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INVITED REVIEW

Pathophysiological implications of mitochondrial oxidative stress mediated by mitochondriotropic agents and polyamines: the role of tyrosine phosphorylation

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Abstract Mitochondria, once merely considered as the "powerhouse" of cells, as they generate more than 90 % of cellular ATP, are now known to play a central role in many metabolic processes, including oxidative stress and apoptosis. More than 40 known human diseases are the result of excessive production of reactive oxygen species (ROS), bioenergetic collapse and dysregulated apoptosis. Mitochondria are the main source of ROS in cells, due to the activity of the respiratory chain. In normal physiological conditions, ROS generation is limited by the anti-oxidant enzymatic systems in mitochondria. However, disregulation of the activity of these enzymes or interaction of respiratory complexes with mitochondriotropic agents may lead to a rise in ROS concentrations, resulting in oxidative stress, mitochondrial permeability transition (MPT) induction and triggering of the apoptotic pathway. ROS concentration is also increased by the activity of amine oxidases located inside and outside mitochondria, with oxidation of biogenic amines and polyamines. However, it should also be recalled that, depending on its concentration, the polyamine spermine can also protect against stress caused by ROS scavenging. In higher organisms, cell signaling pathways are the main regulators in energy production, since they act at the level of mitochondrial oxidative phosphorylation and participate in the induction of the MPT. Thus, respiratory complexes, ATP synthase and transition pore components are the targets of tyrosine kinases and phosphatases. Increased ROS may also regulate the tyrosine phosphorylation of target proteins by activating Src kinases or phosphatases, preventing or inducing a number of pathological states.

Keywords Mitochondria · ROS · Amine oxidases · Polyamines · Mitochondrial medicine · TYR-phosphorylation

Abbreviations

MAO	Monoamine oxidase
AdNT	Adenine nucleotide translocase
RCI	Respiratory control index
P/*O* ratio	Ratio ATP and consumed oxygen
ROS	Reactive oxygen species
MPT	Mitochondrial permeability transition
$O_2^{\cdot-}$	Superoxide anion
RNS	Reactive nitrogen species
FMN	Flavin mono nucleotide
$CoQH_2$/CoQ	Cofactor, ubiquinol/ubiquinone ratio
MnSOD	Mitochondrial superoxide dismutase
$ONOO^-$	Peroxynitrite
MAOs	Monoamine oxidases
FAD	Flavin adenine dinucleotide
·NO	Nitric oxide
mtNOS	Mitochondrial nitric oxide synthase
MDA	Malondialdehyde
HNE	Hydroxynonenal
ALE	Lipoperoxidation

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TPP^+	Triphenylphosphonium
$\Delta\Psi$	Electrical membrane potential
PTPC	Permeability transition pore complex
VDAC	Voltage-dependent anion channel
ANT	Adenine nucleotide translocase
RTK	Receptor tyrosine kinases
NRTK	Non-receptor tyrosine kinases
EGFR	Epidermal growth factor receptor
SFKs	Src family kinases
PTPs	Tyrosine phosphatases

Mitochondria and energy demand by cells

Mitochondria are cytoplasmic organelles forming an integral part of all eukaryotics. They are considered the "powerhouse" of cells, as they convert organic materials into energy. Their two-membrane constitution allows them to build a particularly complex structure, which includes: (1) an outer membrane, surrounding the inner compartments and exhibiting non-specific permeability to metabolites involved in mitochondrial bioenergetics; in particular, the outer membrane contains the enzyme monoamine oxidase (MAO); (2) a small compartment, the intermembrane space, which separates the outer membrane from the inner one; it contains the adenine nucleotide-balancing enzyme, adenylate kinase, and several pro-apoptotic factors; (iii) the inner membrane, containing the protein complexes responsible for energy transduction, with many deep invaginations called cristae (Ernster and Kuylenstierna 1969). The inner membrane surrounds the inner matrix space, rich in soluble proteins and containing, as generally reported, mitochondrial DNA encoding 13 proteins, all of which are located in the inner membrane and form part of the oxidative phosphorylation machinery (Wallace 1997). There is also a recently discovered fourteenth protein, the peptide humanin, which is involved in protection against stress (Guo et al. 2003; Yen et al. 2013). The matrix space also contains enzymes of the Krebs cycle, among many others. Both these membranes come together at particular contact sites and fuse, forming gap junctions. In liver, each mitochondrion contains 100–200 gap junctions, with a diameter of about 20 nm (Hackenbrock 1968).

The inner and outer membranes differ greatly in permeability. The inner membrane is impermeable to all solutes, even the smallest ion, the proton, with the major exception of oxygen, which is soluble in lipid bilayers. Mono- or bi-directional transport of metabolites occurs only via specific transporters and exchangers. Thus, a large number of proteins are involved, including adenine nucleotide translocase (AdNT), which exchanges ATP for ADP (Klingenberg 1979; Klingenberg et al. 1995), the phosphate transporter (Fonyo et al. 1975; Wohlrab 2005), di- and tri-carboxylate transporters and the carnitine shuttle (Palmieri and Klingenberg 1979; LaNoue and Schoolwerth 1979), the polyamine transporter (Toninello et al. 1988, 1992), and the Ca^{2+} uniporter (Carafoli and Crompton 1978; Nicholls and Crompton 1980), etc. All these transporters catalyze the uptake and release of substrates and reaction products involved in oxidative phosphorylation. This process uses electrons predominantly delivered by NADH and also $FADH_2$, derived from macromolecule metabolism or the energy stores of the organism. These electrons flow along the electron transport chain to the final electron acceptor, oxygen, which is reduced to H_2O. The electron transport chain is composed of a sequence of respiratory complexes including NADH-ubiquinone reductase (complex I), succinate dehydrogenase (complex II), ubiquinol cytochrome *c* reductase, also called bc_1 complex (complex III), and cytochrome *c* oxidase (complex IV). Among these complexes, ubiquinone accepts electrons from complexes I and II and transfers them to complex III, cytochrome *c* accepts electrons from complex III and transfers them to complex IV (Slater 1953). Electron transport is coupled to the proton pumping activity of complexes I, III and IV, extruding protons and generating the electrochemical gradient, $\Delta\mu_H^+$, across the inner membrane, also called the proton motive force. $\Delta\mu_H^+$ is used by ATP-synthase (complex V) to synthesize ATP from ADP and phosphate, coupled to a reversal flow of protons from the intermembrane space to the matrix. Mitochondrial-synthesized ATP represents the main energy supply for all cell processes, providing more than 15 times the ATP produced by anaerobic glycolysis (Alberts et al. 2002). The Nobel Prize winner Peter Mitchell, with his chemiosmotic hypothesis, was the first to propose that an electrochemical gradient, generated by the proton pumps coupled to the electron flux through the electron transport chain, is the driving force synthesizing ATP by ATP-synthase (Mitchell 1961). This statement represents the "central dogma" of Mitchell's chemiosmotic theory, universally accepted by the biochemical scientific community and one of the main scientific accomplishments of the 20th century.

Energy homeostasis is one of the main determinants for life in all higher organisms. It has been calculated that a person on average uses 100 kcal/h at rest, corresponding to the production of about 65 kg of ATP/day (Rich 2003). During normal daily exercise, these values may increase many times. Unlike many unicellular organisms, which have evolved devices allowing them to live and grow in anaerobiosis, animals depend entirely on cell respiration. This is because of the presence in the human body of approximately, 10^{14} cells which, although exhibiting very high density, are highly specialized and energy-expensive. These characteristics require efficient supplies of fuel and oxygen and the removal of carbon dioxide through blood

circulation. Thus, the supply of constant energy through glycolysis alone is not feasible in healthy animals: cell respiration must be carried out by mitochondria. The energy demand of cells ranges widely, depending on their function and activity. This means that all higher organisms must make adjustments in energy production to physiological demand. Britton Chance was the first to propose a mechanism for controlling oxidative phosphorylation, called respiratory control (Chance and Williams 1955), which could be measured by calculating the ratio between the rate of oxygen consumption in respiratory state 3 and that in state 4 (the respiratory control index). State 3 is the metabolic state during ATP synthesis, that is, in the presence of ADP and phosphate. State 4 is the resting condition without ATP synthesis, that is, in the absence of added ADP. Another parameter to assess respiratory control is the *P*/*O* ratio, or the ratio between the amount of ATP synthesized and oxygen consumed. These parameters, evaluated in isolated mitochondria, show that oxidative phosphorylation is limited by the availability of ADP and phosphate (substrates for ATP synthesis). In other words, oxidative phosphorylation increases when ATP, used for cell requirements, regenerates ADP and phosphate. It should be noted that ATP production by mitochondria is also regulated by several enzymes of the Krebs cycle which, in turn, are controlled by matrix Ca^{2+} concentration. In this regard, many cell processes are involved in the regulation of Ca^{2+} fluxes across the plasma membrane, endoplasmic reticulum and mitochondrial membrane, to maintain intracellular Ca^{2+} homeostasis. This concerted activity among all the Ca^{2+} transport systems is essential for energy transduction and cell life. Several qualified reviews have described Ca^{2+} transport and its pathophysiological role in mammalian mitochondria (Carafoli 1982, 2003; Gunter et al. 2000; Rizzuto et al. 2000; Gunter and Gunter 2001; Pfeiffer et al. 2001; Salvi and Toninello 2004; Harrington and Murphy 2015).

Mitochondrial Ca^{2+} homeostasis is maintained by the activity of the electrophoretic uniporter sensitive to membrane potential, driving its uptake (Rottenberg and Scarpa 1974) and by the Na^{+}-dependent and Na^{+}-independent electroneutral exchangers responsive for its efflux (Crompton et al. 1976; Puskin et al. 1976). Another mechanism capable of sequestering significant amounts of Ca^{2+} from cytosolic Ca^{2+}, in rapid pulses, called RaM, has also been identified (Sparagna et al. 1995).

Intracellular Ca^{2+} homeostasis is also controlled by interaction between the endoplasmic reticulum (ER), which fine-tunes cytoplasmic Ca^{2+} concentrations, and the mitochondrion, the major Ca^{2+} buffering organelle in cells. The physical ER-mitochondrion interaction is called the mitochondria-associated ER membrane (MAM), the interface between the organelles. MAM plays a pivotal role in several cell functions, including Ca^{2+} signaling, lipid transport, energy metabolism and cell survival, and is in fact vital for regulating Ca^{2+} levels in the mitochondrial matrix. The supply of Ca^{2+} to mitochondria, as mentioned above, is crucial for matching ATP production by the Krebs cycle with ATP demand (Hayashi et al. 2009).

A receptor called sigma-1 receptor (Sig-1R) has been identified in the MAM and regulates the stability of inositol 1,4,5-triphosphate (IP3) receptors to ensure proper Ca^{2+} signaling between the ER and the mitochondrion (Hayashi and Su 2007). Sig-1R also functions against oxidative stress in cells, as evidenced by metabolic and proteomic studies (Pal et al. 2012). Ca^{2+} concentration in the mitochondrial matrix is also sensitive to the presence of natural polyamines, in particular spermine. In the presence of polyamines, mitochondria can enhance Ca^{2+} accumulation, due to activation of the saturable systems of Ca^{2+} uptake. In addition, polyamines can buffer extra mitochondrial Ca^{2+} concentrations to levels similar to those in the cytosol of resting cells (Salvi and Toninello 2004). The mechanism involved is due to a rise in the affinity of the Ca^{2+} transport system, induced by polyamines, most probably exhibiting allosteric behavior. The regulatory site of this mechanism is the S1 binding site of polyamines (Dalla Via et al. 1996, 1999) operating in physiological conditions and located in the energy well between the two peaks present in the energy profile identified for spermine transport (Toninello et al. 2000).

As well as the above-mentioned control mechanisms of mitochondrial bioenergetics, several other levels of control exist, involving the tissue-specific expression of isozymes, allosteric control and cell signaling (Hüttemann et al. 2007). Unlike the first two mechanisms, which are involved in intracellular responses, cell signaling is mainly implicated in higher-order communications between cells and organs. Within the cell there are sequences of enzymes such as kinases and phosphatases, involved in the above process, which add or remove phosphate groups to or from their targets. As pointed out by the above authors, the traditional opinion regarding the presence of linear cascades in signal transduction must be considered an over-simplification, as much cross-talk goes on among the various signaling pathways. This network of signals may hinder the interpretation of experimental results, explaining some reported contrasting results. Mitochondria have come back again as several phosphorylated proteins, kinases and phosphatases have been identified in their inner compartments, suggesting that these organelles play an important role in signaling pathways (Salvi et al. 2002a, b, 2004, 2005a, b, 2007a, b; Horbinski and Chu 2005; Pagliarini and Dixon 2006; Vogt et al. 2007; Tibaldi et al. 2008; Lewandrowski et al. 2008) (see "Tyrosine phosphorylation, ROS, polyamines and mitochondria").

Mitochondria and ROS (oxidant generation by mitochondria)

Mitochondria are the most important source of reactive oxygen species (ROS) in most types of mammalian cells (Andreyev et al. 2005; Turrens 2003; Balaban et al. 2005; Chance et al. 1979; Cadenas and Davies 2000; Raha and Robinson 2000; Adam-Vizi and Chinopoulos 2006; Muller 2000). ROS generation contributes to severe mitochondrial damage in a range of pathologies, but it is also involved in redox signaling from organelles to other cell compartments (Balaban et al. 2005; Chance et al. 1979; Cadenas and Davies 2000; Raha and Robinson 2000; Adam-Vizi and Chinopoulos 2006; Muller 2000; Dröge 2002).

More than 95 % of inhaled oxygen undergoes tetravalent reduction, to produce H_2O by the last reaction of the respiratory chain catalyzed by cytochrome *c* oxidase (complex IV of the chain):

$$O_2 + 4e^- + 4H^+ \rightarrow H_2O.$$

This terminal reaction allows continued electron transport along the chain, together with the proton pumping which generates $\Delta\mu_H^+$ and consequently ATP synthesis. Blocking the electron flux through the respiratory complexes results in dissipation of the proton motive force and a consequent halt in ATP production. This fact shows that one of the peculiar roles of oxygen in all aerobic organisms is to act as a dump for electrons, to maintain the coupling between mitochondrial respiration and ATP synthesis. During two-electron transport along the respiratory chain, there are also alternating one-electron redox reactions, which predispose each transporter to become involved in side-reactions with molecular oxygen.

Mammalian complex I is the entry point for electrons coming from NADH and entering the respiratory chain. The FMN cofactor acts as electron acceptor and transfers the electrons along a chain of iron–sulfur (FeS) centers to the ubiquinone pool. It should be recalled that mitochondria contain a putative ubiquinone at the level of complex I and a large pool of ubiquinone and ubiquinol in the bulk of the membrane but interacting with the components of the bc_1-complex (complex III). In both cases, ubiquinone cycles between the quinone (completely oxidized) to the semiquinone (one-electron reduction product) and to quinol (fully reduced by two electrons), and vice versa. The semiquinone radical may transfer its unpaired electron to oxygen directly, thus generating superoxide anion $O_2^{\cdot-}$ instead of the next electron transporter in the chain. Another mechanism by means of which complex I produces large amounts of $O_2^{\cdot-}$ occurs during reverse electron transport, when electron supply reduces the ubiquinone pool: in the presence of high electrochemical gradients, this forces electrons back from ubiquinol into complex I and reduces NAD^+ to NADH at the FMN site (Adam-Vizi and Chinopoulos 2006; Kudin et al. 2004; Votyakova and Reynolds 2001; Liu et al. 2002).

Similar side-reactions with molecular oxygen may take place in other segments of the respiratory chain with the generation of the toxic $O_2^{\cdot-}$. In particular, several iron–sulfur clusters within the respiratory complexes are also involved. On the basis of these considerations, it is commonly held, as above stated, that mitochondria are the main intracellular source of oxygen radicals in physiological conditions. Thus, it is estimated that 1–4 % of the total oxygen in mitochondria is constitutively converted to superoxide anion. In humans, mean values in the range of 190–355 mmol of $O_2^{\cdot-}$ are produced per day from mitochondrial respiration. There is ongoing debate regarding the exact sites of the production of $O_2^{\cdot-}$. In a review, Murphy (2009) reported the principles which govern $O_2^{\cdot-}$ production in the matrix of mammalian mitochondria. His observations showed that the $O_2^{\cdot-}$ flux is related to the concentration of potential electron donors, the local concentration of O_2, and the rate constant for reactions among them. Mitochondria have two modes of operation in producing significant $O_2^{\cdot-}$ concentrations. The first takes place at the level of Complex I, when mitochondria are not synthesizing ATP and consequently have a high $\Delta\mu_H^+$, very low oxygen consumption, and a greatly reduced CoQ pool. The second mode occurs when there is a high NADH/NAD^+ ratio in the matrix (see also the description above). Instead, when mitochondria are involved in ATP synthesis and have both lower $\Delta\mu_H^+$ and NADH/NAD^+ ratio and low respiration, the extent of $O_2^{\cdot-}$ generation is much lower (for a complete explanation, see Murphy 2009). However, the authors emphasize that all these parameters, together with the $CoQH_2$/CoQ ratio and local O_2 concentrations, vary considerably and are very difficult to measure in vivo. Consequently, it is not possible to determine $O_2^{\cdot-}$ production in in situ mitochondria by applying $O_2^{\cdot-}$ production rates in isolated mitochondria. Such extrapolations reported in the literature are considered misleading (Murphy 2009).

The pioneering works of Chance et al. and others (Chance et al. 1979; Loschen et al. 1971; Boveris and Chance 1973) reported that isolated mitochondria can produce H_2O_2 from the dismutation of superoxide anion generated within mitochondria (Loschen et al. 1974; Forman and Kennedy 1974; Winterbourn et al. 1978). This reaction is catalyzed by mitochondrial superoxide dismutase (MnSOD), which has a rate constant for $O_2^{\cdot-}$ dismutation of the order of 10^9 M^{-1} s^{-1} (Turrens 2003; Cadenas and Davies 2000). The spontaneous dismutation of $O_2^{\cdot-}$ has a rate constant of 10^6 $\mathbf{M^{-1}}$ $\mathbf{s^{-1}}$. However, in the absence of MnSOD, the production of $O_2^{\cdot-}$ decreases by allowing the reverse reaction between superoxide and the electron donor (Winterbourn et al. 1978; Schafer and Buettner 2001).

Another ROS, the hydroxyl radical (OH·) is generated in mitochondria by the interaction of H_2O_2 with Fe^{2+} of the iron–sulfur proteins in the respiratory chain (Fenton reaction). OH· can also be produced by the Haber–Weiss reaction between superoxide anion and hydrogen peroxide. The hydroxyl radical is the most toxic of the ROS, due to its very high reactivity. Its half-life, measured in nano-seconds, is determined by diffusion time, i.e., the time necessary for OH· to interact with the target molecule (see also below). The hydroxyl radical reacts with the biomolecules mainly by means of hydrogen addition or subtraction, during which characteristic products are generated, described as biological markers of oxidative stress. H_2O_2 produced by the dismutation of $O_2^{\cdot-}$, catalyzed by MnSOD, may diffuse out of mitochondria (Boveris 1984; Barja 1999). It has in fact been demonstrated that $O_2^{\cdot-}$ is released from mitochondria through the voltage-dependent anion channel (Han et al. 2003). These authors proposed that $O_2^{\cdot-}$ released into the cytoplasm plays an important role in cell signaling. Cytoplasmic aconitase and other enzymes may also be targets of $O_2^{\cdot-}$. Mitochondria can also produce H_2O_2 by oxidative deamination of biogenic amines by MAOs, a family of FAD-containing enzymes present on the outer membrane of mitochondria (Hauptmann et al. 1996). MAO was discovered in 1928 in liver and was named tyramine oxidase (Hare 1928). In humans, there are two types of MAO: MAO-A and MAO-B. The former is found in neurons, astroglia, liver, gastrointestinal tract, and placenta, and is particularly important in the catabolism of monoamines in food. In detail, MAO-A catabolizes serotonin, norepinephrine, epinephrine and dopamine. MAO-B is mainly found in blood platelets, but also in neurons and astroglia, and oxidizes phenylethylamine and dopamine. Thus, both MAOs are involved in the inactivation of monoaminergic neurotransmitters. Due to this primary role, alterations in their activity are thought to be responsible for several neurological disorders. In this regard, MAO inhibitors are considered to be one of the most important classes of drugs for therapeutic interventions, especially in neurological pathologies such as Parkinson's and Alzheimer's diseases (La Regina et al. 2007; Valente et al. 2011). In conclusion, MAO activities represent an additional source of H_2O_2, not linked to respiration and associated with direct two-electron reduction of O_2 to H_2O_2. It should be noted that the compartmentalization of the source of $O_2^{\cdot-}$ and H_2O_2 is functionally associated with and coordinated by the endogenous safety system composed of MnSOD, catalase and glutathione peroxidases, the activity of which leads to regulation of intramitochondrial levels of the above ROS in aerobic organisms (Chance et al. 1979; Naqui et al. 1986). External ROS produced by MAOs can be scavenged by the systems present in the cytoplasm.

The metabolic state of mitochondria determines the physiological rate of ROS production, associated with the respiratory chain. In particular, respiratory state 4 is characterized by no availability of ADP and slow oxygen consumption. Thus, as also mentioned above, this resting state is associated with a relatively fast rate of superoxide and hydrogen peroxide production, most probably due to the high reduction level of the respiratory chain components. Instead, respiratory state 3, with very high oxygen uptake and high ADP, has a slow rate of ROS production, caused by the highly oxidized level of the respiratory chain carriers. Last, in anoxic state 5, with limited O_2 supply and block of respiration, generation of $O_2^{\cdot-}$ and H_2O_2 does not occur (Loschen et al. 1974; Boveris et al. 1999). Besides the particular metabolic state of mitochondria, ROS generation can be modulated by another physiologically important reactive species, nitric oxide (·NO). ·NO is a highly diffusible free radical, formed by conversion of arginine to citrulline in mitochondria and catalyzed by a specific form of mitochondrial nitric oxide synthase in certain tissues such as liver, brain, heart and thymus (Navarro and Boveris 2008; Alvarez et al. 2003; Haynes et al. 2004). ·NO reversibly inhibits mitochondrial cytochrome *c* oxidase and electron flux along the respiratory chain, preventing the oxidation of ubiquinol to ubisemiquinone radical, with subsequent autoxidation, leading to increased production of superoxide anion (Palacios-Callender et al. 2004). This effect has been proposed as the primary regulatory role of ·NO in mitochondria (Brown and Borutaite 2004). ·NO does maintain relatively higher respiration in very low concentrations of O_2, i.e., physiological levels. This regulatory property is particularly important in hypoxic conditions (Palacios-Callender et al. 2004). It should also be noted that ·NO can react in mitochondria with $O_2^{\cdot-}$, producing peroxynitrite ($ONOO^-$) in a very rapid reaction (Szabó et al. 2007; Packer et al. 1996).

When examining the production of ROS and RNS (reactive nitrogen species) by mitochondria, it is interesting to take into account the diffusion radius and compartmentalization of the different types of radicals, to evaluate their capacity of reacting with targets. It has been reported that oxidants may have different effects, depending on where they are produced. Thus, species like $O_2^{\cdot-}$, with low permeability, are largely restricted to reactions in the compartment in which they are generated, unless there is no channel for their export (Han et al. 2003). Instead, hydrogen peroxide is membrane-permeable and exhibits a diffusion distance for each activity of 1.5 mm, whereas peroxynitrite has a diffusion distance of 50 μM. About OH· diffusion, it should be recalled that in mitochondria it is generated from H_2O_2 in the presence of Fe^{2+} and exhibits a rate constant, for reacting with a substrate, e.g., GSH, of 1×10^{10} M^{-1} s^{-1} (for a

review, see Winterbourn 2008). This means that OH· can be produced far from the site of H_2O_2 generation and exhibits an instantaneous reaction rate.

As well as mitochondria, ROS are also generated in multiple compartments by several enzymes within cells. Contributions come from proteins in the plasma membrane, examples being the family of NADH oxidases (Lambeth 2004), lipid metabolism in peroxisomes, and the activity of various cytosolic enzymes such as cyclooxygenase. The endoplasmic reticulum can also produce ROS. Accumulation of misfolded proteins due to ER stress can release Ca^{2+} from the ER lumen and activate its chaperone machinery (Zhang and Kaufman 2008), producing ROS. This is because correct folding of proteins is an energy-dependent process requiring oxidizing conditions and involving the transfer of reducing equivalents to molecular oxygen (Cuozzo and Kaiser 1999). In addition, Ca^{2+} released from ER is taken up into the mitochondrial matrix, causing collapse of electrical potential, disruption of electron transport, and a further increase in ROS generation (Görlach et al. 2006). Biological membranes are highly sensitive to damage by free radicals, particularly those rich in polyunsaturated fatty acids, and ROS damage their integrity and functions by compromising the capacity of cells to maintain the ionic gradient and affecting the asymmetric distribution of phospholipids. Lipid peroxides formed by the above reactions are degraded to form malondialdehyde (MDA) and hydroxynonenal (HNE). These compounds react with proteins by forming cross-linkages and chemical adducts, known as final products of advanced lipoperoxidation (ALE). Both MDA and HNE adducts with lysine residues have been identified in the lipoproteins of the vascular wall in atherosclerosis and neuronal plates in Alzheimer's disease, supporting the implication of oxidative damage in these diseases (Aliev et al. 2002). Hydroxyl radicals may be involved in reactions with phenylalanine, tyrosine and nucleic acids by forming characteristic hydroxylated derivatives. The levels of other ROS and RNS can be assessed by measuring levels of specific markers of oxidative stress (specific oxidation products).

In particular, nitrotyrosine and ALE are present in elevated amounts in atherosclerotic plates and Alzheimer's disease.

ROS also reacts with carbohydrates to form dicarbonyl compounds which, in turn, react with proteins, forming cross-linkages and adducts, known as glycoxidation products. These compounds are found in large quantities in tissue proteins in diabetes, as a result of hyperglycemia and oxidative stress (Kuroki et al. 2003). The increase in the chemical modifications of proteins by the final products of lipoperoxidation and glycoxidation is implied in the development of vascular, renal and retinal complications.

It is to underline that, H_2O_2 produced within the mitochondrial matrix can interact with peroxiredoxins, catalase and glutathione peroxidase thus underlying a scavenging effect (Rhee et al. 2001; Salvi et al. 2007a, b; Radi et al. 1991; Imai and Nakagawa 2003).

Mitochondria and apoptosis

All the above observations open up new insight on mitochondrial functions, so that, besides being considered as the control centers of energy production, mitochondria are also well-known as the controllers of several other cell activities. They play pivotal roles in apoptosis, necrosis and many other metabolic processes, and are also involved in many human diseases (Di Mauro and Schon 2003; Newmeyer and Ferguson-Miller 2003; Voet and Voet 2004) (see also "Diseases linked to mitochondrial dysfunctions"). These functions account for a large number of agents operating as means of communication to and from mitochondria such as ions, metabolites, hormones, transcription factors, etc. (Butow and Avadhani 2004; Goldenthal and Marín-García 2004; MacDonald et al. 2005). Mitochondria thus behave as centers capable of receiving and integrating cellular signals and transmitting them to other cellular compartments. Apoptosis, or programmed cell death, is a natural process, essential for the physiological development, homeostasis and survival of most multicellular organisms (Schwartzman and Cidlowski 1993) and also particularly important for its function in removing damaged, infected or potentially tumoral cells. Apoptosis is clearly distinct from necrosis. The intact state of the cell membrane is severely compromised or damaged in necrosis, resulting in cell lysis. Necrosis generally occurs in a group of cells or at particular loci of tissues, whereas apoptosis may occur in single cells. During apoptosis, a cell undergoes a series of events, activated by a genetic program, ending in DNA fragmentation and the formation of apoptotic bodies. Despite this, the plasma membrane of early apoptotic cells and bodies remains intact, without losing endogenous materials and thus preventing local tissue damage. Apoptosis may be triggered by a large number of effectors including, as mentioned above, inducers of oxidative stress. ROS generated during normal electron flux are generally scavenged by the anti-oxidant safety systems present in mitochondria. However, the action of these systems may be ineffective, particularly when exogenous mitochondriotropic agents interact with the components of respiratory complexes, resulting in the production of large quantities of ROS. In such cases, ROS may induce oxidative stress, with damage to mitochondrial structures and metabolites.

Oxidative stress may also affect critical thiol groups present on AdNT, with formation of disulphide bridges and induction of the mitochondrial permeability transition (MPT), a phenomenon closely correlated with intrinsic

apoptosis. ROS generation may be involved in other mechanisms, related in various ways to apoptosis. As noted above, mitochondria possess a sophisticated network of signals involving tyrosine kinase and phosphatase activity (Salvi et al. 2002a, b, 2004, 2005a, b, 2007a, b; Horbinski and Chu 2005; Pagliarini and Dixon 2006; Vogt et al. 2007; Tibaldi et al. 2008; Lewandrowski et al. 2008). Several studies have shown that these signals are correlated with triggering of pro-apoptotic pathways. In this case, ROS would have the function of modulating the tyrosine phosphorylation involved in these pathways (see "Tyrosine phosphorylation, ROS, polyamines and mitochondria"). In conclusion, besides having damaging effects, when they are present in mitochondria in not excessive concentrations, ROS may have a dual effect in prompting apoptosis, by activating the intrinsic pathway, and regulating apoptotic signal pathways.

In any case, all the stimuli leading to activation of the pro-apoptotic process appear to converge towards a highly conserved sequence of events, as the morphological and biochemical characteristics of that process are maintained during evolution. Activation of the caspase cascade plays a prominent role in this program (Salvesen and Dixit 1999). Kannan and Jain (2000) noted that, although the initial signal for programmed cell death may be of different origin, its phenotype is uniformly similar and highly conserved during evolution. In other words, multiple signal pathways may converge upstream of a common mechanism of events, resulting in predisposing cells to apoptosis (Schwartzman and Cidlowski 1993; Thompson 1995; Kerr et al. 1972, 1994; Duke et al. 1996). The fact that mitochondria play a central role in apoptosis is now accepted by almost all researchers in the field (Zamzami et al. 1995, 1996; Green and Reed 1988; Susin et al. 1998). There is also the general opinion that mitochondria-mediated apoptosis has wide-ranging implications in cancer research, senescence, and the death of an organism. However, it is also important to evaluate the intensity of the apoptotic process, as slight or excessive apoptosis may lead to different biological consequences (Du et al. 1997). In particular, slight apoptosis is responsible for rheumatoid arthritis and cancer, whereas ischemic heart diseases, AIDS and neurodegenerative diseases are the results of excessive apoptosis (Kerr et al. 1972, 1994; Thompson 1995; Duke et al. 1996; Golstein 1998).

Diseases linked to mitochondrial dysfunctions

As introduced in "Mitochondria and apoptosis", a growing number of mitochondrial pathologies are attributed to mitochondrial dysfunctions. Most of them, known as encephalomyopathies, affect the brain and skeletal muscles, the organs requiring large supplies of energy. In this regard, several myopathies are the result of defects in enzymes such as pyruvate dehydrogenase and carnitine acyl transferase (CoA), in respiratory chain activity (particularly complex I, complex III and ubiquinone) or phosphorylation defects. Many encephalomyopathies are due to single macrodeletions or point mutations in mtDNA: examples are Kearns–Sayre syndrome (KSS) (Kearns and Sayre 1958), mitochondrial encephalomyopathy, lactic acidosis, stroke-like syndrome (Pavlakis et al. 1984), myoclonic epilepsy with ragged red fibers (Fukuhara et al. 1980), and Leber's hereditary optic neuropathy (Leber 1871). As noted above, the field of mitochondrial-associated pathologies has grown exponentially and large numbers of mtDNA mutations, mainly due to ROS activity, have been identified in corresponding clinical phenotypes (Schon et al. 2012). An exhaustive review has recently been published regarding mtDNA defects and their effects on the pathophysiological mechanisms underlying pathologies associated with the central and peripheral nervous systems (Carelli and Chan 2014).

Other very interesting systematic reviews have recently been published, covering such fields as cardiac electrical diseases in KSS and mitochondrial cytopathy (Kabunga et al. 2014), mtDNA aberrations and pathophysiological implications in hematopoietic diseases, chronic inflammatory diseases and cancers (Kim et al. 2015), and the role of mitochondrial oxidative stress on the pathogenesis of target organ damage in hypertension (Rubattu et al. 2014).

In several pathologies such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis, in which defective mitochondria are involved, one still unresolved question is whether mitochondrial dysfunction is a primary cause or a secondary consequence of another essential deficiency within specific neuronal tissues (Scheffler 2008). There is strong emerging consensus by researchers in the field that mitochondrial energy metabolism, oxidative stress, ROS production, apoptosis and neurodegeneration are all part of an intricate picture, in which it will be necessary to discover the definitive cause-and-effect relationship leading to a particular pathology. The problem raised by the above observations and discussions takes into account the possibility that specific mtDNA haplotypes predisposing toward or increasing the risk of triggering a neurodegenerative disease act in combination with nuclear mutations.

Mitochondria as targets of "mitochondrial medicine"

As already noted, ROS and RNS are closely related to degenerative diseases involving neuron death, including

ischemic and hemorrhagic stroke, degenerative cardiac myocyte death, Alzheimer's disease, Parkinson's disease and cancer. The removal of the scavenging effects of these compounds by the mitochondrial safety systems results in structural alterations of organelles, with activation of the cytosolic stress pathway and DNA damage. In particular, membrane cardiolipin is affected, thereby triggering the release of cytochrome *c*, AIF and Smac–Diablo, and activates the intrinsic apoptotic pathway. In addition, neurodegenerative diseases often arise from massive death of neural cells (Mattson 2000), whereas malignant cell transformation should be facilitated by the inhibition of apoptosis. The demonstration that mitochondria play a central role in many pathologies, together with emerging interest in mitochondrial diseases, has led to the creation of a new research field, to identify new pharmacological tools to block (in neurodegenerative diseases) or activate (in cancer) cell death processes. Many new approaches and contexts are involved, as well as renewed interest in the pathologies in which it seems possible to intervene. Thus, gene therapy for diseases due to mitochondrial dysfunctions and antitumoral chemotherapy may be based on MPT inducers or, conversely, MPT inhibitors, anti-oxidant compounds and modulators of apoptosis regulation proteins. "Mitochondrial medicine" aims at developing drugs targeted directly to mitochondria, i.e., mitochondriotropic agents (Weissig 2005; Torchilin 2006). This point is well-illustrated by the poor effectiveness of antioxidants, such as vitamin E, against diseases with partially oxidative etiogenesis such as Parkinson's syndrome. The lack of significant effects in these cases is attributed to poor absorption of compounds and their uniform distribution to all organs, tissues and cells (Murphy and Smith 2007). In this regard, several researchers (Murphy and Smith 2007; Sheu et al. 2006; Cochemè et al. 2007) have turned to mitochondrial antioxidant compounds such as decylubiquinone and α-tocopherol, conjugating them with the triphenylphosphonium group (TPP^+), lipophilic cations which can cross biological membranes and accumulate in compartments with negative potential such as the mitochondrial matrix. This strategy exploits the ratio between accumulated and external TPP^+ concentrations, and is used in bioenergetics to measure the electrical membrane potential, $\Delta\Psi$. Several compounds having these characteristics have been synthesized and are used to study redox signaling in cells and to evaluate their effects on oxidative stress models and apoptosis. Until now, they have demonstrated good bioavailability and, vice versa, little or no toxicity, even after prolonged administration (Murphy and Smith 2007). One important implication is that the TPP^+ group, in itself, is not particularly toxic. It should be emphasized that cationic conjugates such as those mentioned above have sometimes been observed to exhibit certain selectivity for tumoral tissues. The mitochondria of tumor cells have a higher $\Delta\Psi$ than that of normal cells (Chen 1988; Fantin et al. 2006; Fantin and Leder 2006) and may sometimes reach 60 mV, corresponding to an accumulation ratio tenfold higher in cancer cells (Fantin and Leder 2006). In view of this peculiar aspect of the mitochondria of tumor cells, some pioneering pharmacological studies have been performed (Fantin and Leder 2004, 2006; Fantin et al. 2002). Among mitochondriotropic agents, the structure and chemical properties of which were described in an exhaustive review (Horobin et al. 2007), polyphenols are considered among the most appropriate to exploit the above effects at mitochondrial level. The polyphenols constitute a group of about 5000 natural molecules, some belonging to classes with different structures, but they all have non-phenolic functions. They are found in nature and in foods, mainly as glycosylated derivatives or bound to polymer structures such as lignins, and include the flavonoids in fruits and vegetables. They were first described by Albert Szent-Gyorgyi, who discovered vitamin C and observed that polyphenols have a synergic effect on it. The best-known flavonoids are polyphenols with low molecular weight, abundant in nature in ester forms as glycosides and acetyls. Quercetin is one of the best-known flavonoids, occurring not only in onions, apples and citrus fruits, but also in the seeds and peel of fruits and vegetables, in the cortex of some plants, and in wine, tea and chocolate. At therapeutic level, administration of quercetin is suggested against allergies, respiratory deficiency, skin inflammation, uric acid intestinal accumulation, inflammation, and diabetes. Several biological effects of polyphenols, still considered putative, have been ascribed to their antioxidant nature (Jovanovic et al. 1994; Cao et al. 1997a, b; Frei and Higdon 2003; Firuzi et al. 2005), that is, to their capacity to act as reducers and radical scavengers. However, they are also easily oxidized catalytically by transition metals such as Cu^{2+} and Fe^{3+}, leading to the production of hydrogen peroxide and other ROS. In these cases, polyphenols act as oxidative stress inducers, as has been observed in mitochondria treated with some of these compounds. In addition, mitochondria possess a large number of iron–sulfur groups embedded in the respiratory complexes NADH-ubiquinone reductase, succinate ubiquinone reductase and ubiquinol–cytochrome *c* reductase, whereas copper ions are present in cytochrome *c* oxidase. Due to the presence of iron and copper ions in the respiratory chain, mitochondria can behave as ROS producers when they interact with polyphenols and their derivatives.

Genistein, a natural isoflavone occurring in plants, is a major component of soybean. It has been shown to have anti-tumoral, anti-oxidant and anti-inflammatory effects, can also prevent cancer in many organs (Peterson 1995) and has beneficial effects in osteoporosis and cardiovascular diseases (Polkowski and Mazurek 2000; Goldwyn

et al. 2000). Genistein is a potent inhibitor of α-glucosidase (Dong Sun and Sang Han 2001), an enzyme involved in diseases such as diabetes (Jenkins et al. 1981), cancer (Dennis et al. 1987) and viral attachment (Gruters et al. 1987). The widespread consumption of soy in Asian countries very probably accounts for the low incidence of these chronic disorders in those countries, and suggests that it could play an important role in promoting human health (Goldwyn et al. 2000).

One study linking the therapeutic effects of genistein with its activity as a potential pro-apoptotic agent showed that it can induce the MPT in liver mitochondria (Salvi et al. 2002a, b). The event responsible for transition pore opening is oxidative stress triggered by generation of H_2O_2, as a result of the interaction of genistein with the mitochondrial respiratory chain. In particular, genistein reacts with the Fe^{3+} of the b_H heme of bc_1 complex (complex III), following a previously proposed reaction sequence (Cao et al. 1997a, b), with the final production of H_2O_2. MPT induction is well-known to be closely associated with the release of several proteins such as cytochrome *c*, AIF, Smac–Diablo, HtrA2, etc., which prompt the cell toward apoptotic cell death. A clear-cut demonstration of the close correlation between the action of this type of natural compound at mitochondrial level, with triggering of the pro-apoptotic pathway, is that of glycyrrhetinic acid. This compound is the active aglycone of a pentacyclic triterpene glycoside, one of the main components of licorice root. Licorice has long been known as a medicinal plant and its history as a pharmacological drug dates far back into the past (Armanini et al. 2002). Licorice extracts were primarily used as demulcents, expectorants and mild laxatives. Their constituents have several beneficial properties such as anti-inflammatory, anti-hepatotoxic, anti-bacterial, anti-viral and anti-cancer effects (Armanini et al. 2002; WHO 1999). They are also endowed with endocrine properties and, in particular, behave as mineralocorticoids, with estrogenic and antiestrogenic effects (Armanini et al. 2002). However, it should be noted that there is another side in considering flavonoids and polyphenols as antioxidants, since it has been reported that most of these compounds do not act as ROS scavengers but as reducers of reactive nucleophiles or regulators of the signaling pathway (such as Nrf2). In this view, the real antioxidants are α-tocopherol and mitoQ and related compounds (Forman et al. 2014).

It has recently been reported that the compound plastoquinonyl-decyltriphenylphosphonium (SkQ1), at nanomolar concentrations, shows promising biological activity in vivo. SkQ1 can protect against acute pyelonephritis induced by oxidative stress as the result of severe bacterial infections (Plotnikov et al. 2013). Another recent paper has shown that SkQ1 can prevent or slow down some cerebral dysfunctions in accelerated senescence in rats (Stefanova et al. 2014) and suggest that it could be used as a prophylactic to maintain brain health and to treat Alzheimer's disease.

In view of above effects, one particular aspect is that of the natural polyamines, spermine, spermidine and putrescine, known as essential molecules for a large number of physiological processes (for a review, see Tabor and Tabor 1964; Agostinelli et al. 2004; Toninello et al. 2004). Polyamines interact with mitochondrial membranes at specific sites involved in their electrophoretic transport in mitochondria (Grancara et al. 2014). In particular, spermine can also flow from organelles by promoting the cross-membrane cycling which regulates the induction of MPT (Agostinelli et al. 2010). Polyamines in cells are oxidized by cytosolic (Toninello et al. 2004; Agostinelli et al. 2010) and mitochondrial amine oxidases (Stevanato et al. 2011; Bonaiuto et al. 2015) with the production of hydrogen peroxide and aminoaldehydes, both of which are involved in the induction and/or amplification of MPT. However, this phenomenon, which causes loss of the bioenergetic capacity of mitochondria, together with oxidative stress, is strongly inhibited by polyamines in isolated mitochondria (Toninello et al. 2004). Instead, monoamines as well as the diamine agmatine, have an inhibitory effect at higher concentrations, but at low concentrations they behave as inducers. In particular, this dual effect has been observed with agmatine in liver mitochondria (Battaglia et al. 2007). It should also be noted that, in brain mitochondria, unlike liver mitochondria, the only agent able "to protect" against MPT is spermine (Grancara et al. 2012). According to their cytosolic and matrix concentrations, metabolic conditions, presence of effectors, and cell type, polyamines act as inducing, modulating or preventive agents in intrinsic apoptosis. Although their protective effect is due to inhibition by various mechanisms of MPT induction and retention of pro-apoptotic factors, the inducing effect is explained by the production of ROS, as described above, which, in the absence of sufficient protection by the mitochondrial safety systems, trigger MPT induction and release pro-apoptotic proteins. Polyamines, in particular spermine, can also take part in regulating intrinsic cell death pathways by interacting with the mitochondrial tyrosine phosphorylation/dephosphorylation system (see "Tyrosine phosphorylation, ROS, polyamines and mitochondria").

As noted above, spermine and the other polyamines can protect against induction of MPT in isolated mitochondria. Several mechanisms have been suggested to explain this effect, including protein phosphorylation/dephosphorylation (Lapidus and Sokolove 1992) and interaction with a specific permeability transition inhibitor site in the matrix or with anionic head groups of inner membrane phospholipids, in particular, the cardiolipin anular domain of AdNT (Lapidus and Sokolove 1993). It has also been proposed

that spermine increases the affinity of ADP for its inhibitory binding site (Lapidus and Sokolove 1994). Another proposal is that the binding of spermine to site S_1, responsible for polyamine transport, is also competent in inhibiting MPT (Dalla Via et al. 1996). A report on the effect of spermine on MPT induced by the above-mentioned genistein, glycyrrhetinic acid and salicylate provides important information on the protective effect of this polyamine at molecular level (Sava et al. 2006). It demonstrates that these compounds induce oxidative stress, detected by the oxidation of thiol groups, glutathione and pyridine nucleotides, which predispose the membrane to subsequent opening of the transition pore when Ca^{2+} is also added. In these conditions, spermine can prevent oxidation, even when these species are strongly oxidized, i.e., it behaves like a typical free-radical scavenger. This statement is also confirmed by the protective effect exhibited by spermine on lipid peroxidation and protein oxidation. Again, Casero's research group demonstrated in vitro DNA protection by spermine against radical scavenging (Ha et al. 1998). The proposed mechanism for this is that spermine reacts with the hydroxyl radical generated by H_2O_2, producing spermine dialdehyde through a series of intermediates such as bis-*N*-hydroxyspermine and spermine-bis-oxime (Ha et al. 1998). A very recent paper (Giorgio et al. 2013) proposes another mechanism of spermine protection against MPT: the opening of the transition pore by carbenoxolone, a derivative of glycyrrhetinic acid (Salvi et al. 2005a, b), may be induced via the translocator protein TSPO and connexin 43. The authors propose that MPT is triggered by a series of signal translations from cAMP to AdNT through the acyl CoA binding domain containing protein 3 and TSPO. In turn, AdNT can translate the phosphorylation signal to subunit C of F_0-ATP synthase, modulating MPT induction (Azarashvili et al. 2014). It is proposed that carbenoxolone binds to TSPO or the acyl CoA domain, thus increasing the local concentration of cAMP and then inducing TSPO phosphorylation. This event would induce MPT. If spermine can interact with the process of protein phosphorylation, one intriguing proposal is that the polyamine acts at this level to prevent MPT.

Tyrosine phosphorylation, ROS, polyamines and mitochondria

It has become increasingly clear that protein phosphorylation has a profound influence on mitochondria, with protein kinases and phosphatases which regulate the action of critical players involved in the disparate functions of these organelles (Hüttemann et al. 2007; Pagliarini and Dixon 2006; Hebert-Chatelain 2013). More specifically, tyrosine phosphorylation in mitochondria has recently emerged as central to cell biology, several targets being involved in the modulation of virtually all mitochondrial functions, with considerable implications for human disease. Of the substrates found to be tyrosine-phosphorylated, we only mention here: (1) proteins believed to be essential components of the permeability transition pore complex, the multiprotein assembly located at the interface between the outer and inner mitochondrial membranes, including the voltage-dependent anion channel (Lewandrowski et al. 2008), hexokinase type I (Lewandrowski et al. 2008; Pantic et al. 2013), AdNT 1 (Lewandrowski et al. 2008; Feng et al. 2008) and subunit γ of ATP synthase (Di Pancrazio et al. 2006); (2) several subunits of each complex of the respiratory chain (Salvi et al. 2007a, b; Ogura et al. 2012) as well as the electron carrier cytochrome *c* (Sanderson et al. 2013); (3) metabolic enzymes such as pyruvate dehydrogenase kinase and phosphatase (Hitosugi et al. 2011; Shan et al. 2014); (4) the protein chaperone tumor necrosis factor receptor-associated protein 1 (Yoshida et al. 2013), to name just a few. The actors involved in mitochondrial tyrosine phosphorylation are classified as receptor and non-receptor tyrosine kinases (abbreviated as RTK and NRTK, respectively), which may either temporarily migrate from the plasma membrane to mitochondria in response to diverse cues, thereby integrating the mitochondrial "potential" into the overall cell signaling network, as in the case of hepatocytes (Gringeri et al. 2009), or constitutively reside within the different mitochondrial compartments, as in neurons (Tibaldi et al. 2008). In the former case, epidermal growth factor receptor and Met should be recalled among the RTKs (Cao et al. 2011; Lefebvre et al. 2013) and Src family kinases (SFKs) among the NRTKs, although in the latter SFKs are those thought to be abundantly and stably represented within mitochondria. More importantly, both families of tyrosine kinases have been shown to translocate to mitochondria in response to proliferative stimuli (Tibaldi et al. 2008; Gringeri et al. 2009). In this regard, data accumulated over the past few years have aroused considerable interest in SFKs, which have been shown to be crucial players not only in pathological conditions, such as cancer, but also in regenerative processes, such as liver regeneration (Gringeri et al. 2009). It should be borne in mind that their activity is significantly affected by changes in the redox status of the environment, oxidation enhancing their activity, in sharp contrast with tyrosine phosphatases (PTPs), which are inhibited by oxidative stress (Giannoni and Chiarugi 2014). Thus, high levels of ROS lead to elevated tyrosine phosphorylation, with potentially dramatic changes in mitochondrial function (Zheng et al. 2013). Interestingly, SFKs are also keys to reducing ROS levels by phosphorylating a component of complex II of the respiratory chain, i.e., flotillin-1 (Ogura et al. 2014), which again emphasizes the importance of this class of enzymes as both regulators and effectors

in mitochondrial physiology. Because spermine, depending on its concentration and also on the metabolic state of mitochondria, has been found to be both scavenger and producer of ROS (Agostinelli et al. 2007), the existence of a ROS–SFK axis has been hypothesized, which may affect spermine transport: this possibility has been experimentally confirmed, albeit in isolated mitochondria. In fact, the data demonstrate that Src, a member of SFKs, is constitutively present in the brain and is a reasonable candidate for phosphorylation of the spermine channel, as assessed by the use of peroxides, irreversible PTP or SFK inhibitors in rat brain mitochondria (Battaglia et al. 2012). However, the protein channel serving as the spermine transporter has not yet been identified and further research is needed to achieve this structural and functional information, which would pave the way to deeper insights into the connections between ROS, SFKs and spermine transport.

Conclusions

The above observations allow us to postulate that a complex network of signals leading to oxidative and phosphorylative events controls pore opening and intrinsic apoptosis by modulating the activity of cytoplasmic and mitochondrial amine oxidases coupled to kinase signaling pathways. Dysregulation of these events is a sign of several diseases, in particular cancer and neurodegeneration, and the mitochondrial transition pore may represent an intriguing target for pharmacological therapies.

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Conflict of interest The authors declare that they have no conflict of interest.

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