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Oxidative Stress and Mitochondrial Dysfunction in Cardiovascular Diseases

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1. Introduction

Reactive oxygen species (ROS) include a wide variety of molecules and free radicals derived from molecular oxygen. O_2 being highly electrophilic can be reduced by one electron at a time producing relatively stable intermediates, such as superoxide anion ($O_2 \bullet^-$), precursor of most ROS and a relevant mediator in many biological reactions. Dismutation of $O_2 \bullet^-$ produces hydrogen peroxide (H_2O_2), which, in turn, may be fully reduced to water or partially reduced to hydroxyl radical ($\bullet OH$), one of the strongest oxidants in nature. The formation of $\bullet OH$ is catalyzed by reduced transition metals. On the other hand, superoxide anion is able to reduce transition metals and intensify in this way hydroxyl generation. In addition, $O_2 \bullet^-$ may react with other radicals, in particular, with nitric oxide ($\bullet NO$) in a reaction controlled by the rate of diffusion of both radicals. The product, peroxynitrite, is very powerful oxidant. The oxidants derived from $\bullet NO$ have been recently called reactive nitrogen species (RNS). Oxidative stress is an expression used to describe various deleterious processes resulting from an imbalance between the formation and elimination of ROS and/or RNS by antioxidant defenses. While small fluctuations in the steady-state concentration of these oxidants play an important role in intracellular signaling, uncontrolled increase in their concentration produces free radical-mediated chain reactions, which indiscriminately target proteins, lipids, polysaccharides, and DNA. Mitochondria are a major source of ROS, converting as much as 0.2-2% of molecular oxygen to superoxide as a by-product of the electron transfer activity (Wittenberg & Wittenberg, 1989; Alvárez et al., 2003). Other enzymatic sources of ROS include NADPH oxidases, located on the cell membrane of polymorphonuclear cells, macrophages, endothelial and other cells, and cytochrome P450-dependent oxygenases, along with the proteolytic conversion of xanthine dehydrogenase to xanthine oxidase, which produces both $O_2 \bullet^-$ and H_2O_2.

Mitochondria's critical role in cardiomyocyte survival and death has become an exciting finding in the field of cardiac biology. Indeed, it is accepted that mitochondrial dysfunction plays a crucial role in the pathogenesis of multiple cardiac diseases, mainly due to the imbalance of the fine interplay between aerobic metabolism, calcium homeostasis, and ROS production. Reactive oxygen species generated in the mitochondria, unless adequately

neutralized, cause mitochondrial oxidative stress and, through reactions with polyunsaturated fatty acids, form lipid hydroperoxides and unsaturated aldehydes that propagate among cellular compartments and react with proteins and nucleic acids. In the myocardium, the oxidative stress cascade impairs several functions, like mitochondrial biogenesis, fatty acid metabolism, ionic homeostasis, and antioxidant defense mechanisms, leading to diminished cardiac energetic efficiency, altered bioenergetics, apoptosis and degradation. Besides the obvious relevance of mitochondria in energy production, new processes like mitochondrial fusion and fission are reported to be linked to ROS generation and now are included in the cast of key players in cardiac disease. In this chapter, we explore the mechanisms of mitochondrial dysfunction driven by ROS generation associated with the pathophysiology of cardiovascular diseases.

2. Mitochondrial reactive oxygen species generation

The standard reduction potential for the conversion of molecular oxygen to $O_2 \bullet^-$ is -0.160 V (Wood, 1987). Given the highly reducing intramitochondrial environment, various respiratory components, including complexes I and III of the mitochondrial respiratory chain (Paradies et al., 2001; Tompkins et al., 2006), flavoproteins (Prosser et al., 2011), iron-sulfur clusters (Napoli et al., 2006), and ubisemiquinone (Wen & Garg, 2008) are thermodynamically capable to donate one electron to oxygen. Moreover, most steps in the respiratory chain involve single-electron reactions, further favoring the monovalent reduction of oxygen. On the other hand, the mitochondrion possesses various antioxidant defenses designed to eliminate both $O_2 \bullet^-$ and H_2O_2. As a result, the steady state concentrations of $O_2 \bullet^-$ and H_2O_2 have been estimated to be around 1×10^{-10} M and 5×10^{-9} M, respectively (Cadenas & Davies, 2000). Mitochondrial sources of $O_2 \bullet^-$ include several respiratory complexes and individual enzymes. Superoxide formation occurs on the outer mitochondrial membrane, in the matrix, and on both sides of the inner mitochondrial membrane (Table 1, Figure 1). While superoxide anion generated in the matrix is mainly eliminated in that compartment, part of the $O_2 \bullet^-$ produced in the intermembrane space may be carried to the cytoplasm via voltage-dependent anion channels (Han et al., 2003). The relative contribution of every site to the overall $O_2 \bullet^-$ production varies from organ to organ and also depends on whether mitochondria are actively respiring (State 3) or if the respiratory chain is highly reduced (State 4) (Barja, 1999). Complex III appears to be responsible for most of the $O_2 \bullet^-$ produced in heart mitochondria (Turrens & Boveris, 1980; Turrens et al., 1982), although Complex I is thought to be the primary source of ROS in a variety of pathological scenarios ranging from ageing to Parkinson's disease (Betarbet et al., 2002; Sherer et al., 2003a). The rate of $O_2 \bullet^-$ formation by the respiratory chain is controlled primarily by mass action law, increasing both when electron flow slows down (the concentration of electron donors, R• is higher) and when the concentration of oxygen increases[1] (Turrens et al., 1982).

$$d[O_2]/dt = k \, [O_2] \, [R\bullet] \qquad (1)$$

The electron flow through the respiratory chain establishes an H^+ gradient across the inner mitochondrial membrane, used to drive ATP synthesis through the ATP synthase complex (Complex V). In the absence of ADP, the movement of H^+ through ATP synthase is ceased and the H^+ gradient builds up causing slowdown of electron flow and reduction of the

respiratory chain (State 4). As a result, the physiological steady state concentration of $O_2\bullet^-$ formation increases. The formation of $O_2\bullet^-$ may be further increased in the presence of certain inhibitors (for example rotenone, which inhibits Complex I, or antimycin an inhibitor of Complex III), which cause those carriers upstream from the site of inhibition to become fully reduced. In Complex I, the primary source of $O_2\bullet^-$ appears to be one of the iron-sulfur clusters, whereas in Complex III, most of $O_2\bullet^-$ results from ubisemiquinone auto-oxidation, both on the outer and inner sides of the inner mitochondrial membrane (Table 1).

Component	Localization	References
Complex I (NADH dehydrogenase)	Inner membrane/ inner side	(Turrens & Boveris, 1980; Turrens *et al.* 1982; Genova *et al.* 2001; Kushnareva *et al.* 2002)
Complex II (succinate dehydrogenase)	Inner membrane/ inner side	(Zhang *et al.* 1998; Lenaz, 2001)
Complex III (ubiquinol–cytochrome *c* reductase)	Inner membrane/ inner side	(Boveris *et al.* 1976; Cadenas *et al.* 1977; Turrens *et al.* 1985)
Complex III (ubiquinol–cytochrome *c* reductase)	Inner membrane/ outer side	(Han *et al.* 2001; Starkov & Fiskum, 2001)
External NADH dehydrogenase (yeast)	Inner membrane/ outer side	(Fang & Beattie, 2003)
Glycerolphosphate dehydrogenase	Inner membrane/ outer side	(Drahota *et al.* 2002)
Dehydroorotate dehydrogenase	Matrix	(Forman & Kennedy, 1976)
Mono amino oxidase	Outer membrane/ inner side	(Hauptmann *et al.* 1996; Cadenas & Davies, 2000)

Table 1. Compartmental localization of the main mitochondrial sources of superoxide anion. Modified from Turrens, 2003.

Although $O_2\bullet^-$ production increases as the respiratory chain becomes more reduced, not all mitochondrial inhibitors have this effect. Most of the production of $O_2\bullet^-$ by Complex III is actually inhibited if electron flow between the Rieske Fe–S protein and oxygen is blocked, for example by myxothiazol, cyanide or cytochrome *c* depletion (Turrens et al., 1985). This inhibitory effect indicates that $O_2\bullet^-$ must be produced as a result of the autoxidation of semiquinone (Q•), an intermediate produced in Complex III during the Q-cycle (Trumpower, 1990; Figure 2). Coenzyme Q is fully reduced and converted to ubiquinol (QH_2) in the inner side of the mitochondrial membrane and then migrates to the outer side of the inner membrane carrying two protons that become part of the pool needed to sustain ADP phosphorylation. Once on the outer side of the membrane, one electron is transferred to cytochrome *c*1 (via the Rieske Fe–S protein), resulting in the formation of Q•. In a second cycle, a new QH_2 transfers one of its electrons to the iron-sulphur protein (ISP) and then to cytochrome c1, whereas the second electron reduces cytochrome b_{566} and then cytochrome b_{540} (Turrens et al., 2003). This second electron reduces the Q• produced in the first cycle, yielding QH_2. Despite the high efficiency of redox reactions in the Q cycle, some electrons leak and react with oxygen producing $O_2\bullet^-$ (Figure 1).

Another modulator of mitochondrial ROS production is the membrane potential ($\Delta\psi_m$), thus it has been reported that both, uncouplers and uncoupling proteins (UCPs), minimize ROS production by enhancing proton leak and providing a negative feedback loop for

mitochondrial ROS production. A direct impact of ROS on the glutathionylation status of UCPs has been invoked to explain the activation of such proteins (Mailloux & Harper, 2011).
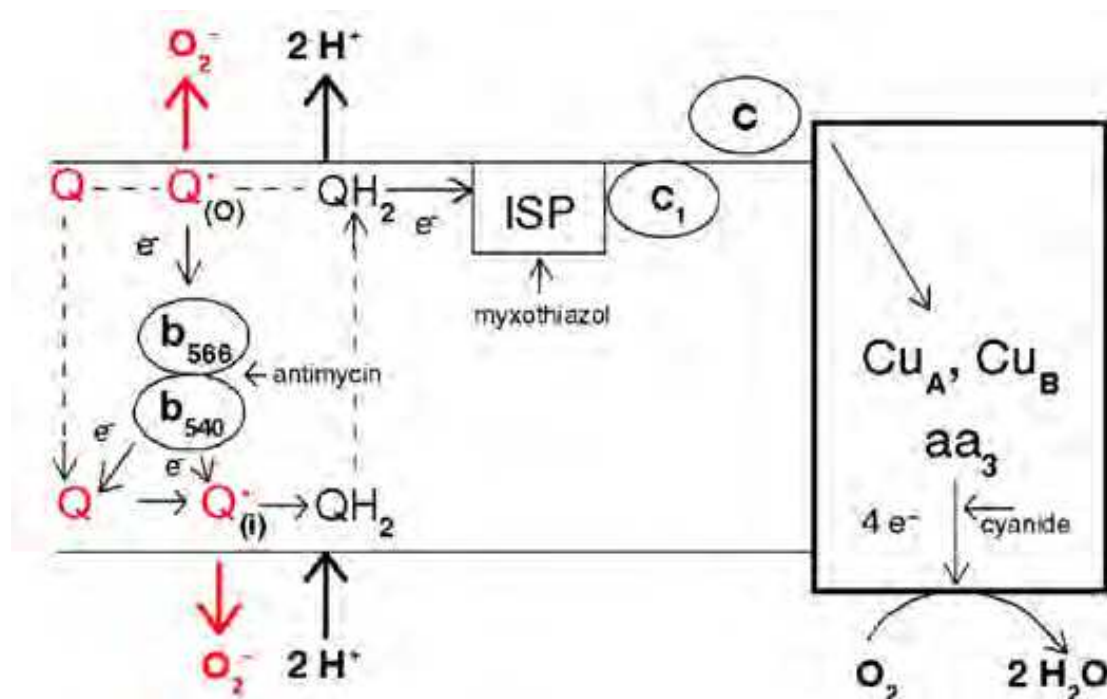

Fig. 1. Electron flow in the Q-cycle. Ubiquinone (Q) is reduced by electrons transferred from NADH or succinate producing ubiquinol (QH_2). The reduced form undergoes a two-cycle reoxidation in which the semiquinone ($Q\bullet$) is a stable intermediate that transfers electrons to oxygen via the Rieske iron-sulfur protein (ISP), cytochrome *c*1 (c1) and cytochrome *c* (c). The terminal oxidase catalyzes the oxidation of reduced cytochrome c and the concomitant four-electron reduction of one O_2 molecule producing water. Superoxide may be produced on both sides of the inner membrane via the autoxidation of Q, but the contribution of each pool has not yet been determined.

Special mention is worth about how Ca^{2+} modulates mitochondrial ROS generation. The primary role of this cation in mitochondria is the stimulation of oxidative phosphorylation by allosteric activation of pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, as well as other proteins of the phosphorylating machinery (Brooks et al., 2004). Thus, the stimulation of oxidative phosphorylation would enhance ROS production, as mitochondria are being forced to work faster and to consume more O_2. Experimental observations are diverse, overall it appears that physiological $[Ca^{2+}]_m$ has no direct effect on respiratory chain function or oxidation/reduction process, however pathological mitochondrial Ca^{2+} overload can lead to ROS increase. Possible mechanisms include Ca^{2+} stimulated increase of metabolic rate; Ca^{2+} stimulated nitric oxide production, which inhibits complex IV; Ca^{2+} induced cytochrome c dissociation that would inhibit the distal respiratory chain; Ca^{2+} induced cardiolipin peroxidation and Ca^{2+} induced mitochondrial permeability transition pore opening (Peng & Jou, 2010).

On the other hand, simultaneous formation of $O_2\bullet^-$ and nitric oxide can produce peroxynitrite, a very strong oxidant and nitrating agent. Nitric oxide is a vasodilator

resulting from the convertion of arginine to citrulline, in a reaction catalyzed by a family of NADPH-dependent enzymes called nitric oxide synthases. It has been reported that the mitochondrial matrix contains a unique form of nitric oxide synthase (Alvarez et al., 2003). Although its physiological role is still unclear, the formation of nitric oxide in mitochondria may have important pathological consequences, as it binds to the heme group of cytochrome oxidase, inhibiting respiration (Poderoso et al., 1996). ROS are also produced within mitochondria at sites other than the inner mitochondrial membrane (Dowrakowski et al., 2008), by proteins such as monoamine oxidase (MAO) and p66Shc (reviewed in Di Lisa et al., 2009).

3. Antioxidant systems in mitochondria

Mammalian mitochondria possess a multi-leveled ROS defense network of enzymes and non-enzymatic antioxidants. Thus, constantly generated ROS, essential for normal cellular physiology and signaling process, are maintained at specific levels by intrinsic antioxidant defenses, avoiding oxidative stress. In healthy mitochondria, ROS contention is driven by manganese-dependent superoxide dismutase (MnSOD), gluthatione peroxidase (GPx), thioredoxin ($TrxSH_2$) and thioredoxin reductase (TrxR), peroxiredoxin (Prx), and glutaredoxin (Grx), as well as water- and lipid-soluble antioxidants, i.e. vitamins C, α-tocopherol (α-toc), reduced glutathione (GSH), and melatonin. It has been proposed that under certain circumstances, the mitochondrial respiratory chain can also contribute to mitochondrial antioxidant defense.

3.1 Non-enzymatic antioxidants

The tripeptide GSH is the main non-protein molecule, containing reactive thiol (-SH) groups, with scavenging properties, that provides an abundant source of reducing equivalents (Stowe & Camara, 2009). GSH reacts with hydroxyl radical (•OH), hypochlorous acid (HOCl), peroxyl radical (RO_2•), carbon-centered radicals, and peroxynitrite anion (ONOO-) producing thiyl radical (GS•), which potentially generates O_2•$^-$ among other ROS (Ježek & Hlavatá, 2005). Despite its exclusive synthesis in the cytosol, GSH is distributed in intracellular organelles, including the endoplasmic reticulum (RE), nucleus, and mitochondrion (Marí et al., 2009; Figure 2). GSH synthesis involves a two step reaction that requires ATP. Glutamate and cysteine are converted to γ-glutamyl-cysteine in a rate-limiting reaction driven by γ-glutamylcysteine synthetase. Then, γ-glutamylcysteine and glycine produce GSH by the action of the enzyme GSH synthetase (Figure 2). The first reaction is inhibited by GSH, a mechanism that regulates cellular GSH concentration (Marí et al., 2009). In the cytosol, GSH concentration is around 11 mM (Griffith & Meister, 1979; Mårtensson et al., 1990) and is transported into the mitochondrial matrix through a non-described high affinity carrier and a low affinity carrier, that could be the mitochondrial oxoglutarate carrier (Coll et al., 2003) or the dicarboxylate carrier (Lash et al., 2002). In mitochondria, GSH levels may fluctuate from 5 to 11 mM (Valko et al., 2007). Mitochondrial GSH plays a critical role in cell survival, as toxic cell death often correlates better with depletion of the mitochondrial GSH pool than with overall intracellular GSH depletion (Orrenius et al., 2007).

The importance of UCPs in the control of mitochondrial ROS generation remains unclear: it is known that UCPs are inner membrane carriers that transfer protons across the mitochondrial inner membrane (MIM), by-passing ATPase (Stuart et al., 2001). A putative
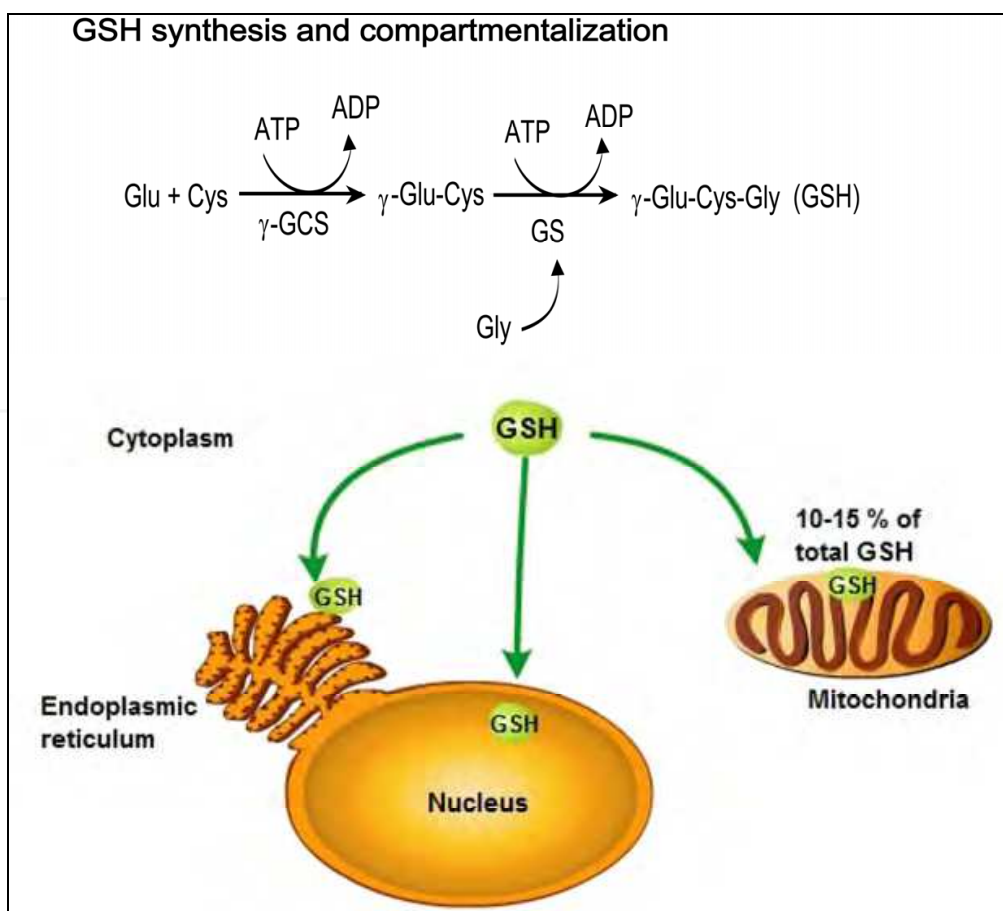
Fig. 2. GSH synthesis and compartmentalization. GSH is synthesized by γ-glutamylcysteine synthetase (γ-GCS) and glutathione synthetase (GS) in the cytoplasm and then redistributed to mitochondria, nucleus, and endoplasmic reticulum.

role in redox homeostasis control has been associated with their capacity to reduce mitochondrial ROS production by modulating Δψ$_m$ (Haines et al., 2010). In fact, UCPs reduce the damaging effects of ROS during cardiac ischemia/reperfusion injury (Barreiro et al., 2009). UCP1 isoform is involved in thermogenesis and expressed specifically in brown adipose tissue mitochondria, in which it confers a regulated proton leak across the inner membrane, whereas the physiologic functions of the other isoforms remain unclear. UCP2 and UCP3 are present in the heart and provide cardioprotection. In rat neonatal cardiomyocytes, UCP overexpression confers tolerance against oxidative stress by a mechanism related with calcium uptake (Sack, 2006b).

α-Tocopherol and melatonin scavenge lipid peroxyl radicals much faster than these radicals can react with adjacent fatty acid side-chains, so they are probably the most important inhibitors of the free-radical chain reaction of lipid peroxidation in animals (Maroz et al., 2009; Gavazza & Catalá, 2009). Aside from its various physiological functions, melatonin could acts as a scavenger of the particularly toxic •OH and carbonate radical, which is important because of their presumed role in mitochondrial damage (Srinivasan et al., 2009, Hardeland & Coto-Montes, 2010). Besides, melatonin could act as an indirect antioxidant promoting *de novo* synthesis of GSH, stimulating the activity of γ-glutamylcysteine synthetase and also through its effects on GPx, Grx, SOD, and CAT gene expression

(Rodríguez et al., 2004), helping in GSH recycling and maintaining a high GSH/GSSG ratio. Melatonin is synthesized and released to the circulation by the pineal gland, and its amphiphilic properties lead to its free access to all compartments in the cell, being concentrated especially in the nucleus and mitochondria (Escames et al., 2010). A direct role of melatonin in regulation of Complex I and IV activity and in other mitochondrial functions has been suggested. This effect, not shared by other antioxidants, would reflect redox interactions with the electron transfer chain complexes, stimulating electron flow, limiting electron leakage, and ROS generation (Escames et al., 2010). Interestingly, it has also been reported that melatonin protects mitochondria from oxidative damage by preventing cardiolipin oxidation (Paradies et al., 2010).

3.2 Enzymatic antioxidants

MnSOD converts superoxide anion ($O_2\bullet^-$) to hydrogen peroxide (H_2O_2) in the matrix side of the inner mitochondrial membrane (Liochev & Fridovich, 2010), while some $O_2\bullet^-$ released into the intermembrane space is partially dismutated by copper-zinc containing superoxide dismutase (CuZnSOD) (Figure 3). Disruption of the MnSOD gene in mice has been
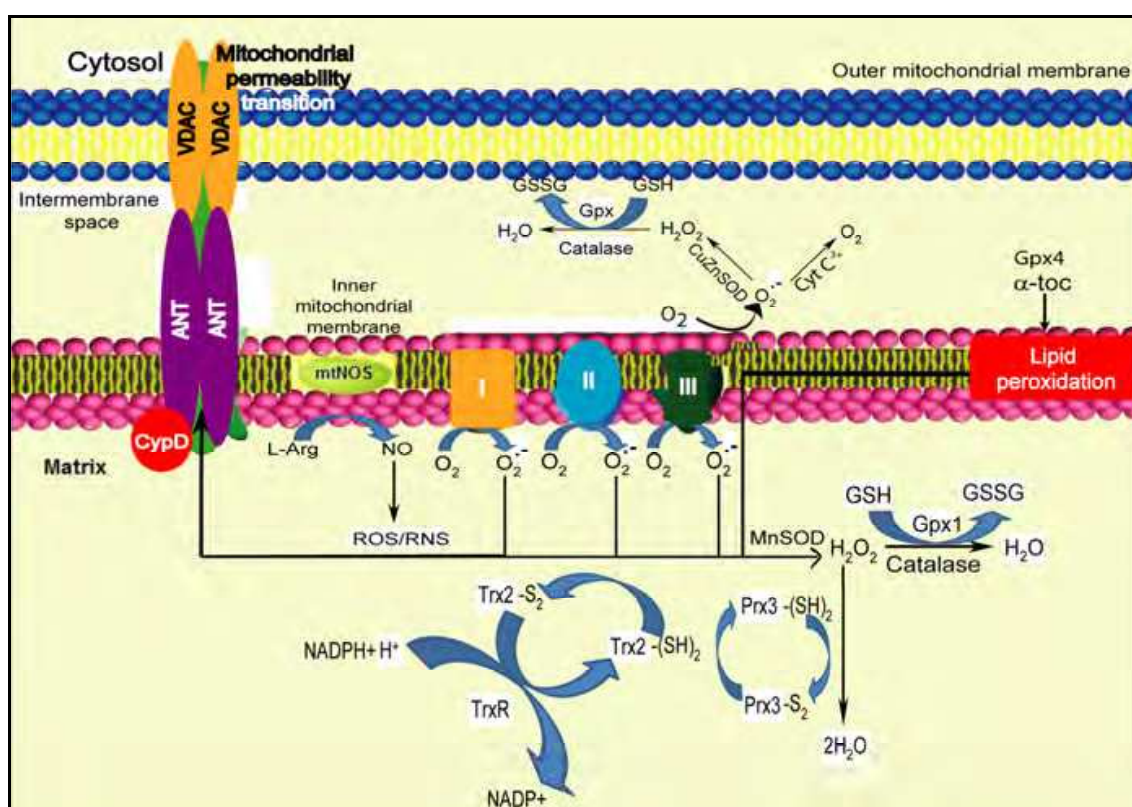

Fig. 3. Mitochondrial antioxidant network. Mitochondria are normally protected from oxidative damage by a network of mitochondrial antioxidant systems. See text for further details. α-toc: α-tocoferol; Cyt c3+ (reduced cytochrome c) ; CuZnSOD: copper-zinc superoxide dismutase; Gpx: glutathione peroxidase; GSH: reduced glutathione, GSSG: oxidized glutathione; L-Arg: L-arginine; mtNOS: mitochondrial nitric oxide synthase, NO: nitric oxide; Prx3-(SH)$_2$: peroxiredoxine 3 reduced, Prx3-S$_2$: peroxiredoxine 3 oxidized; ROS: reactive oxygen species, RNS: reactive nitrogen species; Trx2-(SH)$_2$: tioredoxine 2 reduced, Trx2-S$_2$: tioredoxine oxidized.

associated with early postnatal lethality (Li et al., 1995), while MnSOD overexpression was shown to protect mitochondrial function and block apoptosis (Holley et al., 2010). The activity of MnSOD should be coordinated with H_2O_2-removing enzymes. Thus, H_2O_2 produced by MnSOD could be metabolized by Gpx, Prx, or by catalase that has been found in extremely small amounts in the mitochondrial matrix.

Besides of the importance of GSH as a direct antioxidant, it participates in multiple GSH-linked enzymatic defense systems. Among others, GSH acts as electron donor in the reduction of H_2O_2 and different hydroperoxides by GPx1 and GPx4 (Camara et al., 2011). Five different isoforms of GPx have been identified GPx1, GPx2, GPx3, GPx 4, and GPx 6. GPx1 is the major isoform and is localized predominantly in the cytosol, but a small proportion is also present within the mitochondrial matrix (Orrenius et al., 2007). GPx4 is a unique intracellular antioxidant enzyme that directly reduces peroxidized lipids produced in cell membranes (Nomura et al., 2000). Because GPx4 is membrane-associated, with a fraction localized in the intermembrane space of the mitochondria, possibly at the contact sites of the two membranes, and due to its small size and large hydrophobic surface it can interact with, and detoxify, membrane lipid hydroperoxides much more efficiently than the alternative pathway, phospholipase A_2-GPx1 (PLA2) (Antunes et al., 1995). Hence, GPx4 is considered to be the primary enzymatic defense system against oxidative damage to cellular membranes. Accordingly, GPx4-null mice are embryonically lethal, while the heterozygotes are more sensitive to oxidants than wild type mice (Ran et al., 2003).

Other mitochondrial GSH-linked enzymes are glutaredoxins (Grx), which catalyze glutathione-dependent dithiol reaction, reducing protein disulfides, and monothiol reactions reducing mixed disulfides between proteins and GSH. An interesting member of this family is glutaredoxin 2 (Grx2), which was cloned and found to be present as both mitochondrial and nuclear isoforms (Lundberg et al., 2001). Modeling suggests that the GSH binding site and the hydrophobic surface of Grx2 are similar to those of Grx1 (Lundberg et al., 2001), although Grx2 lacks one of the conserved non-active site cysteine residues of Grx1 (Lundberg et al., 2001), hence it is more resistant to oxidants and oxidized glutathione (GSSG) action. Furthermore, Grx2 can be reactivated directly by thioredoxin reductase (TrxR) as well as by GSH (Johanson et al., 2004). GSSG is reduced by glutathione reductase (GR) with NADPH as a cofactor. In turn, mitochondrial NADPH can be regenerated by matrix dehydrogenases and by reaction of hydride ion transfer, which is proton motive force-dependent, utilizing intramitochondrial NADPH to reduce $NADP^+$. Besides NADPH *per se* can serve directly as a non-enzymatic antioxidant, according to some authors (Kirsch & De Groot, 2001). Another potential source of disulfide reductase activity in mitochondria is the thioredoxin system, which includes thioredoxin 2 (Trx2) and thioredoxin reductase (TrxR2). Trx2 catalyzes the reduction of protein disulfides at much higher rates than Grx (Arner & Holmgren, 2000). This enzyme is important for life, given that disruption of the Trx2 gene in the homozygous mouse causes massive apoptosis and, finally, results in embryonic lethality (Nonn et al., 2003). Specific glutathione S-transferase (GST) isoforms: GSTα1-1, GSTα4-4, and GSTμ1-1, which neutralize reactive molecules such as 4-hydroxy-2-noneal (4-HNE), incorporating GSH to the radical molecule, have been found in mitochondria (Raza et al., 2002).

The intermembrane space of mitochondria contains ~0.7 mM cytochrome *c* (Hackenbrock et al., 1986) capable of superoxide removal. Cytochrome *c* can be alternatively reduced by the

respiratory chain or superoxide. A diminution in its concentration may inhibit the distal respiratory chain and increase ROS production. Indeed, it has been reported that upon cytochrome *c* release during apoptosis initiation, mitochondrial ROS production increases (Cai & Jones, 1998).

As mentioned before, ubiquinone (Q) acts as a pro-oxidant in its semiquinone form, however when fully reduced it acts as an antioxidant. Ubiquinol (UQH_2) contains phenolic hydrogen atoms that can be donated to a carbon- or oxygen centered radical, converting it to a non-radical molecule (Ježek & Hlavatá, 2005). Lipid peroxidation prevention by UQH_2 has been reported (James et al., 2004). Preferential succinate oxidation can provide a tool against excessive accumulation of ROS by increasing the proportion of fully reduced ubiquinone. The antioxidant activity of UQH_2 is independent from the effect of α-tocopherol (Ernster et al., 1992), which acts as a chain-breaking antioxidant, inhibiting the propagation of lipid peroxidation (Maroz et al., 2009). Even more, UQH_2 can efficiently sustain the effect of α-tocopherol by regenerating it from the tocopheroxyl radical, which otherwise must rely on water-soluble agents such as ascorbate (vitamin C) (Ernster & Forsmark-Andree, 1993).

4. Oxidative stress and cardiovascular diseases

The pathological role of increased mitochondrial ROS in heart disease has been established from studies in gene-modified mice with altered mitochondrial antioxidant levels. Deletion of mitochondrial thioredoxin reductase 2 is embryonically lethal in mice because of impaired hematopoiesis and impaired cardiac function (Conrad et al., 2004). Fatal dilated cardiomyopathy in mice is developed after complete deletion of mitochondrial Mn superoxide dismutase (Li et al., 1995). In contrast, attenuated left ventricular remodeling after myocardial infarct was observed in transgenic mice overexpressing mitochondrial peroxiredoxin III (Matsushima et al., 2006) or glutathione peroxidase (Shiomi et al., 2004). Also, mice with a mitochondrial-targeted overexpression of catalase have a prolonged life span and improved cardiac function (Schriner et al., 2005).

A critical role of intracellular Ca^{2+} overload and oxidative stress in the genesis of myocyte dysfunction is well established in cardiovascular diseases like atherosclerosis, hypertension, ischemia/reperfusion damage, cardiac hypertrophy, and heart failure (HF). In general, Ca^{2+} overload can be induced by direct effect of ROS on Ca^{2+} handling proteins or indirectly, by inducing membrane lipid peroxidation (Santos et al., 2011). Recent evidence suggests that redox modification of ryanodine receptor (RyR2) may contribute to abnormal Ca^{2+} handling in disease states. RyR2 dysfunction with an increase in diastolic Ca^{2+} leak from the sarcoplasmic reticulum (SR) may reduce calcium transients and contribute to a reduced contractile force in the failing heart, as well as an increased likelihood of arrhythmia (González et al., 2010). In humans, increased cytosolic calcium ($[Ca^{2+}]_c$) has been related with augmented oxidative stress in atherosclerosis. A growing body of evidence indicates that the production of ROS is tightly linked with Angiotensin II-induced action. In this respect, a causative link between superoxide production and hypertension has been established in experiments in which SOD reduced blood pressure by 50 mm Hg in vascular smooth muscle cells (VSCM) from Angiotensin II-infused rats (Laursen et al., 1997). Much less attention has been paid to other reactions catalyzed by ROS. However, it is known that ATPase activity and inhibition of ATP-independent Ca^{2+} binding are severely depressed in

sarcolemmal membranes exposed to hydrogen peroxide and Fe^{2+} (Kukreja et al., 1992, Kaneko et al., 1989). In addition, augmented levels of iron pool in atherosclerotic lesions suggest that iron-catalyzed formation of free radicals may take place in the development of this pathology (Yuan & Li, 2003). High-fat diets stimulate stress response (heat shock protein 70) and signal transduction genes (Ras, MAPK1), inhibiting SOD and GPx gene expression. These effects could be prevented by scavengers of peroxides and antioxidant supplementation of the high-fat diet and caloric restriction (Rosier & Saes, 2006).

5. Mitochondrial dysfunction

Mitochondrial myopathies were described in the early 1960s, when systematic ultrastructural and histochemical studies revealed excessive proliferation of abnormally looking mitochondria in muscle of patients with weakness or exercise intolerance (Shy & Gonatas, 1964). Mitochondrial dysfunction, reflected in the structure, function and number of mitochondria within the cardiomyocyte, leads to diminished energy production, loss of myocyte contractility, altered electrical properties, and eventual cardiomyocyte cell death (Capetanaki, 2002). In addition, cardiotoxic stimuli often lead to excessive production of ROS and to Ca^{2+} overload in the mitochondrial matrix (Ragoni & Condolini, 2009). Evidences for a pathological role of mitochondrial ROS comes from studies in animal models of myocardial infarction, in which increased mitochondrial ROS production was observed, accompanied by decreases in mtDNA copy numbers, in mitochondrial-encoded gene transcripts, and in related enzymatic activities (complexes I, III, and IV), and from studies of genetically modified animals. Overexpression of Prx-3 (a mitochondrial antioxidant protein) improved post-myocardial infarction left ventricular function by restoring mitochondrial activity and DNA copy numbers (Matsushima et al., 2006). Other examples are studies in mice with complete deletion of mitochondrial MnSOD, which developed severe fatal dilated cardiomyopathy (Li et al., 1995). Decreased vascular SOD activities have also been associated with increased susceptibility to ischemia/reperfusion mediated damage; whereas overexpression of mitochondrial antioxidants increased cardiac tolerance to ischemia (Madamanchi, et al., 2005). Recently, the causal role of mitochondrial ROS in Angiotensin II-induced cardiomyopathy was shown by the observation that mice that overexpress catalase targeted to mitochondria, but not mice that overexpress wild-type peroxisomal catalase, are resistant to cardiac hypertrophy, fibrosis and mitochondrial damage induced by angiotensin II (Dai et al., 2011). Monoamine oxidase (MAO) has been shown to play a prominent role in myocardial injury caused by post-ischemic reperfusion (Bianchi et al., 2005a) and to contribute to the maladaptive evolution from myocardial hypertrophy to heart failure (Kaludercic et al., 2010). MAO-mediated ROS production has been related with serotonin-induced myocyte hypertrophy *in vitro* (Bianchi et al., 2005b) and in mitogenic signaling induction by a process that may involve the activation of the metalloproteinase MMP-2, in smooth muscle cells (Coatrieux et al., 2007).

Mitochondria isolated from hearts of rabbits exposed to hypercholesterolemic diet showed significantly reduced respiration rates (state 3 and state 4) (Kojik et al., 2011), whereas increased cholesterol is related with diminution of the mitochondrial membrane potential and mitochondrial pore opening (Chávez et al., 1998; Martínez Abundis et al., 2007) and activation of apoptosis (Martínez-Abundis et al., 2009).

5.1 Metabolic adaptation

Complex mechanisms have evolved to maintain the balance between myocardial O_2 supply and O_2 consumption under pathological stresses such as hypoxia, ischemia, pressure, and volume overload. These mechanisms induce changes in cardiomyocyte structure and/or function through coordinated changes in gene and protein expression and/or the activities of various proteins. Redox mechanisms are involved in the signaling pathways underlying many of these mechanisms, both via the direct effects of O_2 levels in the cardiomyocyte and through the effects of ROS (Santos et al., 2011). Metabolically, the adult mammalian heart normally uses lipids as the major fuel, and mitochondria supply over 90% of the total ATP through β-oxidation of plasma fatty acids (Opie & Sack, 2002). During hypoxia, under ischemia or settings of increased cardiac workload, there is a substantial increase in glycolytic ATP generation, which may be cardioprotective during ischemia/reperfusion by ensuring an adequate ATP supply for membrane and sarcoplasmic reticulum ion pumps (Opie & Sack, 2002; Correa et al., 2008a). Recent studies suggest that a metabolic shift to glycolysis is related with the redox status in the heart. NADPH has been recognized as a critical modulator of the antioxidant defense through the regeneration of reduced pools of glutathione, while G6PDH activity was shown to be of major importance for the maintenance of redox status, Ca^{2+} homeostasis, and contractile function in cardiomyocytes subjected to oxidative stress (Jain et al., 2003) .

5.2 Mitochondrial biogenesis

Mitochondrial biogenesis can be defined as a chain of events that promote growth and division of preexisting organelles. Mitochondrial biogenesis includes the synthesis, import and incorporation of proteins and lipids, as well as the replication of mitochondrial DNA (mtDNA). Replication and transcription of mtDNA are controlled by mtTFA (mitochondrial transcription factor A), and two specific transcription factors TFB1M and TFB2M (mitochondrial transcription factor B1/B2), an RNA polymerase (POLRMT), and a mitochondrial transcription termination factor (mTERF). The coordination between the expression of mitochondrial and nuclear genes is directed by nuclear respiratory factor (NRF-1 and/or NRF-2), the peroxisome proliferator-activated receptors (PPARs), estrogen receptor (ERR), and co-activators of peroxisome proliferator-activated receptor gamma (PGC-1α) (Scarpulla, 2008). PGC-1α is also involved in regulation of fatty acid oxidation (FAO) and in co-activation of ERRα (Figure 4).

Mitochondrial biogenesis decreases in aging, obesity, insulin resistance, dyslipidemia, and hypertension, co-morbidities associated with cardiovascular diseases. Impaired activation of the renin-angiotensin-aldosterone system (RAAS) has been associated with such pathologies (Cooper et al., 2007). In fact, elevated levels of angiotensin II (Ang II) and aldosterone promote alterations in insulin metabolism, endothelial dysfunction, and loss of myocardial function (Kim et al., 2008; Sowers et al., 2009). RAAS increases the activity of NADPH oxidase and stimulates ROS generation resulting in mitochondrial damage, decreased ATP production, diminished NO availability, and attenuated mitochondrial biogenesis. Clinical and experimental observations report the loss of expression of PGC-1α, and mtTFA NRFs in hypertension (Whaley-Connell et al., 2009). In addition, down-regulation of the mitochondrial biogenesis co-activator PGC-1α and its downstream nuclear

factors have been associated with myocardial contractile dysfunction, intracellular Ca^{2+} mishandling, ROS accumulation, mitochondrial damage, and loss of mitochondrial density and mtDNA content in high-fat diet–induced obesity (Dong et al., 2007).
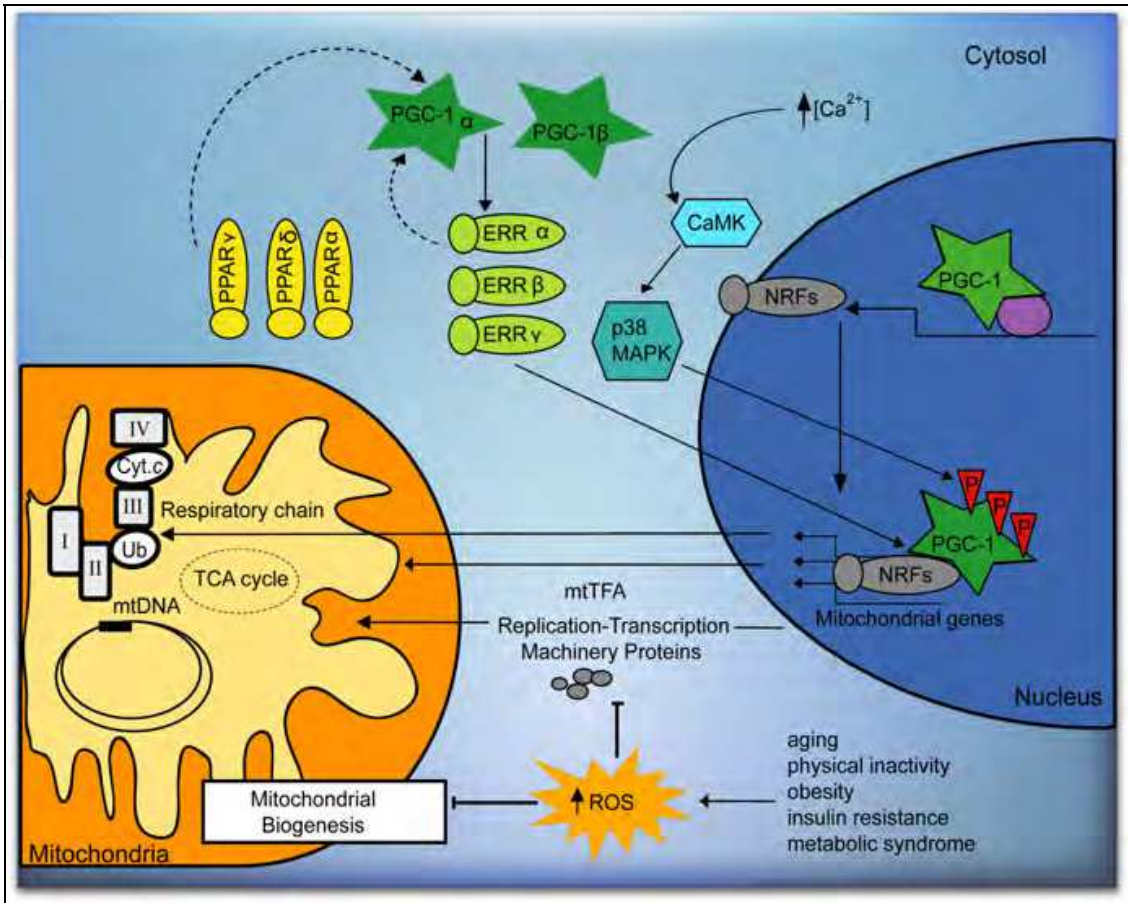

Fig. 4. The transcriptional network that controls mitochondrial biogenesis. See text for further details.

5.3 Mitochondrial ion channels

5.3.1 Permeability transition pore

General membrane damage secondary to ROS-mediated lipid peroxidation is one mechanism by which changes in mitochondrial permeability can occur; however, severe oxidative stress accompanied by calcium overload in the mitochondrial matrix favors the formation of the pathological and non-specific mitochondrial permeability transition pore (mPTP). Opening of the mPTP induces depolarization of the mitochondrial inner membrane (MIM) leading to ATP depletion and further ROS production. The increase in MIM permeability enhances colloidal osmotic pressure in the mitochondrial matrix ultimately leading to matrix swelling and rupture of the mitochondrial outer membrane (MOM). Rupture of the MOM results in release of pro-apoptotic proteins from the mitochondrial intermembrane space to the cytoplasm initiating both caspase-dependent and caspase-independent apoptosis. Permeabilization of the MOM may also occur due to formation of non-selective channels induced by translocation of pro-apoptotic Bcl-2 family proteins to

mitochondria. Due to its central role in cell death triggering, the mPTP represents a potential therapeutic target in some cardiovascular diseases. Studies performed over 20 years have demonstrated that acute cardiac ischemia followed by reperfusion damage is associated with mPTP opening (Arteaga et al., 1992; Griffiths & Halestrap, 1993; Chipuk et al., 2006; Lucken-Ardjomande et al., 2008; Halestrap and Pasdois, 2009). Furthermore, pharmacological and conditional inhibition of mPTP formation significantly improved cardiac function reducing ischemic injury and myocardial infarction size in animal models (Argaud et a., 2005; Hausenloy & Yellon, 2003; Correa et al., 2008b) and in patients (Shanmuganathan et al., 2005; Piot et al., 2008). mPTP opening also causes cell death in isolated endothelial and vascular smooth muscle cells. Indeed, atherosclerosis is exacerbated when mitochondrial antioxidant defenses are hampered and a decrease in mitochondrial ROS formation reduces atherogenesis.

Regulation of the mPTP by a variety of signaling molecules and cellular metabolites and ions is a complex process, and mPTP formation ultimately depends on the balance between factors favoring and inhibiting pore opening (Figure 5). The precise metabolic role of mPTP formation
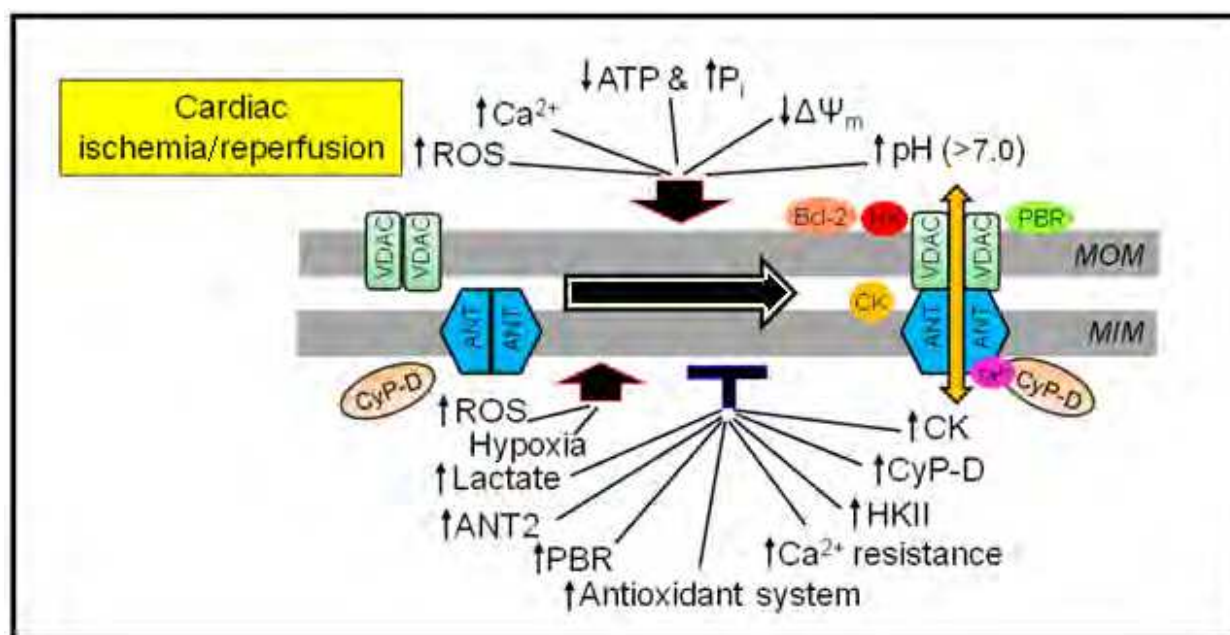

Fig. 5. Metabolic inducers and inhibitors of mPTP opening in cardiac ischemia/reperfusion and tumorigenesis. ANT, adenine nucleotide translocase; CK, creatine kinase; CyP-D, cyclophilin D; HK, hexokinase; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; Pi, inorganic phosphate; PBR, peripheral benzodiazepine receptor; SOD, superoxide dismutase; VDAC, voltage-dependent anion channel.

under physiological conditions is still being debated; reverse pore opening with a low conductance may be attributed to regulation of mitochondrial Ca^{2+}. Low conductive mPT-induced matrix swelling and increased mitochondrial Ca^{2+} can regulate ATP synthesis through the tricarboxylic acid cycle, electron transport chain, and oxidative phosphorylation. Induction of mitochondrial permeability transition (mPT) in response to pathological stresses can also be regulated with the signaling protein kinases: PKA, PKCε

(protein kinase Cε) and GSK-3β (glycogen synthase kinase-3), which interact with the voltage-dependent anion channel (VDAC) (Bera et al., 1995; Baines et al, 2003; Javadov et al., 2009).

5.3.2 Structure of the mPTP complex

Although the crucial role of mPTPs in pathological conditions has been intensively studied in the heart, brain, and liver (Chipuk et al., 2006; Bernardi et al., 2006; Robertson et al., 2009), the actual molecular composition of the mPTP complex remains unclear. Until recently, three proteins had been accepted as key structural components of this megachannel: adenine nucleotide translocase (ANT), cyclophilin D (CyP-D), and the voltage-dependent anion channel (VDAC) located in the MIM, in the matrix and in the MOM, respectively. However, recent studies from different groups have questioned the molecular identity of the mPTP. A new model of the mPTP consisting of a phosphate carrier and ANT has been proposed where Ca^{2+} sensitivity of the pore is regulated by CyP-D binding to the phosphate carrier (Leung et al., 2008). Many studies have provided strong evidence that CyP-D plays a major regulatory role in mPTP formation (Baines et al., 2005; Nakagawa et al., 2005). Mitochondria isolated from CyP-D knockout mice were desensitized to the onset of the mPT, and required much higher concentrations of Ca^{2+} to induce pore opening compared to wild type animals, which is consistent with the role of CyP-D to regulate Ca^{2+}-mPTP interactions (Nakagawa et al., 2005). Recent studies on transgenic mice questioned the role of ANT and VDAC as essential components of the mPTP suggesting a regulatory, rather than structural role in pore formation.

5.3.3 mPTP formation in cardiac Ischemia/Reperfusion (I/R)

mPTP opening has been examined extensively in cardiac pathological conditions, mostly in I/R (Chipuk et al., 2006; Correa et al., 2007; Halestrap & Pasdois, 2009). Acute I/R does not affect the expression of mPTP compounds due to its short duration, although it induces conformational changes in essential mPTP proteins, modifying their interactions with the pore effectors in the cytoplasm and mitochondrial matrix. Although many factors that induce pore opening are present during ischemia, including ATP depletion, Ca^{2+} overload, increased phosphate and ROS levels, it has been demonstrated that pore opening occurs during reperfusion rather than during ischemia (Griffiths and Halestrap, 1995). This is explained, in part, by the acidic conditions resulting from lactate and other acidic intermediates accumulation in the mitochondrial matrix. We and others demonstrated that delayed pHi recovery during reperfusion exerts beneficial effects on post-ischemic cardiac function, associated with improved mitochondrial function and inhibition of mPTP opening (Javadov et al., 2008; Correa et al., 2008a). In this regard, inhibition of the Na^+/H^+ exchanger 1(NHE-1) may be a promising therapeutic strategy against I/R damage (Linz & Busch, 2003; Karmazyn et al., 2001). mPTP opening causes mitochondrial uncoupling, thereby ATP is hydrolyzed rather than synthesized in the post-ischemic heart leading to myocardial death (Correa et al., 2005). mPTP opening also increases in Ca^{2+}-induced cardiomyopathy (Nakayama et al., 2007), in diabetic cardiomyopathy (Oliveira et al., 2003), in heart failure following myocardial infarction (Javadov et al., 2005), and in intracoronary microembolization (Sharov et al., 2007).

5.3.4 Mitochondrial K$_{ATP}$ channels

Mitochondrial ATP-sensitive potassium channels (mK$^+_{ATP}$) were described about 20 years ago in mitoplasts obtained from rat liver mitochondria. First advances in mK$^+_{ATP}$ research demonstrated that different drugs stimulate the opening of mK$^+_{ATP}$ channels decreasing mitochondrial potential ($\Delta\psi_m$) and that mK$^+_{ATP}$ channels are involved in mechanisms regulating cell volume (Garlid, 1988). Latter, it was observed that bradykinin (Yang et al., 2004), opioids (Jang et al., 2008), and adenosine (Kin H, 2005), activate signaling cascades that induce the opening of mK$^+_{ATP}$ and provide cardioprotection against ischemia/reperfusion injury. Recently a causative link between the opening of mK$^+_{ATP}$ and cardioprotection conferred by post-conditioning has been proposed. Garlid et al. (2008) suggested that post-conditioning induces protection via an early redox-sensitive mechanism, followed by persistent mK$^+_{ATP}$ activation. A complex signaling system involving mitochondrial PKCε 1 and 2 may prevent the formation of the mPTP. The putative signaling cascade includes the activation of Gi protein-coupled receptors and cGMP-dependent protein kinase (PKG). At mitochondrial level, this kinase binds to a hypothetic receptor, called "R1" in the mitochondrial outer membrane. This receptor may phosphorylate PKCε 1 (Jaburek et al., 2006), which, in turn, phosphorylates the mK$^+_{ATP}$ channel favoring its opening. Once activated, the entry of K$^+$ may produce alkalinization of the mitochondrial matrix and promote ROS production by Complex I. ROS could activate the mitochondrial matrix PKCε 2, and prevent the formation of mPTP, reducing cell death and infarction size (Costa et al., Garlid et al., 2009) (Figure 6).

Other proposals have been invoked to explain cardioprotection. One hypothesis is that K$^+$ flow into the matrix may depolarize the inner membrane and reduce the driving force that sustains Ca^{2+} overload (Holmuhamedov et al., 1991; Murata et al., 2001). However, although Ca^{2+} reduction in the mitochondrial matrix may reduce mPTP opening in the post-ischemic heart, it is difficult to explain how a small depolarizing effect generated by activation of mK$^+_{ATP}$ channels could avoid Ca^{2+}-overload. Another assumption is that discrete mitochondrial swelling associated with K$^+$ flow would change the architecture and respiratory control of mitochondria, creating a state of mitochondrial "super-efficiency" (Garlid, 2000). Whatever the mechanism involved in mK$^+_{ATP}$ opening, its association with myocardial protection is clear.

6. Programmed cell death and autophagy

ROS/RNS can cause cell death by non-physiological (necrotic) or regulated pathways (apoptotic) in many cardiovascular diseases such as atherosclerosis, ischemic heart disease, heart failure, stroke, hypertension, and diabetes. The mechanisms by which ROS/RNS cause or regulate apoptosis typically are caspase-dependent and include the activation of membrane receptors, Bcl-2 family proteins, and mitochondrial dysfunction. Autophagy, a caspase-independent mechanism of cell death that protects cells against oxidative damage and is involved in the degradation and recycling of oxidized proteins and damaged organelles in cells, yields amino acids for *de novo* protein synthesis or energy provision (Nishida et al., 2009). While programmed cell death participation in cardiovascular diseases is well established, insights into caspase-independent mechanisms of cell death have emerged recently.
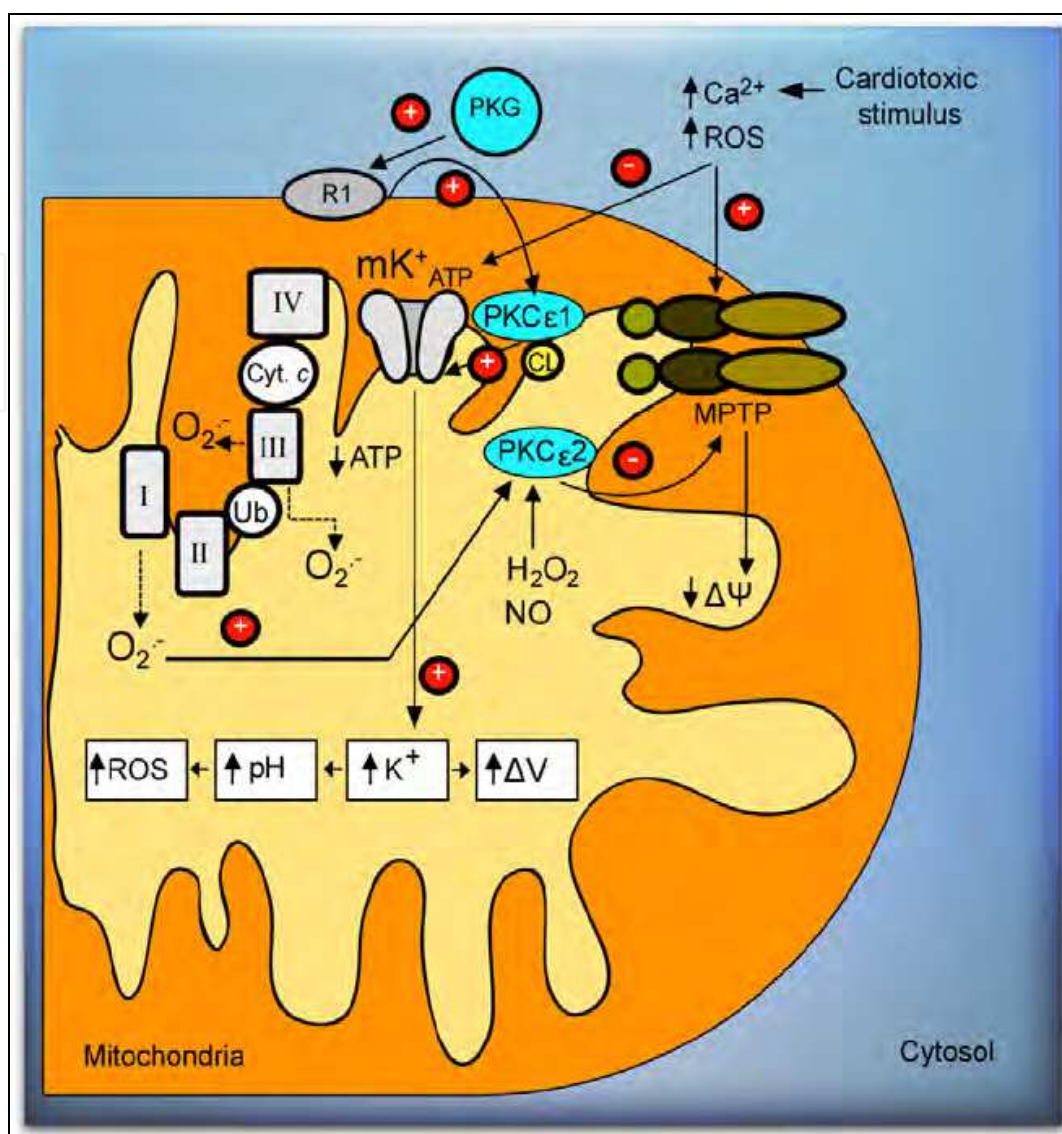
Fig. 6. Intramitochondrial signaling pathways. The pathways leading to mK^+_{ATP} opening, ROS production, and MPT inhibition are shown.

6.1 Apoptotic mitochondrial pathway

Mitochondria contain diverse pro-apoptotic factors within their intermembrane space, such as cytochrome *c*, apoptosis-inducer factor, and Smac / Diablo, which are released and propagate the death cascade. Two different pores have been described as pathways for cytochrome *c* release (reviewed in Kinally & Antonsson, 2007). The first one is the mitochondrial apoptosis-induced channel (MAC) formed by Bax and VDAC proteins (Shimizu et al., 2000). There are also reports indicating that only Bax-forming channels could account for cytochrome *c* release (Kuwana et al., 2002). In this sense, the contribution of Bax channels to cytochrome *c* release after reperfusion has been explored by Bombrun et al., 2003. A second possible pathway described for cytochrome *c* release is the mPTP. These proteins could be assembled into a continued unspecific channel, promoting mitochondrial swelling, MOM rupture, and pro-apoptotic proteins release (Figure 7).
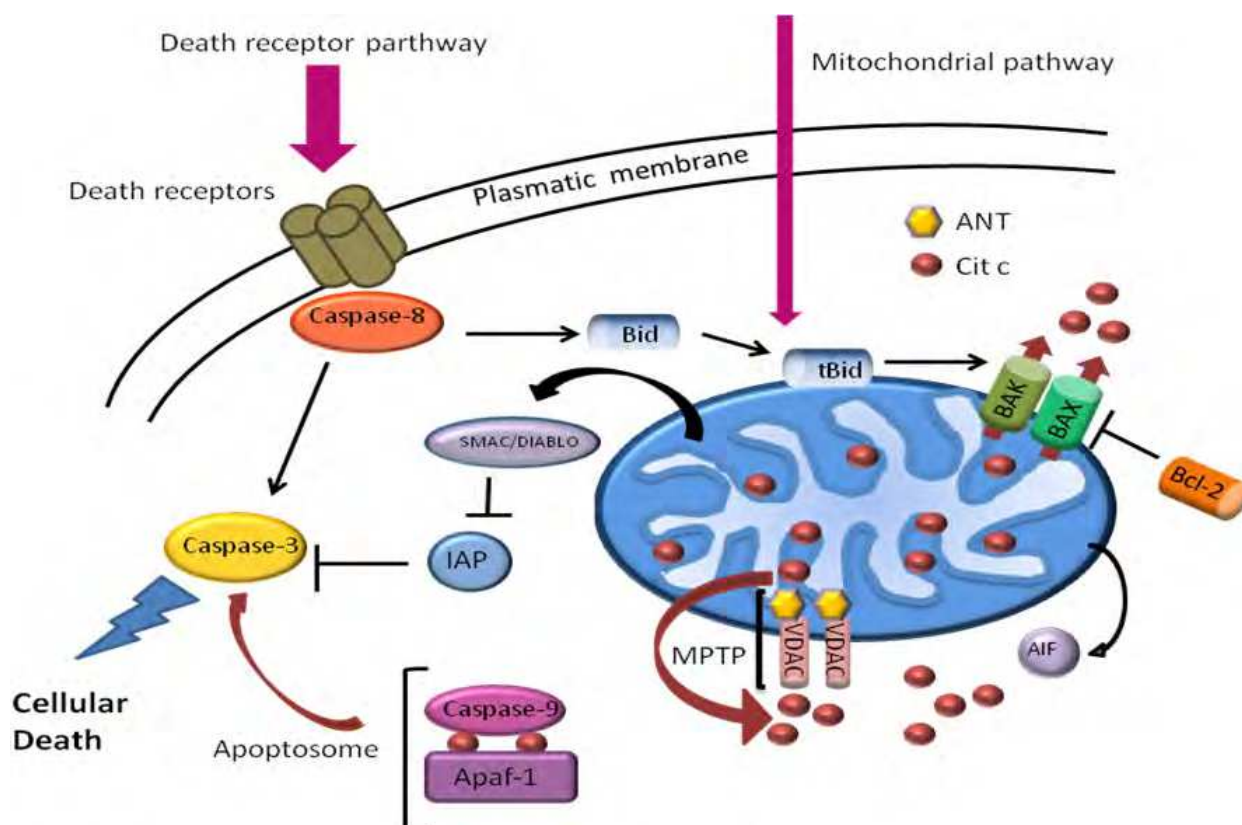
Fig. 7. Simplified model of cellular apoptotic pathways. Specific ligands bind to death receptors, activating initiator and executioner caspases. In the mitochondrial pathway, several stimuli are processed favoring membrane permeabilization and the release of pro-apoptogenic factors as cytochrome *c*, AIF, and Smac/DIABLO to the cytosol, through membrane pore forming proteins, such as BAK/BAX or as a consequence of the mPTP opening. Once in the cytosol, cytochrome c and APAF-1 bind to caspase-9, activating caspase-3. The extrinsic pathway could activate the mitochondrial pathway, through the Bcl-2 family member (Bid) that promotes BAX and BAK oligomerization.

Overexpression of antioxidant proteins in several models sustains the relevance of oxidative stress in cardiomyocyte apoptosis. Catalase, glutathione peroxidase 1, metalothionein, mitochondrial glutaredoxin-2, and peroxiredoxin 2 over- expression reduce apoptosis and improve contractile dysfunction after ischemia/reperfusion injury (Shiomi et al., 2004; Nagy et al., 2008; Zhao et al., 2009). As indicated, oxidative stress may activate the intrinsic apoptotic pathway in cardiomyocytes through multiple mechanisms, such as the induction of mPTP opening, DNA damage-induced, translocation of Bax and Bad to the mitochondria, and caspase activation. However, an alternative mode of oxidative stress-induced activation of the intrinsic apoptotic pathway may also involve induction of the ER stress response, leading to caspase-12 activation and/or Ca^{2+}-dependent opening of the mPTP (Foo et al., 2005). Catecholamines, angiotensin II, prostaglandin $F_{2\alpha}$, or endothelin-1, which interact

with G-protein coupled receptors and induce cardiomyocyte hypertrophy, may also induce apoptosis. A well characterized mechanism is a $G_{\alpha q}$-mediated PKC-dependent transcriptional upregulation of the Bcl-2 family member Nix, which activates the mitochondrial death pathway (Yussman et al., 2002)

6.2 Autophagy

Autophagy is a physiological process that is necessary for cell survival and maintains stable levels of nutrients to sustain cellular homeostasis. It is also involved in various pathophysiological processes, and shows increased activity in response to extracellular and intracellular stimulation such as nutrient starvation and hypoxia. During autophagy, cytoplasmic constituents are sequestered into the autophagosome, a closed double membrane vacuole that eventually fuses with a lysosome. In the new structure, named autolysosome, the contents are degraded and recycled for protein synthesis (Cuervo, 2004). ROS act as signaling molecules in the early events of autophagy induction. If the pro-survival attempt fails, ROS cause cell death which, depending on the experimental context, involves either the autophagic or the apoptotic pathway. Mitochondria are both the major source of intracellular ROS and, at the same time, targets of ROS. An enhanced oxidative stress may activate a signaling cascade involving the PKCβ-dependent phosphorylation of p66 protein and its translocation to the mitochondrial matrix. Damaged mitochondria are degraded by a specialized form of autophagy, called mitophagy, in which mitochondrial calcium plays an active role. Normally calcium homeostasis is tightly regulated and occurs at ER-mitochondria contacts where microdomains of high calcium concentration are present. This event causes a variety of responses depending on the amount of Ca^{2+} increase, from stimulation of metabolism and ATP production to ROS production, mPTP opening, and apoptosis (Figure 8). In this respect, recent data have proposed a role of p66Shc in mediating the response of mitochondria to ROS-induced apoptosis or autophagy (Mammucari & Rizzuto, 2010).

Autophagy increases in response to acute myocardial ischemia (AMI), chronic myocardial ischemia, heart failure, and cardiomyopathy degeneration (Cuervo, 2004). The effect of autophagy upregulation is under debate, as some studies have demonstrated that it leads to myocyte death after ischemia/reperfusion (Valentim et al., 2006), but others indicate that autophagy has a cardioprotective effect during myocardial ischemia. Three mechanisms are invoked to explain protection: 1) generation of free amino acids and fatty acids that contributes to maintain the mitochondrial energy supply, improving cell survival (Matsui et al., 2007), 2) elimination of disordered structural proteins, harmful to cardiac myocytes, and 3) removal of damaged mitochondria. In human myocardial depression associated to endotoxemia, Hickson-Bick et al. (2008) described that mitochondrial biogenesis observed in cardiomyocytes may reflect an effort to replace the mitochondria eliminated by autophagy. In HL-1 cells subjected to oxidative stress, the induction of autophagy by rapamycin suppressed ROS production and protected cells against death (Yuan et al., 2009). These results are consistent with the notion that autophagy is a protective mechanism in this setting and limits the production of harmful ROS, either by removing damaged mitochondria or by supporting *de novo* glutathione biosynthesis through the delivery of amino acids.
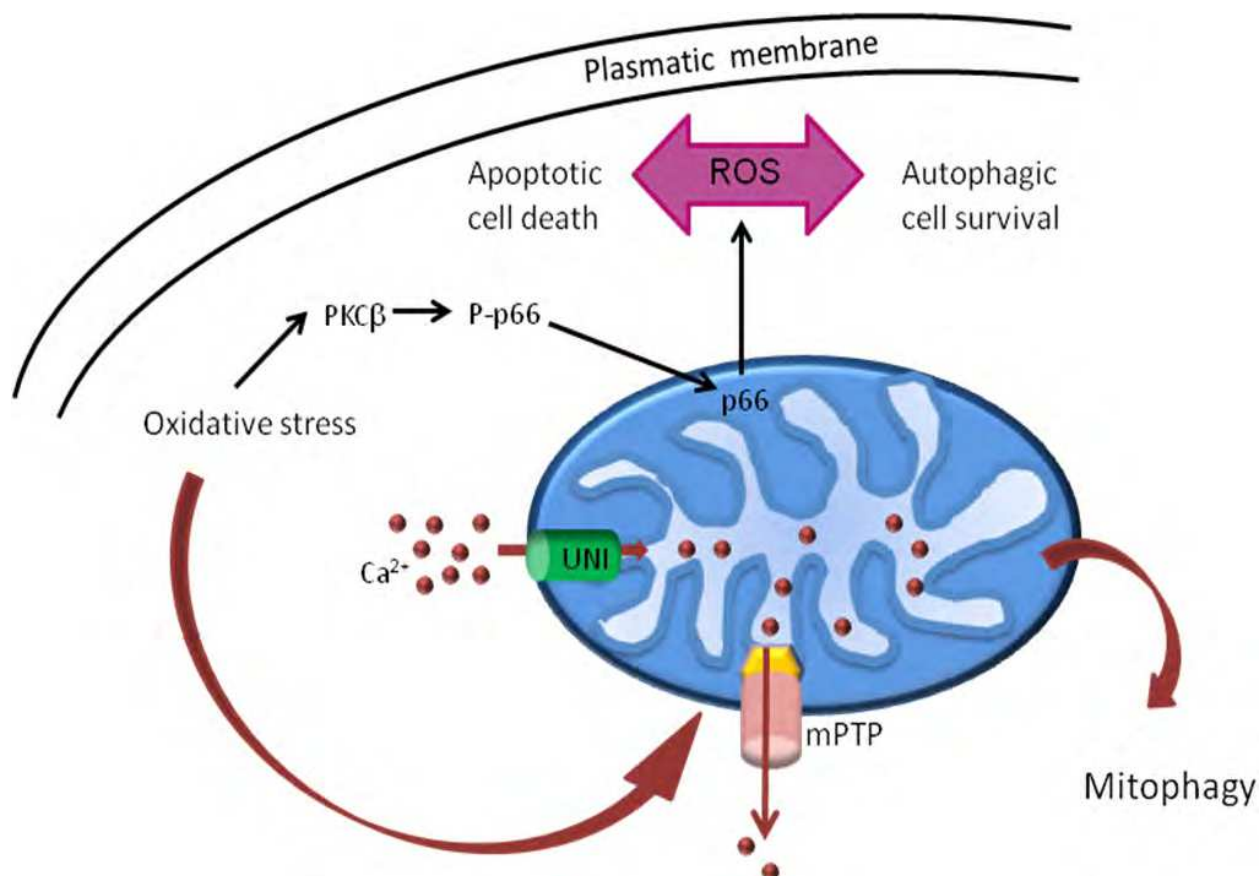
Fig. 8. Signaling pathways, regulating mitochondrial function. ROS production is induced by oxidative stress, which activates a signaling cascade involving the PKCβ-dependent phosphorylation of p66 its translocation to the mitochondrial matrix. Mitochondria are also targets of ROS damage. Damaged mitochondria are removed by mitophagy, a specialized form of autophagy, which is regulated by different pathways.

7. Mitochondrial fission and fusion

Mitochondria are highly dynamic organelles that undergo constantly fusion and fission as part of their normal function (Detmer & Chan, 2007). These two opposite processes are accurately coordinated and necessary for proper morphology and function and are thought to play critical roles during development, cell division, and apoptosis (Cerveny et al., 2007; Chan, 2006). Disruption of mitochondrial fission and fusion has been linked to the development and progression of some diseases.

Mitochondrial fusion facilitates the exchange of materials between mitochondria for the maintenance of functional mitochondria, whereas mitochondrial fission contributes to the elimination of damaged mitochondrial fragments through mitophagy and contributes to the proper distribution of mitochondria in response to the local demand for ATP. Multiple proteins have been identified to mediate mitochondrial fission and fusion processes (Chan, 2006). These two opposing processes are regulated by the mitochondrial fusion proteins, mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2, Fzo1 in yeast) (Eura et al., 2003), and optic atrophy protein 1 (Opa1, Mgm1 in yeast) (Cipolat et al., 2004), and the mitochondrial fission proteins

dynamin-related protein 1 (Drp1, Dnm1 in yeast) (Frank et al., 2001; Smirnova et al., 2001; Ingerman et al., 2005), and human mitochondrial fission protein 1 (hFis1) (Yoon et al., 2003). The balance between mitochondrial fusion and fission within a cell can be disrupted by a myriad of factors, including oxidative stress (Frank et al., 2001) and simulated ischemia (Brady et al., 2006), and has also been linked to aging (Kowald & Kirkwood, 2011) (Figure 9).
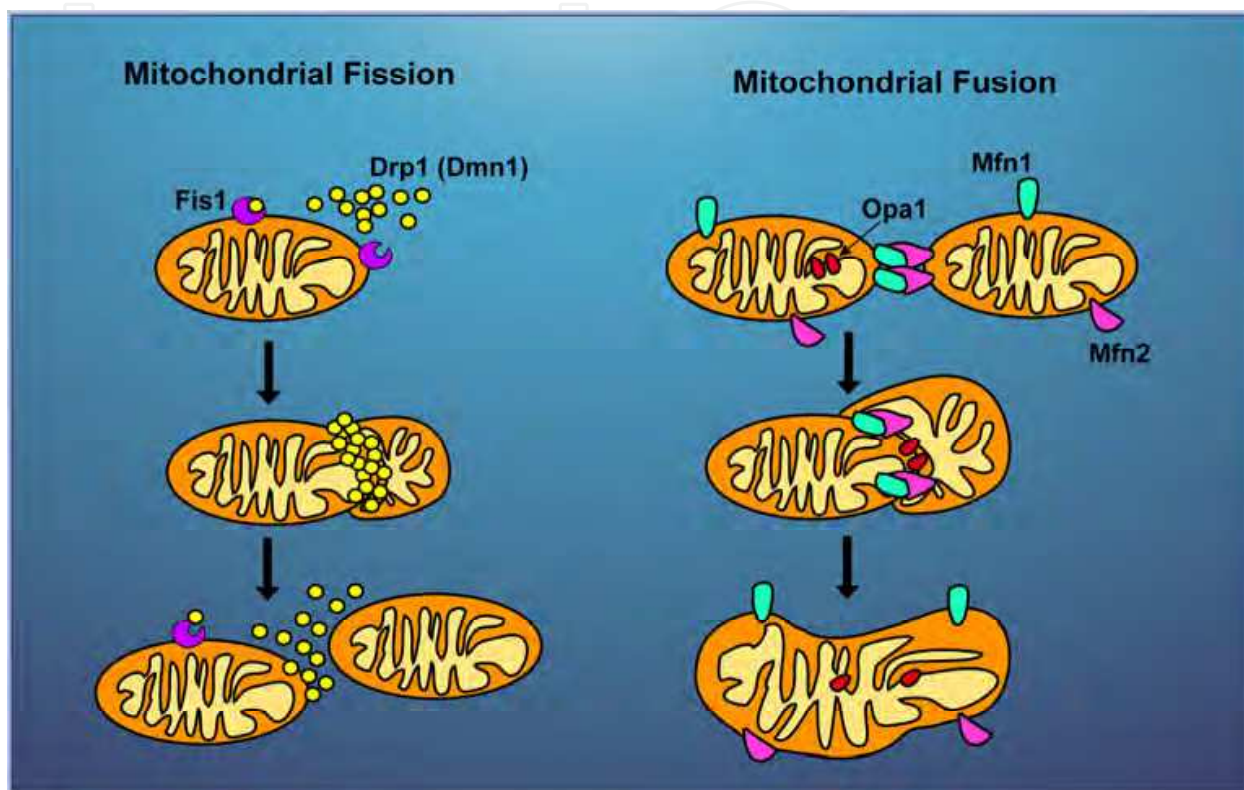

Fig. 9. Mitochondrial fission and fusion. Mitochondrial fission involves the action of Drp1, which can self-assemble into polymeric spirals and is recruited into the mitochondrial membrane by hFis1 and Mdv1/Caf4. Drp1 polymers wrap around the mitochondrion and constrict the membrane until fission occurs. Mitochondrial fusion involves the interaction of Mfn1 and Mfn2 proteins located in the outer mitochondrial membrane of two mitochondria until outer membranes fuse, consequently, inner mitochondrial membrane fusion occurs through interaction of Opa1 proteins. See text for further details.

7.1 Mitochondrial fusion

Data on fission and fusion proteins' role in heart diseases are scarce. Recently it has been reported that cardiac myocyte mitochondria, lacking the fusion protein Mfn2, are pleiomorphic and show enlarged morphopology. Consistent with an underlying mild mitochondrial dysfunction, Mfn2-deficient mice display modest cardiac hypertrophy accompanied by slight functional deterioration (Papanicolau et al., 2011). Expression of OPA1 is decreased in both human and rat failing hearts, which show small and fragmented mitochondria indicative of decreased fusion. OPA1 mRNA levels did not differ between failing and normal hearts, suggesting post-transcriptional control, possibly through degradation by proteases activated by ATP (Baricault et al., 2007).

7.2 Mitochondrial fission

It has been suggested that defects in mitochondrial fusion and fission processes are responsible for abnormal mitochondrial morphologies observed in many cardiac diseases. In cultured neonatal ventricular myocytes, inhibition of mitochondrial fission, by over-expressing a dominant-negative mutant form of Drp1, prevents ROS production, mitochondrial permeability transition pore opening, and subsequent cell death after ceramide treatment (Parra et al., 2008). An increase in the level of cytosolic Ca^{2+} induced by thapsigargin (Tg) causes cardiac mitochondrial fission associated to ROS generation in a Drp1-dependent pathway (Hom et al., 2010). Because calcium overload is a common feature in heart failure (HF), this may increase mitochondrial fission and dysfunction, thus further contributing to the decrease in the metabolic demand of the heart and increasing its injury.

8. Conclusion

Mitochondrial redox signaling is paramount to the maintenance of cardiomyocyte homeostasis. Therefore, oxidative deregulation of mitochondrial key players, like mK^+_{ATP}, mPTP, ionic transporters, metabolism enzymes, and apoptotic machinery, has enormous impact on cardiovascular function. Intense research is devoted to obtain a better understanding of the complex regulatory mechanisms ruling these systems and to enable the development of more specific therapeutic strategies for heart diseases. In addition, fascinating links are beginning to be discovered between mitochondrial function and cardiac physiology and diseases in the context of diverse signaling mechanisms. Besides, proteins with previously known function, like those driving mitochondrial fusion and fission, are now reported to have emergent functions in intracellular calcium homeostasis, apoptosis, and vascular smooth muscle cell proliferation, all, key issues in cardiac disease. These processes broaden the traditional role in energy production undertaken by mitochondria and provide new directions for research in cardiovascular diseases.

9. Acknowledgment

10. References

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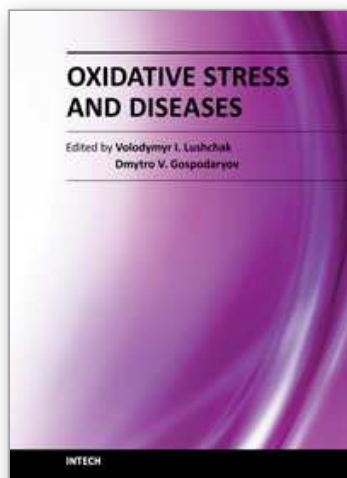
Oxidative Stress and Diseases

Edited by Dr. Volodymyr Lushchak

The development of hypothesis of oxidative stress in the 1980s stimulated the interest of biological and biomedical sciences that extends to this day. The contributions in this book provide the reader with the knowledge accumulated to date on the involvement of reactive oxygen species in different pathologies in humans and animals. The chapters are organized into sections based on specific groups of pathologies such as cardiovascular diseases, diabetes, cancer, neuronal, hormonal, and systemic ones. A special section highlights potential of antioxidants to protect organisms against deleterious effects of reactive species. This book should appeal to many researchers, who should find its information useful for advancing their fields.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

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http://www.intechopen.com/books/oxidative-stress-and-diseases/oxidative-stress-and-mitochondrial-dysfunction-in-cardiovascular-diseases

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