Molecular and structural antioxidant defenses against oxidative stress in animals

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Abstract

The increase in oxygen concentration in the Earth’s atmosphere represented an important selective pressure for living organisms and contributed toward setting up the pace of evolutionary changes in physiological and metabolic systems (69, 119, 129). Despite molecular mechanisms evolving toward the use of oxygen for efficient energy production have been a relevant evolutionary innovation, the reduction-oxidation (or redox) reactions (redox reactions involve the transfer of electrons or hydrogen atoms from one reactant to another) associated with its use have been responsible for the production of free radicals and, more generally, of reactive species (RS). These products of oxygen metabolism may have damaging effects on an organism, in essence, due to oxidation of essential cellular components (88). Consequently, animals have evolved mechanisms to avoid or minimize the production of RS of oxidative metabolism, as well as of antioxidant defense systems needed to limit the prooxidant activity of RS (89). The result has been a high diversity in molecular and structural antioxidant defenses, as well as in redox signaling pathways perfectly integrated in the cellular metabolic machinery. However, the antioxidant machinery is not 100% efficient in mopping RS up, especially when there is a strong imbalance between production of RS and antioxidant response. The consequence of such an imbalance is the generation of oxidative damage to biomolecules. This kind of biochemical stress of cells has been called oxidative stress (88, 195), which may be defined as the rate at which oxidative damage is generated (48). Implicit in this definition is that oxidative stress is a continuous variable that is unlikely to ever be exactly zero since prooxidants are continually produced, and some oxidative damage is always generated. Although the study of oxidative stress physiology has been classically of interest to the medical sciences because of its potential contribution in the progression of many human diseases, recent studies on nonhuman animals suggest that oxidative stress could also represent 1) a universal constraint of life-history evolution, 2) a modulator of phenotypic development, and 3) a physiological correlate of behavioral differences between individuals (3, 17, 41, 44, 47, 50, 64, 132, 136, 174, 178, 217). Therefore, oxidative stress could have
worked as a relevant selective pressure and that is why animal taxa have evolved a myriad of mechanisms to regulate and adjust the redox balance. This high diversity in antioxidant mechanisms also suggests that organisms have a great deal of flexibility in how they deal with RS challenges across time, conditions, and tissue types, as well as that different organisms may have evolved different strategies for dealing with similar oxidative challenges.

In the following paragraphs, we review the high diversity of molecular and structural defenses against RS evolved by animals. In particular, it is our goal to describe physiological mechanisms underlying the production of RS and the biochemical regulation of defense mechanisms, to extend the traditional concept of antioxidant to other structural and functional factors affecting the whole organism, and to link antioxidant defenses to life-history models and maternal/epigenetic inheritance. Finally, we review the state of the art about the link between life span and antioxidant defenses.

We have used the term adaptation in a general way throughout the manuscript, including both real adaptations and exaptations. For a character to be regarded as an adaptation, it must be a derived character that evolved in response to a specific selective agent (p. 13 in Ref. 93). The emergence of an antioxidant mechanism could be maintained by natural selection because it confers current selective advantage, but this is not evidence of why it has been evolved. So it could be considered as an exaptation sensu (81) rather than as an adaptation. This means that the protection against oxidative stress is not the primary reason for why all antioxidant mechanisms have been evolved, but it could have played a role secondarily to provide selective advantage.

Antioxidant Defense Mechanisms as Evolutionary Adaptations to Endogenous Reactive Species Generation

In aerobic organisms, about 85–90% of cellular oxygen is consumed by the mitochondria to produce energy as ATP molecule. A main side-effect of ATP production is the formation of RS (Fig. 1). RS include a variety of molecules, mostly physiologically generated from the metabolic activity (12, 88, 185). RS can induce chemical modifications in other molecules, generating oxidative damage, but also act as second messengers in signal transduction networks.

RS mostly consist of reactive oxygen species (ROS) and reactive carbonyl species (RCS). Superoxide anion, the product of a one-electron reduction of oxygen, is the precursor of most ROS. Dismutation of superoxide anion [either spontaneously or through a reaction catalyzed by superoxide dismutase (SOD) enzymes] produces hydrogen peroxide (H$_2$O$_2$), which, in turn, may be fully reduced to water or in the presence of ferrous or cuprous ions, forms the highly reactive hydroxyl radical. In addition, superoxide anion may react nonenzymatically with other molecules, including nitric oxide (NO) in a reaction controlled by the rate of diffusion of both reactants. The product of the reaction between superoxide anion and NO, peroxynitrite, is also a very powerful oxidant. The oxidants derived from NO have been called reactive nitrogen species (RONS). On the other hand, hydrogen peroxide, although lacking radical properties, can work as a Trojan horse, diffusing away from sites of ROS production to generate the hydroxyl and other reactive radicals at other cellular locations, thereby propagating oxidative damage. In addition to ROS/ RONS, the oxidation of both carbohydrates and lipids [partic-
ularly, polyunsaturated fatty acids (PUFAs) originate a new generation of RS named reactive carbonyl species, or RCS (152). Compared with both ROS and RONS, RCS have a much longer half-life (i.e., minutes to hours instead of microseconds to nanoseconds for most ROS/RONS). Further, the noncharged structure of carbonyls allows them to migrate with relative ease through hydrophobic membranes and hydrophilic cytosolic media, thereby extending the migration distance far from the production site. On the basis of these features, RCS could be more destructive than ROS/RONS and may have far-reaching damaging effects on target sites within or outside membranes.

Diversity in antioxidant mechanisms is thought to be an expression of this high variety in RS and in their molecular consequences. Although not clearly and consistently defined yet, we define antioxidant as “any mechanism, structure and/or substance that prevents, delays, removes or protects against oxidative nonenzymatic chemical modification (damage) to a target molecule.”

Protection against oxidative damage is pivotal for organism function. A major consequence of oxidative damage is the loss of function and structural integrity of modified biomolecules, which have a wide range of downstream functional consequences, such as induction of cellular dysfunctions and tissue damage. Alterations of biochemical and physiological pathways can have a number of detrimental consequences for an individual’s health, potentially decreasing its Darwinian fitness perspectives. Given that environmental conditions (e.g., weather, food quantity and quality, predation risk, and competition) across a range of habitat types, as well as phases of the life-cycle (e.g., reproduction and hibernation) may be associated with oxidative stress threats, individuals are continually under pressure. In this scenario, animals have evolved several antioxidant defenses to control and mitigate the action of RS. Individuals may, therefore, be exposed to relevant physiological costs, mainly expressed in terms of oxidative damage and consumption of energy needed to keep the antioxidant defenses upregulated and to activate repair systems. It is possible that the need to regulate the redox system exposes individuals to a number of trade-offs and, therefore, oxidative stress could have represented a relevant modulator of life-history strategies. Animals show, however, large variation in antioxidant defenses, and it is, therefore, pivotal to review them to figure out the association among oxidative stress, life-history strategies, and response of natural populations to environmental stressors.

Resistance of cellular structural components to oxidative damage. Cellular protection against oxidative damage includes RS elimination and repair/turnover systems. Recent evidence, however, support the notion of another line of defense based on the inherent susceptibility of macromolecules to oxidative damage (155). This susceptibility, defined as the ease with which macromolecules suffer an oxidative injury, is intrinsically linked to the specific structure or chemical composition of carbohydrates, lipids, nucleic acids, and proteins. Available evidence on the differential susceptibility to oxidative damage of biomolecules shows that in living systems:

Glycolytic intermediates (the most unstable and reactive monosaccharides) occur at concentrations in the micromolar range, in contrast to the millimolar range for glucose (the most abundant and stable monosaccharide). It was proposed that glucose emerged as the most important carrier of energy from cell to cell in animal species, because it is the slowest and stable reacting carbohydrate (30) and, consequently, poorly susceptible to oxidation. The lower cellular concentration of highly reactive glycolytic intermediates was, in fact, interpreted as an adaptation to suffer less oxidative stress (137). This intracellular condition is particularly critical for birds, given their high blood glucose levels (101). Available evidence demonstrate that birds possess lower glucose cellular permeability (16) and sensitivity to insulin signaling (67), mechanisms that probably lead to lower intracellular concentration of glycolytic intermediates. So, it is proposed that the high blood glucose level showed by birds in comparison with mammals should be interpreted as a nonreactive and stable functional compartment ready to use exclusively in physiological conditions that require a high energetic demand, thus maintaining cellular integrity against molecular damage.

Highly unsaturated fatty acids (more than 2 double bonds) are on average the least abundant fatty acids in cell membranes and are less abundant in species that live longer (104, 152). Unsaturated fatty acids are the macromolecules most susceptible to oxidative damage in cells, and this sensitivity increases as a function of the number of double bonds they contain (100, 104, 152–153). This means that saturated and monounsaturated fatty acyl chains are essentially resistant to oxidation, whereas polyunsaturates are easily damaged (104, 152).

Another antioxidant adaptive structural system evolved to prevent the oxidation of polyunsaturated fatty acids is linked to the plasmalogens (24, 70, 145). Substantial evidence accumulated in the last decade indicates that plasmalogens, a class of ethanolamine (and choline) phospholipids, could represent a major lipid-soluble antioxidant component based on the ability of the plasmalogens to scavenge several RS, their relatively high concentrations in many animal species (which markedly exceeds the alpha-tocopherol concentrations; see Cellular Protection by Enzymatic and Nonenzymatic Antioxidants) and their subcellular and extracellular locations in close vicinity to oxidizable substrates. The structural peculiarity of plasmalogens lies in the enol ether double bond present within the hydrocarbon chain linked to the C-1 atom of the glycerol backbone of phospholipids.

Guanine is the least abundant nucleotide in mitochondrial DNA (183). Guanine is the nucleobase with the lowest oxidation potential and is thus generally most easily oxidized (18).

Methionine is the amino acid that on average has the smallest percentage presence in cellular proteins (2, 157, 171, 176). Methionine residues from proteins are among the amino acids most susceptible to oxidation by free radicals (204). Consequently, the lower the protein Met content, the higher should be the protein resistance to oxidative damage. In accordance with this, the amino acid compositional analysis of cellular proteins from different tissues (e.g., skeletal muscle, heart, and brain) in birds and mammals reveal that Met is present in the smallest percentage in cellular proteins (2, 157, 171, 176). Selection has, therefore, possibly disfavored the usage of Met in animal tissues, given its higher proneness to be oxidized compared with other amino acids.

Although available evidence suggests that aerobic life has evolved by reducing the relative abundance of those structural components that are highly susceptible to oxidative damage, there still exists great variation among species in body composition (e.g., degree of unsaturation of cell membranes; Refs. 34, 103, 105), whose causes and meaning are still not well
understood, despite that it seems to be a relevant determinant of life span (see Antioxidants and Animal Maximum Life Span). The link between body composition and susceptibility to oxidative stress can, therefore, be ecologically and evolutionary relevant. For example, an environmental factor that can contribute to explain some body composition variation is the diet quality. Diet not only is a source of molecular antioxidants, but it is also a source of nutrients, such as fatty acids and amino acids, which can expose the organism to different oxidative stress threats because of their different molecular susceptibility to be oxidized. Animals can, however, actively make their cell composition through an active regulation of metabolism of nutrients. For example, the lower proportion of n-3 to n-6 polyunsaturated fatty acids in marine bird cardiac membranes compared with their diet suggests a highly selective nature of fatty acid incorporation into membrane lipids (34). Such discrimination of dietary fats is especially important because n-3 polyunsaturated fatty acids are less resistant to oxidative damage than their n-6 counterparts.

Such regulation abilities of cell membrane composition may also be important when the female is depositing nutrients into the egg or milk. Females could influence the future susceptibility to oxidative stress of their offspring not only through the investment of antioxidants, but also through other nutrients that do not have any antioxidant properties. For example, the n-6 polyunsaturated, arachidonic acid, forms between 8% and 19% (wt/wt) of the phospholipid fatty acids of egg yolk of the polyunsaturate, arachidonic acid, which can expose the organism to different oxidative stress threats because of their different molecular susceptibility to be oxidized. Animals can, however, actively make their cell composition through an active regulation of metabolism of nutrients. For example, the lower proportion of n-3 to n-6 polyunsaturated fatty acids in marine bird cardiac membranes compared with their diet suggests a highly selective nature of fatty acid incorporation into membrane lipids (34). Such discrimination of dietary fats is especially important because n-3 polyunsaturated fatty acids are less resistant to oxidative damage than their n-6 counterparts.

From the available evidence, we can, therefore, infer that natural selection operated, possibly through directional selection, reducing the relative abundance of those structural components that are highly susceptible to oxidative damage, thus conferring to the macromolecules a lower susceptibility to oxidative stress and, consequently, a higher structural stability.

Oxidative balance regulatory components. From an evolutionary perspective, it could be expected that natural selection does work to optimize the interaction among mechanisms that regulate production of RS rather than antioxidant molecules only. Hence, an obvious question coming out from this expectation would be: Are there any physiological mechanisms that regulate the rate of mitochondrial free radical generation? Available evidence suggests that this is the case. These mechanisms can be grouped in “internal” and “external”.

INTERNAL MECHANISMS. In animal cells, the major sites of physiological ROS generation are the complex I and III of the mitochondrial electron transport chain, which contain several redox centers (flavins, iron-sulfur clusters, and ubisemiquinone) capable of transferring one electron to oxygen to form superoxide anion (21, 104, 155, 185). It is, therefore, proposed that important internal mechanisms are those inherently linked with the structure and function of the mitochondrial free radical generators: 1) modulation of the absolute content of the mitochondrial electron transport chain, and, very specially, of the complexes I and III; 2) reduction state of complex I; 3) modulation of the free radical production by uncoupling proteins; and 4) potential posttranslational modifications.

A first internal mechanism operates through the decrease in the concentration of the respiratory complex/es responsible for ROS generation. For example, birds have a lower content of complex I than mammals, as well as a lower degree of ROS production (117, 155, 157, 203). Therefore, regulation of the expression of the mitochondrial complexes (and especially complex I) could be part of an adaptive mechanism to adjust ROS production.

A second internal mechanism that controls free radical generation is the regulation of the degree of electronic reduction of these generators: the higher their degree of reduction, the higher will be their rate of ROS production (154–155). It is known that the rate of mitochondrial ROS generation strongly increases with a sigmoidal kinetics when the ratio between the reduced (NADH), an oxidized (NAD+) form of nicotinamide adenine nucleotide, is increased, because this dramatically increases the degree of reduction of the complex I ROS generator (12, 116).

A third internal mechanism is characterized by regulation of uncoupling proteins (UCPs). During oxidation of substrates, the complexes of the mitochondrial electron transport chain reduce oxygen to water and pump protons into the intermembrane space, forming a proton-motive force ($\Delta p$). However, some electrons in the reduced complexes also react with oxygen to produce superoxide. Superoxide can peroxidize membrane phospholipids, forming hydroxynonenal, which induces proton transport through the UCPs and the adenine nucleotide translocase. The mild uncoupling caused by proton transport lowers $\Delta p$ and slightly stimulates electron transport, causing the complexes to become more oxidized and lowering the local concentration of oxygen; both of these effects decrease superoxide production. Thus, the induction of proton leak by hydroxynonenal limits mitochondrial ROS production as a feedback response to overproduction of superoxide by the respiratory chain (21, 68, 186). So, a possible antioxidant physiological function for UCPs has been proposed (68). In this model, UCPs respond to overproduction of matrix super-
oxide by catalyzing mild uncoupling, which lowers proton-motive force and would decrease superoxide production by the electron transport chain (Fig. 1). This negative feedback loop protects cells from RS-induced damage and might represent the ancestral function of all UCPs (21, 68).

Finally, posttranslational modifications (i.e., chemical modifications of a protein after its translation), such as acetylation, S-nitrosation, and glutathionylation, are another important mechanism regulating RS production. For example, glutathionylation of complex I increases superoxide production by the complex itself, and when the mixed disulfides are reduced, superoxide production returns to basal levels (91, 210). S-nitrosation results from the reaction of NO with protein thiols, generating nitrosative stress (28, 58, 76). The implications of nitrosative stress for animal function are, therefore, clear and call for attention by physiologists about its potential contribution to explain individual condition and physiological trade-offs, as stressed in a recent study on effects of immune response on nitrosative stress in birds (197). Finally, recent studies seem to indicate that acetylation can be used as posttranslational modification able to regulate the activity of the mitochondrial electron transport chain complexes by modifying specific subunits (107). In the light of these mechanisms, mitochondrial activity and structure could be under strong selection. For example, mitochondria, being the energy makers of organisms, could expose the individual to energetic constraints because any damage could reduce their efficiency in ATP synthesis. The daily metabolizable energy intake of an animal is potentially limited by either the available feeding time, food availability, or by its capacity to process energy. Oxidative mitochondrial damage could, therefore, increase energy demands of the individual, particularly during demanding phases of the year, such as reproduction or migration. Moreover, organisms can actively respond to environmental stressors, not only through upregulation or downregulation of specific mitochondrial proteins, but also through changes in mitochondrial density, as shown in compensatory responses to changes in physical activity in birds (20) or to cold acclimation in marine invertebrates or fish (87).

EXTERNAL MECHANISMS. The characteristics of the environment (e.g., local oxygen concentration and membrane phospholipid composition, where complexes are embedded) surrounding the mitochondrial electron transport chain complexes can be mentioned as external factors able to modify the free radical generation and, consequently, they can be considered as potential antioxidant mechanisms.

A first mechanism operates through the regulation of the mitochondrial partial pressure of O_2. Normally, animal cells are exposed to quite low oxygen concentrations, likely to minimize oxygen toxicity, which is interpreted as an antioxidant defense (88). When the level of oxygen decreases dramatically and the animal enters a prolonged state of hypoxia, hypoxia-inducible transcription factors (HIFs), especially HIF-1, promote expression of genes encoding proteins that help cells to respond to a hypoxic or anoxic status (72). There exists, however, high variation in how species can tolerate hypoxia. For example, low oxygen flux fauna (e.g., marine organisms) can tolerate prolonged hypoxia without any detrimental consequence for the organism. Also, in high oxygen flux fauna, we can observe special adaptations to hypoxia, such as those described in seals. Seals cope with regular exposure to diving hypoxia by storing oxygen in blood and skeletal muscles (both very rich in hemoglobin and myoglobin, respectively) and by limiting the distribution of blood-borne oxygen to all but the most hypoxia-vulnerable tissues (brain, heart), through cardiovascular adjustments (172).

A second mechanism is dependent on the cardiolipin content of mitochondria. Cardiolipin, a phospholipid located almost exclusively within the inner mitochondrial membrane, is particularly rich in unsaturated fatty acids, but with a low degree of unsaturation (98, 170). This phospholipid plays an important role in mitochondrial bioenergetics by influencing the activity of key mitochondrial inner membrane proteins, including several anion carriers and electron transport complexes I, III, and IV (98). Mitochondrial cardiolipin molecules are potential targets of ROS attack because of their content of unsaturated fatty acids and because of their location in the inner mitochondrial membrane near to the site of ROS production. In this regard, it has been recently demonstrated that mitochondrial-mediated ROS generation affects the activity of complex I (161), as well as complexes III and IV (160, 162), via peroxidation of cardiolipin following ROS-mediated damage to its fatty acid constituents (161). In this context, it is plausible to postulate that cardiolipin was positively selected likely because of its low unsaturation degree that ensures a structural resistance against oxidative damage, as well as a correct functionality for the mitochondrial electron transport chain complexes (152).

Antioxidant diversity is an expression of the variety of reactive species and their molecular consequences. Although defense mechanisms have evolved that regulate the generation of RS, cells continuously produce them, and so their oxidative action can be controlled only if endogenous cellular antioxidants are present. In the following paragraphs, we review briefly the major molecular antioxidant defenses, both enzymatic and nonenzymatic, which have been selected and conserved during animal evolution.

CELLULAR PROTECTION BY ENZYMATIC AND NONENZYMATIC ANTIOXIDANTS. Direct RS scavenging antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (Gpx) and catalase. SOD eliminates superoxide radical by converting it to oxygen and H_2O_2. There are different forms of this enzyme: a Cu,Zn form in the cytosol and in the intermembrane mitochondrial compartment (147), a Mn form in the mitochondrial matrix (MnSOD), and another form in the extracellular compartment (e.g., blood). The dismutation of superoxide by SOD generates the less reactive H_2O_2. Thus, other enzymes (catalase and glutathione peroxidases) work coordinately to eliminate the hydrogen peroxide produced by SOD and other potential sources (Fig. 1). Catalase decomposes H_2O_2 at high rates but shows low affinity for the peroxide and should be most useful during peaks of H_2O_2 production or accumulation. These peaks should occur in vivo, because acatalasemia (i.e., disorder caused by lack of catalase) increases oxidative stress and induces pathologies in humans (80). Gpxs, present in selenium- and nonseleunium-dependent forms, are complementary to catalase, since they decompose H_2O_2 slowly, but with higher affinity. Thus, they are most useful to decompose the small amounts of peroxide continuously and physiologically produced inside cells. At least five selenium-containing glutathione peroxidases have been identified (13), and their activity can be manipulated by changing dietary selenium levels (7). In
animals, these enzymes use the reduced form of GSH and of other thiols (e.g., thioredoxin) to decompose the peroxide.

A phylogenetic analysis of the Gpx family, including plant, fungi, bacteria, invertebrate, and vertebrate taxa, shows complex relationships in this molecular family, suggesting that basal Gpx classes have originated from independent evolutionary events, such as gene duplication, gene losses, and lateral gene transfer (128). In particular, an evolutionary pattern can be recognized in animals leading to the origin of most Gpx isoforms and a second pattern leading to the Gpx4 isozyme, which is found in animals, plants, and fungi. This second cluster would suggest evolutionary convergence mediated by functional pressures, but it is unclear why this pattern was not observed for other Gpx isoforms, as well.

The prooxidant activity of hydrogen peroxide is the result of its one-electron reduction to the hydroxyl radical. This reaction is among the most damaging faced by living systems: formation and reactions of hydroxyl radical are associated with chemical, nonenzymatic reactions, which are beyond any cellular control or antioxidant defense mechanism. Reduction of \( \text{H}_2\text{O}_2 \), as well as of hydroperoxides, occurs, for instance, in the presence of metal ions, such as \( \text{Fe}^{2+} \) or \( \text{Cu}^{+} \) or superoxide radical, which reduce them to highly reactive free radicals, such as hydroxyl, alkoxyl and alkylperoxy radicals. These processes are known as the Haber-Weiss reaction and Fenton reaction, respectively (88) (Fig. 1). This basic chemical process justifies, in evolutionary terms, the need for the sequestration of metal ions, mediated by specific proteins (e.g., ferritin) extensively used by cells, as antioxidant defenses.

In addition to antioxidant enzymes, various types of endogenous nonenzymatic antioxidants are present in animal tissues. Although they are depleted when they react with ROS, their oxidized forms are usually recycled back to the antioxidant form due to reduction by other molecules (e.g., 114). Their low molecular weight can also be an advantage to eliminate ROS at sites not accessible to the much larger enzymes. Furthermore, there are nonenzymatic antioxidants designed to function in either the hydrophilic or lipophilic cellular compartments. The main low-molecular-weight hydrophilic nonenzymatic endogenous antioxidants are glutathione, thioredoxin, and ascorbate. The tripeptide glutathione (188, 196) is particularly abundant in many tissues, where it can reach levels as high as 10 mM in many tissues (e.g., 1 mM in rats) (11). Ascorbate reacts with ROS and is thus converted to oxidized forms, which can be reduced back again by NADPH-dependent (175) or GSH-dependent (127, 221) dehydroascorbate reductases or by a NADH-dependent plasma membrane ascorbate free radical reductase (146). GSH and ascorbate can also interact cooperatively in vivo to cope with RS (130).

Within the lipid compartments, antioxidant protection is apparently less efficient than in the aqueous environments. In fact, there is a dramatic difference in the concentrations and efficiency of antioxidant defense systems between the aqueous and lipidic compartments, pointing to an evolutionary priority for the water-soluble antioxidants. The antioxidants functioning optimally in the lipophilic membrane environment are nonenzymatic components, such as tocopherols and carotenoids, which are both derived from food (60, 208).

At least eight different substances with vitamin E activity have been described: \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \) tocopherol and the corresponding tocotrienols (23, 208, 220). Vitamin E reduces lipid peroxy groups to hydroperoxides, thus inhibiting the propagation of lipid peroxidation in a chain reaction (71). Vitamin E can also reduce lipid alkoxyl radicals to lipid alcohols. Membranes do not contain large amounts of tocopherols: a ratio of one tocopherol per thousand polyunsaturated fatty acid side chains is common (220). The antioxidant activity of vitamin E is due to the reducing capacity of the hydroxyl group of its chromanol ring.

Recent studies on wild birds show that plasma vitamin E is not associated with \( J \) variation in life histories across species, \( 2 \) reproductive success, age, growth and body size within species, and \( 3 \) with diet within and across species (36–37, 120). The authors proposed that this could be due to recycling of vitamin E and short-term variation not easily detectable in large-scale analyses. It is also possible, however, that circulating levels of vitamin E are not very informative, and so we should also look at it in different body compartments.

Together with vitamin E, carotenoids are main lipophilic exogenous antioxidants in animal cells. Hundreds of different carotenoids have been described, although only some of them, like \( \alpha \)- and \( \beta \)-carotene, lutein, lycopene, zeaxanthine, or cryptoxanthine, are present at relevant concentrations in animal tissues and plasma (113, 208). Despite the antioxidant properties, empirical evidence for carotenoids being important antioxidants in vivo is weak. A recent meta-analysis on the effects of carotenoids on biomarkers of oxidative damage (e.g., lipid peroxidation) and antioxidant capacity in birds shows that carotenoids have a contribution to protection against oxidative stress less than 0.002% (45). Further studies published shortly after reinforced this view, showing that the contribution of carotenoids to protection against oxidative stress in birds is also low under stressful conditions (46, 102, 106, 120). In a similar way, many supplementation trials of carotenoids, as well as of other dietary antioxidants (vitamins C and E), administered singly or together in humans (e.g., 86, 122, 143, 211–212) failed to stop disease and aging, and contributed to increased mortality among participants of the trial. These results cast serious doubt on the generalization of the paradigm that availability of antioxidants in the diet is always limiting for the capacity of the organism to cope with oxidative stress and that it can represent an important modulator of life histories. The picture is further complicated by the fact that dietary antioxidants, such as carotenoids or polyphenols, can also exert prooxidant activities, as well as other physiological functions, which are independent from their antioxidant properties (43, 90). For example, the life span extension caused by vitamin E supplementation in mice appeared to be independent of any
antioxidant effect but was likely related to the regulatory role of vitamin E of signal transduction and gene expression (9). However, we cannot rule out that the protective role of carotenoids or vitamin E is specific in terms of tissue or phase of the life cycle rather than general. For example, supplementation of carotenoids may increase parental care of male threespined sticklebacks (Gasterosteus aculeatus) during hypoxic (but not normoxic) conditions (168) or sperm quality in wild male great tits (Parus major), especially in those most deficient in carotenoids (95).

**Cellular Protection by Repair/Turnover and Detoxifying Antioxidant Systems.** Despite this plethora of defense systems, some oxidative damage still occurs in vivo. Animals have so evolved other molecular mechanisms to cope with chemical modifications derived from the interaction between RS and macromolecules. Among them the following ones may be mentioned: enzymes that repair oxidized amino acids (e.g., methionine sulfoxide reductase), systems that degrade oxidatively damaged proteins (e.g., the proteasome), DNA repair enzymes, detoxifying systems against carbonyl compounds derived from carbohydrate and unsaturated fatty acid oxidation, and phospholipid fatty acyl chain removal systems, among others.

All amino acid residues of proteins are susceptible to oxidative modification by one or more forms of RS. Although most amino acids in proteins can be modified by diverse endogenous oxidants, commonly modified residues are lysine (Lys), arginine (Arg), cysteine (Cys), methionine (Met), and tyrosine (59). Oxidation of Met and Cys residues generates methionine sulfoxide and disulfides in proteins, respectively, which may lead to loss of their biological activity, and depletes Met of its function as a methyl donor. Most of the protein oxidative modifications are irreversible, i.e., the only known way to eliminate the protein adduct is to catabolize the protein itself. However, particular oxidative modifications, such as methionine sulfoxide and disulfides, can be repaired by the methionine sulfoxide reductase (Msr) enzyme in a thio-redoxin-dependent reaction (142).

The degradation of nonfunctional, oxidized proteins is an essential part of the antioxidant defenses of cells. Eukaryotic cells have several major pathways for general protein degradation: the lysosomal proteases, calcium-dependent proteases and the proteasomal system. Additionally, many proteins are degraded by specific proteases (e.g., caspases). In healthy cells, the accumulation of oxidatively damaged proteins is prevented by rapid elimination of such proteins before they can begin to aggregate, because oxidative modification of proteins makes them susceptible to proteolysis (83–84). By selectively recognizing and rapidly degrading oxidized proteins, the proteasome constitutes an important part of cellular antioxidant defenses that prevent the build-up of damaged proteins, and their subsequent aggregation. Thus, intracellular proteases responsible for the selective degradation of oxidized proteins function as efficient damage removal and repair systems (85). The removal of physiologically oxidized proteins is an essential function for maintaining cellular homeostasis and to prevent the accumulation of highly oxidized and cross-linked proteins, which are no longer degradable.

Protection of membranes is also achieved by an additional complex system that involves several mechanisms: lipid repair, lipid replacement, scavenging of reactive carbonyl species (as end-products derived from lipid peroxidation), and degradation-removal of modified molecules (104, 152). An important mechanism for removing peroxidized lipids is membrane lipid remodeling. Although the enzyme PHGpx is important in removing peroxidized acyl chains from phospholipids, other enzymes are also involved in the continual decacylation/reacylation of phospholipids, and this turnover of membrane acyl chains is very rapid. Phospholipase A2 is a key enzyme for removing acyl chains from phospholipids, while acyltransferase and transacylase enzymes are responsible for reacylation of phospholipids (73). Thus, for example, when isolated rat liver cells are subjected to oxidative stress, lipid peroxidation is increased; however, there is no change in either the fatty acid composition of, or in the rate of acyl turnover of membrane phospholipids. There is, however, a decrease in the unsaturated fatty acid content of cellular triacylglycerols. It is suggested that rapid constitutive recycling of membrane phospholipids rather than selective in situ repair is responsible for eliminating peroxidized phospholipids, with triacylglycerols providing a dynamic pool of undamaged unsaturated fatty acids for phospholipid resynthesis (78). This mechanism could have evolved because it is less energetically demanding than activation of repair systems, hence possibly less constraining for life-history decisions.

As mentioned above, RCS are compounds produced under oxidative stress conditions. These compounds are detoxified in multiple ways, including conjugation to GSH, oxidation by aldehyde dehydrogenases, or reduction by aldoketoreductases (1, 71, 88, 104, 152, 222). Strongly electrophilic carbonyl compounds induce rapid and significant depletion of intracellular GSH content, suggesting that this is a relevant detoxification mechanism. Once GSH is depleted, cells exhibit an intracellular change in redox status and propagate an oxidative stress response following their production. Reaction of RCS with GSH can proceed in one of two ways: by nonenzymatic conjugation or through GSH transferase-mediated conjugation to form Michael adducts. Glutathione-S-transferases (GSTs) belong to a supergene family of multifunctional enzymes (192), which are particularly involved in the detoxification of highly reactive intermediate aldehydes. Interestingly, protection against oxidative stress is the major driver of positive selection in mammalian GSTs, explaining the overall expansion pattern of this enzyme’s family (75).

Finally, detoxifying mechanisms to protect tissues from RCS damage also include the cytosolic GSH-dependent glyoxalases, thiol- and histidine-containing dipeptides—presumably acting as trapping agents—and ascorbic acid.

**Cellular antioxidant regulatory factors.** Redox regulation is a signaling system used by cells to convey oxidative stress information and coordinate cellular functions, which has remained conserved throughout evolution due to its incredible versatility. Cellular adaptation mechanisms that deserve a special mention are the heat shock response signaling and the antioxidant-response element and Nrf2.

**Heat shock response signaling.** The heat-shock response, through activation of heat-shock transcription factors (HSFs) and the elevated expression of heat-shock proteins and molecular chaperones, protects the cell against the accumulation of nonnative proteins (see e.g., 52, 74, 141, 164, 205). Activation of heat shock factor-1 (HSF1) during this process subsequently induces the expression of a variety of heat shock proteins
Detoxification and antioxidant proteins. Thus, RS can activate the applied stimulus (i.e., the kind of RS). Regulation is governed by both the magnitude and duration of the stimulus.

Antioxidant-Response Element and Nrf2. Redox state is regulated through the action of RS, which act as second messengers in signal transduction networks (77). RS require a receptor site on the target protein, which effectively allows different proteins to function as redox sensors for diverse types of RS. Given that RS can react with any molecule, it is a major challenge to interpret which of these reactions can be considered as redox signaling or as random chemical modifications. In addition, redox signaling reactions vary significantly, depending upon cell type and cellular antioxidant levels, as well as among animal species (77). Nonetheless, it is becoming clear that RS can affect the expression of a vast number of genes and cellular functions, including cell-cell communication, metabolism, structure, motility, proliferation, differentiation, and apoptosis. It is possible to establish some general principles, governing redox regulation (77): 1) oxidant administration to different cell types regulates the expression of a large number of genes, typically between 1% and 5% of the genome; 2) antioxidant levels modulate the effect of RS and also act independently to regulate signaling; 3) changes in the cellular redox balance result in both the upregulation and downregulation of subsets of genes; 4) the expression of individual genes is related to the chemical identity of the regulatory oxidant or antioxidant; and 5) the extent of redox regulation is governed by both the magnitude and duration of the applied stimulus (i.e., the kind of RS).

The response to oxidative stress is characterized by a concerted upregulation of over 100 genes, which encode for detoxification and antioxidant proteins. Thus, RS can activate the “antioxidant response” likely to prevent their accumulation to toxic levels (124, 219). The activation of this antioxidant response by a chemically diverse range of RS indicates that common sensing mechanisms act as generic stress sensors. This signaling cascade culminates in the nuclear translocation of and transactivation by the transcription factor Nrf2 (39, 77, 110) (Fig. 2). A first response acts to protect the cell from foreign toxins. It includes the initiation of metabolic phase I and II enzymes, which are responsible for oxidizing xenobiotics and conjugating them to glucuronol, sulfate, and glucy groups to facilitate their cellular export. A second subset of genes protects the cell from oxidative damage, increasing the cell’s defensive capacity by upregulating antioxidant genes and metabolic enzymes involved in glutathione biosynthesis (39).

Cellular metabolism as an integrated antioxidant system. Inside mitochondria, electrons from reduced substrates move from complexes I and II of the electron transport chain through complexes III and IV to oxygen, forming water and causing protons to be pumped across the mitochondrial inner membrane. When glucose is metabolized through the tricarboxylic acid (TCA) cycle (or fatty acids through β-oxidation), it generates electron donors. The main electron donor is NADH, which gives electrons to complex I. The other electron donor generated by the TCA cycle is FADH2, formed by succinate dehydrogenase, which donates electrons to complex II. The proton-motive force set up by proton pumping drives protons back through the ATP synthase in the inner membrane, forming ATP from their precursors ADP and phosphate (189). The electron transport system is organized in this way so that the level of ATP can be precisely regulated. There are, in this context, two significant main side reactions: electrons leak from the respiratory chain and react with oxygen to form ROS, and pumped protons leak back across the inner membrane, diverting the conserved energy away from ATP biosynthesis into heat. As mentioned above, the major sites of physiological ROS generation are the complex I and III of the mitochondrial electron transport chain.

The rate of mitochondrial ROS generation strongly increases with a sigmoidal kinetics when the NADH/NAD+ ratio is increased, because this dramatically increases the degree of reduction of the complex I ROS generator (12, 116). This effect is also observed when intracellular concentrations of glucose or fatty acids are increased, e.g., in hyperglycemic state like diabetes (27) and insulin resistance (99) and can be reversed upon exposure to agents that act as mitochondrial uncouplers or electron transport chain inhibitors.

The physiological or pathological overproduction of ROS by the mitochondrial electron transport chain decreases the activity of the key glycolytic enzyme GAPDH. The inhibition of GAPDH activity by hyperglycemia does not occur when mitochondrial overproduction of superoxide is prevented by either UCP1 or MnSOD (66). In a step ahead, recent studies have demonstrated that higher intracellular glucose concentration-induced superoxide inhibits GAPDH activity in vivo by modifying the enzyme by ADP-ribosylation (65). By inhibiting mitochondrial superoxide production with either UCP-1 or MnSOD, both modification of GAPDH by ADP-ribose and reduction of its activity are again prevented. Most importantly, both modifications of GAPDH were also prevented by a specific inhibitor of poly(ADP-ribose) polymerase (PARP), the enzyme that makes these polymers of ADP-ribose, establishing a cause-and-effect relationship between PARP activation and the changes in GAPDH (27).
Poly(ADP-ribosyl)ation occurs in almost all nucleated cells of mammals, plants, and lower eukaryotes, but is absent in yeast. It represents an immediate cellular response to DNA damage, as induced by ionizing radiation, alkylating agents, and oxidants (33, 38, 62). In the absence of DNA single- and double-strand breaks, poly(ADP-ribosyl)ation is a very rare event, but it can increase over 100-fold upon DNA damage. Under these conditions, about 90% of poly(ADP-ribose) is synthesized by poly(ADP-ribose) polymerase 1 (PARP-1). PARP-1 is constitutively expressed, but enzymatically activated by DNA strand breaks. It catalyzes the formation of ADP-ribose from the oxidized form of NAD$^+$ by cleavage of the glycosidic bond between nicotinamide and ribose. Glutamate, aspartate, and carboxy-terminal lysine residues of target (“acceptor”) proteins are then covalently modified by the addition of an ADP-ribose subunit, via formation of an ester bond between the protein and the ADP-ribose residue. Poly-(ADP-ribosyl)ation is a posttranslational protein modification linked with genome protection and mammalian longevity (15, 33).

In this scenario, it is plausible to suggest that the inhibitory effect of ADP-ribosylation on GAPDH probably represents a key signal molecule to reduce levels of glycolysis and subsequent flux of metabolites to mitochondria, allowing a decrease in the levels of reducing equivalents and the subsequent mitochondrial ROS production. In addition, this mechanism seems to indicate that the stress-induced block of glycolysis is not the result of a passive oxidative damage, but rather an active cell adaptation programmed via ADP-ribosylation for cell self-defense.

Another class of enzymes that utilize NAD$^+$ as a substrate is that of sirtuins. Sirtuins regulate a wide range of cellular and physiological activities (223). The sirtuin deacetylase activity closely links NAD$^+$ with a variety of cellular functions that are important for regulating development and longevity, including stress resistance, mitochondrial biogenesis, adipogenesis, proliferation, DNA repair, metabolism, and autophagy that collectively are thought to be involved in sustaining healthy cell types and delaying age-related disease states. Therefore, NAD$^+$ may be considered a key signal molecule able to integrate oxidative stress and metabolism to maintain organism health and drive a program of robustness that counteracts or delays aging in a broad multifaceted way.

**Biochemical Integration of the Redox System**

Our overview on antioxidant mechanisms shows that the machinery offering protection against peroxidation is highly complex, involving a large number of gene networks, molecular pathways, and families of molecules. This implicates that the effectiveness of the contribution of one antioxidant component to protection is not totally independent of each other, but a variable degree of biochemical integration can be observed among different antioxidants. Integration (e.g., morphological, metabolic, biochemical, genetic) is a property of biological systems that refers to the degree and extent to which its
components are correlated through functional, structural, developmental, or evolutionary interdependency (29, 51, 79, 112, 134, 173, 191). More specifically, biochemical integration refers to the functional interdependency among biomolecules through a direct or indirect reaction or regulation (e.g., concentration of molecule A depends on concentration of molecule B and vice versa; molecule A affects molecule C through molecule B) (see also Ref. 159). To make rigorous inferences on integration, it is, therefore, important that a basic knowledge of the biochemistry of molecules under study does exist.

Traditionally, redox mechanisms are studied by means of in vitro or ex vivo systems, which focus on the description of specific mechanisms, often in isolation from others. We think that the integration of traditional approaches with the analyses of correlation/covariance matrices (e.g., multivariate analysis, interaction networks) among components of the redox state in in vivo systems could provide a wealth of information on how the system works and why it works the way it does. For example, the application of factor analysis and of cluster analysis on correlation matrix of redox variables can represent a first analytical step to quantify the degree and sign of association among variables and to identify clusters of variables strictly connected to each other (Bruner E, personal communication; see Ref. 51 for an exploratory application on blood redox state in birds). A bootstrap analysis would then be important to quantify the statistical robustness of each node of the generated tree. In case the system under study is governed by nonlinear dynamics, the application of nonlinear dimensionality reduction algorithms (e.g., isometric mapping ISOMAP) would be more appropriate (193).

To determine the degree of covariance (i.e., integration) between redox statuses of two tissue compartments, a two-block partial least squares (PLS) regression could prove valuable. The correlation matrix can finally be used to visualize a network (Fig. 3), i.e., a set of mutually interacting elements whose collective behavior gives rise to emergent properties of the system under study (149, 215). A network is visualized as a set of nodes and links (or edges), where a node represents a molecular component, and a link between two nodes indicates that those two molecules are significantly correlated. The degree of connectedness of the network can be quantified by the coefficient $k$, which is the ratio between the number of links present and the maximum number of possible links in that specific network. As shown in Fig. 3A, the value of $k$ is constant when the redox network remains stable across time. In Fig. 3, B and C, we can observe a decrease and an increase of $k$, respectively, which indicate that the network became less or more integrated, respectively.

Gene and metabolic networks can, for example, become less integrated as the individual ages, hence, becoming noisier and less stable because of a decrease in the effectiveness of communication among functional units (53, 200, 202, 224). Therefore, we can predict that a decrease in $k$ with time may mirror a signal of senescence. On the other hand, we can see an increase in $k$ when the organism is mounting a stress response. In general terms, phenotypic integration can increase with environmental stress, possibly because of the convergence of the various response mechanisms when there is an increased need to promote self-maintenance and survival (e.g., 8, 218). If we look more specifically at the collective behavior of molecules involved in a redox reaction, we can see that the magnitude of correlations among variables can strongly increase under stressful conditions (63), suggesting an increase in integration. The comparison of correlation matrices between different states can, therefore, provide a helpful analytical and conceptual tool to make inferences on the organismal stress response as a whole and to identify factors that modulate its behavior.

The analysis of network may also allow to identify which antioxidant components can constrain the antioxidant response itself. Some antioxidant mechanisms rely on a delicate equilibrium among molecules involved. Hence, a failure of one of them could compromise the effectiveness of antioxidant response. However, it cannot be entirely excluded that maintaining a variety of components of the antioxidant machinery could provide a “fail-safe” to avoid the case that the failure of one antioxidant compromises the whole system. To answer these questions would mean to identify any biochemical constraints present in the network and how these can limit the plasticity of the stress response. This could be done, for example, by experimental removal or downregulation of an arbitrary or most-connected node (i.e., hub) of the network.

A further important outcome of such analytical approach is the description of hierarchical organization of modularity in redox networks (173). A module is a discrete unit of an $x$ number of elements interacting in a tightly integrated way that performs a specific task, separable from the functions of other modules (92, 94). Cluster analysis and PLS regression could be helpful for the identification of these modules, that we could call “redox modules” when they refer to antioxidant-prooxidant units (see Fig. 4 for a schematic illustration).
In this general scenario, what are the fundamental mechanisms of maintenance in long-lived animal species? Available evidence points to two general mechanisms: 1) decrease in the rate of generation of RS and 2) possession of macromolecules less sensitive to oxidative damage.

Concerning the possession of macromolecules less sensitive to oxidative damage, available evidence clearly shows a negative correlation between the maximum life span of the animal species and the content of guanine, methionine, and unsaturated fatty acids in their cellular structural components. In other words, the longer the animal life span, the lower the content-abundance of the structural component susceptible to oxidative stress (2, 102, 104, 108–109, 152–153, 156–157, 171, 176, 183) (Table 1).

The susceptibility of mtDNA to damage could be related to the simplest property of a DNA sequence, i.e., the proportion of adenine (A), cytosine (C), guanine (G), and thymine (T) in the mtDNA molecule. In a recent study (183), analyzing the “light strand” mtDNA sequence in 94 animal species (including invertebrates, birds, and mammals), it was found that short-lived species have higher A and T abundances and lower C abundances than long-lived species, while G was almost uniformly low in all species. The light strand was given this name because it is unusually deficient in G, and data from this study show that this characteristic low G abundance looks to be very common across animal species. This result could suggest that the G abundance has been under strong directional selection. On a more mechanistic ground, it is thought that the asymmetric mode of replication of the mtDNA molecule causes an increase in the G to A transition mutations on the light strand, depletion of G nucleotides. In addition to this finding, it was shown that this pattern of nucleotides affects an important DNA sequence property: the free energy (a physical property of the double-stranded DNA molecule related to the binding energy between the two DNA strands). The more negative the free energy is, the less likely is the spontaneous separation of the two strands through thermal fluctuations, conferring to the mtDNA a greater structural stability and lesser susceptibility to damage (183). In support of these mechanisms, comparative data show a strong relationship between the mtDNA free energy and maximum life span. Thus, the longer the maximum life span of a species, the lower is the free energy of mtDNA. Considering that among the four nucleobases, G has the lowest oxidation potential and is thus generally most easily oxidized (18), it has been proposed that these mtDNA base sequence patterns and derived properties represent an evolutionary adaptation of long-lived species to increase mtDNA resistance to endogenous damage by chemical side-reactions and so to decrease the susceptibility of mtDNA to damage and mutation. Accordingly, in a recent study (144), analyzing the lineage-specific mitochondrial mutation rate across 1,696 mammalian species and comparing it with the nuclear rate, a selected decrease of nuclear base substitution rate was reported in long-lived species, reinforcing the proposal for a causal role of mtDNA mutations in aging. This suggests that natural selection may have decreased the mitochondrial mutation rate in long-lived species. Finally, in accordance with this interpretation, a recent study (109) with a phylogenomic approach to identify the genetic targets of natural selection for increased life span in mammals shows, by comparing the nonsynonymous and synonymous evolution of 5.7 million codon sites across 25 species, genes involved in DNA replication/repair or antioxidation have played no detectable role in the evolution of longevity in mammals, reinforcing the relevance of the association between mitochondrial free radical production and mtDNA oxidation-mtDNA mutations.

Fig. 4. Two examples of network topologies. A: schematic illustration of a highly integrated network, where a modular organization cannot be recognized. B: schematic illustration of a modular network, where three modules (white, gray, and black circles, respectively) can be recognized by the fact that nodes within each module are highly connected, while modules are connected to each other by one link only.

Antioxidants and Animal Maximum Life Span

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From an evolutionary comparative approach, a recent study Aledo et al. (2) address the association between species’ life span and methionine usage in mitochondrial proteins using a
Table 1. Comparative studies of endogenous levels of tissue antioxidants in animal species differing in their maximum life span

<table>
<thead>
<tr>
<th>Compared Species (Common Name)</th>
<th>Antioxidant Source</th>
<th>Correlation With Maximum Life Span</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resistance of cellular structural components to oxidative damage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Hummingbird species, mammalian species</td>
<td>Intracellular glycolytic intermediates</td>
<td>Red blood cells</td>
<td>Negative</td>
</tr>
<tr>
<td>Chicken, mammalian species</td>
<td>Intracellular glycolytic intermediates</td>
<td>Liver, skeletal muscle</td>
<td>Negative</td>
</tr>
<tr>
<td>Mammalian species, bird species, reptile species, insect species</td>
<td>Membrane unsaturation</td>
<td>Heart, liver, skeletal muscle</td>
<td>Negative</td>
</tr>
<tr>
<td>C. elegans (long- vs. short-lived mutants)</td>
<td>Membrane unsaturation</td>
<td>Whole</td>
<td>Negative</td>
</tr>
<tr>
<td>25 mammalian species</td>
<td>Membrane unsaturation</td>
<td>Genomic DNA (genes for fatty acid synthesis)</td>
<td>Negative</td>
</tr>
<tr>
<td>76 mammalian species, 14 bird species, 4 invertebrate species</td>
<td>mtDNA guanine content and free energy per base pair</td>
<td>Complete cytochrome b sequence</td>
<td>Negative</td>
</tr>
<tr>
<td>1,696 mammalian species</td>
<td>mtDNA mutation rate</td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>95 mammalian species, 14 bird species, 10 reptile species, 12 amphibian species, 39 fish species, 24 insect species, 15 crustacean species, 9 arachnid species</td>
<td>mtProtein cysteine content</td>
<td>mtDNA</td>
<td>Negative</td>
</tr>
<tr>
<td>168 mammalian species</td>
<td>mtProtein methionine content</td>
<td>Liver</td>
<td>Negative</td>
</tr>
<tr>
<td>Rat, pigeon</td>
<td>Proteome methionine content</td>
<td>Skeletal muscle</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, rabbit, sheep, pig, cow, horse</td>
<td>Proteome methionine content</td>
<td>Heart</td>
<td>Negative</td>
</tr>
<tr>
<td>Budgerigar, canary, mouse</td>
<td>Proteome methionine content</td>
<td>Brain</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Regulatory mechanisms of free radical generation as antioxidant systems</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat, pigeon, human</td>
<td>Cardiolipin unsaturation</td>
<td>Liver mitochondria</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, sheep, dog, pig, cow, horse</td>
<td>Cardiolipin unsaturation</td>
<td>Liver mitochondria</td>
<td>Negative</td>
</tr>
<tr>
<td>Rat, pigeon</td>
<td>Complex I content</td>
<td>Heart mitochondria</td>
<td>Negative</td>
</tr>
<tr>
<td>Budgerigar, canary, mouse</td>
<td>Complex I content</td>
<td>Brain mitochondria</td>
<td>Negative</td>
</tr>
<tr>
<td>Rat, pigeon</td>
<td>Degree of reduction Complex I</td>
<td>Heart mitochondria</td>
<td>Negative</td>
</tr>
<tr>
<td>Budgerigar, canary, mouse</td>
<td>Degree of reduction Complex I</td>
<td>Heart mitochondria</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Antioxidant diversity is an expression of the variety of reactive species and their molecular consequences: Cellular protection by enzymatic and nonenzymatic antioxidants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, rabbit, cow, human</td>
<td>Ascorbate</td>
<td>Brain</td>
<td>Negative</td>
</tr>
<tr>
<td>Rat, guinea pig, rabbit, pig, cow, rhesus monkey, human</td>
<td>Ascorbate</td>
<td>Liver</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog, trout, toad, canary, pigeon</td>
<td>Ascorbate</td>
<td>Liver</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog, trout, toad, canary, pigeon</td>
<td>Glutathione</td>
<td>Brain, lung</td>
<td>Negative</td>
</tr>
<tr>
<td>Ames dwarf mouse (df/df) vs. normal or transgenic GH mouse</td>
<td>Glutathione</td>
<td>Liver</td>
<td>Negative</td>
</tr>
<tr>
<td>Pig-tailed macaque, rhesus monkey, baboon, chimpanzee, human</td>
<td>Catalase</td>
<td>Liver, kidney</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog, trout, toad, canary, pigeon</td>
<td>Catalase</td>
<td>Brain, liver, lung</td>
<td>Negative</td>
</tr>
<tr>
<td><em>D. melanogaster</em> (long- vs. short-lived lines)</td>
<td>Catalase</td>
<td>Whole</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, naked mole-rat</td>
<td>Catalase</td>
<td>Liver</td>
<td>Negative</td>
</tr>
<tr>
<td>Honey bees (queen vs. worker)</td>
<td>Catalase</td>
<td>Brain, Thorax, Abdomen</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, vampire bat, Mexican free-tailed bat, naked mole-rat, little brown bat</td>
<td>Catalase</td>
<td>Liver</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, squirrel, rabbit, guinea pig, big brown bat, sheep, white-tailed deer, dog, pig, cow, human, Japanese quail, zebra finch</td>
<td>Catalase</td>
<td>Liver, heart, brain</td>
<td>Negative</td>
</tr>
<tr>
<td><em>D. melanogaster</em> (wild-type strains)</td>
<td>Catalase (gene expression)</td>
<td>Whole</td>
<td>Negative</td>
</tr>
<tr>
<td>Marine bivalve species</td>
<td>Catalase</td>
<td>Whole</td>
<td>Negative</td>
</tr>
<tr>
<td>Field mouse, deer mouse, pig-tailed macaque, baboon, human</td>
<td>Glutathione</td>
<td>Brain, liver</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog, trout, toad, canary, pigeon</td>
<td>Glutathione</td>
<td>Liver, lung</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog, trout, toad, canary, pigeon</td>
<td>Glutathione</td>
<td>Brain</td>
<td>NS</td>
</tr>
<tr>
<td>Ames dwarf mouse (df/df) vs. normal or transgenic GH mouse</td>
<td>Glutathione</td>
<td>Liver</td>
<td>Negative</td>
</tr>
<tr>
<td><em>D. melanogaster</em> (long- vs. short-lived lines)</td>
<td>Glutathione</td>
<td>Whole</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Continued*
<table>
<thead>
<tr>
<th>Compared Species (Common Name)</th>
<th>Antioxidant</th>
<th>Source</th>
<th>Correlation With Maximum Life Span</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, naked mole-rat</td>
<td>Glutathione</td>
<td>Liver</td>
<td>NS or Negative</td>
<td>4–5</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog,</td>
<td>Glutathione</td>
<td>Liver</td>
<td>NS</td>
<td>126</td>
</tr>
<tr>
<td>trout, toad, canary, pigeon</td>
<td>reductase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog,</td>
<td>Glutathione</td>
<td>Brain, liver</td>
<td>Negative</td>
<td>10, 167</td>
</tr>
<tr>
<td>trout, toad, canary, pigeon</td>
<td>reductase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog,</td>
<td>Glutathione</td>
<td>Liver, heart, brain</td>
<td>Negative</td>
<td>151</td>
</tr>
<tr>
<td>trout, toad, canary, pigeon</td>
<td>reductase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>House mouse, deer mouse, tree</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Liver, brain, heart</td>
<td>NS</td>
<td>214</td>
</tr>
<tr>
<td>shrew, squirrel monkey, Rhesus monkey, chimpanzee, orangutan, lemur, tamarin, baboon, African green monkey, gorilla, human</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse, rat, hamster, guinea pig, rabbit, dog, sheep, cat, pig, cow, horse</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Brain</td>
<td>Negative</td>
<td>150</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog,</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Liver</td>
<td>NS</td>
<td>126</td>
</tr>
<tr>
<td>trout, toad, canary, pigeon</td>
<td>reductase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ant Lasius Niger (Queen vs. workers and males)</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Whole</td>
<td>Negative</td>
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</tr>
<tr>
<td>Mouse, naked mole-rat</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Liver</td>
<td>Negative</td>
<td>4–5</td>
</tr>
<tr>
<td>Honey bees (queen vs. worker)</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Brain, thorax, abdomen</td>
<td>Negative</td>
<td>40</td>
</tr>
<tr>
<td>Mouse, rat, squirrel, guinea pig, big brown bat, sheep, white-tailed deer, dog, pig, cow, human, Japanese quail, zebra finch</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Liver, heart, brain</td>
<td>Negative</td>
<td>151</td>
</tr>
<tr>
<td>Marine bivalve species</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Whole</td>
<td>Negative</td>
<td>187</td>
</tr>
<tr>
<td>Honey bees (queen vs. worker)</td>
<td>Cu,Zn-superoxide dismutase</td>
<td>Whole</td>
<td>Negative</td>
<td>216</td>
</tr>
<tr>
<td>Mouse, rat, squirrel, guinea pig, big brown bat, sheep, white-tailed deer, dog, pig, cow, human, Japanese quail, zebra finch</td>
<td>Mn-superoxide dismutase</td>
<td>Liver, heart, brain</td>
<td>Negative</td>
<td>151</td>
</tr>
<tr>
<td>D. melanogaster (wild-type strains)</td>
<td>Mn-superoxide dismutase</td>
<td>Whole</td>
<td>Negative</td>
<td>187</td>
</tr>
<tr>
<td>Rat, rabbit, sheep, dog, cow, monkey, horse, human</td>
<td>Base excision repair activities (polymerase β)</td>
<td>Dermal fibroblasts</td>
<td>Negative</td>
<td>26</td>
</tr>
<tr>
<td>D. melanogaster (long- vs. short-lived lines)</td>
<td>Disulfide reductase</td>
<td>Whole</td>
<td>Negative</td>
<td>135</td>
</tr>
<tr>
<td>Snell mouse, C57BL/6NcrlBR mouse, Syrian hamster, Norway rat, Mongolian gerbil, 13-lined ground squirrel, rabbit, guinea pig, big brown bat, Sussex sheep, white-tailed deer, domestic dog, Yorkshire/Hampshire pig, Black angus/Charlet cow, Japanese quail and zebra finch</td>
<td>Glutaredoxin</td>
<td>Liver, heart, brain</td>
<td>Negative</td>
<td>182</td>
</tr>
<tr>
<td>Mouse, rabbit, guinea pig, dog, calf, goldfish</td>
<td>Glutathione peroxidase</td>
<td>Brain, liver</td>
<td>Negative</td>
<td>61</td>
</tr>
<tr>
<td>Hamster, rat, sheep, pig, chicken, human</td>
<td>Glutathione peroxidase</td>
<td>Liver</td>
<td>Negative</td>
<td>121</td>
</tr>
<tr>
<td>Mouse, rat, guinea pig, frog, trout, toad, canary, pigeon</td>
<td>Glutathione peroxidase</td>
<td>Brain, liver, lung</td>
<td>Negative</td>
<td>10, 126, 167</td>
</tr>
<tr>
<td>Mouse, naked mole-rat</td>
<td>Glutathione peroxidase</td>
<td>Liver</td>
<td>Negative</td>
<td>4–5</td>
</tr>
<tr>
<td>Honey bees (queen vs. worker)</td>
<td>Glutathione peroxidase</td>
<td>Brain, Thorax, Abdomen</td>
<td>Negative</td>
<td>40</td>
</tr>
<tr>
<td>Mouse, rat, squirrel, guinea pig, big brown bat, sheep, white-tailed deer, dog, pig, cow, human, Japanese quail, zebra finch</td>
<td>Glutathione peroxidase</td>
<td>Liver, heart, brain</td>
<td>Negative</td>
<td>151</td>
</tr>
<tr>
<td>D. melanogaster (wild-type strains)</td>
<td>Glutathione peroxidase (gene expression)</td>
<td>Whole</td>
<td>Negative</td>
<td>187</td>
</tr>
<tr>
<td>Marine bivalve species</td>
<td>Glutathione peroxidase</td>
<td>Whole</td>
<td>Negative</td>
<td>216</td>
</tr>
<tr>
<td>Honey bees (queen vs. worker)</td>
<td>Glutathione S-transferase</td>
<td>Brain, thorax, abdomen</td>
<td>Negative</td>
<td>40</td>
</tr>
<tr>
<td>Honey bees (queen vs. worker)</td>
<td>Methionine sulfoxide reductase</td>
<td>Brain, thorax, abdomen</td>
<td>Negative</td>
<td>40</td>
</tr>
<tr>
<td>Mouse, naked mole-rat</td>
<td>Proteasome (level and specific activities)</td>
<td>Liver</td>
<td>N.S.</td>
<td>166</td>
</tr>
<tr>
<td>2 Bat species</td>
<td>Proteasome (20S activity)</td>
<td>Liver</td>
<td>Negative</td>
<td>180</td>
</tr>
</tbody>
</table>

Antioxidant diversity as an expression of the variety of reactive species and their molecular consequences: Cellular protection by repair/turnover and detoxifying antioxidant systems.
meta-examination of mitochondrial genomes from 168 species of mammals, encompassing 24 different orders. Results showed that methionine usage and life span exhibit a significant negative correlation. However, species are part of a hierarchically structured phylogeny, raising the possibility that data from different species may not necessarily be statistically independent from one another. Therefore, to correct for non-independence of the individual-species data, phylogenetically independent contrasts were calculated. Interestingly, the association between methionine content and life span became more significant after phylogenetic correction. Extending these findings, Aledo et al. (2) studied the relationship between life span and methionine content within those eutherian orders with 10 or more species present in their compilation. All of the amino acid sequences were analyzed orders, Carnivora, Artiodactyla, Cetacea, and Rodentia; in mitochondrial vs. hydrogenosomal sequences; and in aerobic vs. anaerobic bacteria, archaea, and unicellular eukaryotes; in mitochondrial vs. hydrogenosomal sequences; and in the mitochondria of free-living, aerobic vs. anaerobic-parasitic worms. The association of life span with mitochondrial cysteine depletion persisted after correction for phylogenetic interdependence, but it was uncoupled in helminthic species with predominantly anaerobic lifestyle (139). In contrast, methionine usage in mitochondrially encoded proteins is strikingly higher than in nuclear encoded proteins in the majority of species. Short-lived species synthesize higher levels of low-molecular-weight endogenous antioxidants than long-lived ones (Positive correlation means the contrary).

**Table 1.—Continued**

<table>
<thead>
<tr>
<th>Compared Species (Common Name)</th>
<th>Antioxidant</th>
<th>Source</th>
<th>Correlation With Maximum Life Span</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snell mouse, C57BL/6Ncr1BR mouse, Syrian hamster, Norway rat, Mongolian gerbil, 13-lined ground squirrel, rabbit, guinea pig, big brown bat, Sussex sheep, white-tailed deer, domestic dog, Yorkshire/Hampshire pig, Black angus/Charlet cow, Japanese quail and zebra finch</td>
<td>Proteasome (20S and 26S activities)</td>
<td>Liver, heart, brain</td>
<td>Negative</td>
<td>181</td>
</tr>
<tr>
<td>Honey bees (queen vs. worker)</td>
<td>Thioredoxin peroxidase (mitochondrial)</td>
<td>Brain, thorax, abdomen</td>
<td>Negative</td>
<td>40</td>
</tr>
<tr>
<td>Honey bees (queen vs. worker)</td>
<td>Thioredoxin reductase</td>
<td>Brain, thorax, abdomen</td>
<td>Negative</td>
<td>40</td>
</tr>
<tr>
<td>Snell mouse, C57BL/6Ncr1BR mouse, Syrian hamster, Norway rat, Mongolian gerbil, 13-lined ground squirrel, rabbit, guinea pig, big brown bat, Sussex sheep, white-tailed deer, domestic dog, Yorkshire/Hampshire pig, Black angus/Charlet cow, Japanese quail and zebra finch</td>
<td>Thioredoxin reductase</td>
<td>Liver, heart, brain</td>
<td>Negative</td>
<td>181</td>
</tr>
</tbody>
</table>

**Cellular antioxidant regulatory factors**

- **Mouse, hamster, rat, gerbil, squirrel, rabbit, guinea pig, finch, sheep, deer, pig, cow**
  - Heat shock protein expression (HSP60, HSP70, GRP78, GRP94)
  - Nrf2 (activity)
  - Whole
  - Positive | 209 |
- **D. melanogaster (long-lived for keap1/Nrf2 mutants versus wild type)**
  - Nrf2 (level and activity)
  - Skin-derived fibroblasts
  - Positive | 123 |
- **Mouse (long-lived Snell dwarf mutant vs. wild type)**
  - Nrf2 (activity)
  - Liver, skeletal muscle
  - Positive | 199 |
- **Mouse (long-lived mGsta4 mutant vs. wild type)**
  - Poly(ADP-ribose)polymerase
  - Mononuclear leukocytes
  - Positive | 82 |

**NS, not significant correlation. Negative correlation means that long-lived species constitutively have lower tissue levels of antioxidants enzymes and low-molecular-weight endogenous antioxidants than short-lived ones (Positive correlation means the contrary).**

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The analysis reveals that the frequency with which cysteine is encoded by mitochondrial DNA is a specific and phylogenetically ubiquitous molecular indicator of aerobic life span: long-lived species synthesize respiratory chain complexes, which are depleted of cysteine. Cysteine depletion was also found on a proteome-wide scale in aerobic vs. anaerobic bacteria, archaea, and unicellular eukaryotes; in mitochondrial vs. hydrogenosomal sequences; and in the mitochondria of free-living, aerobic vs. anaerobic-parasitic worms. The association of life span with mitochondrial cysteine depletion persisted after correction for phylogenetic interdependence, but it was uncoupled in helminthic species with predominantly anaerobic lifestyle (139). In contrast, methionine usage in mitochondrially encoded proteins is strikingly higher than in nuclear encoded proteins in the majority of species.
animals (which is the inverse behavior compared with cysteine), and there is no significant correlation between mitochondrial methionine usage and life span (138). The dichotomy methionine accumulation vs. cysteine depletion in mitochondrial respiratory chain complexes probably should be considered in the context of antioxidant protection (for methionine) vs. avoidance of oxidation-derived effects (for cysteine). So, the topological selectivity of longevity-dependent cysteine depletion to the inner mitochondrial membrane might find its most probable explanation in the concurrence of three circumstances that are not to be found elsewhere in the cell: exceptionally high concentrations of multispan transmembrane proteins of outstanding hydrophobicity, their particularly high exposure to free radicals generated in situ, and the unique chemical reactivity of thiols in nonpolar environments. Under these premises, cysteine is avoided to prevent cysteine-catalyzed chain-transfer reactions, and thus, the formation of extended networks of internally cross-linked hydrophobic proteins that might eventually lead to a mitochondrial dysfunction (138).

The mechanisms responsible for the life span-related differences in fatty acid profile can be related, in principle, to the fatty acid desaturation pathway, and the deacylation-recycylation cycle. The available estimates of delta-5 and delta-6 desaturase activities indicate that they are several folds lower in long-lived species than in short-lived ones (152–153). This can explain why 22:6n-3 and 20:4n-6 decrease and 18:2n-6 and 18:3n-3 increase, respectively, from short- to long-lived animals, since desaturases are the rate-limiting enzymes of the n-3 and n-6 pathways synthesizing the highly unsaturated PUFAs 20:4n-6 and 22:6n-3 from their dietary precursors, 18:2n-6 and 18:3n-3, respectively. Thus, desaturation pathways would make available in situ the n-6 and n-3 fatty acids to phospholipid acyltransferases to remodel the phospholipid acyl groups. The fact that acyltransferase/n-6 desaturase activity ratio is about 10:1 in tissues reinforces the idea that regulation of desaturases can be the main limiting factor responsible for the observed membrane unsaturation-longevity relationship. Animals with a high life span have a low degree of membrane fatty acid desaturation based in the redistribution between types of PUFAs without any alteration in the total (%) PUFA content, average chain length, and phospholipid distribution. This may be viewed as an elegant evolutionary strategy, because it decreases the sensitivity to lipid peroxidation and lipoxidation-derived damage to cellular macromolecules without strongly altering fluidity/microviscosity, a fundamental property of cellular membranes for the proper function of receptors, ion pumps, and transport of metabolites. This would occur because membrane fluidity increases acutely with the introduction of the first and less with the second double bond (due to their introduction of “kinks” in the fatty acid molecule), whereas additional (the third and following) double bonds cause a few further variations in fluidity (22). This is so because the kink has a larger impact on fluidity when the double bond is situated near the centre of the fatty acid chain (first double bond) than when it is situated progressively nearer to its extremities (next double bond additions). In the case of the sensitivity to lipid peroxidation, however, double bonds increase it irrespective of their location at the center or laterally on the fatty acids (100). Thus, by substituting fatty acids with four or six double bonds by those having only two (or sometimes three) double bonds, the sensitivity to lipid peroxidation is strongly decreased in long-lived animals, whereas the fluidity of the membrane would be essentially maintained. This hypothesis, reminiscent of membrane acclimation to different environments at PUFAs level in poikilotherms and bacteria, has been denominated homeoviscous longevity adaptation (156). In accordance with this interpretation, a recent study (109) with a phylogenomic approach to identify the genetic targets of natural selection for elongated longevity in mammals has been published. In this work, comparing the nonsynonymous and synonymous evolution of 5.7 million codon sites across 25 species, shows that genes involved in lipid composition (and particularly desaturation system) have collectively undergone increased selective pressure in long-lived species. So, cellular membrane has apparently been the optimized feature.

With reference to mitochondrial free radical generation and maximum life span, all the published investigations about this subject have found that the rate of mitochondrial ROS production is lower in the tissues of long-lived than in those of short-lived animal species independently of their mass-adjusted rates of O₂ consumption (12, 117, 154, 156, 185, 186). In accordance with this low mitochondrial free radical production of long-lived species, there is an adaptive response concerning endogenous cellular antioxidant systems (154–155). Long-lived animal species constitutively have lower (instead of higher) tissue levels of antioxidant enzymes and low-molecular-weight endogenous antioxidants than short-lived ones (Table 1). That characteristic can thus explain why endogenous tissue antioxidants correlate negatively with maximum life span across species: long-lived animals have constitutively low levels of antioxidants because they produce RS at a low rate. If long-lived animals had high rates of ROS production together with their very low levels of endogenous antioxidants, their tissue cells would not be able to maintain oxidative stress homeostasis. Decreasing mitochondrial ROS production instead of increasing antioxidants or repair systems makes sense when considered from the point of view of evolution of life span among species and upon the premise that metabolism is adapted and optimized to achieve balance and economy (131). It would be very inefficient to generate large amounts of ROS and, afterward, try to intercept them before they reach cellular components, or even worse, try to repair after heavily damaging it. This makes even more sense taking into account the high energetic cost of continuously maintaining high levels of antioxidant and repair molecules in cells, considering that availability of energy is limited by both environment (e.g., food availability) and physiological constraints. In agreement with the adaptive response that represents antioxidant defenses, it is relevant to highlight that long-lived species show high (not low) levels/activities of regulatory factors (Table 1), confirming the ability of animals (short- or long-lived) to transitorily induce or repress these protective molecules when needed.

The outcomes of two experimental paradigms deserve also special mention (reviewed in Refs. 154 and 185): 1) experimentally increasing tissue antioxidants through dietary supplementation, pharmacological induction, or transgenic techniques sometimes moderately increases mean life span (life expectancy) but does not change maximum life span; and 2) animals in which genes coding for particular antioxidant enzymes are knocked out can show different pathologies but their rates of aging do not seem to be affected. These findings
are probably due to the activation of negative feedback mechanisms to maintain the oxidative stress homeostasis, which is cell- and species-specific. Other two nonmutually exclusive explanations are that oxidative stress 1) has, in general, a mild effect on aging, but a strong effect on the progression of age-related pathologies (i.e., health span or health aging) and 2) has a significant effect on aging only under stressful environmental conditions (180). The second explanation, in particular, may have significant ecological implications. For example, there may be high heterogeneity in environmental quality in which animals live and there may also be differences in habitat quality among reproductive seasons, particularly evident in seasonal environments. In particular, the interaction between environmental quality and phase of the life cycle (e.g., reproduction, moult, migration, hibernation) of the species may be relevant in setting up the specific needs of antioxidants and the magnitude of the oxidative challenges to which the individual is exposed (19, 49). Future studies will need to take into account the environmental quality as a modulator of effects of oxidative stress on life-history strategies and individual fitness.

In conclusion, we can infer that natural selection tends to decrease structural components that are highly susceptible to oxidative damage, thus conferring to the macromolecules a higher structural stability.

**Perspectives and Significance**

In this review, we have shown that exposure to RS has likely represented an important selective pressure, which led aerobic organisms to reduce the abundance of the structural components that are more highly susceptible to oxidative damage and to evolve a large suite of molecular and structural antioxidant defenses. The high variation in such defenses and the potential energetic costs associated with the activation of antioxidant responses (e.g., upregulation of enzymes and repair systems) have potential implications for life histories trade-offs, such as those among reproduction and self-maintenance, as well as among different components of self-maintenance (e.g., immune response vs. antioxidant response). The incorporation of antioxidant mechanisms into an ecological-evolutionary perspective could also prove valuable for our understanding of intergenerational effects, which could either “program” offspring phenotype to better face their future environment, or conversely constrain or cause a lag in any phenotypic adjustments to current conditions, especially when changes in environmental conditions are not predictable, hence causing a mismatching between developmental and adult environment. Such a scenario is realistic in that many antioxidant molecules, and nutrients affecting body composition and resistance to RS and mitochondria, are maternally inherited. So any perturbation in female oxidative balance may be passed on her offspring.

The complexity and variation of mechanisms underlying the redox physiology make it challenging, however, at this stage to draw general conclusions about the role of oxidative stress as a pace-maker of 1) trade-offs among life-history traits, 2) phenotypic development, and 3) responses of natural populations to environmental changes. Therefore, it will be important to be conservative in drawing general paradigms when mechanisms of redox system are not well known, rather than to translate mechanisms described for specific taxa to all others, in particular, when taxa are phylogenetically unrelated. This last point urgently calls for larger comparative studies than those carried out so far, as soon as additional data on oxidative stress physiology in natural populations is accumulated. Finally, we highlight the importance of defining the biological relevance of measures of oxidative damage, antioxidant status, and gene expression, which is often overlooked in favor of more mechanistic explanations. Consequently, more studies are needed to understand to which extent variation in redox state traits is functionally significant and, if heritable, to which extent this variation may be a target of natural selection.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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Review


Review

ANIMAL ANTIOXIDANT DEFENSES


