress is found, for instance, in cancer cells proliferating under hypoxic conditions, such as cancer cells inside solid tumors and deprived of a proper oxygen supply. In these cells, normal metabolism has changed to accommodate the lack of oxygen, for instance by increasing levels of the enzymes DT-diaphorase and NADPH:cytochrome *c* reductase. These metabolic changes cause a more reducing intracellular environment and have led to the development of so-called "bioreductive drugs," such as mitomycin C. Then again, hypoxia does not rule out the presence of oxidative stressors.

proteins, and also discusses issues related to dietary intake, for instance of the "antioxidant" amino acid methionine, which may extend the maximum life span potential.