Mitochondria and Reactive Oxygen Species

Francesco Addabbo, Monica Montagnani, Michael S. Goligorsky

Fascination by the mitochondria, “the colonial posterity of migrant prokaryocytes, probably primitive bacteria that swam into ancestral precursors of our eukaryotic cells and stayed there,”1 stems from the above-mentioned nebulous endosymbiotic theory of their origin, as well as from the growing realization of a very special role that they play in the pathogenesis of diverse diseases.

These organelles generate energy primarily in the form of the electrochemical proton gradient (ΔμH⁺), which fuels ATP production, ion transport, and metabolism.2 Generation of this universal energy currency, ΔμH⁺, occurs through the series of oxidative reactions conducted by the respiratory chain complexes at the ion-impermeable, almost cholesterol-free inner membrane. Reduced nicotinamide adenine dinucleotide reduces the entry point to the complex I (reduced nicotinamide adenine dinucleotide:ubiquinone reductase), whereas the reduced ubiquinol enters the respiratory chain in the complex III (ubiquinol:cytochrome c [cyt-c] reductase) to reduce cyt-c, the electron carrier to the complex IV, cyt-c oxidase. Each of these steps generates ΔμH⁺ by electrogenic pumping of protons from the mitochondrial matrix to the intermembrane space and is coupled to electron flow, thus generating the electric membrane potential of ~180 mV and a pH gradient of 0.4 to 0.6 U across the inner mitochondrial membrane resulting in the negatively charged matrix side of the membrane and alkaline matrix. Ultimately, accumulated ΔμH⁺ is converted into the influx of protons into the matrix driving ATP synthesis or protein transport. In addition, these end points are necessary for the execution of 2 major enzymatic metabolic pathways within the mitochondrial matrix: the tricarboxylic acid (TCA) oxidation cycle and the fatty acid β-oxidation pathway. This intricate system fueling cellular functions is as elegant as it is vulnerable: practically every component of the system, from the electron transport chain complexes to the permeability properties of the membranes, is a target for various noxious stimuli, some of which can be generated within mitochondria themselves. The list of these noxious stimuli is too long to be recounted here, and the interested reader may refer to a recent excellent review.3 These ancestral oxygen-using proteobacterial invaders carried with them into eukaryotic cells not only evolutionary benefits but also potential side reactions, most dangerous of which are “exothermic oxygen combustion and free radical emission.” This review is focused on one component of the noxious mitochondrial pathway: reactive oxygen species (ROS) from a mitochondrial perspective, which has previously been extensively reviewed.4 Therefore, we shall present the most recent findings but periodically offer historical perspective.

Mitochondrial ROS and Actions

Mitochondrial ROS Generation

Mitochondrial respiration is the major source of ROS, with 0.2% of oxygen consumed being normally converted into superoxide in a quiescent state.5 Unless adequately detoxified, superoxide causes mitochondrial oxidative stress and may contribute to the decline in mitochondrial functions; this general scenario is associated with a wide variety of pathologies. The transfer of electrons to oxygen, generating superoxide, is more likely when these redox carriers are abundantly charged with electrons and the potential energy for transfer is high, as reflected by a high mitochondrial membrane potential. Conversely, ROS generation is decreased when available electrons are few and potential energy for the transfer is low. Mitochondrial enzymes known to generate ROS through the leak of electrons to molecular oxygen include the electron-transport chain (ETC) complexes I, II, and III6–8; the TCA cycle enzymes aconitate 2 and α-ketoglutarate dehydrogenase9; pyruvate dehydrogenase and glycerol-3-phosphate dehydrogenases A and B12,13; and cytochrome c reductase.4

One of the major drawbacks of the previous work in this field was considered to be the lack of in vivo data on ROS production. Therefore, perhaps the most notable breakthrough in the field is related to the recent demonstration of the kinetics of superoxide production in mitochondria of living cells or organs.14 In cultured excitable and nonexcitable cells transfected with a circularly permuted yellow fluorescence protein, a novel biosensor of superoxide that is unaffected by hydrogen peroxide, peroxynitrite, hydroxyl radical, or NO, investigators detected fluorescence flashes arising from individual or a group of functionally coupled mitochondria. Flashes of superoxide production peaked in 3.5 seconds and dissipated with a half-time of 8.6 seconds, occurred randomly, and the frequency and amplitude were reduced by...
a superoxide dismutase (SOD) mimic or by a superoxide scavenger tiron.14 These superoxide flashes were triggered by the transient openings of mitochondrial permeability transition pores (mPTPs) but were unrelated to Ca\(^{2+}\) sparks. Transient mPTP-initiated superoxide flashes required functional ETC and were ATP dependent. Superoxide flashes coincided with transient dissipation of mitochondrial membrane potential, matrix acidosis, and mitochondrial swelling. Anoxia or mild hypoxia decreased the frequency of superoxide flashes in cardiac myocytes, suggesting that elevated ROS levels found under these conditions originate extramitochondrially or are attributed to the defective antioxidant defense. However, on reoxygenation (the period of enhanced vulnerability of the cells), superoxide flash activity increased, and adenosine preconditioning treatment prevented it. This description of quantal, stochastic, and transient superoxide flashes driven by the flickers of mPTPs in quiescent cells should profoundly change the current interpretation of such events as being necessarily pathological, emphasizing the role of graded spatiotemporal ROS production in the normal processes of cell signaling via kinases, phosphatases, channels, and transporters, as well as maintenance of ROS scavenging and detoxifying systems. In addition, these discoveries feed into the deeper appreciation of the complexity of mitochondrial ROS production. Integrating these studies into the broad canvas of the existing knowledge will represent the next challenge. Clearly, the bidirectional effects of ROS generated in the cytosol on mitochondria and ROS generated in mitochondria on cytosolic systems will receive additional details.

### ROS Targets in Mitochondria (Lipids and Proteins)

ROS instability and inability to permeate lipid membranes usually provide an effective shield against propagating damage. However, through reactions with polyunsaturated fatty acids, they generate lipid hydroperoxides and \(\alpha,\beta\)-unsaturated aldehydes, eg, 4-hydroxy-2-nonenal, which are highly electrophilic, stable, readily propagating between cellular compartments, and capable of reacting with proteins and nucleic acids.29 This chain reaction of lipid peroxidation accounts for the role played by ROS in the pathogenesis of atherosclerosis, ischemia/reperfusion injury, and stress-induced premature senescence.

#### Aconitase-2

Aconitase-2 (ACO-2) is a mitochondrial enzyme that catalyzes the isomerization of citrate to isocitrate in the Krebs cycle. It is an Fe-S containing protein. The structural predication of the Fe-S center in ACO-2 to ROS and peroxynitrite20 may, in part, explain its selective vulnerability. ACO-2 has been found previously to be specifically targeted by oxidative damage during aging.31 Prolonged exposure of mitochondria to oxidants results in disassembly of the \([4Fe-4S]\)\(^{2-}\) cluster, carbonylation, and degradation of the enzyme,32 potentially establishing the link between oxidative stress and enzyme inactivation, as detected during cardiac ischemia/reperfusion injury,33 and an eventual reduction in ACO-2 abundance. In a recent study, we established a novel animal model of asymptomatic normotensive endothelial dysfunction by pharmacologically uncoupling eNOS and identified inhibition of the Krebs cycle and reduction in mitochondrial mass as its early manifestations.21

Specifically, a proteome-wide screen of microvasculature using 2D electrophoresis and mass spectroscopy revealed that, in animals treated with subpressor doses of N\(^{\text{n}}\)-methyl-L-arginine (L-NMMA), 2 mitochondrial enzymes, ACO-2 and enoyl coenzyme A (CoA) hydratase-1 (ECHS-1), were depleted in the microvasculature, affecting the Krebs
cycle and leading to the accumulation of lactate. Pharmacological uncoupling of eNOS resulting in enhanced generation of superoxide anion, rather than a complete inhibition of its activity, was found responsible for this phenotype. Considering the uncoupling of eNOS and enhanced mitochondrial generation of ROS (as judged by the MitoSox fluorescence in the case of L-NMMA treatment), it is conceivable that the combination of oxidative stress and reduced NO bioavailability culminates in a unique proteomic profile: reduced mitochondrial mass and selective depletion of ≥2 key enzymes of the Krebs cycle, ACO-2 and ECHS-1. The decrease in ACO-2 should inhibit the Krebs cycle. The excess pyruvate is converted to lactate by lactate dehydrogenase, as is the case in L-NMMA-treated mice. In addition, inhibition of the Krebs cycle is compounded by the depletion of ECHS-1, which catalyzes the second step of β-oxidation in fatty acid metabolism, the process by which fatty acids in the form of acyl-CoA are processed in mitochondria and/or peroxisomes to generate acetyl-CoA, the entry molecule for the Krebs cycle.34 Importantly, this indicates that the observed switch to glycolytic metabolism in the face of relative normoxia makes this metabolic profile similar to the Warburg effect, the phenomenon of preferential glycolysis in tumor cells vide infra. In this context, the role of NO appears to be complex. Acute actions of NO result in "metabolic hypoxia" by inhibition of mitochondrial electron transport. Chronic uncoupling of NOS, on the other hand, confers the same end result through selective depletion of ACO-2 and ECHS-1. We advocate, therefore, that inhibition of the Krebs cycle may herald the development of endothelial dysfunction.

Cardiolipin-Cyt-c and Apoptosis

An anionic phospholipid of the inner mitochondrial membrane (IMM) enriched at contact sites with the outer mitochondrial membrane, cardiolipin, represents another prime target of oxidative stress. Under normal conditions, cardiolipin "fixes" cyt-c to the IMM. Oxidation of the highly unsaturated acyl chains of cardiolipin reduces its binding affinity for cyt-c and liberates it from the IMM. This event, in conjunction with permeabilization of the outer mitochondrial membrane by proapoptotic members of the Bcl-2 family of proteins, results in the release of cyt-c into the cytosol, formation of apoptosisome, and activation of the caspase cascade culminating in apoptosis. Interestingly, contact sites between the outer mitochondrial membrane and IMM also contain hexokinases II and III, bound to the cytoplasmic face of the voltage-dependent anion channel. In this voltage-dependent anion channel–bound state, hexokinases prevent cyt-c release from mitochondria, thus conferring an antiapoptotic effect. This bound state of hexokinases also supports the coupling of intramitochondrial and extramitochondrial metabolism via efficient adenine nucleotide exchange; dissociation of hexokinases from mitochondria promotes uncoupling of mitochondrial metabolism from the cell requirements and facilitates apoptosis.

Consequences of Mitochondrial Oxidative Stress

Oxidative Stress-Mediated Autophagy

The role of mitochondria in cells commitment to caspase-dependent programmed cell death is well established, whereas insights into caspase-independent mechanisms of cell death emerged recently. Autophagy is one of the defense mechanisms protecting cells against oxidative stress. It is a regulated lysosomal pathway involved in the degradation and recycling of oxidized proteins and damaged organelles in the aging cells.39–41 There is accumulating evidence that autophagic processing of damaged or excessive organelles, eg, peroxisomes, endoplasmic reticulum, and mitochondria, can occur in response to ROS.42,43 Excessive ROS in quiescent mitochondria pose the risk to this organelle and to the viability of the cell through opening of mitochondrial membrane channels, including the mPTP and the inner membrane anion channel,44 leading to the collapse of mitochondrial membrane potential and further transient increase in ROS generation by the electron transfer chain.14 These events may result in autophagy, apoptosis, or necrosis.44 In a recent report, Chen et al45 have demonstrated that complex I inhibitor rotenone and complex II inhibitor thienoyl trifluoroacetone induce ROS-mediated autophagy, contributing to cell death in transformed and cancer cell lines. By contrast, in primary astrocytes, rotenone and thienoyl trifluoroacetone failed to induce autophagy. This suggests that the inhibition of mitochondrial ETC selectively induces autophagic cell death, mediated by ROS, in immortalized and cancer cells. These findings led the authors to indicate that the inhibition of the mitochondrial ETC can specifically induce autophagic cell death in cancer cells but not in other cell types, providing a new potential target to fight cancer cells.

Another recent finding has demonstrated that apoptosis and autophagy could be induced by photosensitization of mitochondria or endoplasmic reticulum.46 Sasnauskiené et al47 used low and intermediate energy photodynamic treatments to stimulate mitochondria ROS production in cultured human epidermoid carcinoma A-431 cells. The approach used was based on the visible light excitation of a mitochondria-localized lipophilic cationic photosensitizer, Safranin O, leading to generation of ROS and oxidative damage of cellular components. In this study, low energy photodynamic treatment of cells failed to show hallmarks of apoptosis, necrosis, or senescence, and the detected loss of viability and reduced proliferation of the cells were ascribed to the enhanced autophagy.47

Mitochondrial Biogenesis and “Stress-Response Hormesis”

The former refers to processes regulating mitochondrial mass, whereas the latter refers to the induction of stress-protective mechanisms.48 Interestingly, both processes are interconnected. There are emerging data that a nonlethal oxidative stress may stimulate mitochondrial biogenesis via activation of leucine zipper transcription factors, nuclear factor-E2–related factor and ATF4, which regulate the expression of antioxidant response element–containing genes, eg, glutathi-
one S-transferase, glutathione peroxidase, glutathione reductase (all involved in glutathione biosynthesis and cycling), and heme oxygenase 1, among others. This process is governed, at least in part, by the mitochondrial ROS stimulating nuclear factor-E2–related factor binding to the promoter region (rich in antioxidant response element motifs) of the nuclear respiratory factor 1 in the Akt-dependent derepression of nuclear factor-E2–related factor nuclear translocation. Activation of nuclear respiratory factor 1 is a prerequisite for transcriptional activation of mitochondrial transcription factor A and induction of mitochondrial DNA (mtDNA) replication/transcription and mitochondrial biogenesis. Another recently discovered mechanism stimulating mitochondrial biogenesis in response to the etoposide-induced ataxia telangiectasia mutated–dependent pathway is attributed to AMP-activated protein kinase activation.

Mitochondrial Oxidative Stress in Pathological Conditions

Warburg Effect Is an Early Manifestation of Dysfunctional Endothelial Cells

Since Warburg’s discovery of enhanced glucose uptake by and preferential glycolytic metabolism in tumor cells even during normoxia (aerobic glycolysis), mechanistic explanations of this phenomenon have been offered. These include mutations promoting glycolysis in crucial genes, eg, Ras, Akt kinase, HIF-1, Myc transcription factor, and loss of the tumor suppressor protein p53, to name a few. Therapeutic implications of these investigations using inhibitors of glycolytic enzymes have been incorporated into the antineoplastic arsenal. Thus, the Warburg effect was perceived as a tumor-specific type of metabolic disturbances.

Our in vivo and in vitro findings in vascular endothelial cells subjected to elevated levels of L-NMMA or asymmetrical dimethylarginine showed a similar glycolytic switch. Using the same model of endothelial dysfunction, we have shown that nonpressor doses of NOS inhibitors result in the upregulation of collagen XVIII and its C-terminal fragment, endostatin; microvascular rarefaction readily detectable in the renal medulla; and ultrastructural changes in microvascular endothelia but no appreciable degree of hypoxia, as judged by the lack of pimonidazole retention. Yet, microvasculature obtained from these mice showed markedly depressed expression of the key enzyme of the Krebs cycle, ACO-2; reduced oxidative phosphorylation; and enhanced glycolysis, as judged by the accumulation of lactate in the extracellular fluid and by the inability of cells to survive in the glucose-to-galactose substituted medium (Figure). This is further supported by the fact that α-ketoglutarate, a downstream substrate bypassing mitochondrial enzymatic block at the level of isocitrate formation, partially restored cell viability in the glucose-free medium. Intriguingly, this normoxic glycolysis in dysfunctional endothelial cells was initiated under the circumstances of the eNOS uncoupling and asymmetrical dimethylarginine/L-NMMA-induced mitochondrial oxidative stress, thus expanding the Warburg effect considered previously to be a hallmark of tumors.

Figure. Schematic illustration of the Warburg effect in tumor cells and in early asymptomatic endothelial cell dysfunction. Under normoxic conditions, intact cells metabolize glucose through the TCA cycle. In contrast, tumor cells avidly uptake glucose and exhibit blockade of oxidative phosphorylation at the entry point of conversion of lactate to pyruvate because of the inhibition of pyruvate dehydrogenase kinase-1 (PDK-1). In addition, mutations of p53 result in the reduced signaling through TPS3-induced glycolysis and apoptosis regulator (TIGAR), which normally inhibits the glycolytic pathway. As a result, lactate is produced in excessive amounts, all hallmarks of the Warburg effect. Interestingly, pharmacological inhibitors of lactate dehydrogenase (LDH) may reverse glucose metabolism to oxidative phosphorylation. At the early stages of endothelial cell dysfunction, similar to tumor cells, oxidative phosphorylation is suppressed and glycolysis enhanced, resulting in overproduction of lactate. Under these conditions, blockade of the TCA cycle occurs at the level of the catalyzed by ECHS-1 conversion of acyl-CoA to acetyl-CoA, the point of entry to the cycle, and at the level of the conversion of citrate to isocitrate (because of the depletion of aconitate 2). This block in the TCA cycle can be resolved by bypassing the deficient aconitate 2 with the downstream substrate, α-ketoglutarate (Alpha-KG). These new data on the early metabolic stigmata of dysfunctional endothelia vis-à-vis tumor cells emphasize their similarities and distinctions.

It is interesting that metformin, a widely used antidiabetic drug, inhibits complex I of the respiratory chain and induces glycolysis and lactate production in a p53- and AMP-activated protein kinase-dependent manner. It remains to be elucidated whether this effect is accompanied by signs of endothelial dysfunction.

Oxidative Stress Implications in Diabetes Mellitus and Cardiovascular Complications

The role played by oxidative stress in the development of insulin resistance and metabolic syndrome has been well documented: excessive production of oxidants, decreased NO bioavailability, and decreased local antioxidant capacity in the vasculature. It is important to recognize that excess superoxide itself can directly inhibit critical endothelial enzymes. One such targets is eNOS, an important antiatherogenic enzyme with great relevance to diabetic macrovascular disease: its activity and coupling are dramatically inhibited in animal models of diabetes mellitus and in diabetic patients. Conventional antioxidants are unable to scavenge the hyperglycemia-induced superoxide anion, the rationale being that antioxidants neutralize reactive oxygen molecules on a 1-to-1 basis, thus becoming exhausted by the continuous production of the superoxide. Salvemini et al have shown that a new type of antioxidant, a catalytic antioxidant, eg, a SOD/catalase mimetic, may be more effective because it works...
continuously like the enzymes. Hyperglycemia-induced reactive oxygen overproduction directly reduces eNOS activity in diabetic aortas by 65%. However, when these diabetic animals are treated with a SOD/catalase mimetic, there is no reduction in activity of this antiatherogenic enzyme. These data strongly suggest that therapeutic correction of diabetes mellitus-induced superoxide production may be a powerful new approach for preventing diabetic complications.

Giardino et al. have demonstrated that, in cultured bovine aortic endothelial cells, hyperglycemia increases the production of ROS, and a few years later, in a very elegant fashion, the same group has demonstrated that Krebs cycle is the source of increased ROS generation induced by hyperglycemia. Pharmacological inhibition of electron transport chain complex II with thienoyl trifluoroacetone or abolishing the mitochondrial membrane proton gradient by carbonyl cyanide m-chlorophenylhydrazone, an uncoupler of oxidative phosphorylation, completely prevented hyperglycemia-induced ROS; these results were buttressed using molecular biology tools. One of the most notable findings is that normalizing levels of mitochondrial ROS with each of these agents prevented glucose-induced activation of protein kinase C, formation of advanced glycation end products, sorbitol accumulation, and nuclear factor-κB activation, indicating a single unifying mechanism of induction and a causative role for mitochondrial superoxide in the above-mentioned hyperglycemia-induced metabolic abnormalities. These data provided the basis for the development of new drugs to prevent cardiovascular complications in diabetic subjects.

Another prominent mechanism for the development and perpetuation of mitochondrial dysfunction is related to the induction of an IMM protein, uncoupling protein 2 (UCP2). Oxidative stress results in the accumulation of UCP2 in mitochondria leading to the inward proton leak, which competes with the function of ATP synthase and results in a reduction of ATP synthesis from ADP. This situation can be modeled in vitro by application of rotenone, antimycin A, or diethyldithiocarbamate, all increasing mitochondrial superoxide production, whereas oxidants producing outside mitochondria did not affect UCP2 abundance. This process did not require a parallel increase in ucp2 gene expression or mRNA abundance; rather, it is attributed to enhanced translational efficiency and stabilization of UCP2. Overexpression of UCP2 in cultured pancreatic β-cells reduces glucose-induced insulin secretion, whereas inactivation of the ucp2 gene in vivo enhances insulin secretion. This latter mechanism is critically involved in the development of insulin resistance and type 2 diabetes mellitus.

Aging
Mitochondrial ROS have been proposed to contribute to aging through the accumulation of mtDNA mutations. Human mtDNA is a circular molecule of 16 569 bp that encodes 13 polypeptide components of the respiratory chain, as well as 2 ribosomal RNAs and 22 tRNAs necessary to support intramitochondrial protein synthesis essential for electron transport and ATP generation by oxidative phosphorylation of ADP. mtDNA represents a critical cellular target for oxidative damage that could lead to lethal cell injury through the loss of electron transport, mitochondrial membrane potential, and ATP generation. Furthermore, because of the lack of protective histones and its close proximity to the electron transport chain, mtDNA is especially susceptible to be attacked by ROS (the level of oxidatively modified bases in mtDNA is several-fold higher than that in nDNA). Inherited mutations in mtDNA are known to cause a variety of diseases, most of which affect tissues with high energy requirements, eg, the brain and muscles. Somatic mtDNA mutations acquired during a life span contribute to the physiological decline that occurs with aging. Among the most studied mtDNA mutations accumulating with aging, there are large-scale deletions and point mutations accumulating in specific “hot spots” in the mtDNA control region. The accumulation of these deletions and point mutations with aging correlates with a progressive decline in mitochondrial function. Recently, several groups have addressed the issue of causation generating mtDNA mutations experimentally. In mtDNA-mutator mice, expression of defective mtDNA polymerase resulted in decreased respiratory enzyme activity, ATP production, and life span. Another independent mtDNA-mutator mouse showed a similar marked increase in mtDNA mutations, progeric features, and early mortality. Thus, the effects of mtDNA mutations in these mice do not seem to be mediated through ROS production; rather, these models may shed light on the events subsequent to increased generation of mitochondrial ROS and their effect on mtDNA. It has been demonstrated that enhancing mitochondrial antioxidant defenses can increase longevity. In Drosophila, overexpression of the mitochondrial antioxidant enzymes manganese SOD and methionine sulfoxide reductase prolong life span. This strategy is most successful in short-lived strains of Drosophila but does not guarantee effect in already long-lived strains. However, Schriner et al. generated transgenic overexpressing catalase experimentally targeted to peroxisomes, nuclei, or mitochondria. The mitochondrially targeted construct provided the maximal benefit, increasing median and maximal life span by 20% in an already long-lived murine strain. Catalase overexpression was also associated with a reduction of hydrogen peroxide production and oxidative inactivation of ACO-2 in isolated cardiac mitochondria; DNA oxidation and levels of mitochondrial deletions were reduced in skeletal muscle; and cardiac pathology, arteriosclerosis, and cataract development were delayed.

Hypertension and Mitochondrial ROS
Among many sources of increased vascular ROS production in hypertension, eg, NADPH oxidase, lipoxygenases, cyclooxygenases, xanthine oxidoreductase, cytochrome P450 enzymes, and eNOS, mitochondrial ROS overproduction plays an important role. The subject of mitochondrial ROS and hypertension has been reviewed previously. In spontaneously hypertensive rats, mitochondrial ROS production in the rostral ventrolateral medulla is increased, and administration of coenzyme Q10 restores ETC and attenuates hypertension. A relay mechanism propagating ROS generation from the cytoplasm to the mitochondria has been described in angiotensin II–induced hypertension: increased mitochondrial hydrogen peroxide production could be attenuated by an inhib-
itor of NADPH oxidase or by depleting the p22(phox) subunit of NADPH oxidase, among others. The role played by angiotensin II in developing mitochondrialopathy has been advanced recently by Benigni et al. Deletion of the Agtr-1a gene resulted in the reduced age-related cardio-renal complications, improved mitochondrial biogenesis, and increased longevity in mice.

**Therapeutic Implications**

Pharmacological tools available to regulate mitochondrial respiratory chain complexes and mPTP, both targets of anticancer therapy, are comprehensively reviewed elsewhere and are beyond the scope of this essay. Suffice it to mention that dietary supplement with several metal ion cofactors like selenium, vanadium, chromium, zinc, copper, and manganese, together with vitamins C, A, and E is a necessary prerequisite for successful maintenance of antioxidants. Furthermore, coenzyme Q10 has been shown to reduce mitochondrial oxidative stress while bearing in mind the “antioxidant paradox,” a relatively poor performance of antioxidants in scavenging ROS in vivo. One of the strategies to target antioxidants to mitochondria is based on the selective accumulation of positively charged lipophilic substances, like triphenylphosphonium. By conjugating this carrier to α-tocopherol (MitoVit E) or to ubiquinone (MitoQ), these antioxidants can be effectively delivered into mitochondria.

Another group of mitochondrial antioxidants has been used in studies by Liu et al. These include mitochondrial metabolites acetyl-L-carnitine and lipoid acid and antioxidants α-phenyl-N-t-butyl nitrate (a widely used spin-trapping agent) and N-t-butyl hydroxylamine, which have been shown to improve mitochondrial ultrastructure and function in aged rodents and to delay cell senescence in vitro.

Modest induction (but not a prolonged one) of mitochondrial biogenesis has been shown to promote mitochondrial biogenesis in the heart and can be readily achieved using peroxisome proliferator-activated receptor-γ coactivators. Another compound acting on mitochondrial biogenesis and exhibiting antioxidant properties is the plant-derived polyphenol resveratrol (reviewed in Reference 95). It improves mitochondrial functions in the metabolic syndrome by acting on AMP-activated protein kinase and activating sirtuin 1 and peroxisome proliferator-activated receptor-γ 1a.

Recent studies have demonstrated that nitrite anion results in the reduction of ROS produced at the mitochondrial site by inhibiting complex I activity of ETC under conditions of postischemic oxidative stress (but not under normoxic conditions) and by reduction of activated mPTPs. Nitrite anion acts, most probably, via conversion to NO either through direct reduction or enzymatically by xanthine oxidoreductase, deoxyhemoglobin, or deoxymyoglobin. This approach may represent a valuable pharmacological tool to prevent acute postischemic mitochondrial oxidative stress but may potentially have deleterious effects on chronic administration. There is clearly a long and tortuous path ahead of us in creating highly efficient targeted antioxidants.

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None.

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