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FORUM REVIEW ARTICLE

Mitochondrial ROS Generation and Its Regulation: Mechanisms Involved in H_2O_2 Signaling

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Abstract

Mitochondria are the main source of reactive oxygen species in the cell. These reactive oxygen species have long been known as being involved in oxidative stress. This is a review of the mechanisms involved in reactive oxygen species generation by the respiratory chain and some of the dehydrogenases and the control by thermodynamic and kinetic constraints. Mitochondrial ROS produced at the level of the bc1 complex as well at the level of complex I are discussed. It was recognized more than a decade ago that they can also function as signaling molecules. This signaling role will be developed both in terms of mechanism and in terms of mitochondrial ROS signaling. The notion that hydrogen peroxide acts not only as a damaging oxidant but also as a signaling molecule was proposed more than a decade ago. Hydrogen peroxide signaling can be either direct (oxidation of its target) or indirect (involving peroxiredoxins, for example). The consequences of ROS signaling on crucial biologic processes such as cell proliferation and differentiation are discussed. *Antioxid. Redox Signal.* 14, 459–468.

Introduction

NEITHER DIOXYGEN NOR LIGHT is necessary to life (72). Indeed, we know of organisms that are able to live in a complete anaerobic medium. Moreover, we can hypothesize that life started in the dark trenches of oceans, and therefore the origin of life did not require light. However, for energetic reasons, the switch to light capture occurred. The primitive redox scale available to biologic systems is only −0.5 V/0.0 V (for instance, H^2/H^+ or Sn/S^{2-}), whereas dioxygen formation needs a gap of 1.3 V. Thus light absorption allowing a scale greater than 2.0 V has been a necessary event compatible with dioxygen formation (42).

Whichever the systems for light absorption that were developed about 2 billion years ago, the reactions involved led to the production of reactive oxygen species such as $HO^{\cdot}$ or $O_2^{\cdot-}$. Because of their high reactivity, they were highly poisonous for all early life, and it is likely that the next step in evolution was detoxification rather than dioxygen use. This might suggest that the protective enzymes (*i.e.*, superoxide dismutases and catalases) are ancestral ones (109). Nevertheless, dioxygen and ozone produced by photosynthesis created a protective screen against the damaging solar ultraviolet radiations, allowing the evolution of more-complex organisms. Thus, since the beginning of a rich oxygen atmosphere on earth, life has had to manage with the toxic risks and energetic profits of this very peculiar molecule.

Dioxygen: A Poison for Life

Dioxygen, with two unpaired electrons, each located in a different antibonding orbital, is a biradical (Fig. 1) (89). These two electrons have the same spin quantum number, or, as is often written, they have a parallel spin. This constitutes the most stable state (*i.e.*, ground state) of dioxygen. If a diatomic oxygen molecule attempts to oxidize another atom or molecule by accepting a pair of electrons, both of these electrons must have an antiparallel spin, so as to fit the vacant space in the orbitals. In most cases, electrons forming a pair in an orbital have opposite spins, in accordance with Pauli's principle. This imposes a restriction on electron transfer, which leads dioxygen to accept electrons one at a time. Consequently, dioxygen reacts only very slowly with all nonradicals. Even if, theoretically, the redox potential of the O_2/H_2O couple is the most positive one in the world, oxygen in the air cannot immediately burn living organisms because of the structure of their organic compounds. This spin restriction of dioxygen reactivity allowed the complex evolution of organisms and a metabolic adaptation (*i.e.*, the use of dioxygen as last electron acceptor during catabolism), leading to an efficient energy transfer in cells (46).

However, more-reactive forms of dioxygen, such as two types of singlet oxygens, may be generated by energy input and electronic changes. Even if the singlet oxygens are not always radicals, as strictly defined (presence of unpaired

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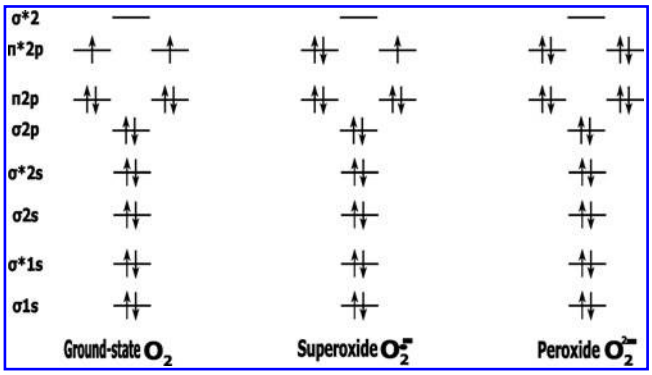

FIG. 1. Bonding in the diatomic oxygen molecule.

electrons on an orbital), the spin restriction is removed, and the oxidative reactivity is largely increased.

When a unique electron is accepted by the ground-state dioxygen, it enters one of the antibonding orbitals and forms the superoxide radical, $O_2^{\cdot-}$. This compound is a very active electron donor, potentially able to generate a toxic cascade of electron-transfer reactions (46). However, at physiologic pH, the main reason for superoxide disappearance in aqueous solution is its dismutation, catalyzed by the superoxide dismutase enzyme. It is often said that spontaneous dismutation is very rapid, but it is worth noting that (a) such a reaction becomes slower as the pH rises; and (b) yeast mutant devoid of superoxide dismutases (sod 1 and sod 2) cannot survive during the stationary phase, when cells switch from fermentation to growth fueled by respiration (67, 68). If dioxygen accepts two electrons, it generates the peroxide ion (*i.e.*, O_2^{2-}), which, in biologic media, is found as hydrogen peroxide (H_2O_2). Hydrogen peroxide is not a radical but is considered a reactive oxygen species because in the presence of a transition metal, it is able to form the hydroxyl radical, $HO^{\cdot}$. In biologic systems, this product can induce a cascade of radical reactions potentially involving any cell compound.

ROS Production by the Respiratory Chain

It is generally believed that the main superoxide producer in the cell is the respiratory chain (Fig. 2). Indeed, two of the respiratory chain complexes (I and III) have been, for a long time, recognized as involved in superoxide production (25, 43). From quantitative data obtained on isolated mitochondria, it has been suggested that about 2–5% of oxygen consumption is due to superoxide anion generation and that about 70–80% of superoxide formation is connected with the operation of the Q cycle in complex III (25). However, such claims are very controversial because superoxide generation varies depending on the cell type and the respiration steady state. More realistic predictions estimate that, under physiologic conditions, the superoxide production is around 0.1% of the respiratory rate (48, 95).

The electron transfer from ubiquinol to cytochrome *c* and the associated proton translocation is catalyzed by complex III or more appropriately termed the ubiquinol- cytochrome *c* oxidoreductase (see Fig. 3). An important pool of ubiquinones and ubiquinols (reduced form: QH_2) exist in the mitochondrial inner membrane in large molar excess over the other components of the respiratory chain. A molecule of ubiquinol from the pool diffuses to a binding-site Q_o close to the cytoplasmic face of the mitochondrial membrane and adjacent to the iron-sulfur protein (Rieske protein). First, an electron is transferred from UQH_2 to the Rieske protein, releasing two protons to the cytoplasm and leaving the free radical semiubiquinone anion species $UQ^{\cdot-}$ at the Q_o site. The electron accepted by the Rieske protein passes down the respiratory chain to cytochrome *c1*, cytochrome *c*, and cytochrome oxidase. The second electron is transferred to the bL heme, which is close to the cytosolic face, and then to the other heme bH site on the same polypeptide. It is believed that UQH_2 and UQ can migrate freely from one side of the hydrophobic core to the other, regardless of proton-motive force, because these hydrophobic carriers are uncharged. A second quinone binding site, Q_i, in the close vicinity of bH, allows the transfer of one electron from the reduced bH with the formation of the semiubiquinone $UQ^{\cdot-}$ (Fig. 3A). The result of this is the presence of a semiquinone firmly bound to Q_i site. In the next part of the Q cycle, a second molecule of UQH2 is oxidized at Q_o, as previously shown (Fig. 3B). Again, one electron passes to cytochrome *c1* and another via bL to bH. This electron achieved the reduction of $UQ^{\cdot-}$ to UQH_2, with two protons coming from the matrix side. The UQH^2 returns to the bulk pool, and the Q cycle is com-

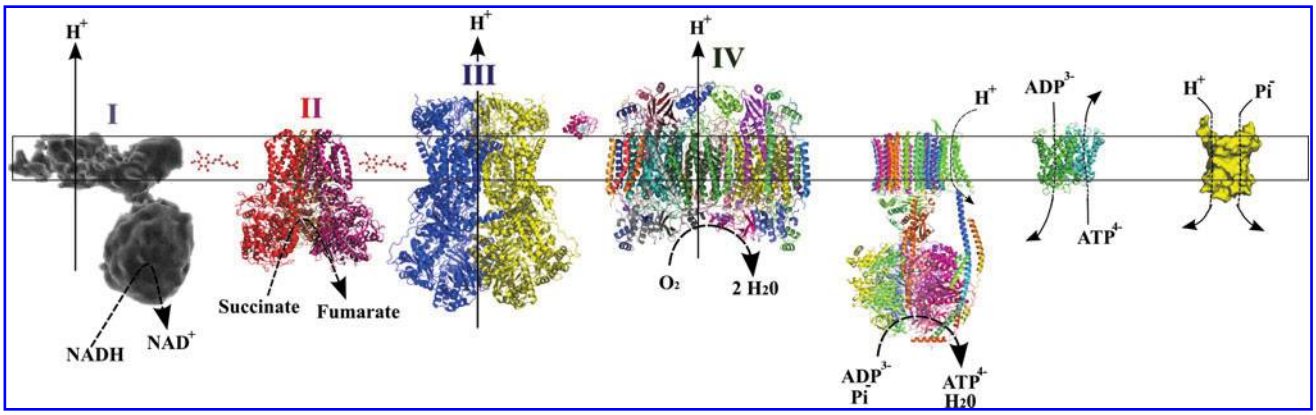

FIG. 2. The mammalian respiratory chain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

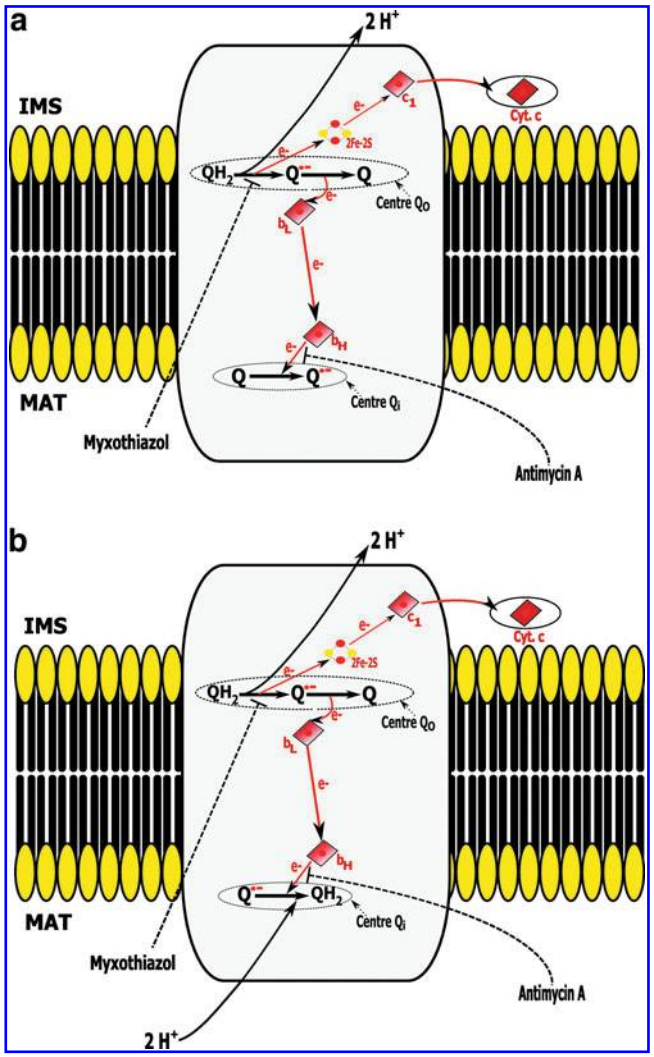

FIG. 3. The Q cycle. (a) Oxidation of the first ubiquinol; **(b)** oxidation of the second ubiquinol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

pleted. Q_o and Q_i are not equivalent in this model: only Q_i binds the semiubiquinone firmly. At Q_o, in the absence of high thermodynamic and/or kinetic constraints, the semiubiquinone has only a transient existence.

Concerning the nature of the superoxide generator at the level of complex III, two components have been proposed to be the autooxidizable electron donors to oxygen: semiubiquinone and the reduced cytochrome *b* (14, 17). To determine which of the possible electron carriers is the direct electron donor to dioxygen, specific inhibitors of the electron transfer through this complex have been used (Fig. 3). Experiments with succinate as an electron donor in the presence of antimycin A in intact rat heart mitochondria showed that myxothiazol, which inhibits the reduction of Rieske iron-sulfur center, prevented the formation of superoxide anions. Similarly, mitochondrial cytochrome *c* depletion inhibited the formation of superoxides, whereas antimycin A, alone, enhanced the production of superoxides. Taken together, these experiments indicate that factors preventing the formation of semiubiquinone also prevent superoxide formation. These observations are consistent with semiubiquinone being the only reduced electron carrier able to reduce dioxygen to superoxide at the level of complex III (100). The Q cycle can produce superoxides on the inner and on the outer surfaces of the inner mitochondrial membrane. Because superoxides do not cross this membrane, it is of importance to know the size of the relative fluxes in the intermembrane space and into the matrix. No study has provided an answer to this question.

The other site in the respiratory chain involved in superoxide formation is complex I. In this large multi-subunit complex, general agreement now indicates that the electron transfer is working at near equilibrium, and thus, superoxide production may be linked to both forward electron transport (FET) and reverse electron transport (RET) (25, 26, 50, 54, 58, 60, 66, 103). It is likely that different sites of superoxide production exist in complex I and that the sites involved are different for FET or in RET (7, 102). The sites of complex I–associated superoxide generation are still controversial. Three main sites have been proposed: the flavine mononucleotide (FMN) (51, 56, 59), the Fe-S clusters (more likely N1a orN2) (44, 49, 58), and a ubiquinone specifically linked to complex I (Q_i site) (61). Rotenone inhibits electron transfer right upstream from the quinine-binding site. Consequently, superoxides that are produced by complex I in the presence of NADH are most likely due to the electron carriers (flavin or Fe/S clusters). However, ROS-generation flux seems mainly linked to reverse electron flux, and the Q_i site could be a major player in this flux. It should be stressed here that unlike ROS production for FET, ROS production from RET can originate either at the actual RET site or at the dehydrogenase level (see later).

Other Processes of ROS Production in Mitochondria

As previously mentioned, the respiratory chain complexes are still the most-studied elements regarding the contribution of mitochondria to ROS production. However, emerging publications describe the possible implication of mitochondrial dehydrogenases in this process.

The α-ketoglutarate dehydrogenase complex (αKGDHC) catalyzes the oxidation of α-ketoglutarate in succinyl-coA with the production of NADH in the Krebs cycle. It is composed of multiple copies of three enzymes: α-ketoglutarate dehydrogenase (E1), dihydrolipoamide succinyl-transferase (E2), and dihydrolipoyl dehydrogenase (Dld or LADH, E3) (70, 87, 104). Starkov *et al.* (91) showed that this complex is able to produce ROS during its catalytic process in mammalian brain mitochondria. This event seems to be linked to the flavin cofactor of the Dld, which can generate superoxide anion (15, 71). Klyachko *et al.* (53) proposed a pH-dependent regulation of the oligomeric state of the Dld to explain this phenomenon, but a recent study showed opposite results, which propose conformational changes with no modification of the oligomeric state (2). This same work demonstrated a downregulation of αKGDHC ROS generation process by lipoic acid. These observations suggest the possibility of ROS generation by other mitochondrial flavoenzymes, such as the pyruvate dehydrogenase complex (PDHC), which also is composed of a Dld (91).

One way to reoxidize the NADH produced during glycolysis is the glycerol-3-phosphate shuttle. This shuttle is made up of two isoforms of the enzyme called glycerol-3-phosphate dehydrogenase (GPDH), which differ by their localization and cofactors. The cytosolic enzyme oxidizes

NADH to NAD^+ with formation of glycerol-3-phosphate, which is converted back to dihydroxyacetone phosphate by the mitochondrial isoform (52). That isoform is located on the external side of the mitochondrial inner membrane and is an FAD-linked enzyme that donates electrons to the respiratory chain via the ubiquinone pool. In mammalians, its level and activity differ depending on the tissues and are inducible by hormones (29, 63, 69). Twenty years ago, the production of ROS during the functioning of the GPDH independent of the respiratory chain complexes (notably by the RET) was suggested by work on guinea pig brain mitochondria (111). These results have been reinforced by more recent work (45, 60, 98). Similar observations have been made in *Drosophilia* mitochondria, in which the muscle glycerol-3-phosphate shuttle is very active (73). Also in *Drosophilia*, topologic studies suggest that GPDH can produce ROS on both sides of the mitochondrial inner membrane in a way similar to that of complex III (74). This ROS production is supposed to be the consequence of the absence of a coenzyme Q binding site in the mitochondrial GDPH, which would diminish the protection of semiubiquinone produced during glycerol-3-phosphate oxidation. This hypothesis is congruent with the fact that the succinodehydrogenase complex (SDHC), which also reduces the ubiquinone pool, possesses a coenzyme Q binding site and that the succinate-induced ROS production is exclusively mediated by complex I through the RET.

Years ago, it was shown that pigeon and rat mitochondrial respiration, with palmitoyl-carnitine as the substrate, could lead to H_2O_2 release (12, 13). St-Pierre *et al*. (90) made the same observations and showed that this H_2O_2 production is independent of the respiratory chain complexes and is on the matrix side. Now the study of the link between ROS generation and lipid metabolism has gained more and more interest with the observation that many pathologies, such as diabetes, feature an alteration in lipid metabolism and oxidative stress (1, 47, 55, 99). For example, in skeletal muscle, evidence exists of a link between insulin resistance and oxidative stress (3, 11). Furthermore, the uncoupling proteins (UCPs) are induced during lipid metabolism (19, 84) and are activated by superoxide (40). A recent study showed that low and physiologic concentrations of palmitoyl-carnitine as the substrate for skeletal muscle mitochondria are sufficient for ROS production with a relatively low dependence on $\Delta\Psi$ (86). In mammals, fatty acids catabolism is located in the mitochondrial matrix and is linked to the electron transport by acetyl-CoA provided to the Krebs cycle and via the flavoprotein ETF (electron-transfer flavoprotein). ETF is reduced by acyl CoA dehydrogenase and passes its electrons to the ubiquinone pool via the ETF CoQ oxidoreductase enzyme (ETF-QO) present on the inner mitochondrial membrane. Earlier studies on the catalytic mechanism of ETF-QO suggest that electrons can escape from the $ETF^{\cdot-}$ or from the semiquinone formed during ETF oxidation (8, 9, 79). Consequently, ETF and/or ETF-QO are supposed to generate ROS with palmitoyl-carnitine as the substrate.

Control of ROS Production Flux by Mitochondria

The redox level of the respiratory chain electron carriers, including semiubiquinone, is thermokinetically controlled. Thus, the forces directly associated with respiratory chain activity, which are the redox potential of the NAD^+/NADH couple and the proton-motive force, are powerful regulators of the steady-state concentration of the free semiubiquinone radical. An increase in electron supply to the respiratory chain or in proton-motive force must lead to an increase in semiubiquinone radical content. Moreover, kinetic constraints exerted downstream of the quinone pool (at the level of cytochrome oxidase, for instance) could further increase the level of free radical semiubiquinones. Conversely, moderate uncoupling will lead to a decrease in force and kinetic constraints and thus effectively decrease superoxide production by the respiratory chain (88). The opposite effect can be observed when the respiratory chain is inhibited (13, 18). Other mechanisms that induce a decrease of the redox proton-pump efficiency (such as slipping) are expected to be less effective because they do not significantly affect the proton-motive force (77, 81, 83, 112). It is well known that the transition state4/state3 or the uncoupling state decrease ROS generation in isolated mitochondria (54). Free fatty acids exert different effects on mitochondria: they inhibit electron flux at the complex I and probably complex III levels, inducing an increase in ROS production associated with forward electron transport in isolated rat heart or liver mitochondria (85). They also induce a slight uncoupling effect, which seems responsible for a large decrease in ROS formation linked to reverse electron transfer. This illustrates the subtle ROS-production response to changes in electron flux through the respiratory chain.

One of the physiologic functions of the UCP family could be the modulation of the proton-motive force in response to an increase in mitochondrial ROS production. Two models have been proposed to explain the control of the activity of UCPs by oxidative stress: first, it has been shown by Klingenberg and co-workers (38, 39) that the proton-pumping activity of UCPs requires quinone and that this interaction influences the regulation of the UCPs by different nucleotides. From this work, it was hypothesized that the UCP activity could be regulated by the quinone redox state (23). However, other authors showed that hydroxynonenal and structurally related compounds (such as *trans*-retinoic acid, *trans*-retinal, and other 2-alkenals) specifically induce uncoupling of mitochondria through the uncoupling proteins UCP1, UCP2, and UCP3. They proposed that hydroxynonenal is not merely toxic, but also may be a biologic signal to induce uncoupling through UCPs and adenine nucleotide translocator (ANT) and thus decrease mitochondrial ROS production (41). Whichever the hypothesis, it is likely that the UCP activities are regulated by ROS either directly or indirectly.

Because it is now established that dehydrogenases are able to produce ROS and that the amount of ROS produced by different dehydrogenases is very different (see earlier), one has to consider the relation between oxidative phosphorylation and ROS production in a more-integrated system. Little literature exists in this field; however, in yeast mitochondria, it has been shown that the two main systems for conveying excess cytosolic NADH to the mitochondrial respiratory chain, which are the external NADH dehydrogenases (Nde1p and Nde2p) and the glycerol-3-phosphate dehydrogenase shuttle, compete for the entrance of electrons in the respiratory chain (16, 78). At saturating concentrations of NADH, the activation of external NADH dehydrogenases completely inhibits glycerol-3-phosphate oxidation. Moreover, by comparing respiratory rates with different respiratory substrates,

we showed that electrons from Nde1p are favored over electrons coming from Ndip (internal NADH dehydrogenase) and that when electrons come from either Nde1p or Nde2p and succinodehydrogenase, their use by the respiratory chain is shared to a comparable extent. This very specific competition for electron entrance into the respiratory chain may depend on the redox status of the quinone pool (82). This competition process is associated with an active leak that is due to the activities of the mitochondrial membrane external dehydrogenases (75) and that could play a role comparable to that of UCPs in mammals (*i.e.*, relieve the redox pressure and decrease both the proton-motive force and ROS production).

ROS and Signaling

ROS from mitochondria and other cellular sources (such as NADPH oxidase) have long been regarded as toxic by-products of metabolism that cause damage to cellular components. As stated earlier, to protect against the damaging effects of ROS, cells possess several antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Thus oxidative stress may be defined as an imbalance between oxidant production and the antioxidant capacity of the cell to prevent oxidative injury. Oxidative stress has been implicated in a large number of human diseases, including cancer, neurodegenerative diseases, and aging (6, 105), yet the relation between oxidative stress and the pathobiology of these diseases is not clear, largely due to a lack of understanding of the mechanisms by which ROS function in both physiologic and pathologic states. More than a decade ago, the notion was proposed that hydrogen peroxide (H_2O_2) acts not only as a damaging oxidant but also as a signaling molecule. Because $O_2^{\cdot-}$ in solution is short lived, in most biologic systems, generation of $O_2^{\cdot-}$ will result in the formation of H_2O_2. H_2O_2 is not a free radical and is a more-stable molecule that is able to diffuse across biologic membranes. Consequently, ROS signaling implies that the molecule involved is H_2O_2. We will suggest some examples of the fact that H_2O_2 is able to function as a signaling agent in both prokaryotes and eukaryotes. The mechanisms involved in H_2O_2 signaling are treated, and evidence that H_2O_2 signaling emerges from mitochondria is shown.

Mechanisms of H_2O_2 Signaling

H_2O_2 signaling in prokaryotes

The transcription factor OxyR is the best-characterized H_2O_2 sensor in prokaryotes (92). It activates the expression of antioxidant defensive activities in response to elevated levels of hydrogen peroxide. The OxyR protein is directly sensitive to oxidation, and only oxidized OxyR is capable of activating transcription. It has been shown that oxidation of OxyR leads to the formation of an intramolecular disulfide bond between cysteine residues 199 and 208 (4, 64, 96, 110) and that OxyR is reduced by enzymatic reduction of this disulfide bond (110). It was determined that only an oxidized form of OxyR was active in stimulating transcription of the oxidative-stress–inducible genes, suggesting that OxyR is oxidized either directly or indirectly in response to H_2O_2, resulting in the activation of the transcription factor (93). An alteration of the contact sites between the protein and DNA occurs with oxidation that suggests that oxidation is accompanied by a substantial conformational change of the protein (97). Addition of H_2O_2 to *Escherichia coli* leads to a transient oxidation and activation of the protein. Reduction and deactivation are catalyzed by *glutaredoxin 1*, a gene that is positively regulated by oxidized OxyR. This transient oxidation occurs even with the addition of low levels of H_2O_2because the rate of reaction of OxyR with H_2O_2 is relatively fast. These quite rapid kinetics result in the complete oxidation of OxyR in 30 s, with addition of just 5 μM H_2O_2 to the cells. These results show that OxyR is able to sense physiologic variations of intracellular H_2O_2 levels.

H_2O_2 signaling in the yeast Saccharomyces cerevisiae

In the budding yeast *Saccharomyces cerevisiae*, the transcription factor Yap1 is a functional homologue to OxyR. Its induction elevates the expression of genes encoding most antioxidants and components of the cellular thiol-reducing pathways (20). Yap1 is translocated to the nucleus with oxidative stress (57). On activation by H_2O_2, Yap1 is oxidized to an intramolecular disulfide bond between Cys^{303} and Cys^{598} (34). A close correlation is found between Yap1 oxidation and its activation that indicates that oxidation is the trigger for activation and suggests that Yap1 is a component of the cellular mechanism sensing H_2O_2. Unlike OxyR, purified Yap1 is not oxidized with 0.1 mM H_2O_2 *in vitro*, indicating that Yap1 is not a direct target of H_2O_2. The oxidation of Yap1 in *S. cerevisiae* results in the transient formation of a minor form of higher molecular mass (35). This species is a mixed disulfide between Cys^{598} of Yap1 and the active site cysteine, Cys^{36}, of hydroperoxidase Gpx3. Thus, the thiol peroxidase Gpx3 is the hydroperoxide sensor that promotes the oxidation of Yap1 to its intramolecular disulfide bond (the activated form of the regulator). This function of Gpx3 uncovers a tight coupling between the mechanisms of hydroperoxide sensing and scavenging (35).

H_2O_2 signaling in mammals

Stimulation of cells with various agonists induces H_2O_2 production, and blockage of H_2O_2 accumulation results in inhibition of signaling by such stimulants (31, 76, 80, 108). In cells stimulated with growth factors such as PDGF or EGF, the propagation of growth-factor signaling requires H_2O_2 (5, 94). Hydrogen peroxide oxidizes critical cystein residues of effector molecules such as protein tyrosine phosphatases. It has been shown that to increase the steady-state level of protein tyrosine phosphorylation in cells, the concomitant inhibition of protein tyrosine phosphatases is required. It seems that the receptor-dependent H_2O_2 production goes through NAPDH oxidase (30, 62, 65, 76, 101). A long-standing question has been the modalities of H_2O_2 signaling in the presence of H_2O_2-scavenging enzymes that are present in the cytosol of most cells. Because peroxiredoxins are much more reactive toward H_2O_2 than protein tyrosine phosphatases, it seems unlikely that these phosphatases are oxidized by H_2O_2. Two interesting sets of data provide a possible answer to this question.

1. PrxI to PrxIV belong to the 2Cys Prx subfamily, which reduce H_2O_2 (24). Prx enzymes have a low K_m toward H_2O_2 and thus are efficient at eliminating low concentrations of H_2O_2 (24). However, they can undergo hyperoxidation of their Cys that results in an inactivation of peroxidase activity and has been proposed to have a mechanism (the floodgate hypothesis) allowing H_2O_2 signaling (10, 106).

2. In a recent article (107), it was shown that PrxI phosphorylation on Tyr^{194} leads to its inactivation in cells stimulated via receptors for growth factors such as PGDF. More important, they show that PrxI phosphorylation is confined to PrxI molecules associated with cell membranes. PrxI present in the cytosol was not phosphorylated. The authors conclude that the spatially confined inactivation of PrxI provides a means for generating favorable H_2O_2 gradients around a submembrane compartment where signaling proteins are concentrated, while minimizing the general accumulation of H_2O_2 to toxic levels and the disturbance of global redox potential (107).

ROS Signaling Emerging from Mitochondria

Mitochondria quality control

Studies depicted earlier are relative to transcription factors that are responsible for the expression of antioxidant defensive activities and rely on the exogenous addition of H_2O_2 to cells. However, the main source of ROS within a cell is the mitochondria, and a recent study showed that H_2O_2 signaling could emerge from mitochondrial dysfunction. In yeast growing on a nonfermentable substrate, there is a growth-yield homeostasis that is due to a tight mitochondrial content adjustment (36). This implies that the biogenesis of the mitochondrial compartment is a tightly controlled process in which it was shown that the Ras/cAMP pathway is involved (32, 33, 37). In the absence of the cAMP-dependent protein kinase Tpk3p, yeast cell growth is altered, with a decrease in the cellular amounts of mitochondria (27). Further, in the absence of this cAMP-dependent protein kinase, yeast mitochondria produce important amounts of ROS (28). This ROS production takes place in the intermembrane space because it is alleviated by the overexpression of SOD1 (copper-zinc superoxide dismutase, expressed both in the cytosol and in the mitochondria intermembrane space). Furthermore, in yeast, nuclear transcription of mitochondrial genes is under the control of the HAP2/3/4/5 transcription factor. The subunits HAP2/3/5 are involved in the gene transcription, whereas the subunit HAP4 is an essential coactivator. It is the only subunit whose expression level is regulated. This subunit is mandatory for the expression of mitochondrial genes, and it has been shown that an increase in mitochondrial ROS production induces a decrease in the cellular amount of this transcriptional coactivator. Consequently, the expression of genes encoding mitochondrial proteins is decreased. This is a very nice regulatory mechanism in which the transcription factors involved in mitochondrial biogenesis are able to sense a mitochondrial dysfunction and then decrease the biogenesis of these dysfunctional mitochondria (28).

Inhibition of preadipocyte proliferation by mitochondrial reactive oxygen species

In white adipose tissue, it was shown that moderate changes in mitochondrial ROS generated by using specific mitochondrial inhibitors such as rotenone or oligomycin decreased cell proliferation. The treatment of 3T3-L1 cells with rotenone reduced cell number as well as [3H]thymidine incorporation. This change in cell growth is due to a decrease in cell proliferation, because no apoptotic or necrotic events were detected. It was shown that both rotenone and oligomycin induced a slight increase in mitochondrial ROS production, which is partly reversed by radical scavengers (BHA and trolox). The inhibition of cell growth parallels the increase in mitochondrial ROS production. Radical scavengers only brought about partial reversion of the effects of rotenone and oligomycin on cell growth, which is consistent with the less than total reversion of ROS generation by these molecules. This strongly suggests that mitochondrial ROS are responsible for inhibition of 3T3-L1 preadipocyte growth. Further, mild uncoupling, by decreasing ROS generation, slightly increased preadipocyte proliferation (21). This shows that mitochondrial ROS are involved in the regulation of preadipocytes proliferation.

Mitochondrial reactive oxygen species control the transcription factor CHOP-10/GADD153 and adipocyte differentiation: a mechanism for hypoxia-dependent effect

Manipulations of mitochondrial ROS generation with low doses of mitochondrial inhibitors demonstrate a very strong and negative correlation between changes in mitochondrial ROS and adipocyte differentiation of 3T3-F442A preadipocytes (22). Moreover, mitochondrial ROS positively and specifically control the expression of the adipogenic repressor CHOP-10/GADD153. Hypoxia (1% O_2) strongly increased ROS generation, hypoxia-inducible factor-1, and CHOP-10/GADD153 expression, and inhibited adipocyte differentiation. All of these hypoxia-dependent effects were partly prevented by antioxidants. HIF-1α was shown not to be required for hypoxia-mediated CHOP-10/GADD153 induction in (HIF-1α)-deficient mouse embryonic fibroblasts. Moreover, the comparison of hypoxia and CoCl2 effects on adipocyte differentiation of wild-type or HIF-1α–deficient mouse embryonic fibroblasts suggests the existence of at least two pathways dependent or not on the presence of HIF-1α. Together, this demonstrates that mitochondrial ROS control CHOP-10/GADD153 expression, are antiadipogenic signaling molecules, and trigger hypoxia-dependent inhibition of adipocyte differentiation (22).

Conclusion

Once living organisms used oxygen as a final electron acceptor for energy-transfer processes, it was unavoidable that ROS were produced. The generation of these species is controlled by multiple parameters. Antioxidant enzymatic and nonenzymatic systems have developed to limit the toxicity of these molecules. Reactive oxygen species are a heterogeneous family with two kinds of molecules with very different status: (a) the radical forms of oxygen that are highly reactive, toxic, and have a very short half-life; and (b) nonradical forms such as hydrogen peroxide, which is a much more stable molecule. Consequently, hydrogen peroxide is the form involved in ROS signaling. A recent study showed that yeast dysfunctional mitochondria that produce important amounts of ROS signal to the nucleus (*i.e.*, a transcription factor coactivator) to downregulate the generation of these dysfunctional mitochondria. This mitochondria quality-control process plays an important role in cell fate.

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Abbreviations Used

3T3-L1 = cell line derived from 3T3cells that is used in biologic research on adipose tissue
αKGDHC = α−ketoglutarate dehydrogenase complex
ANT = adenine nucleotide transporter
BHA = butylated hydroxyanisole
cAMP = cyclic adenosine monophosphate
CoQ = coenzyme Q
Dld = dihydrolipoyl dehydrogenase
EGF = epidermal growth factor
ETF = electron transfer flavoprotein
FET = forward electron transport
GADD153/CHOP-10 = C/EBP homologous protein
GPDH = glycerol-3-phosphate dehydrogenase
Gpx3 = glutathione peroxidase 3
HIF-1α = hypoxia-inducible factor 1-alpha
NAD^+ = nicotinamide adenine dinucleotide, oxidized form
NADH = nicotinamide adenine dinucleotide, reduced form
Nde1p = external NADH dehydrogenase 1
Nde2p = external NADH dehydrogenase 2
Ndip = internal NADH dehydrogenase
OxyR = hydrogen peroxide–inducible genes activator
PDGF = platelet-derived growth factor
PDH C = pyruvate dehydrogenase complex
Prx = peroxiredoxin
Prx I = peroxiredoxin I
Prx IV = peroxiredoxin IV
Q = quinone
Q_0 = ubiquinone binding site toward the outer side of the inner membrane
Q_i = ubiquinone binding site toward the inner side of the inner membrane
RET = reverse electron transport
ROS = reactive oxygen species
SDH C = succinodehydrogenase complex
SOD1 = copper-zinc superoxide dismutase
Tpk3 = Takashi protein kinase gene 3 (cAMP-dependent protein kinase catalytic subunit 3)
UCP = uncoupling protein
UQ = ubiquinone
$UQ^{\cdot -}$ = semiubiquinone
UQH2 = reduced ubiquinone (ubiquinol)
Yap1 = yeast activator protein 1

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