The Emerging Theme of Redox Bioenergetics in Health and Disease

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Mitochondrial function has long been recognized as central to normal physiology and a contributor to a broad range of pathologies. Much of the early research in mitochondrial biology focused on the mechanisms to generate ATP and characterization of mitochondria from highly energetic tissues such as the heart or liver. More recent studies emphasize the role of mitochondria in redox signaling and in less energetic cells such as those in the innate immune system and the vasculature. In this short overview, we discuss some of these recent developments in translational and basic research in mitochondrial pathophysiology. Advanced high throughput analytical techniques are now allowing the assessment of bioenergetic health in human populations and the emergence of the exciting new field of metabolotherapeutics. These have led to the emergence of the new field of redox bioenergetics which encompasses both the canonical aspects of mitochondrial energy production and the organelles' role in cell signaling and disease.
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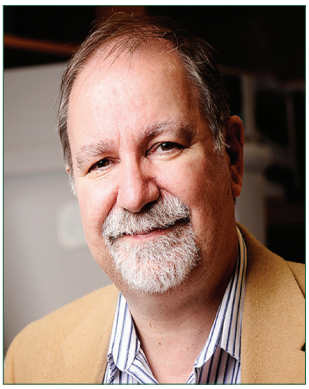
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Mitochondrial biology continues to play a central role in our understanding of the basic biological processes and the pathology of disease. Recent technological advances in cellular respirometry and microscopy have revealed for the first time the dynamic impact of mitochondrial networks, the complexity of mitochondrial metabolism in cells not traditionally viewed as highly energetic, and a completely new pathway for the turnover and removal of "rogue" mitochondria, known as mitophagy.[1-4] Since the discovery of diseases directly attributable to mutations in mitochondrial DNA (mtDNA), the impact of bioenergetic dysfunction has now extended to a broad range of pathologies including diabetes, neurodegeneration, and cardiovascular diseases.[5-11] This, in turn, has ushered onto the stage a broad range of mitochondrial therapeutics which function through different mechanisms, in addition to the recognition that established therapeutics, such as metformin, modulate bioenergetics.[12-17] The role of mitochondria has also developed well beyond simply providing ATP to the cell to encompass a complex retrograde signaling pathway to the nucleus.[18-20] The mechanisms through which this occurs are still not clear, but have been shown in several cases to involve the controlled generation of superoxide and hydrogen peroxide from the respiratory chain.[20-22] Interestingly, among the 13 proteins coded for by the mtDNA are the critical redox centers in the respiratory chain, which offer a mechanism through which mutations in mtDNA could modulate superoxide levels in response to stress and, thus, impact on pathological processes.[23] Taken together, these findings result in the new field of redox bioenergetics.

Our perspective of mitochondrial function is also rapidly changing in response to new findings of cellular bioenergetics in the cells of the innate immune system.[24,25] The early association of cancer cells with an altered bioenergetics metabolism characterized by aerobic glycolysis has now been extended to encompass lymphocytes and monocytes as they adapt to their changing biological functions in normal physiology.[24,26,27] Early studies of mitochondrial function were largely based upon the disruption of tissues such as the heart or liver abundant in the organelle and characterization of the organelles isolated from their cellular milieu. The discovery of a sophisticated molecular postal system which directs proteins to different compartments within the mitochondrion resulted in the widespread notion that the mitochondrial proteome

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could be discrete and defined for a given cell type and context. However, it is now becoming clear from studies in living cells that a more dynamic interaction of cytosolic proteins with the mitochondrion is occurring over minutes to seconds and is responsive to targeted signaling pathways. For example, functional associations of mitochondria with nitric oxide synthases, NADPH oxidase 4, and cytochrome P450 have recently been reported.[28-30] The functional significance of these interactions remains unclear in most cases. In this short overview, we will highlight this theme of redox bioenergetics and the articles supporting these developments from broader literature.

New aspects of mitochondrial function

The classical approach to measuring mitochondrial function has been to prepare the isolated organelle by disrupting its attachment to the cellular milieu and measuring its activity after purification. It is now technically possible to determine mitochondrial function in a cellular context and then, following permeabilization of the plasma membrane, measure oxidative phosphorylation in the mitochondria from the same cell.[31,32] Interestingly, it is increasingly becoming obvious that the activities of oxidative phosphorylation from isolated mitochondria represent only a small fraction of the metabolic activities they exhibit in the intact cell.[2] In addition, it is now becoming possible to measure cells in atypical circumstances including under low oxygen tension, in spheroids, and to model the air–liquid interface typical of lung epithelial cells.[33-37]

The mitochondria are now emerging as a major contributor to a broad range of metabolic diseases and this offers new therapeutic targets both aimed at metabolism and modulating mitochondrial quality control.[2,5,7] Mitochondria are also a target for inflammatory mediators including low levels of HOCl which can inhibit platelet function.[38] Conversely, mitochondrial toxicity underlies many of the dose-limiting problems with therapeutics and mediates the toxicity of numerous xenobiotics, as will be discussed below.[39] Several investigators are developing models for screening for mitochondrial toxicities and one approach has been to substitute galactose for glucose in the media, which forces the cells to rely on mitochondrial function for energetics, thus revealing the bioenergetic defects more readily.[40] These new approaches will bring new insights into how mitochondria behave at the extremes of eukaryotic cellular environments.

Mechanisms of mitochondrial reactive oxygen species and therapeutic targeting

The mitochondria are both a source and target of reactive oxygen species/reactive nitrogen species (ROS/RNS), and the interplay between the regulatory processes in which redox signaling molecules modulate the activity of cytoplasmic signaling cascades (so-called retrograde signaling) is emerging as a major area of interest.[19,41-44] The earliest studies relied heavily on mitochondrial inhibitors to identify the potential sites for mitochondrial ROS formation,[45] but, not surprisingly, it has become clear that this is much more complex. In an elegant comprehensive investigation of the sites of ROS formation in skeletal muscle mitochondria, six independent sites were identified which are differentiated by their relative activity and substrate dependency.[46] How the signals from mitochondrial ROS are transduced to modify cell signaling pathways remains an active area of research. Interestingly, it is now becoming clear that the mitochondrial genetic background is capable of conferring resistance or suceptability to cardiovascular disease by modulating redox signaling.[23] One concept that is gaining acceptance is that it is the modulation of the mitochondrial protein thiol networks which are an essential intermediate in retrograde signaling pathways.[41] Interestingly, except in some specialized cases, protein thiols are not very reactive with hydrogen peroxide. This has led to the suggestion that localized and controlled formation of hydrogen peroxide in the respiratory chain can lead to lipid peroxidation. The resulting reactive lipid species can act as second messengers for cell signaling by modifying protein thiols.[22,47] This opens up the possibility, which is gaining experimental evidence, that electrophile therapeutics and natural products, such as curcumin, are capable of modulating the mitochondrial function through electrophilic signaling.[48,49] An interesting development in this area is the idea that the reaction product of nitric oxide (NO) and superoxide, peroxynitrite, can induce mitochondrial biogenesis through Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) and this, in turn, is modulated by the levels and activity of mitochondrial superoxide dismutase (SOD).[50] Since mitochondria are a potentially controlled site of superoxide production and a target for NO-dependent modulation of respiration, this provides a novel and interesting mechanism for maintenance of mitochondrial quality by stimulating the synthesis of new organelles under conditions of oxidative stress.[51,52] The post-translational modification of proteins mediated by NO remains an area of intense interest, particularly in the mitochondrion.[53] The mitochondrion is potentially particularly susceptible since it contains high concentrations of reactive protein thiols and has long been thought to be a major site for *S*-nitrosation.[41,54-56] Interestingly, under conditions of oxidative stress, degradation of mitochondrial *S*-nitrosothiols appears to be inhibited and this enhances toxicity in the endothelial cells.[57]

From a therapeutic perspective, the need to control mitochondrial ROS and the associated signaling has resulted

in several different strategies to modulate mitochondrial ROS generation both with small molecules and using molecular biology approaches.[12,13,15,58-60] The matrix of the mitochondrion is highly negatively charged and this has been exploited in a series of compounds using a delocalized cationic charge to transport functional pharmacophores into the mitochondrion. The best understood of these compounds is mitochondrially targeted ubiquinone or MitoQ, which is well tolerated in human subjects.[61] It seems likely that the most appropriate therapeutic application of these compounds relates to the signaling pathways modulated by MitoQ which are deranged under conditions of metabolic stress including diabetes, metabolic syndrome, and alcohol-dependent hepatotoxicity.[62-64] The best understood of these targeting molecules is the triphenylphosphonium (TPP+) group which can be coupled to a broad range of pharmacophores and redox-sensitive probes.[16,65,66] These molecules have been used both *in vivo* and in cell culture to probe the mechanisms of redox signaling, and the effects they elicit are generally ascribed to the properties of the functional group and not the carrying molecule. However, it was recently shown that the TPP + group itself can modulate mitochondrial bioenergetics and the effects vary with the linker group used to attach the functional pharmacophore.[67] The concentrations needed to have these effects cannot usually be achieved *in vivo*, but in cell culture, where treatment conditions are not usually constrained by pharmacokinetics, the effects of the carrier group need to be taken into account. Some of the effects ascribed to mitochondrially targeted antioxidants may, in fact, be due to off-target effects such as uncoupling, rather than ROS scavenging.

The adenoviral expression of therapeutic proteins has long been recognized as an important approach to redox therapeutics. Overexpression of the cytosolic isoform of SOD in neurons results in its localization to the mitochondrion, where it is capable of modulating mitochondrial superoxide generation in response to angiotensin II.[68] Importantly, the functional impact of modulating mitochondrial superoxide/hydrogen peroxide is to prevent the angiotensin II–induced neuronal potassium current. Angiotensin II is well known to induce superoxide formation through activation of NADPH oxidase, but it is also becoming clear that mitochondrial superoxide is playing a role in its induction of senescence in vascular smooth muscle cells.[69]

Although the mitochondrion has a complex integrated redox modulatory system, there is a selective pressure against the incorporation the antioxidant enzyme, heme oxygenase into the organelle. In support of this idea, mitochondria-targeted heme oxygenase-1 has recently been reported to suppress the levels of mitochondrial heme proteins and cause oxidative stress and mitochondrial dysfunction in macrophages and other cell types.[70,71]

Mitochondrial quality control and novel modulators of mitochondrial function

The mitochondrial population is in a dynamic equilibrium with a balance maintained between biogenesis and mitophagy.[1,2,72-74] Interestingly, it now appears that polyphenolics may be capable of activating autophagy and may exert their protective effects through this mechanism.[74,75] One of the most important pathways in the cell promoting oxidative stress is the activation of the cytochrome P450 enzymes which are capable of metabolizing a broad range of xenobiotics to form ROS. It is now becoming clear that in addition to the suppression of toxic xenobiotic reactive species by intracellular antioxidants, damaged proteins and organelles must also be removed by the autophagic/lysosomal system.[76,77] One of the commonest causes of liver failure in human subjects is overdose with acetaminophen. Investigation of the mechanisms of toxicity of this compound have revealed that mitochondria are a primary target, and have also revealed a novel mediator of bioenergetic dysfunction, the mitochondrial spheroids.[78] The critical importance of autophagy in bioenergetic dysfunction was further demonstrated in an interesting study in which inhibition of autophagy during chronic alcohol exposure and by several other xenobiotics increased hepatotoxicity, but this was prevented in a cytochrome P450 knockout model.[79] An important alternative mechanism for the removal of oxidized proteins in the mitochondria is their proteolytic degradation mediated by the Lon protease. Under conditions of acute stress, Lon is rapidly induced; but under conditions of chronic stress, aging, and senescence, the levels decline leaving the cell particularly vulnerable to bioenergetic dysfunction.[80]

The oxygen binding site in the mitochondrion at cytochrome *c* oxidase is well known to be the site of interaction with NO.[53] Since the synthesis of NO by nitric oxide synthases also requires oxygen, this establishes an interesting and potentially important biological interaction between oxygen and NO gradients in organs and tissues.[51,53] Interestingly, under conditions of very low oxygen tension or hypoxia, nitrite, a metabolite of NO, can be converted by heme proteins such as myoglobin back to NO, and this can then modulate mitochondrial function.[81]

Hydrogen sulfide (H_2S) is the latest member of the gaseous molecules found to be capable of being generated within cells through metabolic processes. Interestingly, H_2S has the potential to modulate respiration both at cytochrome *c* oxidase and through modulation of the thiol redox state in the mitochondrion.[82] This is clearly an area which is likely to develop rapidly given the emerging

importance of thiol networks in regulating mitochondrial function.[56]

Post-translational modifications that affect the mitochondrial function include phosphorylation and protein acetylation.[83,84] Acetylation of lysines within complex I, II, and V of the electron transport chain and enzymes that control fatty acid oxidation, glycolysis, and amino acid metabolism was shown to reduce the enzyme activity and decrease ATP production. Most often recognized for their role in increasing longevity upon caloric restriction, sirtuins have additionally been shown to control the stress response and, most recently, to mediate the metabolism through direct deacetylation of metabolic enzymes. Mitochondrial Sirt3, specifically, has been shown to sense and control the metabolism through its enzymatic action which is NAD + dependent, and to increase the activity of antioxidant enzymes such as manganese superoxide dismutase (MnSOD).[84] Dysregulation of Sirt3 has pleiotropic effects and is thought to be associated with metabolic syndrome, and other metabolic pathologies including aging, high fat diet, oxidative stress, and ethanol consumption.[84]

Translational bioenergetics

Mitochondrial dysfunction is associated with a broad range of metabolic pathologies including diabetes, neurodegeneration, and cardiovascular diseases.[85] Although the clinical focus with many of these diseases is based upon specific abnormalities in the organ function which impact the patient's health, the effects are systemic and reflected in many tissues including circulating platelets and leukocytes.[86,87] Recognition of this concept has led to the hypothesis that these cells can act as sensors or biomarkers of these pathologies. Testing this hypothesis over the last 20–30 years, most frequently with platelets, has resulted in a robust literature showing that bioenergetics defects can be detected in these cells from patients with diabetes or neurodegenerative disease.[86] The recent development of high-throughput methods to isolate specific cell populations from small quantities of patient's blood and assess their bioenergetics is providing the impetus for the next phase of this field. It is now becoming possible to define a patient's "bioenergetics health" which may be of both prognostic and diagnostic value. Using a mitochondrial stress test, it is now possible to determine bioenergetic health index (BHI) which is a single value that can define the bioenergetic health in the cells isolated from a patient's blood.[88] Importantly, a comparison of the bioenergetics and glycolysis of leukocytes and platelets from the patient conclusively demonstrates that their metabolism is distinct and they can, therefore, act as differential sensors of metabolic defects in human subjects.[87,89]

Interestingly, a mitochondrial involvement in pathologies not directly related to the organelle is beginning to emerge. An interesting example is cystic fibrosis in which it appears that the defects in the cystic fibrosis transmembrane conductance regulator protein CFTR result in perturbations in cellular bioenergetics including suppressed complex I activity.[90,91]

Conclusion

The field of bioenergetics is now rapidly developing to encompass all aspects of redox biology, as summarized in Figure 1. This includes defining how the organelle regulates cell signaling under physiological conditions and how the cells of the innate immune system change their metabolism in response to their evolving role in inflammation. We are now also on the threshold of the emergence of the new field of translational bioenergetics and the applications of mitochondrial therapeutics.

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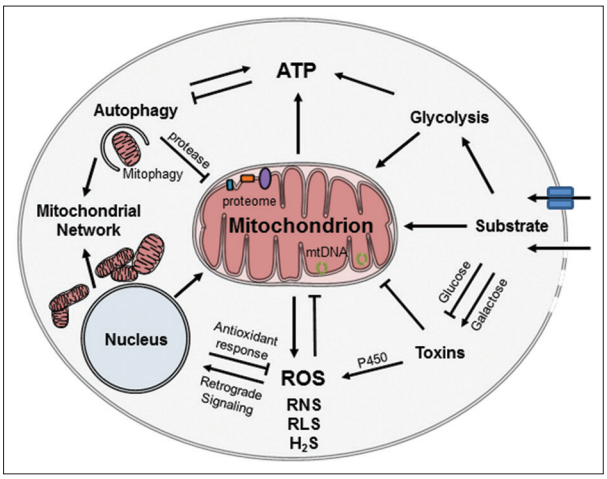

Figure 1: Bioenergetics and its interface with redox biology. Mitochondria are known to generate ATP and reactive oxygen species (ROS) as independently regulated products of metabolism. Critically, substrate availability, toxins, nuclear crosstalk, and mitochondrial biogenesis and degradation all play a role in mitochondrial efficiency and the redox environment. Mitochondrial components such as DNA (mtDNA) and the mitochondrial proteome can be affected by processes such as protease activity, ROS-mediated DNA damage, and mitochondrial dynamics (fission and fusion). Damage resulting to this highly energetic and redox-sensitive organelle can result in an increase in autophagic removal of the mitochondria (mitophagy) and disruption of the mitochondrial network. Other redox active molecules such as reactive nitrogen, lipid species, and hydrogen sulfide have been implicated in oxidative stress and mitochondrial damage. Monitoring mitochondrial function in translational studies can provide insight into the complex interaction between redox biology and cellular bioenergetics.

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