

EDITORIAL

Introduction to Special Issue on Mitochondrial Redox Signaling in Health and Disease

Mitochondria are functional entities that harbor the energy-conservation machinery that supports cell function through the coordination of mitochondrion-derived molecules involved in the regulation of cell signaling and transcription. Conversely, mitochondria are targets of an ever-increasing number of signaling pathways and their activity is also modulated by several transcription factors. The cell's energy-redox homeostasis is primarily a function of mitochondrial oxidative phosphorylation and the formation of O_2^-/H_2O_2 . Notably, other mitochondrion-driven processes contribute to cellular homeostasis, such as mitochondrial biogenesis and dynamics, mitochondrial quality control (autophagy and mitophagy), mitochondrial proteostasis and the role of the mitochondrial unfolded protein response (UPR^{mt}), and redox signaling in the homeostatic control of mitochondrial function. This Free Radical Biology & Medicine special issue on *Mitochondrial Redox Signaling in Health and Disease* covers some aspects of the myriad of processes embraced by mitochondrial biology and physiology, provides mechanistic insights linking mitochondrial function with cell function, and recognizes mitochondrial function as an amenable therapeutic target.

Energy metabolism: Mitochondria and the organelle network

Mitochondrial function cannot be viewed as that originating from isolated organelles, for extensive cross-regulation of organelle function occurs at organelle contact sites, *e.g.*, MAM (mitochondria-associated membranes) between mitochondria and ER [1]. Other organelle interactions involve the ER, Golgi apparatus, nucleus, plasma membrane and others, and are associated with the regulation of several cellular processes. These organelle contact sites acquire further significance when considering that they serve as a platform for cell signaling, as in the case of MAM alterations and processes associated with insulin resistance [2].

The most important function of mitochondria is oxidative phosphorylation with formation of ATP to maintain the cell's energy homeostasis: electrons flowing through the respiratory chain complexes linked with pumping of H^+ across the inner mitochondrial membrane and energy conservation as a protonmotive force drives the synthesis of ATP. The complexity of the oxidative phosphorylation system has revealed the occurrence of supercomplexes with different proportions of complexes I, III, and IV [3, 4], the formation of which is assisted by several assembly factors. The occurrence of two different coenzyme Q pools (one trapped within the supercomplex structure and another free in the inner mitochondrial membrane) serves to reconcile diverging experimental evidences under the plasticity model [5]. It may be surmised

that supercomplex organization is an adaptive mechanism of mitochondria to optimize their function in response to fuel availability and/or oxidant concentration [5]. Mitochondrial translation is essential for the oxidative phosphorylation system biogenesis and, consequently, for cellular energy supply. The synthesis of mtDNA-encoded polypeptide subunits of oxidative phosphorylation complexes is supported by a key quality control factor in mitochondrial translation, the mitochondrial translation factor 4 (mtEF4) [6], which –together with other translational activators– facilitates the crosstalk between mitochondrial translation and cytosolic translation, a process regulated by mTOR signaling [6].

In addition to supercomplex organization, metabolic reprogramming is increasingly recognized among the many enabling features that drive tumorigenesis and required to sustain proliferation and affecting the tumor microenvironment: metabolites from the tricarboxylic acid cycle due to mutations of fumarate hydratase, succinate dehydrogenase, and isocitrate dehydrogenase –termed oncometabolites – drive oncogenic signaling [7].

Plant mitochondria exhibit the highest respiration rates during seed germination, pollen development, and fruit ripening, *i.e.*, mitochondria are key players in plant development and this high energy requirement is met by an increased number of mitochondria [8]. Bypass of the electron-transfer complexes of the respiratory chain by the alternative oxidase does not contribute to ATP generation but plays an important role in stress tolerance in plants, and this activity helps dissipate excess energy thus preventing formation of H₂O₂ [8].

Mitochondrial H₂O₂: The link between energy metabolism and redox biology

The respiratory supercomplexes also have implications for mitochondrial O₂⁻ and H₂O₂ production, *e.g.*, oxidant production by complex I is lower when superassembled with complex III [5]. There are several sources of mitochondrial O₂⁻ and H₂O₂ within the NADH/NAD⁺ isopotential group (*e.g.*, α -ketoglutarate dehydrogenase, pyruvate dehydrogenase, and others) and the QH₂/Q isopotential group (*e.g.*, glycerol-P-dehydrogenase and acyl-dehydrogenase) in complexes I and III of the respiratory chain [9]. The highest rates of O₂⁻/H₂O₂ occur at the QH₂/Q isopotential group; a thorough analysis of the factors that lead to mitochondrial O₂⁻ production suggested reverse electron transport at the level of complex I when the NADH/NAD⁺ ratio is high [10]. This underscores the significance of assessing the cellular and mitochondrial pyridine nucleotide levels by genetically encoded NAD⁺/NADH sensors [11, 12] or fluorescence lifetime imaging microscopy (FLIM) [13]. The latter offers the potential to discriminate between the NAD and NADP pools and provides metabolic information into the changes of time-resolved (NAD(P)H fluorescence in pathophysiological situations [13].

Mitochondrion-derived H₂O₂ is the most likely signal leading to short-term responses (redox

signaling) and long-term responses (redox regulation of transcription) [10]. H₂O₂-mediated redox regulation of signaling and transcription entails thiol/disulfide exchange reactions [14, 15]. In this special issue, mitochondrion-derived H₂O₂: (a) establishes a link between energy metabolism and inflammatory responses [16], acting on redox-sensitive targets such as Nrf2, NFκB, JNK, IIS [17, 18], and stimulates mitochondrial biogenesis [19]; (b) is at the interface between bioenergetics, autophagy, and circadian control [20]; (c) is controlled during cholesterol oxidation in steroidogenic cells and brings together the coordinated activities of peroxiredoxin III –the major H₂O₂ reducing system within mitochondria– sulfiredoxin, which recovers hyperoxidized peroxiredoxin III [21]; (d) is involved in the activation of phospholipases and is an important physiological function associated with O₂ sensing in astrocytes and regulation of breathing [22]; (e) regulates mitogenic cellular signaling in proliferating cells through HIF activation and transcriptional activation of genes required for metabolic adaptations to proliferation and induction of angiogenesis [23]. It has been proposed that transducing an H₂O₂ signal can be accomplished by peroxiredoxin-2 and STAT3 [24].

Mitochondrial H₂O₂ production can be viewed as the integration of an energy component (acetyl-CoA as a metabolic hub, tricarboxylic acid cycle, respiratory chain) and a redox component (mitochondrial NADPH-supported GSH- and thioredoxin-dependent antioxidant networks) into the energy-redox axis [18]. Hepatic GSH levels can be regulated by microRNAs by either targeting glutamate-cysteine ligase (GCL) or downregulating the levels of Nrf2, noting that miR433 is redox sensitive and downregulates the expression of GCL in an Nrf2-independent manner. The liver methionine pathway is also regulated by a specific set of miRNAs [25]. Impairment of the integration of metabolism and redox biology entailed in the energy-redox axis results in mechanisms underpinning removal of damaged organelles (autophagy, mitophagy), *i.e.*, quality control, which is monitored at the protein, organelle and sub-organelle levels [26]. Mitophagy entails the coordinated activity of mitochondrial kinase PINK1 and the ubiquitin ligase Parkin that tag mitochondria for lysosomal degradation; conversely, USP30 deubiquitinase inhibits mitophagy by opposing Parkin-mediated ubiquitination [27]. Essential to mitochondrial quality control is the recognition of receptors that sense different signals and associate the mitophagy machinery with mitochondrial dynamics [26]. Examples of mitophagy receptors are ATG32 (Autophagy-related protein 32), FUNDC1 (FUN14 domain-containing protein 1), and BCL2-L-13 (BCL-2-like protein 13) as well as others. Activation of these receptors is linked to the DRP1-driven mitochondrial fission machinery [26]. Signaling through these receptors can be triggered by bioenergetics deficits, hypoxia, oxidative stress and inflammation [26].

O₂⁻ formation by mitochondria appears to be a requirement for activation of mitochondrial

poly(ADP-ribose) polymerase (mtPARP), thereby catalyzing the poly(ADP-ribosylation) (PARylation) of the electron transfer complexes and decreasing energy production [28]. In plants, H₂O₂ signaling is important in reproductive development, *i.e.*, pollen germination, pollen tube growth and fertilization, and pollen maturation [8].

Another type of mitochondrion-driven signaling, not necessarily redox in nature, is accomplished by mt-DNA-encoded mitochondrial-derived peptides (MDPs), although mechanisms of mitochondrial export are still under investigation [29]. As a mitochondrial signal, MOTS-c (Mitochondrial ORF within the Twelve S rRNA c) has several metabolic targets (folate-methionine cycle and related AMPK and sirtuin signaling), stimulates glucose uptake, regulates fat and muscle metabolism [29].

Mitochondrial function in neurodegenerative disorders

Deficits in cell bioenergetics are a common denominator in several neurodegenerative disorders as well as in aging. Alzheimer's disease [17], Parkinson's disease [30], Friedrich ataxia [31], among others, also share some aspects of impairment of glucose homeostasis and insulin resistance. In this special issue, impairment of oxidative phosphorylation and increased formation of mitochondrial H₂O₂ in Parkinson's disease leads to a compensatory increase in glycolysis (upon oxidant-mediated stabilization of HIF-1) that, in turn, impairs pentose phosphate pathway activity [30]. This metabolic shift and oxidative damage in Parkinson's disease also impairs mitochondrial quality control pathways and leads to the consideration of Parkinson's disease as a metabolic syndrome-like disorder [30]. The interconnected mitochondrial energy- and redox systems in mouse models of Alzheimer's disease [17] are consequences of deficits in brain glucose availability, reduced oxidative phosphorylation, compensatory glycolysis, increased formation of mitochondrial H₂O₂, all leading to the bioenergetics hypothesis of Alzheimer's disease. Thus, redox dysregulation is viewed in this subsection as a common denominator and link to the inflammatory hypothesis, where microglia activation is considered as the driving force for neuroinflammation [17]. The genetic deficiency of the mitochondrial protein frataxin is the cause of Friedreich ataxia. Frataxin deficiency is not associated with cognitive impairment, but with increased oxidative stress with alterations in lipid metabolism, which are discussed as potential therapeutic approaches in Friedreich ataxia [31]. The mitochondrial Lon protease is involved in several neurological disorders, such as hereditary Parkinson's disease, Friedreich ataxia, familial amyotrophic lateral sclerosis, brain ischemia and stroke [32]. In these disorders, the physiological functions of the Lon protease are altered: as a protease, as a chaperone, and as a mtDNA-binding protein [32].

The involvement of mitochondrial dysfunction and oxidative stress in the pathogenesis of

several neurodegenerative diseases represent potential therapeutic targets. Concerning oxidative stress, astrocytes play a key role in providing antioxidant support to neurons [33] through redox regulation of the astrocytic Nrf2 pathway, whereas in neurons the synaptic activity-dependent expression of genes of the GSH and thioredoxin-peroxiredoxin families are the major antioxidant systems. Agonists of the peroxisome proliferation-activated receptor gamma (PPAR γ), a ligand-activated transcription factor involved in the regulation of mitochondrial bioenergetics and turnover, antioxidant defenses, and immune responses, are important therapeutic agents in neurodegenerative diseases [34]. Mitochondrial sirtuins (SIRT3, SIRT4, SIRT5) have a variety of functions, deacetylase, ADP-ribosylase, lipoamidase, succinylation, glutarylation; hence, these sirtuins are involved in the regulation of a broad range of metabolic pathways and, as a corollary, implicated in a range of metabolic diseases, such as neurodegeneration, diabetes, cancer, cardiac dysfunction, and age-related disorders [35].

Mitochondrial genomics in health and disease

Several mechanisms link mtDNA defects to atherosclerosis, and although a causative role of oxidants in the development of plaque formation has been widely reported, oxidative stress is not necessarily required for mtDNA damage to impact energy metabolism, lead to apoptotic cell death, activate inflammatory responses and promote atherogenesis [36]. In addition to mtDNA damage, defects in mt-translation elongation factors have been associated with several human diseases [6]. mtPARP is also involved in the repair of mtDNA by interacting with essential factors of the mtDNA repair machinery however, under oxidative stress conditions, excess of O₂⁻ formation, mtPARP PARylates those essential factors, thereby promoting the disassembly of the mtDNA repair complex [28] and contributing to mtDNA oxidative damage. As mentioned above in mitochondrial signaling, the short open reading frames (sORFs) in mtDNA encode humanin and other small humanin-like peptides [29]. In plants, the highly dynamic nature and diversity of mtDNA has profound implications for response to stresses as well as development [8].

This Free Radical Biology & Medicine Special Issue on *Mitochondrial Redox Signaling in Health and Disease* covers only a small part of the many biological processes in which mitochondria operate as a cellular hub. The significance of mitochondrial function is increased because of the large number of mitochondrion-centric mechanisms accounting for pathophysiological situations as well as the development of mitochondrial pharmacology [31, 34, 37]. The invited review articles in this Special Issue will be of interest to researchers in the field of mitochondrial redox signaling, including postdoctoral fellows and graduate students. The

Editors' long-term objective is to provide readers with informed updates of this research field every 3-4 years in Free Radical Biology & Medicine.

Acknowledgements

References

- [1] Bravo-Sagua, R.; Torrealba, N.; Paredes, F.; Morales, P. E.; Pennanen, C.; López-Crisosto, C.; Troncoso, R.; Criollo, A.; Chiong, M.; Hill, J. A.; Simmen, T.; Quest, A. F.; Lavandero, S. Organelle communication: Signaling crossroads between homeostasis and disease. *Int. J. Biochem. Cell Biol.* **50**:55-59; 2014.
- [2] Sebastián, D.; Hernández-Alvarez, M. I.; Segalés, J.; Sorianello, E.; Muñoz, J. P.; Sala, D.; Waget, A.; Liesa, M.; Paz, J. C.; gopalacharyulu, P.; Oresic, M.; Pich, S.; Burcelin, R.; Palacín, M.; Zorzano, A. Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. *Proc. Natl. Acad. Sci. USA* **109**:5523-5528; 2012.
- [3] Chaban, Y.; Boekema, E. J.; Dudkina, N. V. Structures of mitochondrial oxidative phosphorylation supercomplexes and mechanisms for their stabilization. *Biochim Biophys Acta* **1837**:418-426; 2014.
- [4] Enriquez, J. A. Supramolecular organization of respiratory complexes. *Annu. Rev. Physiol.* **78**:533-561; 2016.
- [5] Moreno-Loshuertos, R.; Enriquez, J. A. Respiratory supercomplexes and the functional segmentation of the CoQ pool. *Free Radic Biol Med* **in press**; 2016.
- [6] Cao, X.; Qin, Y. Mitochondrial translation factors reflect coordination between organelles and cytoplasmic translation via mTOR signaling: Implication in disease. *Free Radic Biol Med* **in press**; 2016.
- [7] Sciacovelli, M.; Frezza, C. Oncometabolites: Unconventional triggers of oncogenic signalling cascades. *Free Radic Biol Med* **in press**; 2016.
- [8] Liberatore, K. L.; Dukowic-Schulze, S.; Miller, M. E.; Chen, C. C.; Kianian, S. F. The role of mitochondria in plant development and stress tolerance. *Free Radic Biol Med* **in press**; 2016.
- [9] Brand, M. D. Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radic Biol Med* **in press**; 2016.
- [10] Murphy, M. P. How mitochondria produce reactive oxygen species. *Biochem. J.* **417**:1-13.; 2009.
- [11] Bilan, D. S.; Belousov, V. V. Genetically encoded probes for NAD⁺/NADH monitoring. *Free Radic. Biol. Med.* **in press**; 2016.
- [12] Zhao, Y.; Yang, Y. Real-time and high-throughput analysis of mitochondrial metabolic states in living cells using genetically NAD⁺/NADH sensors. *Free Radic Biol Med* **in**

- press**; 2016.
- [13] Blacker, T. S.; Duchon, M. R. Investigating mitochondrial redox state using NADH and NADPH autofluorescence. *Free Radic Biol Med* **in press**; 2016.
- [14] Murphy, M. P. Mitochondrial thiols in antioxidant protection and redox signaling: distinct roles of glutathionylation and other thiol modifications. *Antioxid. Redox Signal.* **16**:476-495.; 2012.
- [15] Yin, F.; Boveris, A.; Cadenas, E. Mitochondrial energy metabolism and redox signaling in brain aging and neurodegeneration. *Antioxid. Redox Signal.* **20**:353-371; 2014.
- [16] Yin, F.; Sancheti, H.; Patil, I. Y.; Cadenas, E. Energy metabolism and inflammation in brain aging and Alzheimer's disease. *Free Radic Biol Med* **in press**; 2016.
- [17] Yin, F.; Sancheti, H.; Liu, Z.; Cadenas, E. Mitochondrial function in ageing: coordination with signalling and transcriptional pathways. *J Physiol.* **594**:2025-2042; 2016.
- [18] Yin, F.; Jiang, T.; Cadenas, E. Metabolic triad in brain aging: mitochondria, insulin/IGF-1 signalling, and JNK signalling. *Biochem. Soc. Trans.* **41**:101-105; 2013.
- [19] Lee, H. C.; Wei, Y. H. Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. *Int. J. Biochem. Cell Biol.* **37**:822-834; 2005.
- [20] Wende, A. R.; Young, M. E.; Chatham, J. C.; Zhang, J.; Rajasekaran, N.; Darley-Usmar, V. M. Redox biology and the interface between bioenergetics, autophagy, and circadian control of metabolism. *Free Radic Biol Med* **in press**; 2016.
- [21] Rhee, S. G.; Kil, I. S. Mitochondrial release of H₂O₂ is controlled by the concerted action of peroxiredoxin III and sulfiredoxin: linking mitochondrial function to circadian rhythm. *Free Radic Biol Med* **in press**; 2016.
- [22] Angelova, P. R.; Abramov, A. Y. Functional role of mitochondrial reactive oxygen species in physiology. *Free Radic Biol Med* **in press**; 2016.
- [23] Diebold, L.; Chandel, N. S. Mitochondrial ROS regulation in proliferating cells. *Free Radic Biol Med* **in press**; 2016.
- [24] Sobotta, M. C.; Liou, W.; Stocker, S.; Talwar, D.; Oehler, M.; Ruppert, T.; Scharf, A. N.; Dick, T. P. Peroxiredoxin-2 and STAT3 form a redox relay for H₂O₂ signaling. *Nat Chem Biol* **11**:64-70; 2015.
- [25] Lu, S. C.; Mato, J. M.; Espinosa-Diez, C.; Lamas, S. MicroRNA-mediated regulation of glutathione and methionine metabolism and its relevance for liver disease. *Free Radic Biol Med* **in press**; 2016.
- [26] Wu, H.; Wei, H.; Sehgal, S. A.; Liu, L.; Chen, Q. Mitophagy receptors sense stress signals and couple mitochondrial dynamic machinery for mitochondrial quality control. *Free Radic Biol Med* **in press**; 2016.
- [27] Bingol, B.; Sheng, M. Mechanisms of mitophagy: PINK1, Parkin, USP30 and beyond. *Free Radic Biol Med* **in press**; 2016.
- [28] Brunyanszki, A.; Szczesny, B.; Virág, L.; Szabo, C. Mitochondrial poly(ADP-ribose)

- polymerase: The wizard of Oz at work. *Free Radic Biol Med* **in press**; 2016.
- [29] Lee, C.; Kim, K. H.; Cohen, P. MOTS-c: A novel mitochondrial-derived peptide regulating muscle and fat metabolism. *Free Radic Biol Med* **in press**; 2016.
- [30] Requejo-Aguilar, R.; Bolaños, J. P. Mitochondrial control of cell bioenergetics in Parkinson's disease. *Free Radic Biol Med* **in press**; 2016.
- [31] Tamarit, J.; Obis, E.; Ros, J. Oxidative stress and altered lipid metabolism in Friedreich ataxia. *Free Radic Biol Med* **in press**; 2016.
- [32] Bota, D. A.; Davies, K. J. A. Mitochondrial Lon protease in human disease and aging: Including an etiologic classification of Lon-related diseases and disorders. *Free Radic Biol Med* **in press**; 2016.
- [33] Baxter, P. S.; Hardingham, G. E. Adaptive regulation of the brain's antioxidant defences by neurons and astrocytes. *Free Radic Biol Med* **in press**; 2016.
- [34] Corona, J. C.; Duchen, M. R. PPAR γ as a therapeutic target to rescue mitochondrial function in neurological disease. *Free Radic Biol Med* **in press**; 2016.
- [35] Osborne, B.; Bentley, N. L.; Montgomery, M. K.; Turner, N. The role of mitochondrial sirtuins in health and disease. *Free Radic Biol Med* **in press**; 2016.
- [36] Yu, E.; Bennett, M. R. The role of mitochondrial DNA damage in the development of atherosclerosis. *Free Radic Biol Med* **in press**; 2016.
- [37] Smith, R. A.; Hartley, R. C.; Cochemé, H. M.; Murphy, M. P. Mitochondrial pharmacology. *Trends Pharmacol. Sci.* **33**:341-352; 2012.

Juan P. Bolaños

Institute of Functional Biology and Genomics,
University of Salamanca, Salamanca, Spain

Enrique Cadenas

Pharmacology & Pharmaceutical Sciences, School of Pharmacy,
University of Southern California, California, USA

Michael R. Duchen

Department of Cell and Developmental Biology,
University College London, London, UK

Mark B. Hampton

Centre for Free Radical Research, Department of Pathology,
University of Otago, Christchurch, New Zealand

Giovanni E. Mann

Cardiovascular Division, Faculty of Life & Health Sciences,
King's College London, London, UK

Michael P. Murphy

Medical Research Council, Mitochondrial Biology Unit,

