Oxidative Stress

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Abstract

Oxidative stress is two sided: Whereas excessive oxidant challenge causes damage to biomolecules, maintenance of a physiological level of oxidant challenge, termed oxidative eustress, is essential for governing life processes through redox signaling. Recent interest has focused on the intricate ways by which redox signaling integrates these converse properties. Redox balance is maintained by prevention, interception, and repair, and concomitantly the regulatory potential of molecular thiol-driven master switches such as Nrf2/Keap1 or NF-κB/IκB is used for system-wide oxidative stress response. Nonradical species such as hydrogen peroxide (H_2O_2) or singlet molecular oxygen, rather than free-radical species, perform major second messenger functions. Chemokine-controlled NADPH oxidases and metabolically controlled mitochondrial sources of H_2O_2 as well as glutathione- and thioredoxin-related pathways, with powerful enzymatic back-up systems, are responsible for fine-tuning physiological redox signaling. This makes for a rich research field spanning from biochemistry and cell biology into nutritional sciences, environmental medicine, and molecular knowledge-based redox medicine.

Contents

INTRODUCTION

Oxidation–reduction (redox) homeostasis, like pH control, is central to life. Redox processes pervade practically all fundamental processes, from bioenergetics to metabolism and life functions (see **Figure 1** as overview). As in acid–base regulation, spatiotemporal control operates at different set points. Although it is commonly accepted that one cannot speak of the pH of a cell because of differences by orders of magnitude in H^+ concentration in various subcellular compartments, similar awareness seems to be less widespread regarding redox biology: There is no overall cellular

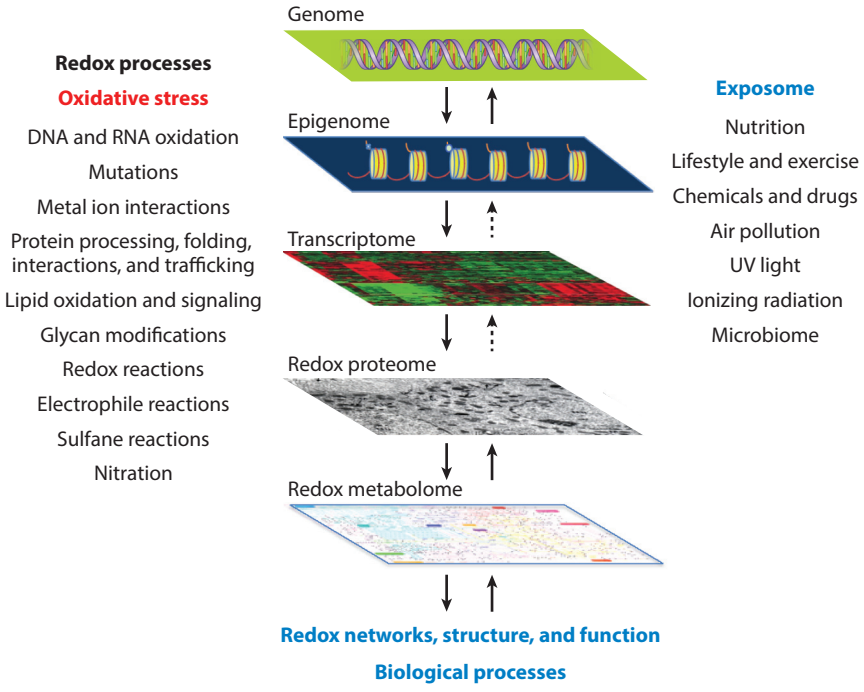

Figure 1

Redox processes have fundamental implications in biology. Oxidative stress, either as reversible oxidative eustress in redox regulation or as a cause of damage to biomolecules (oxidative distress), pervades all principal levels, from genome integrity and maintenance to the redox metabolome. Endogenous metabolism and exogenous impact, denoted as the exposome, affect biological processes through redox networks.

redox state. In various compartments within and outside of cells, a given physiological or pathophysiological situation is characterized by hugely different set points of redox systems operating concurrently [an example for different set points is the reduced nicotinamide adenine dinucleotide (NADH) system, discussed below in **Figure 3*a***]. Biological redox reactions are manifold and organized according to principles of the Redox Code (1).

Biological Redox Steady States Are Nonequilibria

Biological redox equilibria do not denote, as a matter of fact, true thermodynamic equilibria but instead are nonequilibria as defined by steady state (2). As long as there is flux through reactions, there is a deviation from thermodynamic equilibrium, and the deviation is proportional to the magnitude of flux (3). It is useful to consider these fundamental facts before considering the multitude of biological redox reactions in cells and tissues, because the set point of the redox potential of a given reaction may be quite different at different locations within the same cell (e.g., the NADH or glutathione systems; see 1).

Contemporary Relevance of Oxidative Stress

Before going into detail discussing oxidative stress, it may be mentioned at the outset that deviations from the set point in metabolic steady states are utilized for redox signaling, and

Oxidative stress: an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage (15)

Redox signaling: transmission of a redox signal via an essential redox element from a source to a target

sophisticated mechanisms of redox control are in place. More pronounced deviations (e.g., toward oxidation) may ultimately cause damage to biomolecules and can modulate, and even disrupt, physiological redox signaling. Because newer research tools permit examination of redox signaling pathways in sufficient chemical detail, it is now clear that redox processes are likely as important in biology as are phosphorylation–dephosphorylation reactions. Interestingly, these two fundamental chemical ways of modulation and control are linked at particular junctions. Similarly, acetylation/deacetylation and methylation/demethylation, central mechanisms for controlling the genome and epigenome, provide fundamental chemical links to central redox processes.

The obvious existence of endogenous and exogenous oxidants, on one hand, and the systems of counteraction known as enzymatic and nonenzymatic antioxidants, on the other hand, has attracted widespread interest throughout the research disciplines, from chemistry through cell biology all the way to health sciences. There have been considerable advances in knowledge, but there has been a dilemma: Sound molecular understanding of reactions and their often complicated interrelations was outpaced by applications, even in health care and lifestyle recommendations. Respectable health institutions and businesses initiated costly human studies, and outcomes were often questionable, with a disconnect to basic redox biology. A bewildering explosion of interest in oxidative stress occurred in many research fields, and excitement by the public outran progress in sound knowledge.

Against this background, we propose to identify the basis of oxidative stress in molecular terms, provide a glimpse into the developing field of redox signaling and redox control, and address what is being called redox medicine. Although returning to some of the roots of this burgeoning field, we focus largely on recent development of concepts, illustrated by selected examples, rather than attempting to cover all current literature.

THE CONCEPT OF OXIDATIVE STRESS

Prologue on Stress, Resilience, and Allostasis

In 1936, Hans Selye proposed the concept of "stress" in studying adrenal adaptive responses (**Figure 2**) (4). According to Selye's 1976 article: "Stress is the nonspecific response of the body to

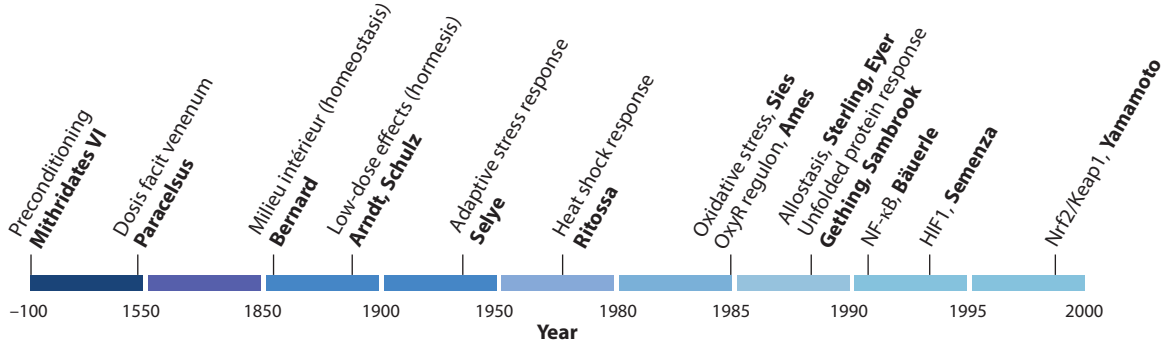

Figure 2

Timeline showing the concepts of stress and adaptive stress responses. Mithridates VI (see 210) and Paracelsus (see 211) had early insights. Bernard's concept of milieu intérieur (see 212) received the name "homeostasis" (213), and the Arndt–Schulz rule (see 214) received the name "hormesis" (215, 216). The twentieth century brought the adaptive stress response (4), heat shock response (217), oxidative stress (12), OxyR (218), allostasis (7), unfolded protein response (219), NF-κB (128), HIF1 (220), and Nrf2/Keap1 (122). Abbreviations: HIF1, hypoxia-inducible factor 1; Keap1, Kelch-like ECH-associated protein 1; NF-κB, nuclear factor κB; Nrf2, nuclear factor-E2-related factor 2.

any demand" (5, p. 53). The idea of homeostasis and deviations therefrom goes back to Paracelsus and to Claude Bernard's "milieu intérieur" and more recently has found attraction with the terms "resilience" and "allostasis." Resilience denotes the power or ability to return to the original form, position, etc., after being bent, compressed, or stretched, whereas allostasis refers to the active process by which the body responds to daily events and maintains homeostasis, literally meaning to achieve stability through change (6, 7). Both these terms, as well as the term "adaptive homeostasis" (8), and the literature about them overlap with Selye's concept of adaptive stress response.

Definition of Oxidative Stress

The early use of the term seems to begin with rubber chemistry in 1956 (9). In 1970, "cells were subjected to oxidative stress" was used to describe the addition of H_2O_2 to erythrocytes (10, p. 456). Since 1985, the term denoted oxidative damage to cells and organs (11).

The concept of oxidative stress was first formulated in the introductory chapter of the 1985 book entitled *Oxidative Stress* as "a disturbance in the prooxidant-antioxidant balance in favor of the former" (12, p. 1). The review entitled "Biochemistry of Oxidative Stress" (13) presented the knowledge on prooxidants and antioxidants and their endogenous and exogenous sources and metabolic sinks. With the emergence of the role of redox signaling, there was a need for updating the concept (14), which culminated in the definition provided here (15).

As useful as the term oxidative stress may be in research, there has been an inflationary development (i.e., the term has been overstressed). Warrantable cautionary words were voiced regarding the translation of the concept into clinical applications and the general use of the terms oxidative stress and reactive oxygen species (16, 17; for a historical perspective see 18).

Given the enormous variety and range of prooxidant and antioxidant enzymes and compounds, attempts have been made to classify subforms of oxidative stress (15) and to conceptually introduce intensity scales ranging from physiological oxidative stress (eustress) to excessive and toxic oxidative burden (distress) (19, 20). Acute, chronic, and repetitive oxidative stress have been examined (21). Redox homeostasis has been phrased as "the golden mean of healthy living" (22, p. 1). **Table 1** provides a list of subforms of oxidative stress (modified from Reference 23).

OXIDATION–REDUCTION (REDOX) SPECIES IN BIOLOGY

Before the advent of oxygen in the atmosphere at appreciable amounts approximately 600 million years ago as the result of photosynthetic activity (24), redox reactions took place in various forms of anaerobic life, relying to a large extent on sulfur redox chemistry. The present discussion focuses on aerobic metabolism (i.e., oxygen-related redox reactions). **Table 2** presents the so-called reactive oxygen species (ROS), illustrating the enormous range of reactivity and of chemical nature. Note that ROS is a term that should be replaced by the name of the specific chemical species under consideration whenever possible. Some of the reduction products of oxygen are of a free-radical nature, having a free electron (e.g., the superoxide anion radical and the hydroxyl radical), whereas hydrogen peroxide, the two-electron reduction product, is not a radical and as such is a chemically stable molecule. Electronically excited states comprise singlet molecular oxygen and excited carbonyl compounds, which are also nonradicals. Further biologically important reactive species are chlorine and bromine species such as hypohalous acids, hypochlorite, and chloramines. Detailed chemical properties of these molecules have been compiled in textbooks and reviews. Suffice it to say here that the chemical reactivities of the various reactive species shown in **Table 2** vary by up to 11 orders of magnitude when assayed against a given target; an example is shown in **Table 3**.

Table 1 Oxidative stress: classification according to intensity, specific forms, related terms, and related biological responses

Category	Term
Classification	
Intensity	Basal oxidative stress (oxidative eustress, physiological oxidative stress, positive oxidative stress)
	Low-, intermediate-, high-intensity oxidative stress Low: oxidative eustress High: oxidative distress
	Acute, chronic, repetitive oxidative stress
Specific forms	Physiological oxidative stress (eustress)
	Nutritional, dietary, postprandial oxidative stress
	Glyco-oxidative stress (AGE)
	ER stress, proteotoxic stress, disulfide stress
	Photooxidative stress [ultraviolet (UV-A, UV-B), visible, infrared-A]
	Radiation-induced oxidative stress
	Nitrosative stress
	Nanoparticle-induced oxidative stress
	Environmental stress (sulfur dioxide, nitrogen dioxide, ozone)
Related terms	Oxidant stress, prooxidant stress
	Shear stress
	Redox stress, electrophilic stress
	Reductive stress, hypoxic stress
	Energy stress
Related responses of stress signaling	Heat shock response
	Unfolded protein response
	Cell proliferation
	Autophagy, apoptosis, necroptosis, ferroptosis
	Inflammation and danger signals, DAMP

Abbreviations: AGE, advanced glycation end products; DAMP, damage-associated molecular patterns; ER, endoplasmic reticulum; UV-A, ultraviolet A; UV-B, ultraviolet B.

In addition to ROS, further important reactive species have notable impacts on redox biology and, consequently, on oxidative stress. These include the reactive nitrogen species (RNS) (25), summarily named here as nitric oxide, nitrogen dioxide (both free radicals), peroxynitrite, and nitrite/nitrate. Reactive sulfur species (RSS) (26) include the various forms of cysteine and methionine, plus some low-molecular-mass compounds such as glutathione, trypanothione, or mycothiol (27, 28). Reactive carbonyl species (RCS) include various forms of metabolically generated aldehydes and electronically excited (triplet) carbonyls (13). Finally, various reactive selenium species (RSeS) include low-molecular-mass compounds and, importantly, selenocysteine and selenomethionine residues in proteins (29).

Other oxidants can be generated by one-electron reduction of suitable compounds, notably of quinone nature. The resultant semiquinone can autoxidize, generating the superoxide anion radical by electron transfer. Thus, the regenerated quinone may undergo another round of one-electron reduction in a process termed redox cycling (30). Alternatively, the quinone can undergo a two-electron reduction step to form the hydroquinone, catalyzed by reduced nicotinamide adenine dinucleotide phosphate (NADPH)–quinone oxidoreductase (NQOR). The hydroquinone can be glucuronidated and excreted.

Table 2 Reactive species: diversity of chemical nature

Free radicals	Nonradicals
Reactive oxygen species	
Superoxide anion radical ($O_2^{-\bullet}$)	Hydrogen peroxide (H_2O_2)
Hydroxyl radical ($OH^\bullet$)	Organic hydroperoxide (ROOH)
Peroxyl radical ($ROO^\bullet$)	Singlet molecular oxygen ($O_2\,^1\Delta_g$)
Alkoxyl radical ($RO^\bullet$)	Electronically excited carbonyls (RCO)[a]
	Ozone (O_3)
Reactive chlorine/bromine species	
Atomic chlorine ($Cl^\bullet$)	Hypochlorite (OCl^-)
Atomic bromine ($Br^\bullet$)	Chloramines (RNHCl)
	Hypobromite (OBr^-)
Reactive nitrogen species	
Nitric oxide = nitrogen monoxide ($NO^\bullet$)	Nitrite (NO_2^-)
Nitrogen dioxide ($NO_2^\bullet$)	Nitroxyl anion (NO^-)
	Peroxynitrite ($ONOO^-$)
	Peroxynitrate (O_2NOO^-)
	Nitrosoperoxycarbonate ($ONOOCO_2^-$)
Reactive sulfur species	
Thiyl radical ($RS^\bullet$)	Thiol (RSH), thiolate (RS^-) [e.g., glutathione (GSH), thioredoxin (Trx)]
	Disulfide (RSSR) [e.g., GSSG, mixed disulfide (protein SSG)]
	Sulfenate (RSO^-)
	Sulfinate (RSO_2^-)
	Sulfonate (RSO_3^-)
	Hydrogen sulfide (H_2S)
	Polysulfide (H_2S_x), x = 2 or higher; RSSH
Reactive carbonyl species	
	Acetaldehyde
	Acrolein
	Methylglyoxal
	4-Hydroxy-nonenal
	Electronically excited (triplet) carbonyls
Reactive selenium species	
	Selenite
	Selenate
	Selenocysteine
	Selenomethionine

[a]All oxygen radicals are reactive oxygen species, but not all reactive oxygen species are oxygen radicals. In fact, most reactive oxygen species are not free radicals. See Reference 139 for a more complete list.

OXIDANT AND ANTIOXIDANT SYSTEMS

Archaea (31) and bacteria (32) evolved batteries of antioxidant enzymes, and eukaryotes did likewise (33). The major oxidant and antioxidant systems (i.e., the sources and sinks of H_2O_2 as major reactive species) are presented in **Table 4**. The burden of antioxidant defense is primarily provided

Table 3 Oxidant range of reactivity: A range of 11 orders of magnitude for the second-order rate constants in the reaction of various oxidants with methionine (at neutral pH, in water)

Oxidant	Rate constant L mol^{-1} s^{-1}
Hydroxyl radical ($HO^{\bullet}$)	7×10^9
Hypochlorous acid (HOCl)	4×10^7
Singlet molecular oxygen	2×10^7
$CCl_3OO^{\bullet}$ (in H_2O/isopropanol)	3×10^7
Ozone (O_3)	5×10^6
Peroxynitrous acid (ONOOH)	2×10^3
Various chloramines	$4 \times 10^1 - 2 \times 10^2$
Superoxide anion ($O_2^{-\bullet}$)	<0.3
Peroxyl ($HOO^{\bullet}$)	$<5 \times 10^1$
Peroxynitrite ($ONOO^-$)	2×10^{-1}
Hydrogen peroxide (H_2O_2)	2×10^{-2}

Data compiled from Reference 63.

by enzymes, not by low-molecular-mass compounds (34). This consideration is important because the general public as well as part of the research community do not always acknowledge this fact. Complex networks of oxidant and antioxidant activities need to be considered. There seem to be no enzymes capable of deactivating electronically excited states, for example, singlet molecular oxygen and electronically excited (triplet) states of carbonyls, which are generated in the processes of photoexcitation and chemiexcitation. This domain is covered by low-molecular-mass compounds, notably carotenoids.

The enzymes and their reactants listed in **Table 4** are present simultaneously within cells and tissues in widely ranging proportions and activities. What turns them on or off? They are dependent, of course, on the ambient O_2 concentration and on exogenous cues of various natures,

Table 4 Major oxidant and antioxidant systems: enzymatic hydrogen peroxide (H_2O_2) sources and sinks

Sources	Sinks
NADPH oxidases	**Catalases** (catalatic and peroxidatic)
NADH oxidases	**Thioredoxin system**
Mitochondrial complexes I, II, and III	**Glutathione peroxidases (GPx 1–8)**
2-Oxoacid dehydrogenases	**Peroxiredoxins (Prx 1–6)**
Superoxide dismutases (SOD1 and SOD2)	Eosinophil peroxidase
Extracellular SOD (SOD3)	Myeloperoxidase
	Lactoperoxidase
Cytochromes P-450	
Monoamine oxidases	
Xanthine oxidase	
Glycolate oxidase	Transport systems
L-α-hydroxyacid oxidase	**Aquaporins, peroxiporins**
Aldehyde oxidase	Glutathione disulfide transport
D-aminoacid oxidase	

Bold indicates these systems are discussed in the text.

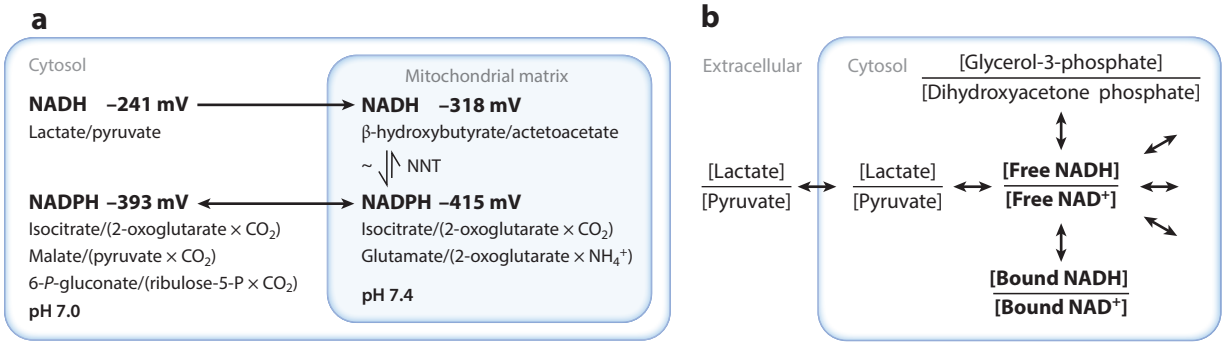

Figure 3

(*a*) Nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) redox potentials in the cytosol and the mitochondrial matrix. The major redox indicator metabolite systems are indicated. Energy-linked nicotinamide nucleotide transhydrogenase (NNT) connects mitochondrial matrix NADH and NADPH systems as exemplified in liver. (*b*) Intracellular and extracellular NADH redox communication. Cytosolic NADH binding sites buffer the free NADH concentration to approximately 1 μM (pNADH = 6). The right-hand arrows depict redox equilibration with other dehydrogenase substrate couples. Data compiled from Reference 37.

physical or chemical. Furthermore, they are dependent on the specific type of individual cell or tissue or organ, depending on the control of gene expression (**Figure 1**). Thus, like a given optimum pH exists for a given cellular or subcellular space, an optimum pattern of prooxidants and antioxidants exists for a given physiological condition. This is denoted as peroxide tone (35), carbonyl tone, or sulfide tone. The sulfide tone serves as a useful example: For glutathione (GSH), reactions can depend upon the GSH concentration (e.g., GSH *S*-transferase reaction); the ratio of GSH to its major disulfide form, GSSG (e.g., protein glutathionylation); or the redox potential of the 2-electron couple, 2 GSH/GSSG (e.g., platelet activation) (36).

Backbone Role of NADH and NADPH Systems

Catabolism and energy capture are funneled through the NADH system, whereas the NADPH system drives not only reductive anabolism but also enzymatically controlled oxidative reactions such as NADPH oxidases, nitric oxide synthases (NOS), cytochrome P-450-dependent hydroxylations, and so forth (see 1). Cytosolic NADH operates at a much more positive redox potential than mitochondrial NADH owing to powerful cytosolic NADH binding sites (**Figure 3*a***), the set point of free NADH being buffered to 1 μM (i.e., pNADH = 6) (37). Redox biology has focused on these relationships (38), for example, in view of the NAD^+/sirtuin pathway (39). Control of the level of cellular NAD has repercussions on energy metabolism (40). In the mitochondria, NADH and NADPH are interlinked by the enzyme, energy-linked nicotinamide nucleotide transhydrogenase (NNT), which uses the proton motive force to transfer electrons from NADH to $NADP^+$, thus backing up NADPH-related antioxidant defense. This reaction is reversible, so that under pathological metabolic demand NADPH is consumed to support NADH and ATP production, which weakens antioxidant defense. Reversed flux through NNT causes oxidative stress, as shown in heart failure (41).

NONINVASIVE ASSAY SYSTEMS

Analysis of redox systems in biological context is faced with major challenges. Given the spatiotemporal compartmentation characterized by substantial subcellular differences in redox potential and

also by intercellular heterogeneity in an organ, grinding up samples in an oxygen-rich atmosphere may generate artifacts. The issue of sample preparation continues to be an important one. Even in 1928, Otto Warburg noted in his book, *On the Catalytic Actions of the Living Substance*, that one should study enzymes under the most natural conditions of action, in the living cell itself (42). This is the mindset behind the current use of noninvasive methods selectively sensing and reporting ligands or reactants such as H_2O_2 (see 43).

Redox Indicator Metabolites

Redox equilibration occurs by the activity of powerful dehydrogenase systems. The redox potential of the nicotinamide adenine dinucleotide (NAD) system in the cytosol is reflected by the extracellular lactate/pyruvate concentration ratio (**Figure 3*b***). The concept of redox indicator metabolite ratios was introduced into redox biology early on (44). In particular, the cytosolic (45) and mitochondrial (46) NAD systems are amenable to analysis using the lactate/pyruvate and ß-hydroxybutyrate/acetoacetate ratios, respectively. The redox state of the NADP systems has been analyzed in a similar fashion, though it is not accessible noninvasively because the reactants are not permeable to the extracellular space. This research field lay dormant during past decades, but in view of rising interest in examination of integrated systems, novel applications might arise.

Biophysical Methods

Specific noninvasive readout of endogenous molecules of interest is afforded by biophysical methods. The redox state of heme proteins can be monitored continuously by following characteristic absorbance bands. Subtle redox changes may be accessible for analysis with further development of organ redox imaging techniques. Noninvasive spectrophotometric analysis of the redox state of mitochondrial and extramitochondrial cytochromes has become a field of interest. Likewise, fluorescence emission of endogenous components (e.g., NADH) has been employed successfully. Aside from the prominent Soret band, catalase compound I has an absorbance peak in the difference spectrum in the near infrared (charge transfer band), at 660 nm, which permitted the first identification of H_2O_2 noninvasively in an intact respiring organ, the perfused liver (47). Redox-sensitive two-photon microscopy and other techniques are being developed for spatiotemporal analysis (see 48).

Direct and indirect (spin trap) measurements of free radical components (e.g., the superoxide anion radical) by electron spin resonance (ESR) [or electron paramagnetic resonance (EPR)] methods have been developed for noninvasive detection (e.g., in accessible sites such as human skin). EPR is considered the gold standard for the detection and identification of radicals in biological systems (49).

Genetically Encoded Fluorescent Protein Indicators

Although the aforementioned techniques measure endogenously occurring components for readout of their respective redox states, an exciting new field of redox research utilizes properties of known redox sensor proteins as coupled with fluorescent proteins. These indicator systems can be genetically encoded at sites in cells and organs to monitor changes in redox states during metabolic activity, which hopefully is progressing undisturbed. A landmark example is the fluorescent probe HyPer (50), which consists of circularly permuted yellow fluorescent protein (cpYFP) inserted into the regulatory domain of the prokaryotic H_2O_2-sensing protein OxyR, an H_2O_2-inducible gene regulator. Several types of redox-sensitive proteins have been developed.

By inserting a redox-active pair of cysteines into the structure of green fluorescent protein (GFP) or YFP, a new class of redox-sensitive fluorescent proteins was created, generically called roGFP (51). Fusion with human glutaredoxin-1 led to a probe suitable for real-time imaging of the intracellular glutathione redox system (52). Real-time monitoring of basal H_2O_2 levels became possible with peroxiredoxin-based probes (53). Redox indicator mice stably expressing genetically encoded roGFP serve to noninvasively decipher subcellular redox dynamics (54).

Redox regulation: control of enzymes and processes by oxidation–reduction

Nonredox Exogenous Probes

Whereas the fluorescent protein probes are actively emitting fluorescence upon a redox-based conformational change, another approach utilizes fluorescence emitters that are cloaked and transported into specific cellular target spaces until they are activated. Using boronate-based chemistry (55), a nonredox exogenous probe compound is administered to the intact organism or cell. Subsequently, at the target site, the compound is transformed in vivo to a diagnostic fluorescent compound or to an exomarker, which is analyzable, for example, by mass spectrometry (56).

Other Fluorescent Probes

Members of a group of small-molecule fluorescent probes are dihydrorhodamine, dihydroethidium, luminol, and lucigenin, and each has its specific merits and pitfalls (see 57 for detailed discussion). Therefore, these compounds should be used with caution (57). One example is 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), which some investigators claimed measured cellular peroxides or ROS: This probe does not, however, measure cellular peroxides directly but rather detects radicals to which peroxides are converted by transition metal ions (58).

REDOX REGULATION

Redox reactions control almost all aspects of life. Regulation can take place via control of single enzymatic activity or at the transcriptional level. Redox control of transcription factors was an early discovery (see 59). Redox regulation of transcriptional activity is mediated by DNA binding of Fos and Jun as the heterodimer, activator protein-1 (AP-1) (60). Central FoxO transcription factors are also redox sensitive (61). Thus, several signaling mechanisms themselves are redox regulated. The role of redox regulation of protein kinases has developed beyond that of protein tyrosine phosphatases to a more general role in regulating tyrosine kinases in fine-tuning the duration and amplification of the phosphorylation signal (62).

Protein oxidation is not necessarily deleterious. The mechanism of redox signaling and control involves, to a large extent, the oxidative modification of amino acid side chains in proteins by hydrogen peroxide: cysteine, methionine, proline, histidine, and tryptophan (in decreasing order of reactivity and biological reversibility) (see 13). Other oxidants play a role as well (63). The activity of enzymes can be modulated by reversible thiol/disulfide redox changes of specific amino acids (64, 65). Hexose transport (66) and photosynthesis (67) were the first biological processes to be identified as being thiol redox–regulated. Thiols undergo different reversible oxidative modifications beyond disulfide formation, such as sulfenic acid, nitrosation, or *S*-glutathionylation (68). Most of these oxidatively modified residues are reduced by thioredoxins and glutaredoxins (69). Physiological processes such as embryonic development are thioredoxin/glutaredoxin-dependent (70). Knowledge regarding the mechanisms involved in oxidizing protein thiols is accumulating. For example, macrophage responses to metabolic cues from the extracellular environment are

mediated by protein *S*-glutathionylation (71). Distinguishing between deleterious and beneficial protein oxidation is often difficult.

Redox sensing: perception of a specific oxidation–reduction state by a sensor molecule

Hydrogen Peroxide in Redox Sensing and Redox Signaling

Hydrogen peroxide, an uncharged molecule, is well suited for redox sensing and redox signaling (72–74). Although the molecule reacts relatively sluggishly with biomolecules (second-order rate constants approximately 1 $M^{-1}s^{-1}$), there are notable exceptions, for example, certain cysteinyl residues in peroxiredoxins or selenocysteinyl residues in glutathione peroxidases (10^7 $M^{-1}s^{-1}$) (75). Because of this sluggishness, H_2O_2 can diffuse away from the site of its generation to reach a more reactive target at a certain distance, whereas highly reactive oxidants such as the hydroxyl radical cannot.

The concept that thiol peroxidases can act as H_2O_2 sensors and transducers has been corroborated in recent studies. H_2O_2 is a major component in redox signaling (76), as schematically represented in **Figure 4**. One example of a redox sensing strategy is that peroxiredoxin-2 acts as an ultrasensitive primary H_2O_2 receptor that specifically transmits oxidative equivalents to the redox-regulated transcription factor STAT3, thus forming a redox relay for H_2O_2 redox signaling (77). A different strategy to allow for spatiotemporal control is that peroxiredoxin cysteinyl residues become hyperoxidized by H_2O_2 to the sulfinic acid, which results in inactivation of the peroxidase. It has been suggested (78) that, as a result, H_2O_2 builds up locally at target sites, likened to the opening of a floodgate, which allows the oxidation of specific target proteins. The functional loop is closed by sulfiredoxins, which are capable of reducing the hyperoxidized peroxiredoxins back to restore functionality (79). Thus, when confined near the physiological range of H_2O_2 concentration at approximately 10 nM (43, 80), which is well below toxic levels, H_2O_2 is being utilized

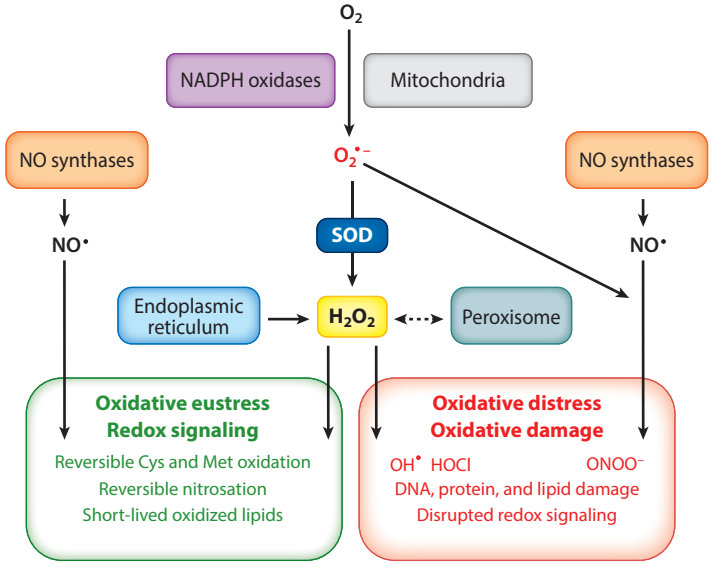

Figure 4

Role of H_2O_2 and NO in redox signaling (oxidative eustress) and in oxidative damage (oxidative distress). Major endogenous H_2O_2 sources via superoxide radical formation include NADPH oxidases (NOX enzymes) and mitochondria. Other enzymes and organelles contribute to H_2O_2 formation as well. NO is generated by NO synthases (NOS enzymes). Abbreviations: Cys, cysteine; Met, methionine; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; SOD1, superoxide dismutase-1.

Table 5 Hydrogen peroxide (H_2O_2) production rate in intact organ

Substrates or inhibitors	H_2O_2 production rates (nmol H_2O_2/min/g of liver wet weight)
L-lactate, 2 mM; pyruvate, 0.3 mM	49
+ octanoate, 0.3 mM	170
+ oleate, 0.1 mM	66
+ glycolate, 3 mM	490
+ antimycin, 8 μM	75

Isolated hemoglobin-free perfused liver data were obtained by methanol titration of catalase compound I; organ photometry data from Reference 81. (For discussion, see References 43, 80.)

as a suitable second messenger in redox biology. H_2O_2 production rates in the intact organ are of the order of 50 nmol H_2O_2 per min per gram, assayed noninvasively in perfused liver, which is approximately 2% of total oxygen uptake at steady state (**Table 5**) (43, 81). How much of this total cellular production is generated by various sources of H_2O_2 at a given metabolic situation in the cell needs to be examined.

Aquaporins as peroxiporins. It had long been assumed that hydrogen peroxide diffusion through biological membranes occurs at a sufficient rate for metabolic purposes. However, in 2000 it was shown that H_2O_2 uses water channels, the aquaporins, to cross the cell membrane more rapidly than by simple diffusion across a lipid bilayer (82). This discovery opened an exciting field of membrane transport of hydrogen peroxide. Specific aquaporins, referred to as peroxiporins, allow H_2O_2 diffusion (83). Mitochondrial aquaporin-8 knockdown in human hepatoma HepG2 cells caused loss of viability (84). Cellular stress conditions reversibly inhibit the permeability of aquaporin-8, providing a novel mechanism for regulation of cell signaling (85). Aquaporin-3 was shown to mediate H_2O_2 uptake to regulate downstream signaling (86). **Figure 5** illustrates the role of aquaporins in the plasma membrane to capture H_2O_2 from the extracellular space for intracellular H_2O_2 signaling. Chloroplasts, which produce H_2O_2, also contain aquaporins, so that they are likely functioning as peroxiporins (87). Additional knowledge of the peroxiporin activity of aquaporins is likely to enhance understanding of the mechanistic details of oxidative stress in different compartments.

Role of NADPH oxidases. The initial product of the oxidase carrying out the respiratory burst in leukocytes is superoxide (88); the reducing equivalents come from NADPH. Human fibroblasts release superoxide under the control of cytokines (89). The enzyme family responsible is that of NADPH oxidases (Nox) (90), shown to have abundant functions in physiology and pathophysiology (91) (see **Figure 4**). Although one major function is in host defense (i.e., in a prooxidant role), important cellular functions are in redox signaling. For example, Nox4 has a protective vascular function, and its deletion causes apoptosis (92). The constitutive contribution of Nox activity to cellular H_2O_2 production can be significant: Nox4 contributes approximately one-third of cellular H_2O_2 formation in vascular endothelium (92). Targeted redox inhibition of protein phosphatase-1 by Nox4 regulates eukaryotic initiator factor 2α–mediated stress signaling (93). Conversely, overexpression of Nox4 induces cardiac arrhythmic phenotype, shown in zebrafish (94). Nox enzymes are constituents of redox-active endosomes, termed redoxosomes (95), which may be targets for disease-specific therapies (96).

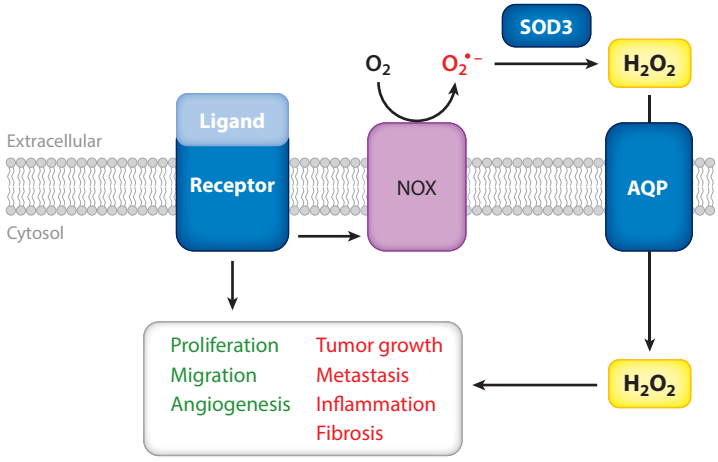

Figure 5

Role of aquaporins as peroxiporins in growth factor receptor signaling. NOX enzymes are activated upon ligand–receptor interaction, and extracellular SOD3 captures the superoxide, providing H_2O_2 for import by aquaporins (peroxiporins). Ligand–receptor pairs, for example, can be TNFα–TNFR or EGF–EGFR. Green denotes physiological processes, and red denotes pathophysiological processes. Abbreviations: AQP, aquaporin; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; NOX, NADPH oxidase; SOD3, superoxide dismutase 3; TNFα, tumor necrosis factor α; TNFR, tumor necrosis factor receptor.

Role of mitochondria. Shortly after cellular production of H_2O_2 in intact cells was established in 1970 (47), isolated mitochondria were identified as a major source of H_2O_2 (97, 98). Mitochondria produce superoxide and hydrogen peroxide in two major categories: mitochondrial inner membrane complexes I, II, and III (99, 100) and the mitochondrial matrix and/or inner membrane–bound dehydrogenases (101, 102). A dozen mitochondrial sources of O_2^-/H_2O_2 have been identified, as depicted in **Figure 6** (see 101, 103). A total of 31 cellular hydrogen peroxide–generating enzymes have been compiled (104). A crucial question is how mitochondrial redox switches operate in the intact cell (105). Whether H_2O_2 is released from the mitochondria into the cytosol in intact cells has yet to be demonstrated. Work with HyPerRed indicated that mitochondrial matrix H_2O_2 does not spread to the intermembrane space and the cytosol (106). Redox signals might use other avenues, for example, via glutathionylation (107). Mitochondrial functions include a multitude of effects, modulating neuroendocrine, metabolic, inflammatory, and transcriptional responses to acute psychological stress (108). Mitochondrial redox signaling is involved in triggering responses to hypoxia (109). Mitochondria were also found to contribute significantly to the handling of extramitochondrial oxidants (110), underlining a mitochondrial role in compartment-specific control of oxidants (111).

Hydrogen Sulfide, Nitric Oxide, and Peroxynitrite

Although much information speaks for H_2O_2 as a prime signaling molecule (72–74), there is also evidence that free radicals (one-electron reactions via the thiyl radical) play a role in thiol-based redox signaling (112, 113). In fact, it has been suggested that reactive sulfide species are prevalent in intracellular redox signaling (27). The role of biologically active sulfane–sulfur-containing compounds and organic and inorganic persulfides is being elucidated and has been recognized in cellular redox regulation (114, 115).

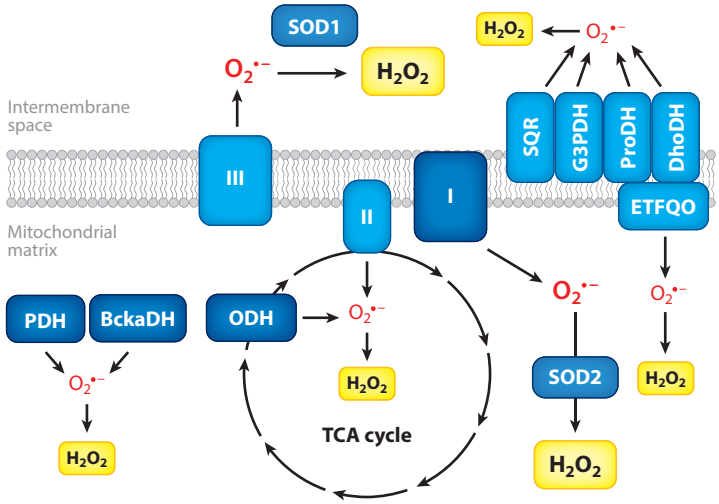

Figure 6

Mitochondrial H_2O_2 sources. The mitochondrial inner membrane complexes I, II, and III as well as a number of flavin-dependent enzymes, membrane bound or in the matrix space, produce superoxide (*dark blue*, NADPH-dependent enzymes; *turquoise blue*, quinone-dependent enzymes), which is rapidly converted to H_2O_2 by SOD1 in the intermembrane space and by SOD2 in the mitochondrial matrix space. Abbreviations: BckaDH, branched-chain ketoacid dehydrogenase; DhoDH, dihydroorotate dehydrogenase; ETFQO, electron transfer flavoprotein oxidoreductase; G3PDH, glycerol-3-phosphate dehydrogenase; ODH, 2-oxoglutarate dehydrogenase; PDH, pyruvate dehydrogenase; ProDH, proline dehydrogenase; SOD1, superoxide dismutase-1; SQR, succinate:quinone reductase; TCA cycle, tricarboxylic acid cycle.

Nitric oxide, which is produced by NOS, is a physiological messenger molecule with well-characterized signaling properties. As a free radical, it reacts rapidly with the superoxide anion radical to form peroxynitrite (116). Peroxynitrite, in turn, is used in nitration reactions to form 3-nitrotyrosine residues in proteins. Peroxynitrite acts as biological oxidant, affecting mitochondrial functions and triggering cell death via oxidation and nitration reactions.

Redox Proteomes

The thiol group in proteins undergoes reversible and irreversible oxidation reactions with impact on protein structure and function (117). These include multiple oxidation states (sulfenic, sulfinic, sulfonic), adduct forms (glutathionylation, nitrosylation, cysteinylation, persulfidation, etc.), and metal ion coordinations (Zn, Fe, Cu, etc.). The refined methodology of protein analysis using mass spectrometry, redox imaging, immunoassays, and manipulation with molecular biology have allowed for identification of redox patterns and compartmentation, with increasing capabilities to analyze thiol/disulfide redox states in signaling and sensing (118, 119). Major limitations include the propensity for oxidative artifact as mentioned above, the cost associated with detailed studies, and the relatively sparse coverage that tends to measure cysteines in peptides from relatively high abundance proteins. The cysteine redox proteome has been widely studied (for review, see 104).

In addition to signaling functions, the redox proteome serves more general functions to control and integrate functional systems. Studies of cultured cells and animal organs without imposed oxidant or impaired antioxidant systems show that the median percentage oxidation of cysteine residues in the proteome is between 6% and 14%. Ongoing oxidation is balanced by reduction; thus, oxidative eustress is ongoing. Pathway and network studies show that cysteine residues within

functional pathways share similar percentages of oxidation (119). This is evident for cysteines in many actin-associated proteins, nuclear import proteins, and mRNA processing enzymes.

The methionine redox proteome is increasingly viewed as part of a highly regulated machinery controlling signaling factors under oxidative stress (120). Although enzymes are mostly inhibited or inactivated by methionine oxidation, examples of enzyme activation have also been found. Oxidation of the sulfur atom in methionine introduces an asymmetric center, and specific enzyme systems linked to sulfur- and selenium-containing proteins are needed to reverse the R- and S-forms (121).

MOLECULAR REDOX SWITCHES: MASTER REGULATORS

Molecular redox switches serve two functions: to control activation/deactivation cycles (i.e., in redox signaling) and to modulate or integrate activity of systems (i.e., in redox sensing). Redox-sensitive transcription factors as master regulators (e.g., Nrf2/Keap1, NF-κB) control very broad ranges of biological functions. A central issue is to develop methods to identify a master redox set point, with oscillation about that set point to maintain homeostasis.

Nrf2/Keap1 and NF-κB

Nrf2 (nuclear factor-E2-related factor 2) belongs to a small family of transcription factors inducing a set of antioxidant and detoxication enzymes (122). Keap1 (Kelch-like ECH-associated protein 1) subjects Nrf2 to rapid ubiquitination and degradation and thus suppresses the transcriptional activity of Nrf2 under unstressed conditions. Under oxidative or electrophilic stress, specific cysteinyl residues of Keap1 are modified and Keap1 loses its ability to ubiquitinate Nrf2, allowing it to accumulate in the nucleus to induce expression of its target genes. This redox stress–sensing adaptive response system has been studied widely in terms of molecular mechanism and biological significance. Activation of the Nrf2/Keap1 system is clearly protective, but overactivation can be counterproductive, for example, in cancer and in resistance to chemotherapy (123). Interestingly, the selenoprotein thioredoxin reductase 1 (TrxR1) has been proposed as a potent regulator of Nrf2 (**Figure 7**), permitting fine-tuning of this master switch (124). Nrf2 undergoes translocational oscillations (125). Regulatory fine-tuning of Nrf2 also includes p62 and TRIM21 utilizing ubiquitination mechanisms (126). Trapping of Nrf2 in the nucleus at the nuclear periphery by a mutant form of lamin A, progerin, leads to impaired Nrf2 signaling and chronic oxidative stress, which contributes to the premature aging phenotype (127).

Nuclear factor κB (NF-κB) is a multisubunit transcription factor that can rapidly activate the expression of genes involved in inflammatory, immune, and acute phase responses. Oxidation by H_2O_2 leads to activation, causing the release of the inhibitory subunit of the NF-κB inhibitor (IκB) (128). Like Nrf2, DNA-binding subunits of NF-κB contain redox-sensitive cysteine residues, which inhibit activity upon oxidation (129). Activity is stimulated by nuclear Trx1 (130) and inhibited by increased nuclear H_2O_2 production (131), indicating that the nuclear peroxide tone is an important determinant of function. As with Nrf2/Keap1, NF-κB oscillations occur (132), allowing synchronization to a variety of periodic external perturbations. Transcription factor oscillatory dynamics have been proposed as a means of segmenting time to provide renewing opportunity windows for decision (132).

PHOTOOXIDATIVE STRESS: ROLE OF SINGLET OXYGEN

Sunlight photochemistry played important roles in the origin of life, although, paradoxically, it was also one of the main threats to the persistence of early life forms (133). The generation of oxidants

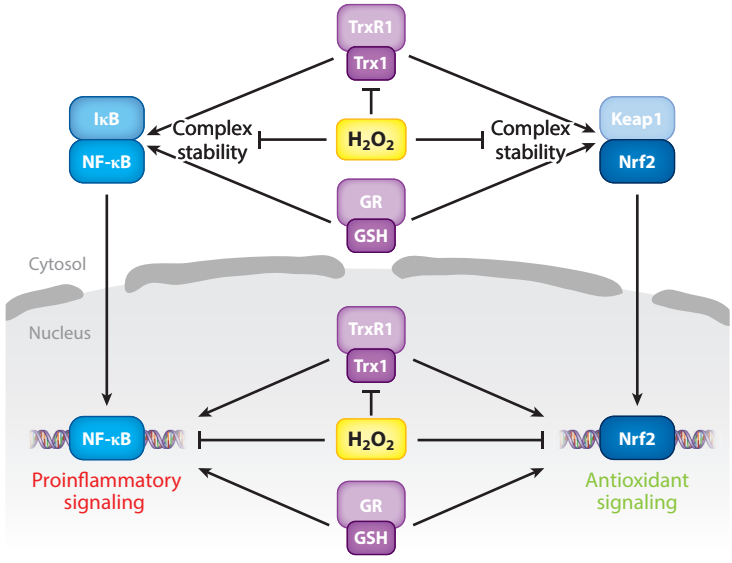

Figure 7

Thioredoxin reductase and glutathione reductase maintain complex stability of NF-κB/IκB and Nrf2/Keap1. Cytosolic H_2O_2 leads to dissociation of the complexes, allowing for transport of NF-κB and Nrf2 through nuclear pores to the sites of DNA binding. Note that gene activation in the nucleus needs reductive conditions. Abbreviations: GR, glutathione disulfide reductase; GSH, glutathione; IκB, NF-κB inhibitor; Keap1, Kelch-like ECH-associated protein 1; NF-κB, nuclear factor κB; Nrf2, nuclear factor-E2-related factor 2; Trx1, thioredoxin-1; TrxR1, thioredoxin reductase-1.

by electronic excitation through impinging light represents a major part of photobiology (for an overview of phototrophic organisms, see 134). Photoexcitation of endogenous or exogenous sensitizer molecules (photosensitization) leads to formation of reactive species, notably singlet molecular oxygen (**Figure 8**), electronically excited carbonyls, and superoxide anion radicals; these may cause molecular damage. The wavelength ranges of biological importance are ultraviolet B and ultraviolet A, but visible light and even infrared-A are also known to generate photobiological responses. The extensive literature on this topic in plant sciences is not covered here. In human health, exposed tissues such as the skin and eyes are most studied. Singlet oxygen mediates the photoaging-associated mitochondrial common deletion (135). Dietary micronutrients such as carotenoids and polyphenols can provide nutritional protection against damage from sunlight (136). Of the carotenoids, lycopene has the highest rate constant in the reaction with singlet molecular oxygen (137). The second-order rate constants for the carotenoids with singlet oxygen are near diffusion control. In physical quenching, the carotenoids come out unchanged, obviating the need for an extra enzyme detoxifying singlet oxygen (**Figure 8**). However, in addition to the physical reaction, bleaching of the carotenoid occurs to some small degree as a chemical reaction. Other cellular components, such as melanin derivative–induced DNA photoproducts after chemiexcitation, can contribute to damage (138).

OXIDATIVE DAMAGE AND REPAIR

Early in the description of the biochemistry of oxidative stress, damage to biomolecules and its consequences became a focus of interest (13). An enormous literature has since accumulated (see

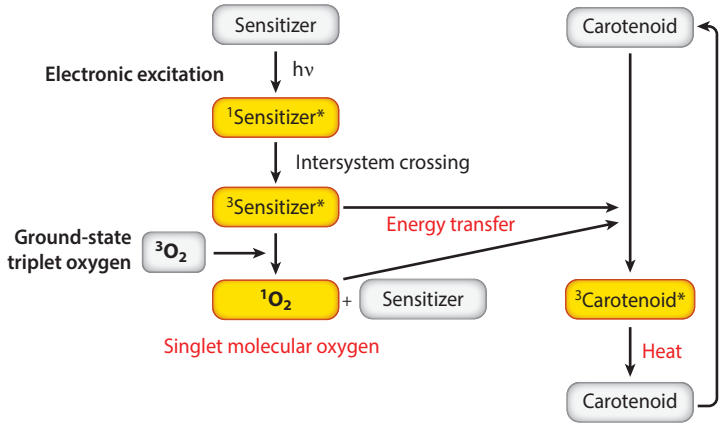

Figure 8

Singlet molecular oxygen and physical quenching by carotenoids. Photoexcitation or chemiexcitation of a sensitizer molecule leads to electronically excited states. The triplet sensitizer reacts with oxygen to generate singlet molecular oxygen, regenerating the ground-state sensitizer (type II reaction). The energy is transferred to carotenoids with their system of conjugated double bonds that, in turn, release the energy as heat to the solvent, regenerating the carotenoid unchanged. Thus, physical quenching of singlet oxygen by carotenoids obviates the need for an enzyme.

139). In this section, we discuss only some recent aspects. The main message is that oxidatively modified biomolecules not only are counteracted by direct repair or restoration responses but also can act as bona fide signaling molecules.

Nucleic Acids

DNA oxidation, along with DNA hydrolysis and DNA methylation, is a major contributor to instability and decay of the genome. Spontaneous mutagenesis under aerobic conditions is larger than under anaerobic conditions, and deletion of the OxyR regulon in bacteria, which counteracts DNA damage, enhances spontaneous mutations significantly (140). Among the DNA bases, guanine is most susceptible to oxidative damage. The major mutagenic lesion is 8-oxo-7,8-dihydroguanine (also called 8-oxoguanine or 8-hydroxyguanine), which base pairs with adenine rather than with cytosine and thus generates transversion mutations after replication (141). A multitude of DNA damage reactions and their breakdown products have been studied (142). The accumulation of 8-oxoguanine causes mitochondrial dysfunction and is oncogenic (143), and the enzyme human mutT homolog (MTH1) that detoxifies oxidized nucleotides is a potential target in cancer therapy (144).

RNA is subject to oxidation as well, with implications for disease processes (145). MicroRNAs are noncoding RNAs, approximately 18–25 nucleotides in length, which bind to target mRNAs at the 3′-UTR and either affect mRNA degradation or inhibit protein translation. A subset of microRNAs that regulates redox pathways has been termed redoximiRs (146). Oxidative modification of microRNA-184 enables it to target the mRNA of the B cell lymphoma proteins Bcl-xL and Bcl-w, thereby blocking their translation, which leads to apoptosis (**Figure 9**) (147). Another illustrative example is microRNA-15b, which regulates mitochondrial superoxide production through sirtuin-4 translation (148).

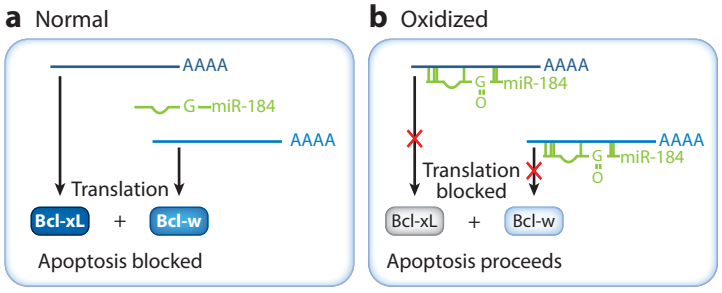

Figure 9

Oxidized microRNA (miR) can change mRNA specificity. Native miR-184 does not bind to the 3′-UTRs of Bcl-xL and Bcl-w, allowing their translation, which leads to blocked apoptosis (*a*). A guanine base oxidized in miR-184 causes misrecognition of the 3′-UTRs of the B cell lymphoma proteins Bcl-xL and Bcl-w. This results in their downregulation, which leads to apoptosis (*b*). See text.

Proteins

Protein oxidation forms a multitude of oxidation products in amino acid side chains (63, 149, 150). As reversible oxidative modifications, some protein damage products support signaling events. The endoplasmic reticulum (ER) is a major site of oxidative protein folding, introducing intra- and intermolecular disulfide bonds into proteins via oxidative processes (151). These processes include the activity of the PDI family of dithiol-disulfide oxidoreductases, the pathways of ER oxidoreductin 1 (Ero1), peroxiredoxin IV, glutathione peroxidase 7 and 8 (GPx7 and GPx8), and vitamin K epoxide reductase (VKOR). Stringent reduction in the ER lumen is required, because a molecule of H_2O_2 is produced for each disulfide bond formed by Ero1. GPx8 prevents the leakage of H_2O_2 from the ER (**Figure 10*a***) (152). Likewise, GPx7 has been identified as a novel oxidative stress sensor/transmitter (153). The transit of H_2O_2 across the ER membrane is not sluggish (154). Accumulation of unfolded proteins in the ER lumen (ER stress) activates the unfolded protein response (UPR). Studies in *Caenorhabditis elegans* have revealed that chronic proteotoxic stress is associated with a shift toward more reducing conditions in the ER and more oxidized conditions in the cytosol (155). Thus, there is an integration of redox signals and chaperones (156) in ER stress, ultimately leading to reestablishment of redox homeostasis or cell death (**Figure 10*b***) (157, 158).

Lipids

Biologically significant oxidation products of lipids play a central role in oxidative signaling. The understanding of oxidative lipidomics has been richly developed, with major classes being lipid hydroperoxides, lipid hydroxides and epoxides (including cholesterol oxidation products), isoprostanes, malondialdehyde (MDA) and other aldehydes and ketones, and more (159, 160). Lipid oxidation products are ligands for the peroxisome proliferator-activated receptor (PPAR) (161).

Carbohydrates: glycans, glycosylation, glycation, GlcNAcylation. Relations of carbohydrates with oxidative stress are manifold. Oxidative damage to the sugar backbone of nucleic acids causes strand breaks. Free carbohydrates generate oxidants such as reactive carbonyls (162). Nonenzymic glycosylation, the initial stage of the Maillard reaction, generates glycoxidation products that accumulate in tissue with age (163), and growing evidence shows that advanced glycation

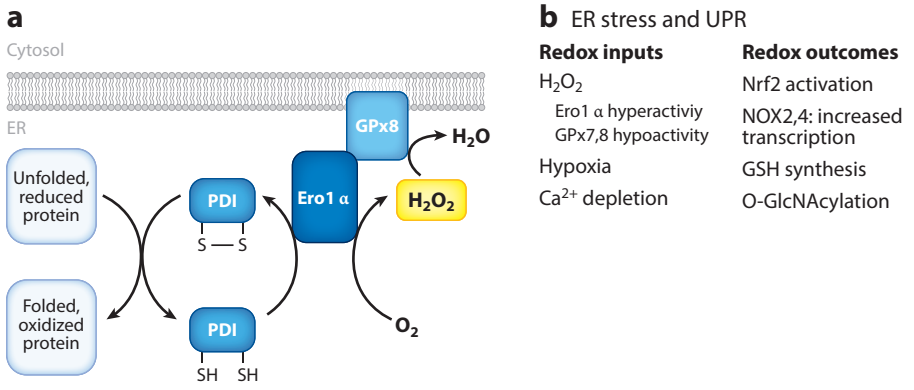

Figure 10

(*a*) H_2O_2 production in the ER is coupled to generation of disulfide bonds. Each insertion of a disulfide bond via PDI leads to generation of one H_2O_2 molecule. This H_2O_2 is reduced by GPx8, which thereby is essential in control of the ER lumen redox state. (*b*) ER stress and UPR. In response to various redox inputs, UPR signaling stimulates redox effector processes. Abbreviations: ER, endoplasmic reticulum; Ero1, ER oxidoreductin 1; GPx8, glutathione peroxidase-8; GSH, glutathione; NOX, NADPH oxidases; Nrf2, nuclear factor-E2-related factor 2; O-GlcNAcylation, O-linked *N*-acetylglucosamine at serines or threonines; PDI, protein disulfide isomerase; UPR, unfolded protein response.

end products (AGE) and the receptor (RAGE) interaction elicit oxidative stress (glyco-oxidative stress). O-linked *N*-acetylglucosamine (O-GlcNAc) is a posttranslational protein modification (GlcNAcylation) employed by cells to respond to stress (164). The bisecting GlcNAc modification of β-site amyloid precursor protein-cleaving enzyme-1 (BACE1) stabilizes this protein under oxidative stress conditions, leading to increased amyloid-β generation (165). Ammonia-induced O-GlycNAcylation of astrocyte proteins may contribute to the pathophysiology of hepatic encephalopathy (166).

BIOMARKERS

Numerous biomarkers of oxidative stress have been employed (167), and the clinical relevance of such biomarkers has been examined (168). Major biomarkers include protein carbonyls and AGE; 3-nitrotyrosine; oxidized low-density lipoprotein; other lipid oxidation products such as 4-hydroxy-nonenal and MDA; F2-isoprostanes (for a recent discussion, see 169); DNA/RNA oxidation products such as 8-oxoguanine; glutathione, protein thiols, and methionine sulfoxide; and others. **Figure 11** shows a cluster analysis of such biomarkers in disease (168). Specialized biomarkers relating to specific conditions exist. One of these biomarkers is the length of telomeres, which decreases with oxidative stress (170). Another is the comet assay, utilized to assay DNA damage in cells (171). GSH/GSSG and cysteine/cystine redox couples in plasma have been used, and high cystine levels have been linked to death as an outcome in cardiovascular disease (172). Biomarkers of inflammatory disease states involve damage-associated molecular patterns, including oxidation-related products (173).

Epigenetic systems, immune cell selection, and other mechanisms allow an individual to adapt during the lifespan; these adaptive responses decrease flexibility and contribute to aging and disease. An understanding of the impact of episodic oxidative stress on exposure memory will be important. Methods to measure exposure memory and its contribution to disease are needed.

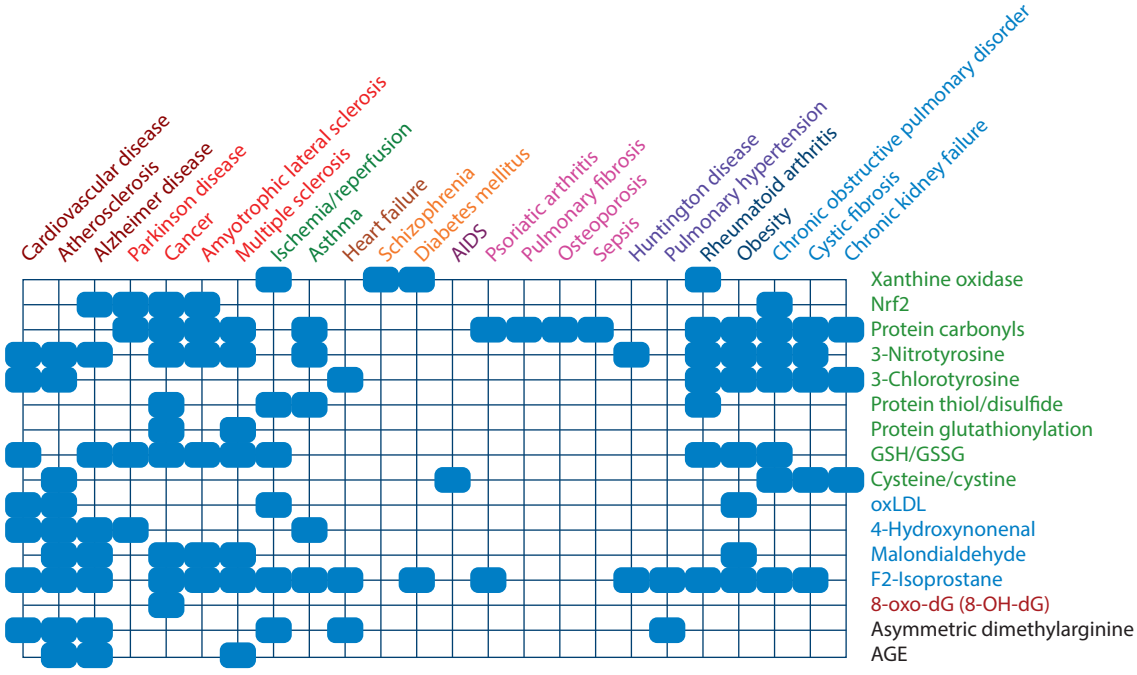

Figure 11

Clinical relevance of biomarkers of oxidative stress. Protein (*green*), lipid (*blue*), and DNA (*red*) biomarkers were analyzed in various diseases listed across the top. Color coding on top illustrates the result of cluster analysis. Abbreviations: AGE, advanced glycation end products; GSH, glutathione; GSSG, glutathione disulfide; Nrf2, nuclear factor-E2-related factor 2; oxLDL, oxidized low-density-lipoprotein. Data compiled from Reference 168.

STRATEGIES OF ANTIOXIDANT DEFENSE

Given the enormous range of reactivity of oxidants with a particular target (e.g., **Table 2**) and, conversely, given the similarly diverse range of targets, multiple strategies by living cells and organisms counteract oxidative challenge, called antioxidant defense. Protection is categorized into prevention, interception, and repair (33).

Prevention

Strategies to evade oxidative stress have evolved. Minimization of exposure to photooxidative stress occurs in skin by melanin synthesis, in plankton simply by descending from the surface of the seawater to lower levels to evade solar irradiation, or in DNA by packaging the DNA into chromatin with protein to provide alternate targets to shield genetic material. Antioxidant defense in multicellular organisms can be more efficient than in unicellular organisms. *Escherichia coli* can defend colonial but not individual cells against hydrogen peroxide (i.e., unicellular organisms have multicellular defense), which was proposed to be a driving force for the evolution of multicellular organisms (174). Microbial communities embedded in self-produced matrices constitute biofilms. Mechanisms activated by biofilms in response to oxidative stress have implications in ecology and medicine (175).

Regarding radical formation, enzymes are ingeniously designed to avoid release of radicals; a prominent example is the heme iron–containing and copper-containing cytochrome *c* oxidase.

Many enzymes control the level of reactive species (see **Table 4**) in cells and body fluids. Biologically reactive electrophilic intermediates generated in metabolic pathways, for example, by the cytochrome P-450s or in quinone metabolism, can be detoxified by glutathione *S*-transferases or by quinone reductases, respectively. This represents a broad field in toxicology and pharmacology.

Prevention by diversion is another strategy, which consists of channeling an attacking species into a less harmful or more readily repairable product, lowering the risk of further damage. In the extreme, this strategy can involve subcellular structures or whole cells—in other words, elimination by autophagy, apoptosis, or related pathways. These processes involve oxidative stress for a biological purpose.

Interception

Once reactive species are formed, powerful enzymatic systems maintain a physiological level. This category includes the superoxide dismutases, catalase, glutathione peroxidases, and peroxiredoxins, which directly counteract by chemical reduction of oxidants. Other enzymes, such as glutamate:cysteine ligase, which is needed for glutathione synthesis, indirectly serve the purpose. Yet other proteins, such as the aquaporins described above or the GSSG exporters, facilitate transport of reactive species or their products across biological membranes. All of these are under transcriptional, translational, and posttranslational control, forming the basis of the adaptive responses to oxidative stress (see **Table 4**).

Nonenzymatic interception occurs by low-molecular-mass antioxidants, such as α-tocopherol (vitamin E), a chain-breaking antioxidant in lipid peroxidation, or the water-soluble ascorbate (vitamin C). Other bioactives include polyene quenchers for singlet molecular oxygen, notably carotenoids and oxocarotenoids (176). Macromolecular barriers, such as melanin in pigmented epithelium and stratum corneum of mammalian skin, also exist. A high content of cysteine and methionine residues in mammalian proteins has been proposed to include decoys to protect critical active sites from oxidative damage.

Repair

As prevention and interception are not perfect, particularly over extended periods of time, molecular damage occurs, which is subject to powerful repair machinery. As discussed above, this concerns all classes of biomolecules and is not dealt with in further detail here.

REDOX MEDICINE

As a term, redox medicine has the propensity to be overused, similar to oxidative stress. Fertilization, birth, and cell death all have redox components. Most diseases, at early or late stages, also have a redox component. Whether redox processes are the cause or the consequence of disease states is not readily discernible. Paradoxical roles of antioxidant enzymes in health implications have been noted (177–179). In this section, we outline some of the principles that might lead to a future redox medicine in terms of oxidative stress. This task similarly applies to inflammation as a central phenomenon in disease (180, 181). For instance, in atherosclerosis, oxidative modifications were classified as an oxidative response to inflammation—in other words, inflammation as primary process and oxidative stress as secondary event (182). Turning off inflammatory processes includes resolution as a process with mediators including the resolvins, a class of specifically oxygenated polyunsaturated fatty acids (183).

Approaches Increasing Oxidative Stress (Therapeutic Prooxidants)

One aspect may be to categorize medical approaches utilizing measures to increase oxidative stress versus those that decrease or attenuate it. However, the distinction is not clear-cut: Even in a given disease state, say infection, an initial strategy combats the virus or bacterium by increasing oxidative challenges through host defense, whereas at a later stage activation of antioxidant enzyme batteries minimizes tissue damage. Similarly, in cancer there is tumor initiation, promotion, and progression, with vastly different roles of oxidants and antioxidants; further, the tumor-stroma microenvironment plays a role (184). Thus, the pathophysiology of disease development is multifaceted, considering the timeline and involvement of different cells, tissues, and organ systems in the response of the whole organism. Attempts have been made to assemble current knowledge in this area (185, 186).

Ionizing radiation. As in host defense against invading cells or organisms, a targeted strategy to eliminate potentially deleterious cells is straightforward. This applies to a large segment of current medicine: Radiotherapy applies oxidative stress through generation of the indiscriminately toxic hydroxyl radical. Thus, radiation biology is applied redox chemistry (187) and is one of the earliest examples of redox medicine.

Anticancer drugs. Major current anticancer drugs follow a similar strategy, targeting DNA (e.g., bleomycin) (188). A variety of redox-directed cancer therapeutics (221) have been evaluated clinically (222). Of the many other types of approach, we mention that the relationship between high-dose vitamin C and cancer is being revisited for colon cancer. At high doses, ascorbate acts as a prooxidant, not as an antioxidant (189). At pharmacological doses, ascorbate acts as a radiosensitizer, as shown for pancreatic cancer (190).

Photodynamic therapy. Tumors tend to selectively take up precursors of porphyrins, which are utilized as photosensitizers to generate cytotoxic singlet molecular oxygen (i.e., exert photooxidative stress). Apart from cell killing by apoptosis, there are numerous cell biological effects of photodynamic therapy, such as immunological and inflammatory responses to the localized oxidative stress (see 191 for review).

Wound healing and regeneration. At high concentration, H_2O_2 kills cells, whereas the process of wound healing and of cell proliferation during regeneration of tissue requires H_2O_2. H_2O_2 is one of the transcription-independent damage signals in the initiation of wound healing (192). Nonthermal plasma is being studied as well (193). The bacterial load on chronic wounds in patients was decreased using cold argon plasma (194).

Approaches Decreasing Oxidative Stress (Therapeutic Antioxidants)

In the past and unfortunately still at present, unspecific antioxidant treatment has been used to treat a variety of diseases linked to oxidative stress, indicating that "it is still widely assumed that antioxidant administration will always provide benefit. This is naive thinking. Antioxidants can protect or increase injury depending on the situation and therefore their use should always be made with a full appreciation of the situation" (16, p. 5).

The conundrum of antioxidant action is illustrated, for example, by the observation that glutathione and thioredoxin pathways synergize to drive cancer initiation and progression (195). Depending on tumor type, substantial differences in thioredoxin and glutathione systems exist,

further illustrating the complexity (196). In general, mounting of the antioxidant defense systems, for example, by activation of Nrf2-dependent transcription of ARE-responsive genes (see above), seems to be a strategy for chemoprevention (197). However, cancer cells may also profit from increased Nrf2-controlled enzyme activity, for example, by detoxification of cancer drugs.

Neurological disorders. Among the various neurological disorders, long-term neurodegenerative diseases such as Alzheimer disease, Parkinson disease, and Huntington disease as well as multiple sclerosis are associated with significant oxidative components. An emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases is given by the Nrf2/Keap1 pathway (198, 199). Dimethylfumarate (DMF), a drug used in multiple sclerosis, has potential in neuroprotection and immunomodulation, both by inhibiting NF-κB and by inducing Nrf2/Keap1 (200). DMF may have therapeutic potential against synucleinopathy in Parkinson disease (201). Likewise, amyotrophic lateral sclerosis relates to oxidative stress by virtue of mutations in the superoxide dismutase-1 (SOD1) gene (202). Regarding the role of astrocytes in neuroprotection and neurodegenerative diseases, gap–junctional intercellular communication by connexins is essential for their resistance to oxidative stress (203). Osmotic and oxidative/nitrosative stress play a prominent role in ammonia toxicity and hepatic encephalopathy (204).

Metabolism: diabetes and obesity. Redox processes play essential roles in carbohydrate and lipid metabolism, evidenced by an insulin-like effect of H_2O_2 (66, 205). Reactive oxygen species enhance insulin sensitivity (206). Conversely, hyperglycemia-related formation of reactive oxygen species has been shown to induce glucotoxicity (162). Postprandial oxidative stress is a base for metabolic disorders such as diabetes and obesity (207). There is mounting evidence of oxidative components in diabetes and diabetic complications (208).

Other. Owing to space limitation, other major diseases and health conditions cannot be covered here. These include circulation, arteriosclerosis, gastrointestinal disorders, infectious diseases, immunology, comorbidity/multimorbidity, hearing loss, sleep deprivation (chronobiology), and the flourishing fields of research into aging and senescence, among others. Some of these areas are covered in Reference 1. A synoptic view of oxidative stress in terms of endogenous and exogenous factors and its role in redox signaling and oxidative damage is given in **Figure 12**.

OUTLOOK

The substantial advances in oxidative stress research of recent times allow us to formulate, in a nutshell, an outlook of further development. Discrimination of oxidative eustress, a fundamental process in maintaining health, from oxidative damage will improve clarity in developing redox medicine. Dramatic advances in methodologies for redox imaging, redox proteomics, and redox metabolomics facilitate specific as opposed to global analysis (209). These tools support improved quantitation and spatiotemporal resolution and a return to mechanistic studies of key molecules rather than poorly defined measures provided by nonspecific kits. Application along with other advanced -omics methods, including genomics, epigenomics, and exposomics, will provide refined understanding of health and disease processes by characterizing redox components and their functional organization within entire biological systems. The emerging big data era offers new opportunities for development of oxidative stress knowledge bases. Search engines to extract and integrate this knowledge with individual genetic makeup, nutrition, exposure, and lifestyle data will create important new opportunities for personalized redox medicine.

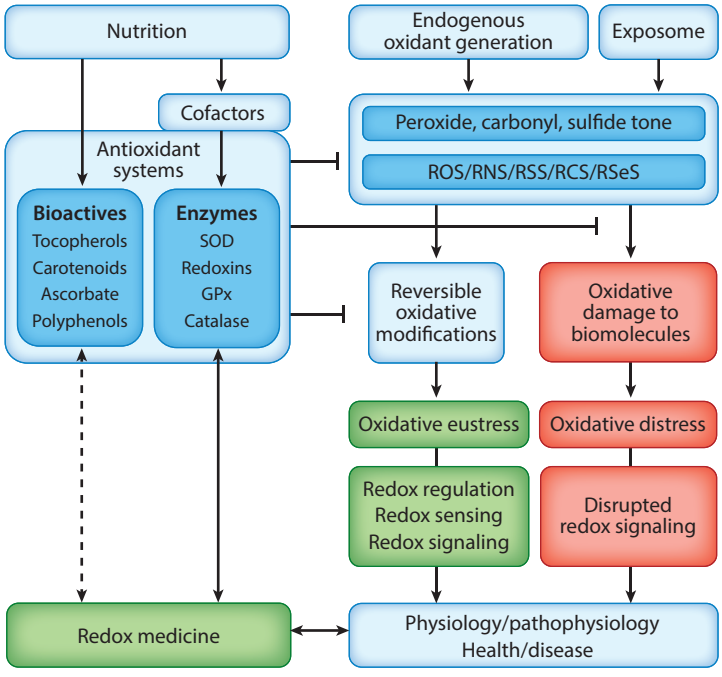

Figure 12

Synoptic view of oxidative stress and its relation to nutrition and redox medicine. Cofactors include micronutrients and metal ions. Redoxins are the thioredoxin, glutaredoxin, and peroxiredoxin systems. Endogenous oxidant generation includes respiratory chain, lipid oxidations, and others. Abbreviations: GPx, glutathione peroxidase; RCS, reactive carbonyl species; RNS, reactive nitrogen species; ROS, reactive oxygen species; RSeS, reactive selenium species; RSS, reactive sulfur species; SOD, superoxide dismutase.

DISCLOSURE STATEMENT

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