Repositorio Institucional de la Universidad Autónoma de Madrid

https://repositorio.uam.es

Esta es la **versión de autor** del artículo publicado en:
This is an **author produced version** of a paper published in:

Biochimica et Biophysica Acta 1837. 7 (2014): 1099-1112

DOI: 10.1016/j.bbabio.2014.03.010

Copyright: © 2014 Elsevier

El acceso a la versión del editor puede requerir la suscripción del recurso
Access to the published version may require subscription

The H^+-ATP synthase: a gate to ROS-mediated cell death or cell survival

Inmaculada Martínez-Reyes and José M. Cuezva*

Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid (CSIC-UAM), Centro de Investigación Biomédica en Red de Enfermedades Raras CIBERER-ISCIII, Instituto de Investigación Hospital 12 de Octubre, Universidad Autónoma de Madrid, 28049 Madrid, Spain.

Short title: The H^+-ATP synthase signals cell fate

Keywords: Mitochondria, Reactive oxygen species (ROS), H^+-ATP synthase, ATPase Inhibitory Factor 1 (IF1), Cancer, ROS signaling.

*, To whom correspondance should be addressed:

Prof. José M. Cuezva,

Centro de Biología Molecular Severo Ochoa,

Universidad Autónoma de Madrid,

28049 Madrid, Spain.

Phone: 34-91 196 4618; Fax: 34-91 196 4420; E-mail: jmcuezva@cbm.uam.es

Summary

Cellular oxidative stress results from the increased generation of reactive oxygen species (ROS) and/or the dysfunction of the antioxidant systems. Most intracellular ROS derive from superoxide radical although the majority of the biological effects of ROS are mediated by hydrogen peroxide. In this contribution we overview the major cellular sites of ROS production, with special emphasis in the mitochondrial pathways. ROS regulate signaling pathways involved in promoting survival and cell death, proliferation, metabolic regulation, the activation of the antioxidant response, the control of iron metabolism and Ca^{2+} signaling. The reversible oxidation of cysteines in ROS transducers is the primary mechanism of regulation of the activity of these proteins. Next, we present the mitochondrial H^{+}-ATP synthase as a core hub in energy and cell death regulation, defining both the rate of energy metabolism and the ROS-mediated cell death in response to chemotherapy. Two main mechanisms that affect the expression and activity of the H^{+}-ATP synthase down-regulate oxidative phosphorylation in prevalent human carcinomas. In this context, we emphasize the prominent role played by the ATPase Inhibitory Factor 1 (IF1) in human carcinogenesis as an inhibitor of the H^{+}-ATP synthase activity and a mediator of cell survival. IF1 promotes metabolic rewiring to an enhanced aerobic glycolysis and the subsequent production of mitochondrial ROS. The generated ROS are able to reprogramme the nucleus to support tumor development by arresting cell death. Overall, we discuss the cross-talk between ROS signaling and mitochondrial function that is crucial in determining the cellular fate.

List of abbreviations: AIF, apoptosis inducing factor**;** Akt, v-Akt murine thymoma viral oncogene**;** AP-1, activator protein 1; ARE, antioxidant responsive element**;** ASK1, apoptosis signal-regulated kinase 1**;** ATM, ataxia teleangiectasia mutated**;** Duox, Dual oxidase enzymes**;** DUSP3, dual-specific phosphatase 3**;** ETC, electron transport chain; GPXs, glutathione peroxidases; GSH, glutathione; GST, glutathione S-transferase; HIF1α, Hipoxia Inducible Factor 1; HO1, heme oxygenase-1; IER3, immediate early response gene; InsP3R, InsP3 receptor; IF1, ATPase Inhibitory Factor 1; IRE, iron-responsible elements; IRP, iron regulatory protein; JNK1, c-Jun N-terminal kinase 1, monoamine oxidase (MAO); mROS, mitochondrial reactive oxygen species; NFkB, nuclear factor kappa-light-chain-enhacer; NOX, NADPH oxidase; Nrf2, NFE2-like 2; O_2^{-}, superoxide radical, $^{\cdot}OH$, hydroxyl radical; OONO, peroxynitrite; OXPHOS, oxidative phosphorylation, p66Shc, 66 kDa proto-oncogene; SERCA, sarco/endo-plasmic reticulum Ca^{2+} -ATPase; Src homologous-collagen homologue (Shc) adaptor, PI3K, phosphoinositide-3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PP2A, protein phosphatase 2A ; PRXs, peroxiredoxins; PTEN, phosphatase and tensin homolog; PTP, permeability transition pore; PTP1b, phosphotyrosine protein phosphatase; Ref-1, redox factor-1; RNS, reactive nitrogen species; ROS, reactive oxygen species; RyR, ryanodine receptor; SODs, superoxide dismutases; UTR, untranslated region; VHL, von Hippel-Lindau; $\Delta\psi m$, mitochondrial membrane potential.

1. Introduction

Oxidative stress is a phenotypic trait of many tumors. Main causes of this phenotype are the increased generation of reactive oxygen species (ROS) and the dysfunction of the antioxidant systems in cancer cells. ROS generation and scavenging are tightly connected to the metabolic state of the cell and especially to the activity of mitochondria. Nowadays, it is accepted that the roles played by cellular ROS are highly dependent on the level at which they are being produced. In this regard, it has been reported that high levels of ROS lead to increased cell death inhibiting tumorigenesis and metastasis [1], whereas low levels of ROS have an effect in promoting tumorigenesis by activating the signaling pathways that regulate proliferation, angiogenesis and metastasis [2, 3], stressing the relevance of ROS as important signaling molecules that regulate cell fate. In this review we will briefly summarize: (i) the sites of production, mechanism of action and signaling pathways that are activated by ROS and (ii) the role of the mitochondrial H^+-ATP synthase in ROS-signaling cell death or cell survival paying, in the latter case, especial attention to the new physiological function unveiled for the ATPase Inhibitory Factor 1 (IF1) as a main regulator of the oncogenic phenotype in some prevalent carcinomas.

2. ROS dynamics and signaling.

2.1. Major cellular sites of ROS production.

Most intracellular ROS are derived from the superoxide radical (O_2^-), which is the product of the one electron reduction of O_2 (Fig. 1). Superoxide is then converted to hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD1, SOD2 and SOD3) (Fig. 1). The enzymes peroxiredoxins (PRXs), glutathione peroxidases (GPXs) and catalase are responsible for removing cellular H_2O_2 (Fig. 1), a process that is tightly regulated [4]. H_2O_2 can also react with iron to generate hydroxyl radicals ($^{\cdot}OH$) that are main drivers of the modifications in proteins, lipids and DNA that result in oxidative stress (Fig. 1).

Several enzymes produce superoxide radical in the cell. Among them, NADPH oxidase is the best described enzymatic source of superoxide that uses NADPH as an electron donor (Fig. 1) [5, 6]. NADPH oxidases include the Nox family members (Nox1-5) and the Dual oxidase enzymes (Duox1-2) that are expressed in numerous

tissues [6-8]. These enzymes play important roles in cell signaling, regulation of gene expression, cell death, differentiation and growth [9]. Nox enzymes have developed different regulatory mechanisms depending of their function [6, 8, 10-12]. ROS produced by Nox proteins can act both intra- and extra-cellularly. These enzymes generate superoxide at the plasma membrane, in endosomes and in the endoplasmic reticulum [13, 14]. ROS produced by Nox2 have a main physiological role in the respiratory burst that occurs in phagocytes. Nox1 in the colon and Duox1 and 2 in the lung also play important roles in host defense [15]. However, ROS derived from Nox also participate in signaling as they can specifically and reversibly alter the activity, localization and half-life of proteins in response to various stimuli [9]. The phosphoinositide-3-kinase (PI3K) [16] and nuclear factor kappa-light-chain-enhancer of activated cells (NFkB) [13] pathways are two important signaling routes in which NADPH oxidases are involved. Fibroblasts over-expressing Nox1 displayed increased levels of superoxide and exhibited a transformed phenotype [17]. Moreover, it has been described that Nox1 signals angiogenic and tumorigenic effects through hydrogen peroxide [18]. Excess ROS produced by Nox5 have also been related to cancer [19, 20].

A substantial portion of cellular ROS is generated in mitochondria. There are eight sites in mitochondria that have the ability to produce ROS [21]. The mitochondrial electron transport chain (ETC) is the major site of non-enzymatic formation of superoxide radical (Fig. 2). The ETC is composed of four multiprotein complexes (I-IV) located in the inner mitochondrial membrane. Complexes I, II and III have the ability to produce superoxide as a result of the flux of electrons through the ETC. Complexes I, II and III produce ROS within the mitochondrial matrix whereas complex III also generates ROS and releases it into the intermembrane space (Fig. 2) [22]. Importantly, ROS generated in the intermembrane space are supposed to access the cytosol in a faster way what may confer them signaling advantages [3, 23]. ROS are released to the cytosol through voltage-dependent channels that are constituents of the permeability transition pore (PTP) and by the inner membrane anion channel (IMAC) [24, 25]. The transition of ROS from mitochondria to the cytosol is crucial in the regulation of programmed cell death geared by mitochondria [25, 26].

Other important sources of mitochondrial ROS (mROS) are p66Shc and monoamine oxidase (MAO) (Fig. 2). The protein p66Shc plays key roles in the oxidative stress response by inducing apoptosis under stressful conditions (Fig. 2) [27].

p66Shc acts as a redox protein due to its capability to interact and oxidize cytochrome c (Fig. 2) [28]. MAO is a flavoenzyme bound to the outer mitochondrial membrane that catalyzes the oxidative deamination of neurotransmitters and monoamines. MAO represents a significant source of ROS production in brain mitochondria where it has been shown to generate ROS in a much higher amount than the respiratory chain [29]. In fact, MAO is involved in multiple neuropathologies and myocardial diseases and its inhibition is likely to provide a promising target for the relief of the oxidative stress that is associated with these pathologies [30].

The overproduction of ROS in response to metabolic stress triggered by hypoxia or chemotherapy promotes an oxidative stress that has been invariably linked to multiple pathologies including neurodegenerative diseases, diabetes, cancer and premature aging [3]. Nowadays, it is indubitable that mROS are important signaling intermediates in the communication of the organelle with other compartments and cellular processes for maintenance of homeostasis under different conditions and for adaptation to stress [3]. In fact, mROS can balance between survival and cell death in a process that is highly dependent on the levels at which they are being produced [31]. For instance, the mitochondrial release of H_2O_2 in hypoxia activates the transcription factor hypoxia inducible factor 1 (HIF1α), which is required for metabolic adaptation under low oxygen tension [32, 33]. In addition, the mitochondrial release of H_2O_2 has been reported to activate key signaling proteins such as c-Jun N-terminal kinase 1 (JNK1), p53 and NFkB [34-36]. Numerous reports highlighting the importance of mROS-dependent signaling in a variety of systems and processes have emerged in the literature of the last decade [3, 31, 37-39].

It is well established that mROS production is highly dependent on the proton motive force as has been shown by titration of the mitochondrial membrane potential ($\Delta\psi$m) with uncouplers [40-42]. Perhaps, the best characterized site of ROS production in mitochondria is at complex III of the ETC (Fig. 2) [43]. The production of superoxide during forward electron transfer at complex III is low but significantly increases by reverse electron flow under conditions of hypoxia [44] or cellular toxicity [40, 42] from substrates that feed electrons to complex II. The fact that mitochondria produce more ROS under low oxygen levels is intriguing because it seems to contradict the dependence of mROS formation on the availability of oxygen that was observed in isolated mitochondria [43, 45]. It is likely that mROS generated in cells in response to

hypoxia depend on additional factors of the hypoxic cellular environment by mechanisms that remain to be elucidated (see [43] for details). In general, cellular conditions that slow-down the rate of electron transfer to molecular oxygen in the respiratory chain (Fig. 2) at high values of $\Delta\psi m$ favor the generation of superoxide radical in mitochondria. One such situation is provided by inhibition of the back-flow of H^+ through the H^+-ATP synthase (Fig. 2) that is mediated by the over-expression of IF1 in human carcinomas [38, 46-48] (see following section). In this situation, $\Delta\psi m$ provides a link between energy metabolism, ROS production and cell fate [47].

2.2. Cysteine oxidations regulate the activity of ROS transducers.

The regulation of signaling pathways by ROS is exerted by their ability to promote reversible posttranslational modifications of proteins [42, 49, 50]. Hydrogen peroxide is more stable than superoxide radical and is also capable of crossing biological membranes so the protein modifications mediated by ROS are predominantly H_2O_2-dependent. However, superoxide itself has been shown to be involved in aging related processes [51]. H_2O_2 can reversibly oxidize thiol groups (-SH) of redox-reactive cysteine (Cys) residues on proteins to form disulfide bonds (-S-S-) or sulfenic acid (−SOH), the latter can be further oxidize to sulfinic ($-SO_2H$) and sulfonic ($-SO_3H$) acid (Fig. 3) [31, 52]. Sulphenic acid (-SOH) can react with glutathione (GSH) to become glutathionylated (-SSG) (Fig. 3). These oxidative modifications result in changes in the structure of the targeted proteins thereby affecting its activity in the signaling pathways in which they are involved. In addition, the generation of intermolecular disulfide bonds can promote the homo- and/or heterodimerization of proteins that lead to the activation or repression of the signal transducers. Thiol groups can also react with reactive nitrogen species (RNS) forming S-nitrosothiol groups (-SNO) (Fig. 3). With the exception of sulfonic and sulfinic acid that are essentially irreversible reactions the modification of cysteines is reversible by the action of the reducing systems of the cell such as glutathione, thioredoxin and peroxiredoxin (Fig. 3) that are critical elements in redox sensing and signaling (Fig. 3) [53-55]. Phosphatases such as phosphotyrosine protein phosphatase (PTP1b), phosphatase and tensin homolog deleted on chromosome ten (PTEN) and MAPK phophatase are known examples of proteins involved in signaling pathways inactivated by H_2O_2 oxidation of cysteines [56, 57]. Superoxide also reacts with nitric oxide (NO), forming highly reactive and potentially damaging peroxynitrite (OONO−) [58]. The formation of peroxynitrite from O_2^- can lead to

reversible glutathionylation of proteins on reactive cysteines, as has been reported for the Na^+-K^+ ATPase [59]. The inactivation of aconitase by reaction of superoxide with $(FeS)_4$ clusters provides an example of the inhibition of the activity of a metabolic enzyme mediated by the interaction of O_2^- and iron [60, 61].

2.3. Overview of signaling pathways regulated by ROS.

Low or transient ROS levels can activate kinases and/or inhibit phosphatases involved in a wide variety of cell signaling processes by oxidizing critical cysteine residues of the proteins (Fig. 4) [62, 63]. Proteinases and matrix metalloproteins have also been described as ROS targets [64]. Thioredoxin that catalyzes the reversible reduction of disulfides to a dithiol in ROS targeted proteins (Fig. 3) interacts in its reduced state with apoptosis signal-regulated kinase 1 (ASK1) that is activated under oxidative stress (Fig. 4) [65]. Thioredoxin-ASK1 interaction blocks oligomerization of ASK1 and its subsequent activation [66, 67]. When thioredoxin is oxidized by ROS, it disassociates from ASK1 allowing protein oligomerization and subsequent activation through auto-phosphorylation [68]. This kinase mediates apoptosis by regulating the JNK and p38 MAPK pathways [69]. The regulation of differentiation [70] and immune signaling [71] mediated by ASK1 through the p38 MAPK pathway are other important biological effects triggered by ROS. Importantly, thioredoxin is also involved in the regulation of AMPK activity by preventing the oxidation of cysteine residues in the α subunit of the metabolic stress kinase [72]. This elegant study links oxidative stress and metabolism demonstrating that a reducing enzyme is a critical cofactor controlling the activation of AMPK, a key regulator of metabolism and cell survival in situations of energy stress [72] (Fig. 4).

The best-characterized example of ROS-mediated inactivation of phosphatases by the oxidation of the active site cysteine residue of the enzyme is that of protein tyrosine phosphatase 1b (PTP1b). The inactivation of this enzyme results in the promotion of MAPK and growth factor signaling pathways initiated from different stimulus (Fig. 4) [62, 73-76]. Additionally, ROS can inactivate the dual-specific phosphatase 3 (DUSP3) that is another protein tyrosine phosphatase involved in the dephosphorylation of ERK1/2 causing the sustained activation of the ERK1/2 signaling pathway [77] that play an essential role in cell proliferation, differentiation, invasion, and apoptosis [78-80].

The PI3K pathway which is important for cellular growth, survival and proliferation (Fig. 4) [81] can be affected by the redox state of the cell through different mechanisms [82]. For example, Akt that is a main protein kinase downstream in the pathway has been shown to be activated by H_2O_2 [83]. The target of ROS in the PI3K pathway is the tumor suppressor PTEN, a phospholipid phosphatase that converts PIP3 back to PIP2 thus acting as a negative regulator of the pathway [82, 84]. PTEN is inhibited by hydrogen peroxide through disulfide bond formation between the active site cysteine (Cys124) and a vicinal cysteine residue [85-87]. Through PTEN, the PI3K pathway is subject to reversible redox regulation by ROS generated by growth factor stimulation [88]. PTEN oxidation is reversed by cytoplasmic peroxiredoxin II that eliminates the H_2O_2 generated in response to growth factors [85]. Mitochondrial-generated ROS can also inhibit PTEN affecting the angiogenesis process [89]. Protein phosphatase 2A (PP2A) which is involved in the dephosphorylation of Akt [90] and the inactivation of the PI3K/Akt pathway [91] is another redox sensitive phosphatase. By inhibiting these important phosphatases the AKT signaling pathway is deregulated promoting uncontrolled cellular proliferation and enhanced survival and growth. Ataxia-teleangiectasia mutated (ATM) protein (Fig. 4) is a PI3K-like serine/threonine protein kinase that is activated under stressful conditions and phosphorylates various proteins involved in cellular proliferation, death, survival and DNA repair [92, 93]. ATM is preferentially activated by DNA double strand breaks and also acts as a sensor of oxidative stress [94]. ATM protein is also regulated by a redox sensitive mechanism via the formation of active ATM dimers through intermolecular disulfide bond formation (Fig. 3) [95].

The hypoxia inducible transcription factor HIF1α is also a target of ROS (Fig. 4) [23]. HIF1α is responsible for the coordination of the cellular responses to decreased oxygen availability [96, 97]. During normoxia prolyl hydroxylation of HIF1α promotes its association with the von Hippel-Lindau (VHL) tumor suppressor that targets the protein for ubiquitination and degradation. However, during hypoxia the hydroxylation of proline in HIF1α is inhibited due to the inactivation of prolyl hydroxylases. mROS are involved in the stabilization of HIF1α to promote the transcriptional activity of the protein [32, 98-101]. The stabilization of HIF1α under low oxygen conditions requires the generation of ROS in complex III of the ETC [102]. In other words, the mitochondrial respiratory chain acts as an O_2 sensor that activates a signaling cascade to

stabilize HIF1α through the production of ROS. First evidences illustrating that mROS are involved in the regulation of HIF1α aroused from studies in cells depleted of mitochondrial DNA (ρ^o cells) that failed to stabilize HIF1α under hypoxia [32]. HIF1α stabilization is blunted by treating the cells with mitochondrial antioxidants emphasizing that mitochondria are the source of ROS under hypoxic conditions [32, 33]. Moreover, inhibitors of the mitochondrial electron transport chain that block HIF1α activation in hypoxia have also been described [103]. The mROS mediated regulation of HIF1α is implicated in regulating tumorigenesis by controlling genes involved in metabolism, angiogenesis, and metastasis [97, 104].

As discussed previously mROS produced in p66Shc play relevant roles in the activation of the apoptotic pathway and in the regulation of life span [27]. The production of mROS by this protein leads to mitochondrial damage and apoptosis under oxidative or genotoxic stress conditions [105]. The mechanisms that mediate mitochondrial translocation of p66Shc and its proapoptotic activity seem to be controlled by protein phosphorylation [106], although it has also been suggested that ROS production and the initiation of apoptosis by p66Shc is also redox sensitive by formation of two disulfide bonds in the protein (Fig. 4) [107]. The interaction observed between p66Shc with the TOM-TIM protein import complexes [105, 108] and with cytochrome c [28] are also mechanisms involved in the proapoptotic function of the protein (Fig. 2).

An important point in the cellular response to increased levels of ROS is the redox regulation of transcription factors that activate the antioxidant defense system (Fig. 4). The transcription factor redox factor-1 (Ref-1) is a multifunctional protein that translocates to the nucleus upon exposure to genotoxic agents and H_2O_2 and initiates a protective response of the cell from DNA and oxidative damage [109, 110]. Ref-1 regulates the transcriptional activity of several key transcription factors involved in cellular defense such as activator protein 1 (AP-1), p53, NFkB and HIF1α by its redox sensitive cysteine residues [111-113]. The antioxidant genes glutathione S-transferase (GST) [114], NADPH quinone oxidoreductase-1 (NQO1) [115] and heme oxygenase-1 (HO1) [116, 117] are regulated by an enhancer termed the antioxidant responsive element (ARE) [118]. H_2O_2 activates transcription of these genes via the ARE element and involves a complex set of redox regulated proteins [119]. The primary transcription factor involved in ARE activation is the redox-sensitive transcription factor NFE2-like 2

(Nrf2) [120] (Fig. 4) that is translocated into the nucleus under oxidative stress [121]. In the nucleus Nrf2 dimerizes with the small Maf proteins and binds the ARE to activate ARE-dependent transcription of target genes in ROS homeostasis [118, 122]. Interestingly, the transcriptional activity of Nrf2 is also regulated by Ref-1 [123] indicating the collaboration of these proteins in the detoxification of the cell under oxidative stress.

Extensive literature has related ROS and Ca^{2+} signaling and their effects on apoptosis, aging and cardiovascular diseases [124] (Fig. 4). ROS can modify the properties and activities of some of Ca^{2+} channels and transporters [125, 126]. In fact, some of the proteins involved in Ca^{2+} signaling such as the InsP3 receptor (InsP3R) [127], the ryanodine receptor (RyR) channels [128] and the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) [129] have been shown to be sensitive to ROS.

In addition, ROS signaling is also important for the maintenance of iron homeostasis (Fig. 4). Iron is an essential element that plays crucial roles in cell proliferation and metabolism as it represents a functional constituent of various enzymes. Excessive levels of free iron can generate ROS via the Fenton reaction [130, 131] promoting deleterious oxidative stress to the cells (Fig. 1). Iron regulatory protein-1 and -2 (IRP1 and IRP2) regulate the expression of many genes involved in iron transport and storage at the posttranscriptional level by interacting with iron-responsive elements (IRE) in the 5′- or 3′-untranslated region (UTR) of the mRNAs. IRP1 and IRP2 have redox sensitive target sites that are subjected to redox regulation by H_2O_2 and nitric oxide [132-135]. Taken together, the IRE–IRP regulatory system is also regulated by ROS to elicit a defense mechanism against iron-catalyzed oxidative stress.

3. Old and new functions of the H^+-ATP synthase.

3.1. The H^+-ATP synthase, a core hub in energy and cell death regulation.

The oxidation of glucose in the cytoplasm and the subsequent oxidation of pyruvate in mitochondria provide the energy, reducing power and carbon skeletons required for the maintenance of cellular homeostasis and proliferation [47, 136]. Normoxic cells oxidize most of the pyruvate to CO_2 in mitochondria and the electrons collected onto NADH and $FADH_2$ are transferred to the complexes of the respiratory chain to generate the proton electrochemical gradient that is used for the synthesis of

ATP in oxidative phosphorylation (OXPHOS) (Fig. 2). ATP is synthesized by the mitochondrial H^+-ATP synthase, a reversible engine of the inner mitochondrial membrane that provides most of the ATP that is required to maintain cellular activities in normal aerobic differentiated cells [47]. The mammalian H^+-ATP synthase consists of two main domains: a membrane-bound hydrophobic FO portion, which contains the proton channel, and the soluble catalytic F1 portion that encloses the adenine nucleotide binding sites at the α/β subunit interface [137, 138]. Both regions are linked together by a central and a peripheral stalk. In normal aerobic cells under phosphorylating conditions, the re-entrance of protons into the mitochondrial matrix (Fig. 2) triggers the rotation of the c-ring in FO and of the attached central stalk to induce the conformational changes in the β-F1-ATPase subunit that drive the synthesis of ATP (Fig. 2).

The cellular availability of ATP, NADH and some metabolic intermediates coordinate at short-term the flux of glucose consumption by regulating the activity of key enzymes of the glycolytic pathway and mitochondrial dehydrogenases, to limit the production of biological energy as it is being demanded [47]. In other words, the efficient production of biological energy by OXPHOS determines the rate of glucose consumption, which is nowadays formulation of the Pasteur Effect [139]. When the cells have a limited supply of oxygen or have a genetic or epigenetic impairment that restrains OXPHOS, glycolysis is enhanced [47, 140]. When short-term regulation of enzyme activities of energy metabolism is not enough to cope with the energetic demand cells onset the gene expression programs required for adaptation. Examples that are relevant in this regard are the induction of glycolysis during adaptation to hypoxia [141], the rewiring of metabolism in cancer [142] and in dedifferentiation of somatic cells [143], the onset of the bioenergetic function of mitochondria during adaptation of mammals to the aerobic extrauterine environment [144, 145] and the metabolic reprogramming that accompanies stem cell differentiation [146, 147].

3.2. The H^+-ATP synthase in signaling cell death.

Down-regulation of oxidative phosphorylation (OXPHOS) and the concurrent activation of aerobic glycolysis is a hallmark of proliferating cancer cells [140, 148]. Whereas the increase of glycolysis in the majority of carcinomas is nowadays out of question, the role of OXPHOS modifications in tumor development and progression is

still debated [47]. Nevertheless, it has been shown that a dysfunctional OXPHOS promotes cellular proliferation and invasion [47, 149] whereas an increase in oxidative metabolism halts cellular proliferation and tumor progression [47, 140, 150, 151]. In this regard, the activity of OXPHOS has been demonstrated to be specifically required for the execution of cell death [47, 152-154]. In particular, molecular components that participate in OXPHOS, such as cyt c, AIF and subunits of the H^+-ATP synthase are needed for the execution of cell death [40, 155-158]. Hence, bioenergetics and cell death are two master tasks of mitochondria that are molecularly and functionally integrated [47].

The impairment of mitochondrial energy production by metabolic stress and/or in response to chemotherapy leads to increased ROS generation through respiratory chain electron leakage. ROS can signal mitochondrial geared cell-death pathways or activate transcription programs aimed at cell survival, two opposite cellular fates that largely depend on the intensity of the ROS signal [1, 31]. The induction of cell death by different stressful conditions promotes $\Delta\psi$m collapse that is preceded by transient mitochondrial hyperpolarization [40, 156, 159] and the subsequent production of mROS which is highly dependent on $\Delta\psi$m [40, 42, 160]. It has been suggested that mROS produced in response to cell death stimulation occurs by reverse electron flow from complex II-linked respiratory substrates into complex I of the ETC (Fig. 2) because its production can be inhibited with rotenone [40, 42, 161]. Consistent with a role for mROS in the execution of cell death [1, 162], extensive protein carbonylation of cellular proteins as well as covalent modifications in mitochondrial proteins have been reported in response to staurosporine treatment [40]. The generation of mROS preceded the release of cyt c, the activation of caspase 3 and cell death [40]. Upon inhibition of mitochondrial respiration with staurosporine [163] it is suggested that the hydrolysis of glycolytic ATP by reverse functioning of the H^+-ATP synthase maintains $\Delta\psi$m [40, 164-166]. In this situation, the inhibition of the activity of the H^+-ATP synthase with oligomycin blunted mitochondrial hyperpolarization and ROS production, prevented the oxidation and modification of mitochondrial proteins, delayed the release of cyt c and the execution of cell death [40, 164].

In contrast to these findings, the 1,4-benzodiazepine-derivative Bz-423 signals apoptosis by the induction of ROS production from the mitochondrial respiratory chain as a result of the inhibition of the H^+-ATP synthase [167]. The antagonistic effects on

apoptosis of oligomycin and Bz-423 seem to be dependent on the different mechanism by which these compounds inhibit the enzyme [167]. Similarly, the drug 3,3`-diindolylmethane also promotes ROS-mediated cell death by inhibiting the H^+-ATP synthase [168]. In any case, these findings support a role for the activity of the H^+-ATP synthase in controlling the extent of oxidative damage to mitochondrial constituents that will effectively swamp the cells into death [40]. Consistently, the cell death response to different chemotherapeutic agents varies largely depending upon the relative activity of the pathways that sustain energy metabolism (Fig. 5) [40, 169]. In fact, highly glycolytic cells with negligible contribution of OXPHOS for ATP provision have a cell-death resistant phenotype because mROS signaling after chemotherapeutic targeting is blunted (Fig. 5) [40, 169]. Overall, the down-regulation of the H^+-ATP synthase, and thus of OXPHOS, is part of the molecular strategy adapted by cancer cells to avoid reactive oxygen species-mediated cell death.

Interestingly, it has been shown that the activity of the H^+-ATP synthase *per se* is inhibited by ROS [170, 171]. The impact of ROS on the activity of the complex is also observed in chloroplasts where the γ subunit seems to be a main target for ROS oxidation [172]. These findings further emphasize the tightly regulated connections that exist between the H^+-ATP synthase, energy production, ROS generation and cell death.

The point of no return in cell death is the permeabilization of the inner mitochondrial membrane to low molecular weight solutes, the so-called PTP opening [173-175]. Although the molecular composition of the PTP remains unknown recent findings support that a critical component of the high-conductance channel is subunit c of the H^+-ATP synthase [176]. Moreover, it has been shown that dimers of the H^+-ATP synthase form a channel with electrophysiological properties identical to those of the PTP [177]. Non-specific ROS-mediated modifications of mitochondrial constituents could represent a critical point of regulation of the mitochondria-geared cell death pathway. Protein oxidation could define the threshold value of irreversible damage of the mitochondria and the set-point for the release of the mitochondrial arsenal that controls cell death (Fig. 5) [40]. Indeed, oxidative stress promotes cell death by increasing the susceptibility of the opening of the PTP [178]. PTP opening is linked to oxidative stress since it has been shown to be dependent on the NADPH redox state [179, 180] and promoted by thiol oxidation [181, 182]. Consistently, it has been reported that PTP opening can be stimulated by the addition of exogenous sources of

ROS and prevented by antioxidants in pro-apoptotic conditions [180]. Interestingly, PTP opening also increases mitochondrial ROS production illustrating a retro-amplification cascade when the decision to execute cell-death has been taken [183].

Consistent with the tumor suppressor function of mitochondrial activity there is a large body of data supporting that OXPHOS, both under basal conditions or in response to chemotherapeutic agents abolishes tumorigenicity (see [47] for updated review). A likely mechanism that explains the preferential death of cancer cells when forced to oxidize mitochondrial substrates is the overproduction of superoxide radical as a result of the stimulation of mitochondrial metabolism [40, 47, 184]. Both genetic [185] and pharmacological [186] studies have shown that the PGC1α-mediated improvement of mitochondrial activity and metabolism restrains cancer progression by increasing ROS-mediated apoptosis in cancer cells [185].

A diverse set of genetic, epigenetic and environmental mediated mechanisms interfere with mitochondrial bioenergetics of the cancer cell [47, 187]. One such mechanism, which is observed in many prevalent human carcinomas, is the down-regulation of the expression of the catalytic subunit of the mitochondrial H+-ATP synthase (β-F1-ATPase) relative to the enzyme of glycolysis glyceraldehyde-3-phosphate dehydrogenase [140, 188]. This finding has been confirmed and extended to other carcinomas (see [140] for other studies), providing a "bioenergetic signature" of cancer [140, 188] of clinical applicability. Indeed, as assessed in large cohorts of colon [188-190], lung [191, 192], breast [193] and ovarian carcinomas [194] and in cells of acute myeloid leukemia patients [195] the altered bioenergetic signature of the tumors predicts a worst overall and/or disease-free survival for the patients. Down-regulation of the bioenergetic signature is also functionally linked to the resistance to chemotherapy in many different cancer cells [47, 196, 197], in colon cancer patients [190] and chronic [198] and acute [195] leukemia patients. In general, the bioenergetic signature represents a functional index of metabolic activity of the cells because it correlates, both *in vitro* [199] and *in vivo* [192], with the rate of glucose utilization. Overall, these findings emphasize that a diminished bioenergetic activity of mitochondria in the cancer cell predisposes to cancer onset and progression, highlighting the emerging role that the H^+-ATP synthase plays as a master regulator of cell death [40, 156, 177, 200, 201]. Consistently, cancer progression requires the silencing of the bioenergetic activity of mitochondria [199] not only by down-regulating the content of the H^+-ATP synthase as

above discussed but also by over-expressing the natural physiological inhibitor of the enzyme, the so-called ATPase Inhibitory Factor 1 (IF1) [38, 46, 47].

3.3. The ATPase Inhibitory Factor 1 (IF1).

In mitochondria, ATP hydrolysis by the H^+-ATP synthase is inhibited by the ATPase Inhibitory Factor 1 (IF1) that reversibly binds to the enzyme (Fig. 6) [202, 203]. The natural inhibitor of the H^+-ATP synthase is a low molecular weight (~10kDa) mitochondrial protein which inhibited the soluble ATPase, but did not interfere with its coupling activity [204]. IF1 is encoded in the nuclear ATPIF1 gene located on chromosomes 1 and 4 in human and mouse, respectively. IF1 has been described in mammals, birds, amphibious, nematodes, yeast and plants and shows considerable sequence identity among various eukaryotes [205]. It is absent in bacteria and chloroplasts. Three different isoforms of the human protein are produced by alternative splicing. Isoform 1 is the longest one, it codifies for a protein of 106 amino acids (12,2 kDa; pI=10) which is, by far, the protein more abundantly expressed in most human tissues [46, 47]. Isoform 2 and 3 codify for proteins of 71 (7,9 kDa; pI=8.5) and 60 (6,6 kDa; pI=9.3) amino acids, respectively. The inhibitor protein has an N-terminal presequence of 25 residues for targeting the protein into the mitochondrial matrix which is cleaved off after import [206, 207].

The interaction between IF1 and the H^+-ATP synthase depends on the pH of the mitochondrial matrix and is affected by changes in the $\Delta\psi m$ [202, 207]. IF1 binds β, α and γ subunits of the H^+-ATP synthase due to its activation under low pH conditions blocking ATP hydrolysis and preventing a useless waste of energy [207]. The substitution of histidine 49 in the IF1 sequence by a lysine renders a mutant form of IF1 (H49K) that is as active as IF1 in the inhibition of the ATP hydrolase activity but less sensitive to the regulation by pH [138, 202, 203, 208, 209]. The participation of E26 in the pH regulated inhibitory activity of bovine IF1 has also been suggested [210]. Bovine IF1 has been shown to have oligomeric states, tetramer and dimer, favored by pH values above and below 6.7, respectively [202]. Dimerization and activation of IF1 occurs by formation of an antiparallel α-helical coiled-coil in its C-terminal region which places the N-terminus (inhibitory regions) of the monomers at opposite ends of the dimer, allowing the dimeric IF1 to bind two domains of F1-ATPase simultaneously [202]. The structure of the inhibited F1-ATPase complex with bound IF1 in the presence of ATP

has been solved and confirms that the N-terminal region of the dimeric inhibitor is bound to F1-ATPase [138]. The inhibitory 42L-58K segment of IF1 has been shown to interact with α/β pair of subunits of the F1-ATPase domain. It is suggested that this interaction inhibits the conformational inter-conversions of the catalytic sites involved in ATP hydrolysis and hence rotatory catalysis [138]. Hence, the mechanism of inhibition of the ATPase by IF1 arises from the disruption of the catalytic site.

The physiological function ascribed to IF1 in normal hypoxic cells is to inhibit the hydrolase activity of the H^+-ATP synthase [202, 203, 211, 212]. Until recently, the role of IF1 in preserving cellular ATP in myocardial ischemia [209, 211] and in ischemic preconditioning [209] have been the most extensively studied functions of IF1. However, the transformation of mitochondria from ATP producers into ATP consumers under depolarizing conditions has been recently questioned [213]. Upon mitochondrial depolarization IF1 has been identified as an essential gene to promote PARK2 recruitment onto mitochondria to establish the selective autophagic program of mitophagy [214]. More recent findings support a relevant role for IF1 in controlling the ferrochelatase activity of mitochondria, and hence heme biosynthesis, in erythroid cells [215]. Surprisingly, a recently ill-defined knockout IF1 mouse model has revealed no alterations in phenotype [216]. The absence of IF1 is known to occur in Luft's disease, a mitochondrial myopathy of the striated muscle [209, 217, 218]. The disorder is characterized by a hypermetabolic state, mitochondria with densely packed cristae and a loosely coupled OXPHOS [209, 217, 219], suggesting additional functional roles for IF1 in the regulation of the H^+-ATP synthase of muscle mitochondria. It has been shown that long rows of dimers of the H^+-ATP synthase promote the high local curvature of the inner membrane at cristae ridges [220-223]. Interestingly, ageing seems to melt-down the inner-membrane *cristae* of mitochondria by age-dependent dissociation of ATP synthase dimers [224]. It has been suggested that IF1 regulates the oligomeric state of the H^+-ATP synthase increasing the density of cristae and the formation of dimeric ATP synthase complexes [212, 225-227]. However, this suggestion has been questioned [228-230].

3.4. IF1 is a master regulator of energy metabolism in cancer and in stem cells.

A differential expression level of IF1 has been reported between different mouse [231] and human tissues [46, 48]. Moreover, cardiomyocytes of low heart rate species

(human) have a higher expression level of IF1 than cardiomyocites of fast heart rate species (rat, mouse) [209, 211], what might explain the differential preservation of cellular ATP upon sublethal ischemic episodes. Interestingly, normal colon, lung and breast tissue have negligible expression of IF1 [38, 46, 48]. In contrast, mitochondria of almost all lung, colon, breast and ovarian carcinomas analyzed in large cohorts of cancer patients show an overwhelming increase in the expression of IF1 [48]. We have demonstrated that the over-expression of IF1 results in the inhibition of the ATP synthetic activity of the H^+-ATP synthase and the switch of the cells to an enhanced aerobic glycolysis [38, 46]. On the contrary, silencing of IF1 enhances the H^+-ATP synthase activity and reduces aerobic glycolysis, strongly supporting a crucial role for IF1 in mediating the metabolic switch experienced by cancer cells [38, 46]. Likewise, IF1 is present in human mesenchymal stem cells (hMSCs) as well as in stem cells of the prostate and in the Lieberkühn crypts of the colon [147]. Consistent with a master role for IF1 in the regulation of energy metabolism we have shown that the regulated degradation of IF1 triggers metabolic rewiring from aerobic glycolysis to a predominant OXPHOS in the differentiation process of hMSCs into osteocytes [147], once again stressing the biological relevance of this protein in regulating energy metabolism of proliferating cells.

3.5. The role of IF1 in ROS signaling cell survival.

The IF1-mediated inhibition of the H^+-ATP synthase results in mitochondrial hyperpolarization and the subsequent production of superoxide radical in colon [38] and other cancer [48] cells (Fig. 6). Therefore, besides the role of IF1 in rewiring energy metabolism [46, 232], the over-expression of IF1 also triggers a retrograde ROS signal to the nucleus to establish the appropriate adaptive cellular program needed to support tumor development [38, 48]. IF1 mimics the inhibitory effect of oligomycin in reprogramming energy metabolism and in inhibiting apoptosis [40, 156]. Remarkably, it has been demonstrated that the ROS-mediated response in colon cancer cells in response to the over-expression of IF1 signals to the nucleus an NF-κB-dependent adaptation that includes enhanced proliferation, invasion and cell survival [38]. An IF1 ROS-mediated resistance to cell death has also been demonstrated in other cancer cells [48]. The ROS-signaling pathways (Fig. 4) activated in response to IF1 over-expression that arrest cell death in many prevalent human cancer cells still remain to be investigated [48]. An alternative, not mutually exclusive mechanism of action of IF1 to

prevent cell death is that it might contribute to stabilize the oligomerisation state of the H^+-ATP synthase to preserve mitochondrial *cristae* impeding in this way the release of cyt c [233]. In this regard, IF1 has been suggested to increase the density of mitochondrial cristae by the formation of dimeric ATP synthase complexes [212]. In this situation, IF1 would represent a negative regulator of PTP opening [177] contributing to the evasion of cell death. Consistently, recent findings in a conditional transgenic mouse expressing H49K (a gain-of-function mutant of human IF1) in neurons that also inhibits the H^+-ATP synthase supports both a ROS-mediated metabolic and structural pathways to prevent neuronal cell death *in vivo* after an excitotoxic insult [234]. Overall, these findings strongly support that IF1 plays a master role in the regulation of energy metabolism and in retrograde communication to the nucleus other features of the oncogenic phenotype such as cell survival [47, 187].

3.6. Regulation of IF1 activity and expression and other IF1 paradoxes.

In silico analysis of the promoter region of the human ATPIF1 gene reveal the existence of potential *cis*-acting responsive elements for transcription factors involved in cancer. Data from high-throughput ChiP-sequencing confirmed the binding of several transcription factors involved in the regulation of cell cycle (NF-YB, NF-YA, Ini1), proliferation (c-FOS, Sp1, c-MYC) inflammation and cell death (NFκB, TAF1) in the proximal promoter region of the ATPIF1 gene. However, the regulation of IF1 expression in human carcinomas is exerted at post-transcriptional levels [48]. In fact, IF1 has a very short half-life (~2h) in colon cancer cells [48] being degraded by a mitochondrial protease [147]. However, the mitochondrial protease involved in IF1 degradation has not been identified despite our recent attempts using a large siRNA screen [147]. It has been suggested that the hypoxia regulated transcription factor HIF1α participates in controlling IF1 expression (Fig. 4) [235]. Moreover, it has been shown that the immediate early response gene (IER3) binds the C-terminus of IF1 to render the protein prone to proteolytic digestion in HeLa cells [236]. However, recent findings in different cancer cell lines seem to exclude the participation of HIF1α [48] and of IER3 [48] in controlling IF1 expression in colon, lung and breast carcinomas.

Paradoxically, tissues with very high activity of OXPHOS (liver, muscle, neurons) show a very high content of IF1 [38, 46, 48, 234], what would imply the partial IF1-mediated inhibition of the H^+-ATP synthase in these tissues, which is

obviously not the case. As recently pointed out [48], these findings suggest that besides to the well characterized pH controlled binding of IF1 to β-F1-ATPase [202], an additional mechanism should regulate the biological activity of IF1 specially in tissues that naturally over-express the protein. It has been described that IF1, in addition to binding subunits of the H^+-ATP synthase, also binds other membrane proteins of mitochondria in a pH and $\Delta\psi m$ independent manner [237]. Specifically, it has been shown that the binding to one of these membrane proteins (~ 5-6 kDa) hampers the activity of IF1 as an inhibitor of the ATPase [237]. Hence, it is conceivable that tissues that normally over-express IF1 could also express a putative receptor that might act as a negative regulator of IF1 in order to counterbalance its inhibitory activity, contributing in this way to the fine-tuning of OXPHOS. Alternatively, tissue-specific post-translational modifications of IF1 [238] could explain its activity on the H^+-ATP synthase. The biological relevance of these modifications and the proteins and signals involved are unknown but they are likely to be relevant to understand IF1 expression and activity.

Interestingly, the study of IF1 expression in tumors of large cohorts of breast and colon cancer patients support that it represents a marker of clinical outcome [48]. Surprisingly, colon and breast cancer patients with high tumor expression of IF1 have a better prognosis in terms of time of disease relapse [48] what suggests that cancer cells with a low expression of IF1 should be more likely to metastasize [48]. The molecular bases of this paradox are presently unknown.

4. Final Remarks

We have outlined the main pathways that lead to superoxide production in mitochondria, the primary mechanism by which ROS modulate the activity of proteins involved in signaling pathways and their biological consequences in the cell response. The mitochondrial H^+-ATP synthase is presented as a key transducer in controlling energy metabolism, ROS-mediated cell death or the retrograde ROS response that allows the acquisition of an enhanced proliferation and the resistance to cell death, key features of the phenotype observed in carcinomas. ROS signaling by mitochondria is important in cancer onset and progression and it might represent the remnant mechanism that allowed the successful symbiosis of the two organisms that gave rise to the aerobic eukaryotic cell. A master regulator of mitochondrial ROS production with

clear effects in promoting the malignant phenotype of cancer cells is the ATPase Inhibitory Factor 1. Characterizing the regulation of its expression and/or activity are critical issues to understand the regulation of OXPHOS in different mammalian tissues and of many of the hallmarks of the cancer phenotype. The development of tissue specific mouse models with regulated expression of IF1 will contribute to portray its functional role in cellular physiology and pathophysiology. Overall, we can conclude that bioenergetics, ROS production and cell death are master tasks of mitochondria that are molecularly and functionally integrated. Unveiling the mechanisms that mediate these signaling networks will bring up new opportunities for cancer therapies.

Acknowledgements

The authors gratefully acknowledge the work, support and ideas of many colleagues and collaborators, especially to Drs. María Sánchez-Aragó and Laura Formentini and to the excellent technical support provided by M. Chamorro and C. Nuñez de Arenas over all these years. Work in the authors' laboratory was supported by grants from the Ministerio de Educación y Ciencia (BFU2010-18903), by the Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII and by Comunidad de Madrid (S/2011-BMD-2402), Spain. The CBMSO receives an institutional grant from Fundación Ramón Areces. We apologize to authors whose work or primary references could not be cited owing to space limitations.

References

[1] S. Orrenius, V. Gogvadze, B. Zhivotovsky, Mitochondrial oxidative stress: implications for cell death, Annu Rev Pharmacol Toxicol 47 (2007) 143-183.

[2] P. Storz, Reactive oxygen species in tumor progression, Front Biosci 10 (2005) 1881-1896.

[3] L.A. Sena, N.S. Chandel, Physiological roles of mitochondrial reactive oxygen species, Mol Cell 48 (2012) 158-167.

[4] C.C. Winterbourn, M.B. Hampton, Thiol chemistry and specificity in redox signaling, Free Radic Biol Med 45 (2008) 549-561.

[5] Y.A. Suh, R.S. Arnold, B. Lassegue, J. Shi, X. Xu, D. Sorescu, A.B. Chung, K.K. Griendling, J.D. Lambeth, Cell transformation by the superoxide-generating oxidase Mox1, Nature 401 (1999) 79-82.

[6] J.D. Lambeth, NOX enzymes and the biology of reactive oxygen, Nat Rev Immunol 4 (2004) 181-189.

[7] T. Kawahara, M.T. Quinn, J.D. Lambeth, Molecular evolution of the reactive oxygen-generating NADPH oxidase (Nox/Duox) family of enzymes, BMC Evol Biol 7 (2007) 109.

[8] H. Sumimoto, Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species, FEBS J 275 (2008) 3249-3277.

[9] D.I. Brown, K.K. Griendling, Nox proteins in signal transduction, Free Radic Biol Med 47 (2009) 1239-1253.

[10] M. Geiszt, J.B. Kopp, P. Varnai, T.L. Leto, Identification of renox, an NAD(P)H oxidase in kidney, Proc Natl Acad Sci U S A 97 (2000) 8010-8014.

[11] T. Ago, T. Kitazono, H. Ooboshi, T. Iyama, Y.H. Han, J. Takada, M. Wakisaka, S. Ibayashi, H. Utsumi, M. Iida, Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase, Circulation 109 (2004) 227-233.

[12] A.N. Lyle, N.N. Deshpande, Y. Taniyama, B. Seidel-Rogol, L. Pounkova, P. Du, C. Papaharalambus, B. Lassegue, K.K. Griendling, Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells, Circ Res 105 (2009) 249-259.

[13] Q. Li, M.M. Harraz, W. Zhou, L.N. Zhang, W. Ding, Y. Zhang, T. Eggleston, C. Yeaman, B. Banfi, J.F. Engelhardt, Nox2 and Rac1 regulate H2O2-dependent recruitment of TRAF6 to endosomal interleukin-1 receptor complexes, Mol Cell Biol 26 (2006) 140-154.

[14] J.D. Van Buul, M. Fernandez-Borja, E.C. Anthony, P.L. Hordijk, Expression and localization of NOX2 and NOX4 in primary human endothelial cells, Antioxid Redox Signal 7 (2005) 308-317.

[15] T.L. Leto, M. Geiszt, Role of Nox family NADPH oxidases in host defense, Antioxid Redox Signal 8 (2006) 1549-1561.

[16] S. Dong-Yun, D. Yu-Ru, L. Shan-Lin, Z. Ya-Dong, W. Lian, Redox stress regulates cell proliferation and apoptosis of human hepatoma through Akt protein phosphorylation, FEBS Lett 542 (2003) 60-64.

[17] R.S. Arnold, J. Shi, E. Murad, A.M. Whalen, C.Q. Sun, R. Polavarapu, S. Parthasarathy, J.A. Petros, J.D. Lambeth, Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1, Proc Natl Acad Sci U S A 98 (2001) 5550-5555.

[18] J.L. Arbiser, J. Petros, R. Klafter, B. Govindajaran, E.R. McLaughlin, L.F. Brown, C. Cohen, M. Moses, S. Kilroy, R.S. Arnold, J.D. Lambeth, Reactive

oxygen generated by Nox1 triggers the angiogenic switch, Proc Natl Acad Sci U S A 99 (2002) 715-720.
[19] S.S. Brar, Z. Corbin, T.P. Kennedy, R. Hemendinger, L. Thornton, B. Bommarius, R.S. Arnold, A.R. Whorton, A.B. Sturrock, T.P. Huecksteadt, M.T. Quinn, K. Krenitsky, K.G. Ardie, J.D. Lambeth, J.R. Hoidal, NOX5 NAD(P)H oxidase regulates growth and apoptosis in DU 145 prostate cancer cells, Am J Physiol Cell Physiol 285 (2003) C353-369.
[20] A.S. Kamiguti, L. Serrander, K. Lin, R.J. Harris, J.C. Cawley, D.J. Allsup, J.R. Slupsky, K.H. Krause, M. Zuzel, Expression and activity of NOX5 in the circulating malignant B cells of hairy cell leukemia, J Immunol 175 (2005) 8424-8430.
[21] M.D. Brand, The sites and topology of mitochondrial superoxide production, Exp Gerontol 45 (2010) 466-472.
[22] F.L. Muller, Y. Liu, H. Van Remmen, Complex III releases superoxide to both sides of the inner mitochondrial membrane, J Biol Chem 279 (2004) 49064-49073.
[23] T. Klimova, N.S. Chandel, Mitochondrial complex III regulates hypoxic activation of HIF, Cell Death Differ 15 (2008) 660-666.
[24] D. Han, F. Antunes, R. Canali, D. Rettori, E. Cadenas, Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol, J Biol Chem 278 (2003) 5557-5563.
[25] N.R. Brady, A. Hamacher-Brady, H.V. Westerhoff, R.A. Gottlieb, A wave of reactive oxygen species (ROS)-induced ROS release in a sea of excitable mitochondria, Antioxid Redox Signal 8 (2006) 1651-1665.
[26] M. Crompton, The mitochondrial permeability transition pore and its role in cell death, Biochem J 341 (1999) 233-249.
[27] E. Migliaccio, M. Giorgio, S. Mele, G. Pelicci, P. Reboldi, P.P. Pandolfi, L. Lanfrancone, P.G. Pelicci, The p66shc adaptor protein controls oxidative stress response and life span in mammals, Nature 402 (1999) 309-313.
[28] M. Giorgio, E. Migliaccio, F. Orsini, D. Paolucci, M. Moroni, C. Contursi, G. Pelliccia, L. Luzi, S. Minucci, M. Marcaccio, P. Pinton, R. Rizzuto, P. Bernardi, F. Paolucci, P.G. Pelicci, Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis, Cell 122 (2005) 221-233.
[29] N. Hauptmann, J. Grimsby, J.C. Shih, E. Cadenas, The metabolism of tyramine by monoamine oxidase A/B causes oxidative damage to mitochondrial DNA, Arch Biochem Biophys 335 (1996) 295-304.
[30] F. Di Lisa, N. Kaludercic, A. Carpi, R. Menabo, M. Giorgio, Mitochondrial pathways for ROS formation and myocardial injury: the relevance of p66(Shc) and monoamine oxidase, Basic Res Cardiol 104 (2009) 131-139.
[31] T. Finkel, Signal transduction by mitochondrial oxidants, J Biol Chem 287 (2012) 4434-4440.
[32] N.S. Chandel, E. Maltepe, E. Goldwasser, C.E. Mathieu, M.C. Simon, P.T. Schumacker, Mitochondrial reactive oxygen species trigger hypoxia-induced transcription, Proc Natl Acad Sci U S A 95 (1998) 11715-11720.
[33] R.D. Guzy, P.T. Schumacker, Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia, Exp Physiol 91 (2006) 807-819.

[34] N.S. Chandel, W.C. Trzyna, D.S. McClintock, P.T. Schumacker, Role of oxidants in NF-kappa B activation and TNF-alpha gene transcription induced by hypoxia and endotoxin, J Immunol 165 (2000) 1013-1021.

[35] N.S. Chandel, M.G. Vander Heiden, C.B. Thompson, P.T. Schumacker, Redox regulation of p53 during hypoxia, Oncogene 19 (2000) 3840-3848.

[36] S. Nemoto, K. Takeda, Z.X. Yu, V.J. Ferrans, T. Finkel, Role for mitochondrial oxidants as regulators of cellular metabolism, Mol Cell Biol 20 (2000) 7311-7318.

[37] Y. Collins, E.T. Chouchani, A.M. James, K.E. Menger, H.M. Cocheme, M.P. Murphy, Mitochondrial redox signalling at a glance, J Cell Sci 125 (2012) 801-806.

[38] L. Formentini, M. Sánchez-Aragó, L. Sánchez-Cenizo, J.M. Cuezva, The mitochondrial ATPase Inhibitory Factor 1 (IF1) triggers a ROS-mediated retrograde pro-survival and proliferative response, Mol. Cell 45 (2012) 731-742.

[39] G.Y. Liou, P. Storz, Reactive oxygen species in cancer, Free Radic Res 44 (2010) 479-496.

[40] G. Santamaria, M. Martinez-Diez, I. Fabregat, J.M. Cuezva, Efficient execution of cell death in non-glycolytic cells requires the generation of ROS controlled by the activity of mitochondrial H+-ATP synthase, Carcinogenesis 27 (2006) 925-935.

[41] T.V. Votyakova, I.J. Reynolds, DeltaPsi(m)-Dependent and -independent production of reactive oxygen species by rat brain mitochondria, J Neurochem 79 (2001) 266-277.

[42] M.D. Brand, C. Affourtit, T.C. Esteves, K. Green, A.J. Lambert, S. Miwa, J.L. Pakay, N. Parker, Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins, Free Radic Biol Med 37 (2004) 755-767.

[43] M.P. Murphy, How mitochondria produce reactive oxygen species, Biochem J 417 (2009) 1-13.

[44] N.S. Chandel, D.S. McClintock, C.E. Feliciano, T.M. Wood, J.A. Melendez, A.M. Rodriguez, P.T. Schumacker, Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing, J Biol Chem 275 (2000) 25130-25138.

[45] D.L. Hoffman, J.D. Salter, P.S. Brookes, Response of mitochondrial reactive oxygen species generation to steady-state oxygen tension: implications for hypoxic cell signaling, Am J Physiol Heart Circ Physiol 292 (2007) H101-108.

[46] L. Sanchez-Cenizo, L. Formentini, M. Aldea, A.D. Ortega, P. Garcia-Huerta, M. Sanchez-Arago, J.M. Cuezva, Up-regulation of the ATPase inhibitory factor 1 (IF1) of the mitochondrial H+-ATP synthase in human tumors mediates the metabolic shift of cancer cells to a Warburg phenotype, J Biol Chem 285 (2010) 25308-25313.

[47] M. Sanchez-Arago, L. Formentini, J.M. Cuezva, Mitochondria-mediated energy adaption in cancer: the H(+)-ATP synthase-geared switch of metabolism in human tumors, Antioxid Redox Signal 19 (2013) 285-298.

[48] M. Sanchez-Arago, L. Formentini, I. Martinez-Reyes, J. Garcia-Bermudez, F. Santacatterina, L. Sanchez-Cenizo, I.M. Willers, M. Aldea, L. Najera, A. Juarranz, E.C. Lopez, J. Clofent, C. Navarro, E. Espinosa, J.M. Cuezva, Expression, regulation and clinical relevance of the ATPase inhibitory factor 1 in human cancers, Oncogenesis 2 (2013) e46.

[49] M.P. Murphy, Mitochondrial thiols in antioxidant protection and redox signaling: distinct roles for glutathionylation and other thiol modifications, Antioxid Redox Signal 16 (2012) 476-495.
[50] T. Finkel, Oxidant signals and oxidative stress, Curr Opin Cell Biol 15 (2003) 247-254.
[51] B.S. Fleenor, J.S. Eng, A.L. Sindler, B.T. Pham, J.D. Kloor, D.R. Seals, Superoxide signaling in perivascular adipose tissue promotes age-related artery stiffness, Aging Cell (2013) doi:10.1111/acel.12196.
[52] C.C. Winterbourn, D. Metodiewa, Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide, Free Radic Biol Med 27 (1999) 322-328.
[53] G. Roos, J. Messens, Protein sulfenic acid formation: from cellular damage to redox regulation, Free Radic Biol Med 51 (2011) 314-326.
[54] D. Barford, The role of cysteine residues as redox-sensitive regulatory switches, Curr Opin Struct Biol 14 (2004) 679-686.
[55] H.J. Forman, M. Torres, J. Fukuto, Redox signaling, Mol Cell Biochem 234-235 (2002) 49-62.
[56] S.G. Rhee, Y.S. Bae, S.R. Lee, J. Kwon, Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation, Sci STKE 2000 (2000) pe1.
[57] N.K. Tonks, Redox redux: revisiting PTPs and the control of cell signaling, Cell 121 (2005) 667-670.
[58] G. Ferrer-Sueta, R. Radi, Chemical biology of peroxynitrite: kinetics, diffusion, and radicals, ACS Chem Biol 4 (2009) 161-177.
[59] G.A. Figtree, C.C. Liu, S. Bibert, E.J. Hamilton, A. Garcia, C.N. White, K.K. Chia, F. Cornelius, K. Geering, H.H. Rasmussen, Reversible oxidative modification: a key mechanism of Na+-K+ pump regulation, Circ Res 105 (2009) 185-193.
[60] A. Panov, S. Dikalov, N. Shalbuyeva, G. Taylor, T. Sherer, J.T. Greenamyre, Rotenone model of Parkinson disease: multiple brain mitochondria dysfunctions after short term systemic rotenone intoxication, J Biol Chem 280 (2005) 42026-42035.
[61] P.R. Gardner, I. Raineri, L.B. Epstein, C.W. White, Superoxide radical and iron modulate aconitase activity in mammalian cells, J Biol Chem 270 (1995) 13399-13405.
[62] T.C. Meng, T. Fukada, N.K. Tonks, Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo, Mol Cell 9 (2002) 387-399.
[63] W.C. Barrett, J.P. DeGnore, Y.F. Keng, Z.Y. Zhang, M.B. Yim, P.B. Chock, Roles of superoxide radical anion in signal transduction mediated by reversible regulation of protein-tyrosine phosphatase 1B, J Biol Chem 274 (1999) 34543-34546.
[64] H.J. Zhang, W. Zhao, S. Venkataraman, M.E. Robbins, G.R. Buettner, K.C. Kregel, L.W. Oberley, Activation of matrix metalloproteinase-2 by overexpression of manganese superoxide dismutase in human breast cancer MCF-7 cells involves reactive oxygen species, J Biol Chem 277 (2002) 20919-20926.
[65] K. Tobiume, A. Matsuzawa, T. Takahashi, H. Nishitoh, K. Morita, K. Takeda, O. Minowa, K. Miyazono, T. Noda, H. Ichijo, ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis, EMBO Rep 2 (2001) 222-228.

[66] M. Saitoh, H. Nishitoh, M. Fujii, K. Takeda, K. Tobiume, Y. Sawada, M. Kawabata, K. Miyazono, H. Ichijo, Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1, EMBO J 17 (1998) 2596-2606.
[67] G. Fujino, T. Noguchi, A. Matsuzawa, S. Yamauchi, M. Saitoh, K. Takeda, H. Ichijo, Thioredoxin and TRAF family proteins regulate reactive oxygen species-dependent activation of ASK1 through reciprocal modulation of the N-terminal homophilic interaction of ASK1, Mol Cell Biol 27 (2007) 8152-8163.
[68] K. Tobiume, M. Saitoh, H. Ichijo, Activation of apoptosis signal-regulating kinase 1 by the stress-induced activating phosphorylation of pre-formed oligomer, J Cell Physiol 191 (2002) 95-104.
[69] H. Ichijo, E. Nishida, K. Irie, P. ten Dijke, M. Saitoh, T. Moriguchi, M. Takagi, K. Matsumoto, K. Miyazono, Y. Gotoh, Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways, Science 275 (1997) 90-94.
[70] T.G. Choi, J. Lee, J. Ha, S.S. Kim, Apoptosis signal-regulating kinase 1 is an intracellular inducer of p38 MAPK-mediated myogenic signalling in cardiac myoblasts, Biochim Biophys Acta 1813 (2011) 1412-1421.
[71] A. Matsuzawa, K. Saegusa, T. Noguchi, C. Sadamitsu, H. Nishitoh, S. Nagai, S. Koyasu, K. Matsumoto, K. Takeda, H. Ichijo, ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity, Nat Immunol 6 (2005) 587-592.
[72] D. Shao, S. Oka, T. Liu, P. Zhai, T. Ago, S. Sciarretta, H. Li, J. Sadoshima, A Redox-Dependent Mechanism for Regulation of AMPK Activation by Thioredoxin1 during Energy Starvation, Cell Metab 19 (2014) 232-245.
[73] C. Persson, T. Sjoblom, A. Groen, K. Kappert, U. Engstrom, U. Hellman, C.H. Heldin, J. den Hertog, A. Ostman, Preferential oxidation of the second phosphatase domain of receptor-like PTP-alpha revealed by an antibody against oxidized protein tyrosine phosphatases, Proc Natl Acad Sci U S A 101 (2004) 1886-1891.
[74] H. Kamata, S. Honda, S. Maeda, L. Chang, H. Hirata, M. Karin, Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases, Cell 120 (2005) 649-661.
[75] K.A. Robinson, C.A. Stewart, Q.N. Pye, X. Nguyen, L. Kenney, S. Salzman, R.A. Floyd, K. Hensley, Redox-sensitive protein phosphatase activity regulates the phosphorylation state of p38 protein kinase in primary astrocyte culture, J Neurosci Res 55 (1999) 724-732.
[76] R.M. Liu, J. Choi, J.H. Wu, K.A. Gaston Pravia, K.M. Lewis, J.D. Brand, N.S. Mochel, D.M. Krzywanski, J.D. Lambeth, J.S. Hagood, H.J. Forman, V.J. Thannickal, E.M. Postlethwait, Oxidative modification of nuclear mitogen-activated protein kinase phosphatase 1 is involved in transforming growth factor beta1-induced expression of plasminogen activator inhibitor 1 in fibroblasts, J Biol Chem 285 (2010) 16239-16247.
[77] C.C. Wentworth, A. Alam, R.M. Jones, A. Nusrat, A.S. Neish, Enteric commensal bacteria induce extracellular signal-regulated kinase pathway signaling via formyl peptide receptor-dependent redox modulation of dual specific phosphatase 3, J Biol Chem 286 (2011) 38448-38455.
[78] S. Meloche, J. Pouyssegur, The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition, Oncogene 26 (2007) 3227-3239.

[79] X. Wang, J.L. Martindale, N.J. Holbrook, Requirement for ERK activation in cisplatin-induced apoptosis, J Biol Chem 275 (2000) 39435-39443.
[80] G. Pages, P. Lenormand, G. L'Allemain, J.C. Chambard, S. Meloche, J. Pouyssegur, Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation, Proc Natl Acad Sci U S A 90 (1993) 8319-8323.
[81] L.C. Cantley, The phosphoinositide 3-kinase pathway, Science 296 (2002) 1655-1657.
[82] N.R. Leslie, C.P. Downes, PTEN: The down side of PI 3-kinase signalling, Cell Signal 14 (2002) 285-295.
[83] S. Nemoto, T. Finkel, Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway, Science 295 (2002) 2450-2452.
[84] L.C. Cantley, B.G. Neel, New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway, Proc Natl Acad Sci U S A 96 (1999) 4240-4245.
[85] J. Kwon, S.R. Lee, K.S. Yang, Y. Ahn, Y.J. Kim, E.R. Stadtman, S.G. Rhee, Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors, Proc Natl Acad Sci U S A 101 (2004) 16419-16424.
[86] S.R. Lee, K.S. Yang, J. Kwon, C. Lee, W. Jeong, S.G. Rhee, Reversible inactivation of the tumor suppressor PTEN by H2O2, J Biol Chem 277 (2002) 20336-20342.
[87] N.R. Leslie, D. Bennett, Y.E. Lindsay, H. Stewart, A. Gray, C.P. Downes, Redox regulation of PI 3-kinase signalling via inactivation of PTEN, EMBO J 22 (2003) 5501-5510.
[88] J.H. Seo, Y. Ahn, S.R. Lee, C. Yeol Yeo, K. Chung Hur, The major target of the endogenously generated reactive oxygen species in response to insulin stimulation is phosphatase and tensin homolog and not phosphoinositide-3 kinase (PI-3 kinase) in the PI-3 kinase/Akt pathway, Mol Biol Cell 16 (2005) 348-357.
[89] K.M. Connor, S. Subbaram, K.J. Regan, K.K. Nelson, J.E. Mazurkiewicz, P.J. Bartholomew, A.E. Aplin, Y.T. Tai, J. Aguirre-Ghiso, S.C. Flores, J.A. Melendez, Mitochondrial H2O2 regulates the angiogenic phenotype via PTEN oxidation, J Biol Chem 280 (2005) 16916-16924.
[90] L.C. Trotman, A. Alimonti, P.P. Scaglioni, J.A. Koutcher, C. Cordon-Cardo, P.P. Pandolfi, Identification of a tumour suppressor network opposing nuclear Akt function, Nature 441 (2006) 523-527.
[91] C.R. Yellaturu, M. Bhanoori, I. Neeli, G.N. Rao, N-Ethylmaleimide inhibits platelet-derived growth factor BB-stimulated Akt phosphorylation via activation of protein phosphatase 2A, J Biol Chem 277 (2002) 40148-40155.
[92] Y. Shiloh, ATM and related protein kinases: safeguarding genome integrity, Nat Rev Cancer 3 (2003) 155-168.
[93] P.J. Hurley, F. Bunz, ATM and ATR: components of an integrated circuit, Cell Cycle 6 (2007) 414-417.
[94] A. Barzilai, G. Rotman, Y. Shiloh, ATM deficiency and oxidative stress: a new dimension of defective response to DNA damage, DNA Repair (Amst) 1 (2002) 3-25.
[95] Z. Guo, S. Kozlov, M.F. Lavin, M.D. Person, T.T. Paull, ATM activation by oxidative stress, Science 330 (2010) 517-521.

[96] J.D. Gordan, M.C. Simon, Hypoxia-inducible factors: central regulators of the tumor phenotype, Curr Opin Genet Dev 17 (2007) 71-77.
[97] G.L. Semenza, Targeting HIF-1 for cancer therapy, Nat Rev Cancer 3 (2003) 721-732.
[98] F.H. Agani, P. Pichiule, J.C. Chavez, J.C. LaManna, The role of mitochondria in the regulation of hypoxia-inducible factor 1 expression during hypoxia, J Biol Chem 275 (2000) 35863-35867.
[99] J.K. Brunelle, E.L. Bell, N.M. Quesada, K. Vercauteren, V. Tiranti, M. Zeviani, R.C. Scarpulla, N.S. Chandel, Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation, Cell Metab 1 (2005) 409-414.
[100] R.D. Guzy, B. Hoyos, E. Robin, H. Chen, L. Liu, K.D. Mansfield, M.C. Simon, U. Hammerling, P.T. Schumacker, Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing, Cell Metab 1 (2005) 401-408.
[101] K.D. Mansfield, R.D. Guzy, Y. Pan, R.M. Young, T.P. Cash, P.T. Schumacker, M.C. Simon, Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-alpha activation, Cell Metab 1 (2005) 393-399.
[102] E.L. Bell, T.A. Klimova, J. Eisenbart, C.T. Moraes, M.P. Murphy, G.R. Budinger, N.S. Chandel, The Qo site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production, J Cell Biol 177 (2007) 1029-1036.
[103] X. Lin, C.A. David, J.B. Donnelly, M. Michaelides, N.S. Chandel, X. Huang, U. Warrior, F. Weinberg, K.V. Tormos, S.W. Fesik, Y. Shen, A chemical genomics screen highlights the essential role of mitochondria in HIF-1 regulation, Proc Natl Acad Sci U S A 105 (2008) 174-179.
[104] S.A. Patel, M.C. Simon, Biology of hypoxia-inducible factor-2alpha in development and disease, Cell Death Differ 15 (2008) 628-634.
[105] F. Orsini, E. Migliaccio, M. Moroni, C. Contursi, V.A. Raker, D. Piccini, I. Martin-Padura, G. Pelliccia, M. Trinei, M. Bono, C. Puri, C. Tacchetti, M. Ferrini, R. Mannucci, I. Nicoletti, L. Lanfrancone, M. Giorgio, P.G. Pelicci, The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential, J Biol Chem 279 (2004) 25689-25695.
[106] P.D. Ray, B.W. Huang, Y. Tsuji, Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling, Cell Signal 24 (2012) 981-990.
[107] M. Gertz, F. Fischer, D. Wolters, C. Steegborn, Activation of the lifespan regulator p66Shc through reversible disulfide bond formation, Proc Natl Acad Sci U S A 105 (2008) 5705-5709.
[108] F. Orsini, M. Moroni, C. Contursi, M. Yano, P. Pelicci, M. Giorgio, E. Migliaccio, Regulatory effects of the mitochondrial energetic status on mitochondrial p66Shc, Biol Chem 387 (2006) 1405-1410.
[109] G. Tell, A. Zecca, L. Pellizzari, P. Spessotto, A. Colombatti, M.R. Kelley, G. Damante, C. Pucillo, An 'environment to nucleus' signaling system operates in B lymphocytes: redox status modulates BSAP/Pax-5 activation through Ref-1 nuclear translocation, Nucleic Acids Res 28 (2000) 1099-1105.
[110] C.V. Ramana, I. Boldogh, T. Izumi, S. Mitra, Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals, Proc Natl Acad Sci U S A 95 (1998) 5061-5066.

[111] G. Tell, F. Quadrifoglio, C. Tiribelli, M.R. Kelley, The many functions of APE1/Ref-1: not only a DNA repair enzyme, Antioxid Redox Signal 11 (2009) 601-620.
[112] L.J. Walker, C.N. Robson, E. Black, D. Gillespie, I.D. Hickson, Identification of residues in the human DNA repair enzyme HAP1 (Ref-1) that are essential for redox regulation of Jun DNA binding, Mol Cell Biol 13 (1993) 5370-5376.
[113] S. Xanthoudakis, T. Curran, Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity, EMBO J 11 (1992) 653-665.
[114] T. Nguyen, H.C. Huang, C.B. Pickett, Transcriptional regulation of the antioxidant response element. Activation by Nrf2 and repression by MafK, J Biol Chem 275 (2000) 15466-15473.
[115] A.K. Jaiswal, Regulation of genes encoding NAD(P)H:quinone oxidoreductases, Free Radic Biol Med 29 (2000) 254-262.
[116] J. Zhang, T. Ohta, A. Maruyama, T. Hosoya, K. Nishikawa, J.M. Maher, S. Shibahara, K. Itoh, M. Yamamoto, BRG1 interacts with Nrf2 to selectively mediate HO-1 induction in response to oxidative stress, Mol Cell Biol 26 (2006) 7942-7952.
[117] S.M. Keyse, L.A. Applegate, Y. Tromvoukis, R.M. Tyrrell, Oxidant stress leads to transcriptional activation of the human heme oxygenase gene in cultured skin fibroblasts, Mol Cell Biol 10 (1990) 4967-4969.
[118] T. Nguyen, P.J. Sherratt, C.B. Pickett, Regulatory mechanisms controlling gene expression mediated by the antioxidant response element, Annu Rev Pharmacol Toxicol 43 (2003) 233-260.
[119] E.L. MacKenzie, P.D. Ray, Y. Tsuji, Role and regulation of ferritin H in rotenone-mediated mitochondrial oxidative stress, Free Radic Biol Med 44 (2008) 1762-1771.
[120] K. Itoh, N. Wakabayashi, Y. Katoh, T. Ishii, K. Igarashi, J.D. Engel, M. Yamamoto, Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain, Genes Dev 13 (1999) 76-86.
[121] M.C. Jaramillo, D.D. Zhang, The emerging role of the Nrf2-Keap1 signaling pathway in cancer, Genes Dev 27 (2013) 2179-2191.
[122] K. Itoh, T. Chiba, S. Takahashi, T. Ishii, K. Igarashi, Y. Katoh, T. Oyake, N. Hayashi, K. Satoh, I. Hatayama, M. Yamamoto, Y. Nabeshima, An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements, Biochem Biophys Res Commun 236 (1997) 313-322.
[123] K. Iwasaki, E.L. Mackenzie, K. Hailemariam, K. Sakamoto, Y. Tsuji, Hemin-mediated regulation of an antioxidant-responsive element of the human ferritin H gene and role of Ref-1 during erythroid differentiation of K562 cells, Mol Cell Biol 26 (2006) 2845-2856.
[124] R.P. Ureshino, K.K. Rocha, G.S. Lopes, C.B. Trindade, S.S. Smaili, Calcium signaling alterations, oxidative stress and autophagy in aging, Antioxid Redox Signal (2014). [Epub ahead of print]
[125] G. Csordas, G. Hajnoczky, SR/ER-mitochondrial local communication: calcium and ROS, Biochim Biophys Acta 1787 (2009) 1352-1362.
[126] A.V. Zima, L.A. Blatter, Redox regulation of cardiac calcium channels and transporters, Cardiovasc Res 71 (2006) 310-321.

[127] M. Madesh, B.J. Hawkins, T. Milovanova, C.D. Bhanumathy, S.K. Joseph, S.P. Ramachandrarao, K. Sharma, T. Kurosaki, A.B. Fisher, Selective role for superoxide in InsP3 receptor-mediated mitochondrial dysfunction and endothelial apoptosis, J Cell Biol 170 (2005) 1079-1090.
[128] A.A. Voss, J. Lango, M. Ernst-Russell, D. Morin, I.N. Pessah, Identification of hyperreactive cysteines within ryanodine receptor type 1 by mass spectrometry, J Biol Chem 279 (2004) 34514-34520.
[129] V.S. Sharov, E.S. Dremina, N.A. Galeva, T.D. Williams, C. Schoneich, Quantitative mapping of oxidation-sensitive cysteine residues in SERCA in vivo and in vitro by HPLC-electrospray-tandem MS: selective protein oxidation during biological aging, Biochem J 394 (2006) 605-615.
[130] E.L. MacKenzie, K. Iwasaki, Y. Tsuji, Intracellular iron transport and storage: from molecular mechanisms to health implications, Antioxid Redox Signal 10 (2008) 997-1030.
[131] A.J. Ghio, Disruption of iron homeostasis and lung disease, Biochim Biophys Acta 1790 (2009) 731-739.
[132] X. Brazzolotto, J. Gaillard, K. Pantopoulos, M.W. Hentze, J.M. Moulis, Human cytoplasmic aconitase (Iron regulatory protein 1) is converted into its [3Fe-4S] form by hydrogen peroxide in vitro but is not activated for iron-responsive element binding, J Biol Chem 274 (1999) 21625-21630.
[133] E. Soum, X. Brazzolotto, C. Goussias, C. Bouton, J.M. Moulis, T.A. Mattioli, J.C. Drapier, Peroxynitrite and nitric oxide differently target the iron-sulfur cluster and amino acid residues of human iron regulatory protein 1, Biochemistry 42 (2003) 7648-7654.
[134] A. Stys, B. Galy, R.R. Starzynski, E. Smuda, J.C. Drapier, P. Lipinski, C. Bouton, Iron regulatory protein 1 outcompetes iron regulatory protein 2 in regulating cellular iron homeostasis in response to nitric oxide, J Biol Chem 286 (2011) 22846-22854.
[135] K.B. Zumbrennen, M.L. Wallander, S.J. Romney, E.A. Leibold, Cysteine oxidation regulates the RNA-binding activity of iron regulatory protein 2, Mol Cell Biol 29 (2009) 2219-2229.
[136] R.J. DeBerardinis, J.J. Lum, G. Hatzivassiliou, C.B. Thompson, The biology of cancer: metabolic reprogramming fuels cell growth and proliferation, Cell Metab 7 (2008) 11-20.
[137] P.D. Boyer, The ATP synthase. A splendid molecular machine, Annu. Rev. Biochem. 66 (1997) 717-749.
[138] E. Cabezon, M.G. Montgomery, A.G. Leslie, J.E. Walker, The structure of bovine F1-ATPase in complex with its regulatory protein IF1, Nat Struct Biol 10 (2003) 744-750.
[139] A. Lehninger, Biochemistry, Worth Publishers, New York (1970).
[140] J.M. Cuezva, A.D. Ortega, I. Willers, L. Sanchez-Cenizo, M. Aldea, M. Sanchez-Arago, The tumor suppressor function of mitochondria: translation into the clinics, Biochim Biophys Acta 1792 (2009) 1145-1158.
[141] G.L. Semenza, Hypoxia-inducible factors in physiology and medicine, Cell 148 (2012) 399-408.
[142] A.D. Ortega, M. Sanchez-Arago, D. Giner-Sanchez, L. Sanchez-Cenizo, I. Willers, J.M. Cuezva, Glucose avidity of carcinomas, Cancer Lett 276 (2009) 125-135.
[143] C.D. Folmes, T.J. Nelson, A. Martinez-Fernandez, D.K. Arrell, J.Z. Lindor, P.P. Dzeja, Y. Ikeda, C. Perez-Terzic, A. Terzic, Somatic oxidative bioenergetics

transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming, Cell Metab 14 (2011) 264-271.
[144] J.M. Cuezva, M. Sanchez-Arago, S. Sala, A. Blanco-Rivero, A.D. Ortega, A message emerging from development: the repression of mitochondrial beta-F1-ATPase expression in cancer, J Bioenerg Biomembr 39 (2007) 259-265.
[145] R.A. Breckenridge, I. Piotrowska, K.E. Ng, T.J. Ragan, J.A. West, S. Kotecha, N. Towers, M. Bennett, P.C. Kienesberger, R.T. Smolenski, H.K. Siddall, J.L. Offer, M.M. Mocanu, D.M. Yelon, J.R. Dyck, J.L. Griffin, A.Y. Abramov, A.P. Gould, T.J. Mohun, Hypoxic regulation of hand1 controls the fetal-neonatal switch in cardiac metabolism, PLoS Biol 11 (2013) e1001666.
[146] C.T. Chen, Y.R. Shih, T.K. Kuo, O.K. Lee, Y.H. Wei, Coordinated changes of mitochondrial biogenesis and antioxidant enzymes during osteogenic differentiation of human mesenchymal stem cells, Stem Cells 26 (2008) 960-968.
[147] M. Sanchez-Arago, J. Garcia-Bermudez, I. Martinez-Reyes, F. Santacatterina, J.M. Cuezva, Degradation of IF1 controls energy metabolism during osteogenic differentiation of stem cells, EMBO Rep 14 (2013) 638-644.
[148] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, Science 324 (2009) 1029-1033.
[149] G. Amuthan, G. Biswas, S.Y. Zhang, A. Klein-Szanto, C. Vijayasarathy, N.G. Avadhani, Mitochondria-to-nucleus stress signaling induces phenotypic changes, tumor progression and cell invasion, EMBO J 20 (2001) 1910-1920.
[150] T. McFate, A. Mohyeldin, H. Lu, J. Thakar, J. Henriques, N.D. Halim, H. Wu, M.J. Schell, T.M. Tsang, O. Teahan, S. Zhou, J.A. Califano, N.H. Jeoung, R.A. Harris, A. Verma, Pyruvate dehydrogenase complex activity controls metabolic and malignant phenotype in cancer cells, J Biol Chem 283 (2008) 22700-22708.
[151] S. Bonnet, S.L. Archer, J. Allalunis-Turner, A. Haromy, C. Beaulieu, R. Thompson, C.T. Lee, G.D. Lopaschuk, L. Puttagunta, S. Bonnet, G. Harry, K. Hashimoto, C.J. Porter, M.A. Andrade, B. Thebaud, E.D. Michelakis, A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth, Cancer Cell 11 (2007) 37-51.
[152] R. Dey, C.T. Moraes, Lack of oxidative phosphorylation and low mitochondrial membrane potential decrease susceptibility to apoptosis and do not modulate the protective effect of Bcl-x(L) in osteosarcoma cells, J Biol Chem 275 (2000) 7087-7094.
[153] J.Y. Kim, Y.H. Kim, I. Chang, S. Kim, Y.K. Pak, B.H. Oh, H. Yagita, Y.K. Jung, Y.J. Oh, M.S. Lee, Resistance of mitochondrial DNA-deficient cells to TRAIL: role of Bax in TRAIL-induced apoptosis, Oncogene 21 (2002) 3139-3148.
[154] A. Tomiyama, S. Serizawa, K. Tachibana, K. Sakurada, H. Samejima, Y. Kuchino, C. Kitanaka, Critical role for mitochondrial oxidative phosphorylation in the activation of tumor suppressors Bax and Bak, J Natl Cancer Inst 98 (2006) 1462-1473.
[155] X. Wang, The expanding role of mitochondria in apoptosis, Genes Dev 15 (2001) 2922-2933.
[156] S. Matsuyama, Q. Xu, J. Velours, J.C. Reed, The Mitochondrial F0F1-ATPase proton pump is required for function of the proapoptotic protein Bax in yeast and mammalian cells, Mol Cell 1 (1998) 327-336.

[157] D.R. Plas, C.B. Thompson, Cell metabolism in the regulation of programmed cell death, Trends Endocrinol Metab 13 (2002) 75-78.
[158] N. Vahsen, C. Cande, J.J. Briere, P. Benit, N. Joza, N. Larochette, P.G. Mastroberardino, M.O. Pequignot, N. Casares, V. Lazar, O. Feraud, N. Debili, S. Wissing, S. Engelhardt, F. Madeo, M. Piacentini, J.M. Penninger, H. Schagger, P. Rustin, G. Kroemer, AIF deficiency compromises oxidative phosphorylation, EMBO J 23 (2004) 4679-4689.
[159] G. Kroemer, P. Petit, N. Zamzami, J.L. Vayssiere, B. Mignotte, The biochemistry of programmed cell death, FASEB J. 9 (1995) 1277-1287.
[160] B. Herrera, A.M. Alvarez, A. Sanchez, M. Fernandez, C. Roncero, M. Benito, I. Fabregat, Reactive oxygen species (ROS) mediates the mitochondrial-dependent apoptosis induced by transforming growth factor (beta) in fetal hepatocytes, FASEB J 15 (2001) 741-751.
[161] A.J. Lambert, M.D. Brand, Superoxide production by NADH:ubiquinone oxidoreductase (complex I) depends on the pH gradient across the mitochondrial inner membrane, Biochem J 382 (2004) 511-517.
[162] M.P. Mattson, G. Kroemer, Mitochondria in cell death: novel targets for neuroprotection and cardioprotection, Trends Mol Med 9 (2003) 196-205.
[163] A.C. Rego, S. Vesce, D.G. Nicholls, The mechanism of mitochondrial membrane potential retention following release of cytochrome c in apoptotic GT1-7 neural cells, Cell Death Differ 8 (2001) 995-1003.
[164] S. Matsuyama, J. Llopis, Q.L. Deveraux, R.Y. Tsien, J.C. Reed, Changes in intramitochondrial and cytosolic pH: early events that modulate caspase activation during apoptosis, Nat Cell Biol 2 (2000) 318-325.
[165] K. Buchet, C. Godinot, Functional F1-ATPase essential in maintaining growth and membrane potential of human mitochondrial DNA-depleted rho degrees cells, J Biol Chem 273 (1998) 22983-22989.
[166] D.G. Nicholls, M.W. Ward, Mitochondrial membrane potential and neuronal glutamate excitotoxicity: mortality and millivolts, Trends Neurosci 23 (2000) 166-174.
[167] K.M. Johnson, X. Chen, A. Boitano, L. Swenson, A.W. Opipari, Jr., G.D. Glick, Identification and validation of the mitochondrial F1F0-ATPase as the molecular target of the immunomodulatory benzodiazepine Bz-423, Chem Biol 12 (2005) 485-496.
[168] Y. Gong, H. Sohn, L. Xue, G.L. Firestone, L.F. Bjeldanes, 3,3'-Diindolylmethane is a novel mitochondrial H(+)-ATP synthase inhibitor that can induce p21(Cip1/Waf1) expression by induction of oxidative stress in human breast cancer cells, Cancer Res 66 (2006) 4880-4887.
[169] M. Sanchez-Arago, J.M. Cuezva, The bioenergetic signature of isogenic colon cancer cells predicts the cell death response to treatment with 3-bromopyruvate, iodoacetate or 5-fluorouracil, J Transl Med 9 (2011) 19.
[170] Y. Zhang, O. Marcillat, C. Giulivi, L. Ernster, K.J. Davies, The oxidative inactivation of mitochondrial electron transport chain components and ATPase, J Biol Chem 265 (1990) 16330-16336.
[171] G. Lippe, M. Comelli, D. Mazzilis, F.D. Sala, I. Mavelli, The inactivation of mitochondrial F1 ATPase by H2O2 is mediated by iron ions not tightly bound in the protein, Biochem Biophys Res Commun 181 (1991) 764-770.
[172] F. Buchert, Y. Schober, A. Rompp, M.L. Richter, C. Forreiter, Reactive oxygen species affect ATP hydrolysis by targeting a highly conserved amino acid cluster

in the thylakoid ATP synthase gamma subunit, Biochim Biophys Acta 1817 (2012) 2038-2048.
[173] L. Galluzzi, K. Blomgren, G. Kroemer, Mitochondrial membrane permeabilization in neuronal injury, Nat Rev Neurosci 10 (2009) 481-494.
[174] F. Di Lisa, A. Carpi, V. Giorgio, P. Bernardi, The mitochondrial permeability transition pore and cyclophilin D in cardioprotection, Biochim Biophys Acta 1813 (2011) 1316-1322.
[175] P. Bernardi, The mitochondrial permeability transition pore: a mystery solved?, Front Physiol 4 (2013) 95.
[176] M. Bonora, A. Bononi, E. De Marchi, C. Giorgi, M. Lebiedzinska, S. Marchi, S. Patergnani, A. Rimessi, J.M. Suski, A. Wojtala, M.R. Wieckowski, G. Kroemer, L. Galluzzi, P. Pinton, Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition, Cell Cycle 12 (2013) 674-683.
[177] V. Giorgio, S. von Stockum, M. Antoniel, A. Fabbro, F. Fogolari, M. Forte, G.D. Glick, V. Petronilli, M. Zoratti, I. Szabo, G. Lippe, P. Bernardi, Dimers of mitochondrial ATP synthase form the permeability transition pore, Proc Natl Acad Sci U S A 110 (2013) 5887-5892.
[178] P. Bernardi, A. Krauskopf, E. Basso, V. Petronilli, E. Blachly-Dyson, F. Di Lisa, M.A. Forte, The mitochondrial permeability transition from in vitro artifact to disease target, FEBS J 273 (2006) 2077-2099.
[179] E.B. Zago, R.F. Castilho, A.E. Vercesi, The redox state of endogenous pyridine nucleotides can determine both the degree of mitochondrial oxidative stress and the solute selectivity of the permeability transition pore, FEBS Lett 478 (2000) 29-33.
[180] A.J. Kowaltowski, R.F. Castilho, A.E. Vercesi, Mitochondrial permeability transition and oxidative stress, FEBS Lett 495 (2001) 12-15.
[181] P. Costantini, B.V. Chernyak, V. Petronilli, P. Bernardi, Selective inhibition of the mitochondrial permeability transition pore at the oxidation-reduction sensitive dithiol by monobromobimane, FEBS Lett 362 (1995) 239-242.
[182] P. Costantini, B.V. Chernyak, V. Petronilli, P. Bernardi, Modulation of the mitochondrial permeability transition pore by pyridine nucleotides and dithiol oxidation at two separate sites, J Biol Chem 271 (1996) 6746-6751.
[183] L.I. Gordon, M.A. Burke, A.T. Singh, S. Prachand, E.D. Lieberman, L. Sun, T.J. Naik, S.V. Prasad, H. Ardehali, Blockade of the erbB2 receptor induces cardiomyocyte death through mitochondrial and reactive oxygen species-dependent pathways, J Biol Chem 284 (2009) 2080-2087.
[184] E.D. Michelakis, G. Sutendra, P. Dromparis, L. Webster, A. Haromy, E. Niven, C. Maguire, T.L. Gammer, J.R. Mackey, D. Fulton, B. Abdulkarim, M.S. McMurtry, K.C. Petruk, Metabolic modulation of glioblastoma with dichloroacetate, Sci Transl Med 2 (2010) 31-34.
[185] I. D'Errico, L. Salvatore, S. Murzilli, G. Lo Sasso, D. Latorre, N. Martelli, A.V. Egorova, R. Polishuck, K. Madeyski-Bengtson, C. Lelliott, A.J. Vidal-Puig, P. Seibel, G. Villani, A. Moschetta, Peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1alpha) is a metabolic regulator of intestinal epithelial cell fate, Proc Natl Acad Sci U S A 108 (2011) 6603-6608.
[186] X. Wang, C.T. Moraes, Increases in mitochondrial biogenesis impair carcinogenesis at multiple levels, Mol Oncol 5 (2011) 399-409.
[187] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646-674.

[188] J.M. Cuezva, M. Krajewska, M.L. de Heredia, S. Krajewski, G. Santamaria, H. Kim, J.M. Zapata, H. Marusawa, M. Chamorro, J.C. Reed, The bioenergetic signature of cancer: a marker of tumor progression, Cancer Res 62 (2002) 6674-6681.

[189] M. Aldea, J. Clofent, C. Nunez de Arenas, M. Chamorro, M. Velasco, J.R. Berrendero, C. Navarro, J.M. Cuezva, Reverse phase protein microarrays quantify and validate the bioenergetic signature as biomarker in colorectal cancer, Cancer Lett 311 (2011) 210-218.

[190] P.C. Lin, J.K. Lin, S.H. Yang, H.S. Wang, A.F. Li, S.C. Chang, Expression of beta-F1-ATPase and mitochondrial transcription factor A and the change in mitochondrial DNA content in colorectal cancer: clinical data analysis and evidence from an in vitro study, Int J Colorectal Dis 23 (2008) 1223-1232.

[191] J.M. Cuezva, G. Chen, A.M. Alonso, A. Isidoro, D.E. Misek, S.M. Hanash, D.G. Beer, The bioenergetic signature of lung adenocarcinomas is a molecular marker of cancer diagnosis and prognosis, Carcinogenesis 25 (2004) 1157-1163.

[192] F. Lopez-Rios, M. Sanchez-Arago, E. Garcia-Garcia, A.D. Ortega, J.R. Berrendero, F. Pozo-Rodriguez, A. Lopez-Encuentra, C. Ballestin, J.M. Cuezva, Loss of the mitochondrial bioenergetic capacity underlies the glucose avidity of carcinomas, Cancer Res 67 (2007) 9013-9017.

[193] A. Isidoro, E. Casado, A. Redondo, P. Acebo, E. Espinosa, A.M. Alonso, P. Cejas, D. Hardisson, J.A. Fresno Vara, C. Belda-Iniesta, M. Gonzalez-Baron, J.M. Cuezva, Breast carcinomas fulfill the Warburg hypothesis and provide metabolic markers of cancer prognosis, Carcinogenesis 26 (2005) 2095-2104.

[194] E. Hjerpe, S. Egyhazi Brage, J. Carlson, M. Frostvik Stolt, K. Schedvins, H. Johansson, M. Shoshan, E. Avall-Lundqvist, Metabolic markers GAPDH, PKM2, ATP5B and BEC-index in advanced serous ovarian cancer, BMC Clin Pathol 13 (2013) 30.

[195] X. Xiao, J. Yang, R. Li, S. Liu, Y. Xu, W. Zheng, Y. Yi, Y. Luo, F. Gong, H. Peng, M. Pei, M. Deng, G. Zhang, Deregulation of mitochondrial ATPsyn-beta in acute myeloid leukemia cells and with increased drug resistance, PLoS One 8 (2013) e83610.

[196] Y.K. Shin, B.C. Yoo, H.J. Chang, E. Jeon, S.H. Hong, M.S. Jung, S.J. Lim, J.G. Park, Down-regulation of mitochondrial F1F0-ATP synthase in human colon cancer cells with induced 5-fluorouracil resistance, Cancer Res 65 (2005) 3162-3170.

[197] E. Hernlund, E. Hjerpe, E. Avall-Lundqvist, M. Shoshan, Ovarian carcinoma cells with low levels of beta-F1-ATPase are sensitive to combined platinum and 2-deoxy-D-glucose treatment, Mol Cancer Ther 8 (2009) 1916-1923.

[198] R.J. Li, G.S. Zhang, Y.H. Chen, J.F. Zhu, Q.J. Lu, F.J. Gong, W.Y. Kuang, Down-regulation of mitochondrial ATPase by hypermethylation mechanism in chronic myeloid leukemia is associated with multidrug resistance, Ann Oncol 7 (2010) 1506-1514.

[199] M. Sanchez-Arago, M. Chamorro, J.M. Cuezva, Selection of cancer cells with repressed mitochondria triggers colon cancer progression, Carcinogenesis 31 (2010) 567-576.

[200] K.N. Alavian, H. Li, L. Collis, L. Bonanni, L. Zeng, S. Sacchetti, E. Lazrove, P. Nabili, B. Flaherty, M. Graham, Y. Chen, S.M. Messerli, M.A. Mariggio, C. Rahner, E. McNay, G.C. Shore, P.J. Smith, J.M. Hardwick, E.A. Jonas, Bcl-x(L) regulates metabolic efficiency of neurons through interaction with the mitochondrial F(1)F(O) ATP synthase, Nat Cell Biol 13 (2011) 1224-1233.

[201] S. Chivasa, D.F. Tome, J.M. Hamilton, A.R. Slabas, Proteomic analysis of extracellular ATP-regulated proteins identifies ATP synthase beta-subunit as a novel plant cell death regulator, Mol Cell Proteomics 10 (2011) M110 003905.
[202] E. Cabezon, P.J. Butler, M.J. Runswick, J.E. Walker, Modulation of the oligomerization state of the bovine F1-ATPase inhibitor protein, IF1, by pH, J Biol Chem 275 (2000) 25460-25464.
[203] J.R. Gledhill, M.G. Montgomery, A.G. Leslie, J.E. Walker, How the regulatory protein, IF(1), inhibits F(1)-ATPase from bovine mitochondria, Proc Natl Acad Sci U S A 104 (2007) 15671-15676.
[204] M.E. Pullman, G.C. Monroy, A Naturally Occurring Inhibitor of Mitochondrial Adenosine Triphosphatase, J Biol Chem 238 (1963) 3762-3769.
[205] M.S. Lebowitz, P.L. Pedersen, Regulation of the mitochondrial ATP synthase/ATPase complex: cDNA cloning, sequence, overexpression, and secondary structural characterization of a functional protein inhibitor, Arch Biochem Biophys 301 (1993) 64-70.
[206] J.E. Walker, N.J. Gay, S.J. Powell, M. Kostina, M.R. Dyer, ATP synthase from bovine mitochondria: sequences of imported precursors of oligomycin sensitivity conferral protein, factor 6, and adenosinetriphosphatase inhibitor protein, Biochemistry 26 (1987) 8613-8619.
[207] J.E. Walker, The ATP synthase: the understood, the uncertain and the unknown, Biochem Soc Trans 41 (2013) 1-16.
[208] R. Schnizer, G. Van Heeke, D. Amaturo, S.M. Schuster, Histidine-49 is necessary for the pH-dependent transition between active and inactive states of the bovine F1-ATPase inhibitor protein, Biochim Biophys Acta 1292 (1996) 241-248.
[209] D.W. Green, G.J. Grover, The IF(1) inhibitor protein of the mitochondrial F(1)F(0)-ATPase, Biochim Biophys Acta 1458 (2000) 343-355.
[210] C. Ando, N. Ichikawa, Glutamic acid in the inhibitory site of mitochondrial ATPase inhibitor, IF(1), participates in pH sensing in both mammals and yeast, J Biochem 144 (2008) 547-553.
[211] W. Rouslin, C.W. Broge, Mechanisms of ATP conservation during ischemia in slow and fast heart rate hearts, Am J Physiol 264 (1993) C209-216.
[212] M. Campanella, E. Casswell, S. Chong, Z. Farah, M.R. Wieckowski, A.Y. Abramov, A. Tinker, M.R. Duchen, Regulation of mitochondrial structure and function by the F1Fo-ATPase inhibitor protein, IF1, Cell Metab 8 (2008) 13-25.
[213] C. Chinopoulos, Mitochondrial consumption of cytosolic ATP: not so fast, FEBS Lett 585 (2011) 1255-1259.
[214] V. Lefebvre, Q. Du, S. Baird, A.C. Ng, M. Nascimento, M. Campanella, H.M. McBride, R.A. Screaton, Genome-wide RNAi screen identifies ATPase inhibitory factor 1 (ATPIF1) as essential for PARK2 recruitment and mitophagy, Autophagy 9 (2013) 1770-1779.
[215] D.I. Shah, N. Takahashi-Makise, J.D. Cooney, L. Li, I.J. Schultz, E.L. Pierce, A. Narla, A. Seguin, S.M. Hattangadi, A.E. Medlock, N.B. Langer, T.A. Dailey, S.N. Hurst, D. Faccenda, J.M. Wiwczar, S.K. Heggers, G. Vogin, W. Chen, C. Chen, D.R. Campagna, C. Brugnara, Y. Zhou, B.L. Ebert, N.N. Danial, M.D. Fleming, D.M. Ward, M. Campanella, H.A. Dailey, J. Kaplan, B.H. Paw, Mitochondrial Atpif1 regulates haem synthesis in developing erythroblasts, Nature (2012) 608-612.

[216] J. Nakamura, M. Fujikawa, M. Yoshida, IF1, a natural inhibitor of mitochondrial ATP synthase, is not essential for the normal growth and breeding of mice, Biosci Rep 33 (2013) 735-741.
[217] R. Luft, D. Ikkos, G. Palmieri, L. Ernster, B. Afzelius, A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical, and morphological study, J Clin Invest 41 (1962) 1776-1804.
[218] D. Faccenda, M. Campanella, Molecular Regulation of the Mitochondrial F(1)F(o)-ATPsynthase: Physiological and Pathological Significance of the Inhibitory Factor 1 (IF(1)), Int J Cell Biol 2012 (2012) 367934.
[219] S. DiMauro, E. Bonilla, C.P. Lee, D.L. Schotland, A. Scarpa, H. Conn, Jr., B. Chance, Luft's disease. Further biochemical and ultrastructural studies of skeletal muscle in the second case, J Neurol Sci 27 (1976) 217-232.
[220] P. Paumard, J. Vaillier, B. Coulary, J. Schaeffer, V. Soubannier, D.M. Mueller, D. Brethes, J.P. di Rago, J. Velours, The ATP synthase is involved in generating mitochondrial cristae morphology, EMBO J 21 (2002) 221-230.
[221] M. Strauss, G. Hofhaus, R.R. Schroder, W. Kuhlbrandt, Dimer ribbons of ATP synthase shape the inner mitochondrial membrane, EMBO J 27 (2008) 1154-1160.
[222] K.M. Davies, M. Strauss, B. Daum, J.H. Kief, H.D. Osiewacz, A. Rycovska, V. Zickermann, W. Kuhlbrandt, Macromolecular organization of ATP synthase and complex I in whole mitochondria, Proc Natl Acad Sci U S A 108 (2011) 14121-14126.
[223] K.M. Davies, C. Anselmi, I. Wittig, J.D. Faraldo-Gomez, W. Kuhlbrandt, Structure of the yeast F1Fo-ATP synthase dimer and its role in shaping the mitochondrial cristae, Proc Natl Acad Sci U S A 109 (2012) 13602-13607.
[224] B. Daum, A. Walter, A. Horst, H.D. Osiewacz, W. Kuhlbrandt, Age-dependent dissociation of ATP synthase dimers and loss of inner-membrane cristae in mitochondria, Proc Natl Acad Sci U S A 110 (2013) 15301-15306.
[225] J.J. Garcia, E. Morales-Rios, P. Cortes-Hernandez, J.S. Rodriguez-Zavala, The inhibitor protein (IF1) promotes dimerization of the mitochondrial F1F0-ATP synthase, Biochemistry 45 (2006) 12695-12703.
[226] F. Minauro-Sanmiguel, S. Wilkens, J.J. Garcia, Structure of dimeric mitochondrial ATP synthase: novel F0 bridging features and the structural basis of mitochondrial cristae biogenesis, Proc Natl Acad Sci U S A 102 (2005) 12356-12358.
[227] E. Bisetto, M. Comelli, A.M. Salzano, P. Picotti, A. Scaloni, G. Lippe, I. Mavelli, Proteomic analysis of F1F0-ATP synthase super-assembly in mitochondria of cardiomyoblasts undergoing differentiation to the cardiac lineage, Biochim Biophys Acta 1827 (2013) 807-816.
[228] I. Wittig, H. Schagger, Supramolecular organization of ATP synthase and respiratory chain in mitochondrial membranes, Biochim Biophys Acta 1787 (2009) 672-680.
[229] M. Fujikawa, H. Imamura, J. Nakamura, M. Yoshida, Assessing the actual contribution of IF1, an inhibitor of mitochondrial FoF1, to ATP homeostasis, cell growth, mitochondrial morphology and cell viability, J Biol Chem 287 (2012) 18781-18787.
[230] L. Tomasetig, F. Di Pancrazio, D.A. Harris, I. Mavelli, G. Lippe, Dimerization of F0F1ATP synthase from bovine heart is independent from the binding of the inhibitor protein IF1, Biochim Biophys Acta 1556 (2002) 133-141.

[231] D.J. Pagliarini, S.E. Calvo, B. Chang, S.A. Sheth, S.B. Vafai, S.E. Ong, G.A. Walford, C. Sugiana, A. Boneh, W.K. Chen, D.E. Hill, M. Vidal, J.G. Evans, D.R. Thorburn, S.A. Carr, V.K. Mootha, A mitochondrial protein compendium elucidates complex I disease biology, Cell 134 (2008) 112-123.
[232] M. Sanchez-Arago, L. Formentini, J. Garcia-Bermudez, J.M. Cuezva, IF1 reprograms energy metabolism and signals the oncogenic phenotype in cancer, Cell Cycle 11 (2012) 2963-2964.
[233] D. Faccenda, C.H. Tan, A. Seraphim, M.R. Duchen, M. Campanella, IF1 limits the apoptotic-signalling cascade by preventing mitochondrial remodelling, Cell Death Differ 20 (2013) 686-697.
[234] L. Formentini, M.P. Pereira, L. Sanchez-Cenizo, F. Santacatterina, J.J. Lucas, C. Navarro, A. Martínez-Serrano, J.M. Cuezva, In vivo inhibition of the mitochondrial H+-ATP synthase in neurons promotes metabolic preconditioning, EMBO J (2014) (Epub ahead of print).
[235] L.J. Huang, I.C. Chuang, H.P. Dong, R.C. Yang, Hypoxia-inducible factor 1alpha regulates the expression of the mitochondrial ATPase inhibitor protein (IF1) in rat liver, Shock 36 (2011) 90-96.
[236] L. Shen, L. Zhi, W. Hu, M.X. Wu, IEX-1 targets mitochondrial F1Fo-ATPase inhibitor for degradation, Cell Death Differ 16 (2009) 603-612.
[237] C. Lopez-Mediavilla, H. Vigny, C. Godinot, Docking the mitochondrial inhibitor protein IF1 to a membrane receptor different from the F1-ATPase beta subunit, Eur J Biochem 215 (1993) 487-496.
[238] X. Zhao, I.R. Leon, S. Bak, M. Mogensen, K. Wrzesinski, K. Hojlund, O.N. Jensen, Phosphoproteome analysis of functional mitochondria isolated from resting human muscle reveals extensive phosphorylation of inner membrane protein complexes and enzymes, Mol Cell Proteomics 10 (2011) M110.000299.

Figure Legends.

Fig. 1. The metabolism of oxygen. Superoxide is mainly produced by NADPH oxidases and by the mitochondrial respiratory chain. Superoxide is converted to hydrogen peroxide (H_2O_2) by superoxide dismutases (SODs). H_2O_2 is converted to water (H_2O) by glutathione peroxidases (GPX), peroxiredoxins (PRX) or catalase. H_2O_2 is the main player in ROS cellular signaling because it can promote posttranslational modifications in proteins by thiol oxidation. The reaction of H_2O_2 with iron (Fe^{2+}) generate hydroxyl radicals ($^{\cdot}OH$) that are responsible for lipid, protein and DNA damage, promoting oxidative stress.

Fig. 2. Overview of mitochondrial ROS production. The scheme shows the relevant sites of ROS production by mitochondria. The transfer of electrons obtained by the oxidation of NADH and $FADH_2$ to molecular oxygen by respiratory complexes in the inner mitochondrial membrane is depicted by continuous red lines. The formation of the proton gradient generated by respiration and its utilization for the synthesis of ATP by the H^+-ATP synthase in oxidative phosphorylation is indicated. Complexes I, II and III produce superoxide (discontinuous red lines, ROS) and release it in the matrix. Complex III also releases ROS into the intermembrane space. The activity of the H^+-ATP synthase also modulates the levels of ROS produced by mitochondria. ROS are also generated by the redox protein p66Shc which interacts with TOM-TIM protein import complexes and with cytochrome c (cyt c). MAO, which is located in the outer mitochondrial membrane, represents an additional source of ROS in mitochondria.

Fig. 3. Regulation of protein activity by ROS. Proteins are regulated by ROS through the oxidation of thiol groups (-SH) of redox-reactive cysteine residues. Oxidation of these residues by ROS can form reactive sulfenic acid (-SOH) that can undergo further oxidation to sulfonic ($-SO_3H$) acid or form disulfide bonds with nearby cysteines (-SS-). These modifications lead to the inactivation of the proteins. Disulfide bonds can be reduced by thioredoxin (TRX) reductase that recovers the activity of the protein. Sulphenic acid (-SOH) can also react with glutathione (GSH) becoming glutathionylated (-SSG). Protein activity can be recovered by the action of glutaredoxin (GRX) that recognizes glutathionylated substrates and utilizes glutathione (GSH) for the reduction of the –SSG groups. Thiol groups can also react with reactive nitrogen species (RNS) to form S-nitrosothiol groups (-SNO) that also trigger protein inactivation.

Fig. 4. Signaling pathways regulated by ROS. ROS regulate several signaling pathways with key roles in cellular processes by affecting the activity of critical molecules. ROS can mediate survival, proliferation, metabolism and cell death by regulating the activity of proteins involved in MAPK pathways such as, ASK1, PTP1b, and DUSP3. Proteins participating in the PI3K pathway such as AKT, PTEN, PP2A and ATM are also direct targets of ROS. HIF1α, p66Shc and AMPK are also regulated by ROS. ROS modulate the antioxidant response through the action of REF1 and NRF2. The iron regulatory proteins IRP1 and IRP2 that are involved in iron homeostasis and InsP3R, RyR and SERCA that participate in the regulation of $Ca2^{+}$ signaling are also targeted by ROS.

Fig. 5. Energy metabolism determines the cell-death pathway of cancer cells. Susceptibility of cancer cells to death stimulus is highly dependent on their metabolic phenotype. The rapid dismantling and fragmentation of the mitochondrial tubular network into small mitochondria (green) occurs as a first response to a death stimulus to chemotherapeutic agents. Tumor cells with a significant activity of oxidative phosphorylation (OXPHOS) will produce high levels of ROS contributing to the oxidation of mitochondrial proteins (red dots) and the release of the mitochondrial arsenal involved in the execution of cell death (fragmented nucleus in yellow). Cells with a diminished activity of OXPHOS and thus an increased activity of the glycolytic pathway will not generate ROS under conventional chemotherapy what results in a resistant cell death phenotype.

Fig. 6. IF1 mediates the oncogenic phenotype by ROS signaling. The illustration shows a schematic organization of the mitochondrial H^{+}-ATP synthase. Several subunits are color-coded and labeled. In tumor cells, the ATPase Inhibitory Factor 1 (IF1) (in red) is highly over-expressed and binds the catalytic α /β interface. This interaction prevents synthesis of ATP triggering metabolic reprogramming towards an enhanced glycolytic phenotype. Inhibition of the H^{+}-ATP synthase also promotes an increase in the mitochondrial membrane potencial ($\Delta\psi$m) and the subsequent increase in superoxide radical production (ROS). ROS signaling will activate proliferation and survival pathways in the nucleus of the cell. All these events represent hallmarks of cancer as they promote tumorigenesis and metastasis.

Figure 1.

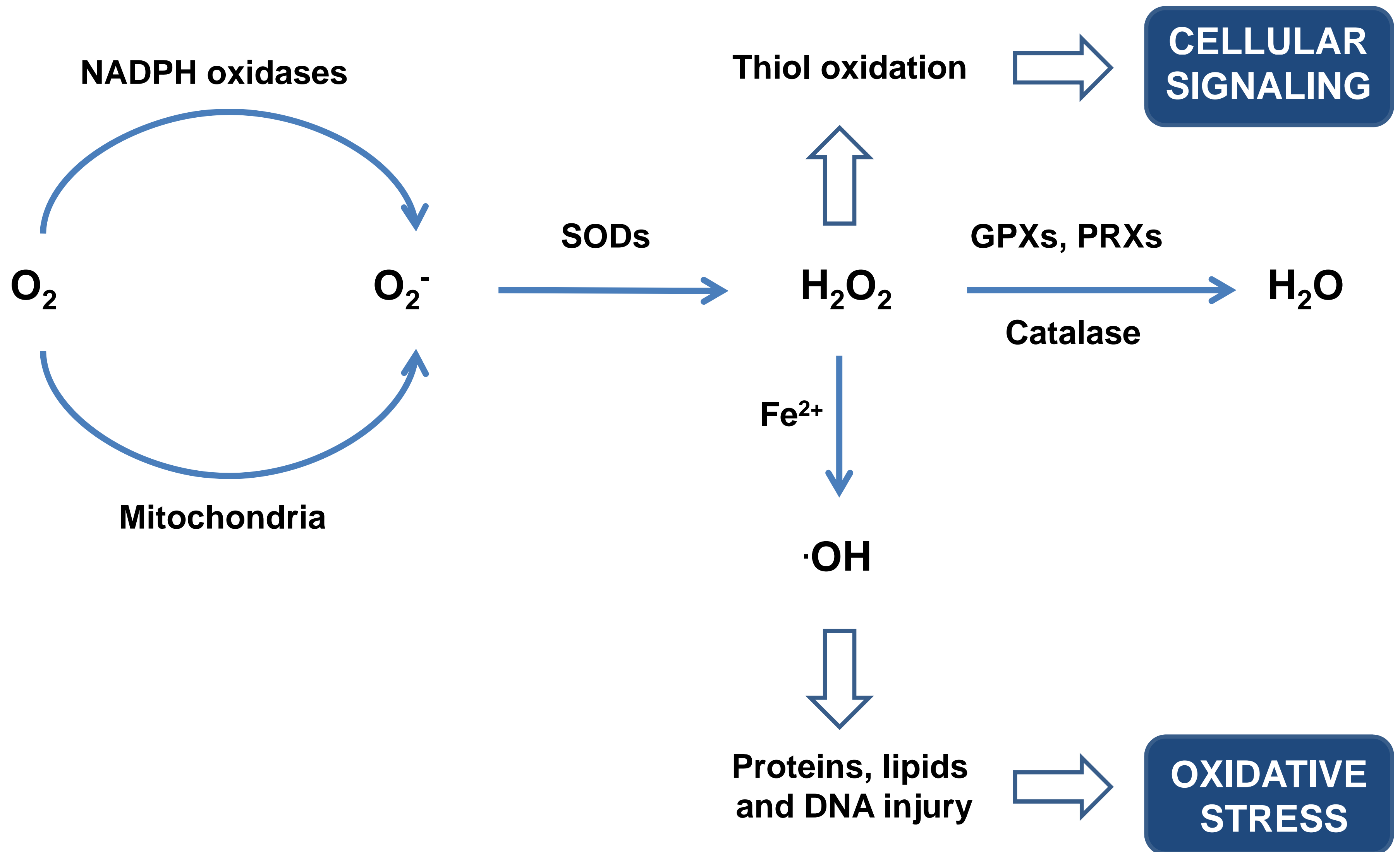

Figure 2.

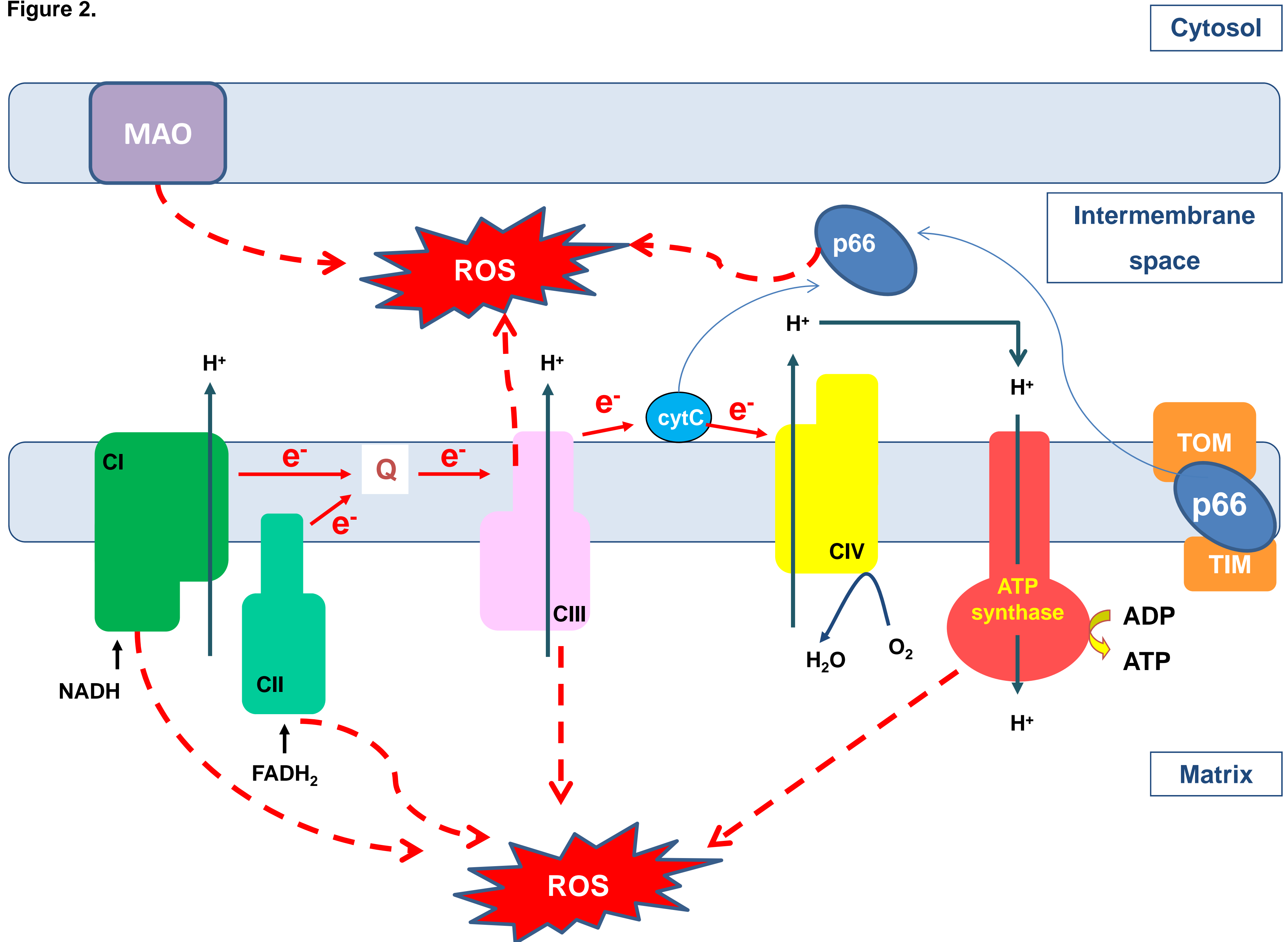

Figure 3.

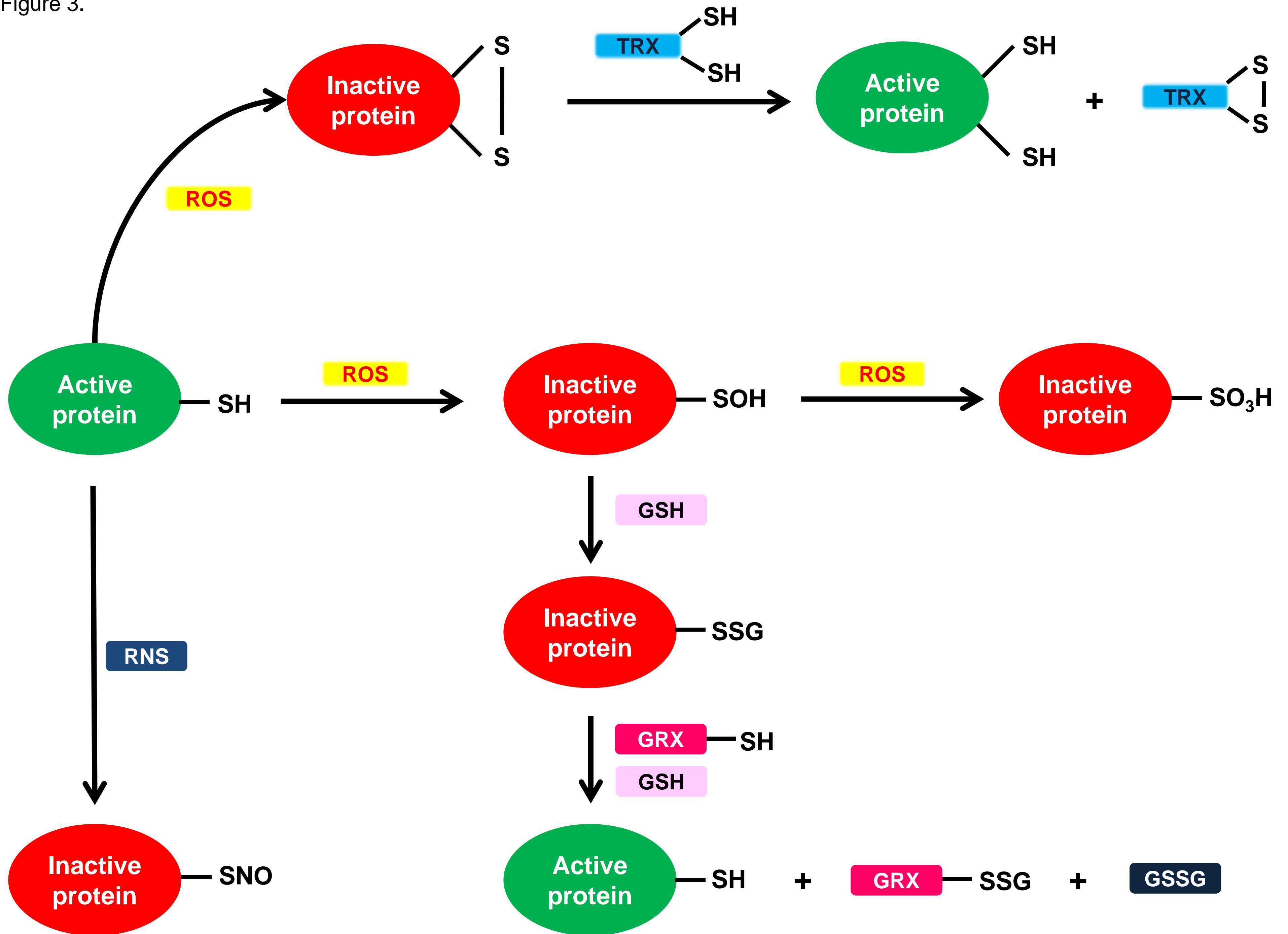

Figure 4.

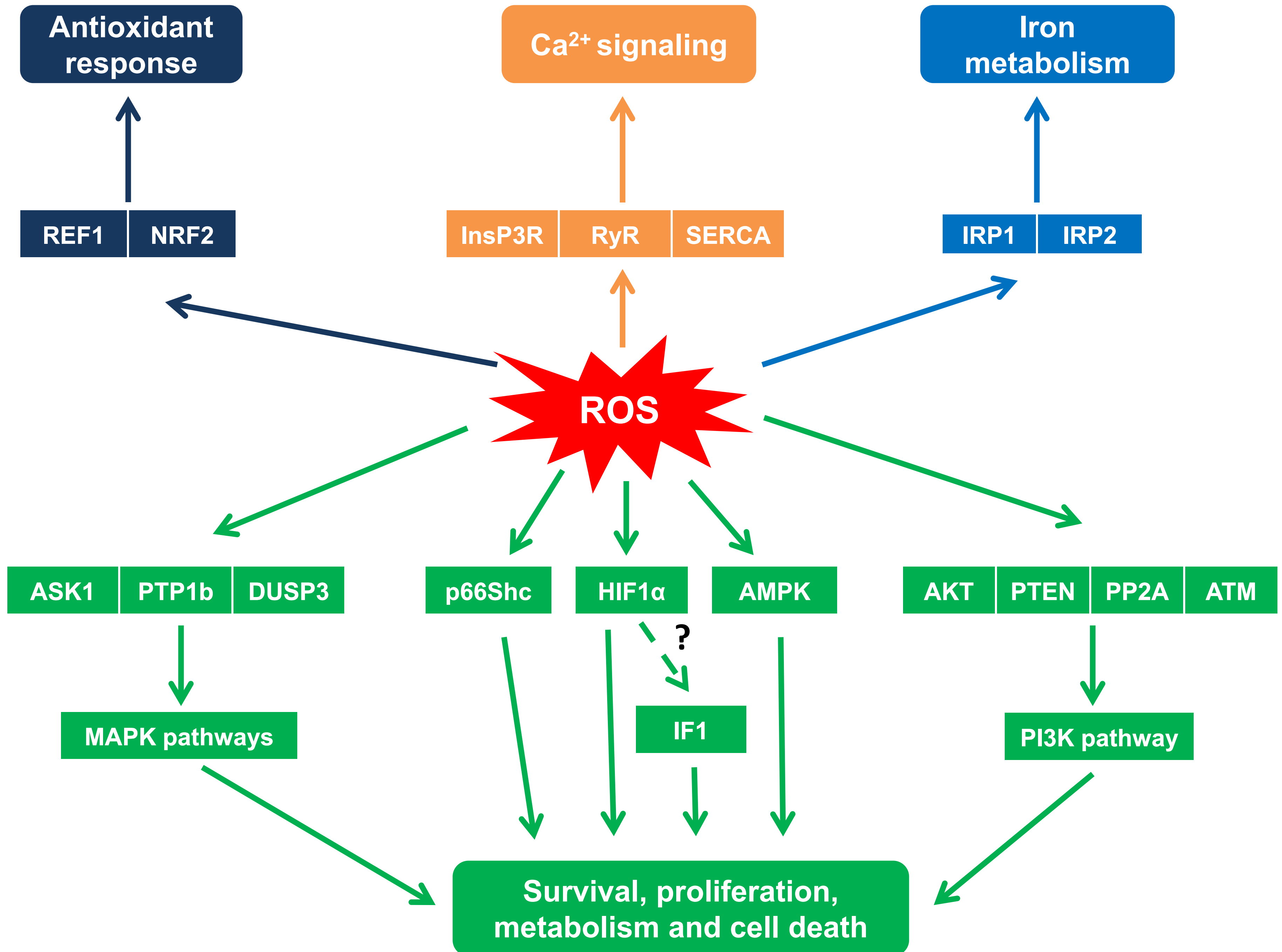

Figure 5.

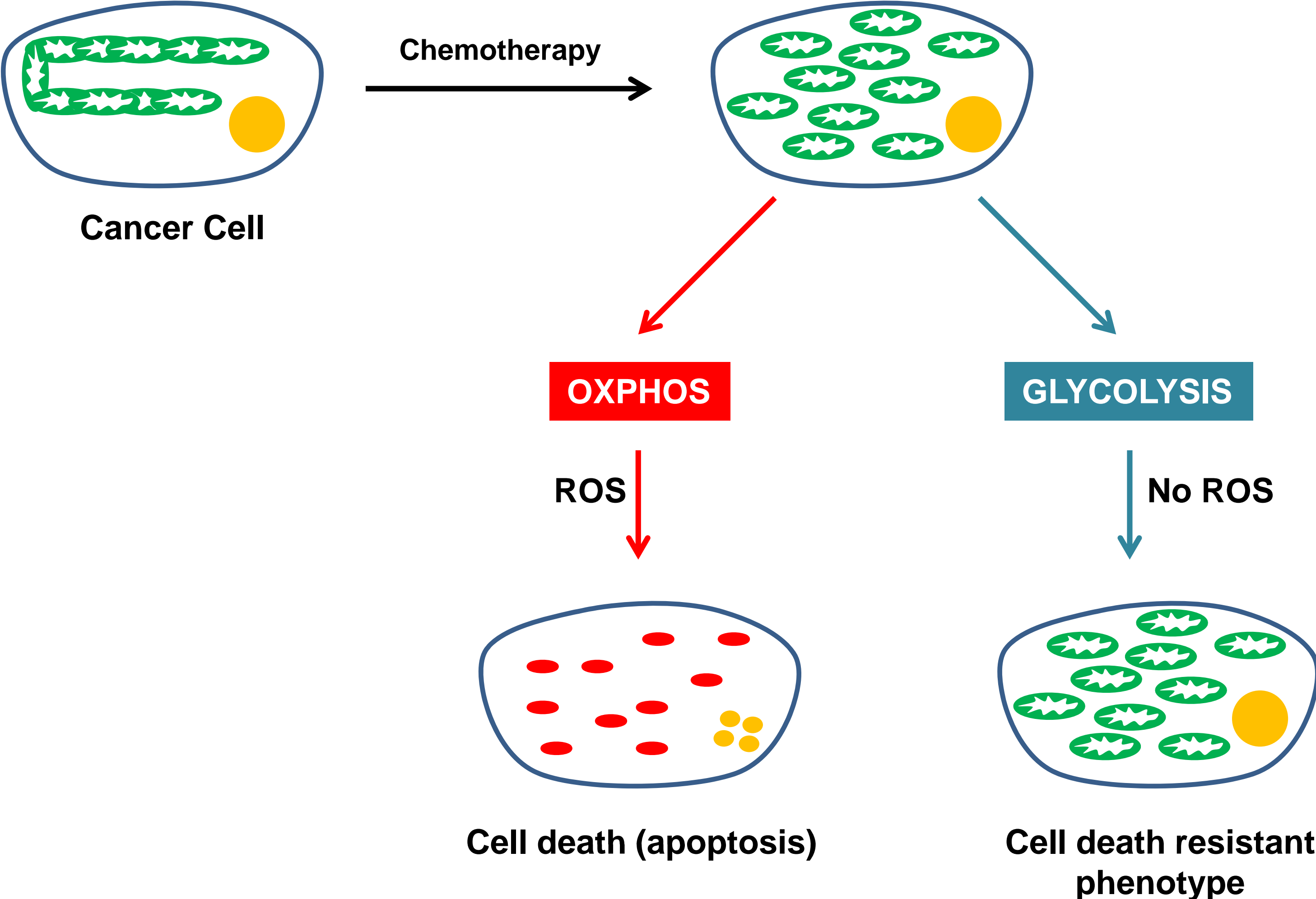

Figure 6.