Chapter 9

Mitochondria in Cell Life and Death

Diego De Stefani • Paolo Pinton • Rosario Rizzuto

Introduction

The mitochondrion represents a unique organelle within the complex endomembrane systems that characterize any eukaryotic cell. It is realistic to state that complex life on earth has been made possible through the "acquisition" of mitochondria which provide an adequate supply of substrates for energy-expensive tasks. Higher multicellular organisms have indeed high-energy requirements necessary to carry out complex functions, such as muscle contraction, hormones and neurotransmitters synthesis and secretion, in addition to basal cellular metabolism (biomolecules synthesis and transformation, maintenance of ionic gradients across membrane, cell division). Mitochondria can fulfill this huge energy demand thanks to their extraordinary biosynthetic capacities: every day, mitochondria of a single human being can recycle up to 50 Kg of ATP. To further underline the relevance of these subcellular structures, one can also consider how these organelles have affected the physiology of the whole organism: lungs, heart, and circulatory system have evolved essentially to provide molecular oxygen to mitochondria, which consume about 98% of the total O2 we breathe. However, beyond the pivotal role they play in ATP production, a whole new mitochondrial biology has emerged in the last few decades: mitochondria have been shown to participate in many other aspects of cell physiology such as amino-acid synthesis, iron-sulphur clusters assembly, lipid metabolism, Ca2+ signaling, reactive oxygen species (ROS) production, and cell death regulation. Hence, it is consequent that any mitochondrial dysfunction will inevitably lead to disease. Indeed, many pathological conditions are associated with organelle failure, including neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's), motoneuron disorders (amyotrophic lateral sclerosis, type 2A Charcot-Marie-Tooth neuropathy), autosomal dominant optic atrophy, ischemia-reperfusion injury, diabetes, aging, and cancer.

Understanding how mitochondria can sense, handle, and decode vari-

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ous signals from the cytosol and other subcellular compartments represents a new exciting challenge in biomedical sciences.

Mitochondria: The Basics

The mitochondrion is a double membrane-bounded organelle thought to be derived from an a-proteobacterium-like ancestor, presumably due to a single ancient invasion occurring more than 1.5 billion years ago. The basic evidence of this endosymbiont theory [1] is the existence of the mitochondrial DNA (mtDNA), a 16.6-Kb circular, double-stranded DNA molecule with structural and functional analogies to bacterial genomes (gene structure, ribosome). This mitochondrial genome encodes only 13 proteins (in addition to 22 tRNAs and 2 rRNAs necessary for their translation), all of which are components of the electron transport chain (mETC) complexes (I, III, and IV), while the whole mitochondrial proteome consists of more than 1,000 gene products. Thus, one critical step in the transition from autonomous endosymbiont to organelle has been the transfer of genes from the mtDNA to the nuclear genome. At the same time, eukaryotes had to evolve an efficient transport system to deliver nuclear-encoded peptides inside mitochondria: TIM (Transporters of the Inner Membrane), TOM (Transporters of the Outer Membrane) and mitochondrial chaperones (such as hsp60 and mthsp70) build up the molecular machinery that allows the newly-synthesized unfolded proteins to enter mitochondrial matrix [2].

Mitochondria are defined by two structurally and functionally different membranes: the plain outer membrane, mostly soluble to ions and metabolites up to 5,000 Da, and the highly selective inner membrane, characterized by invaginations called cristae which enclose the mitochondria matrix. The space between these two structures is traditionally called intermembrane space (IMS), but recent advances in electron microscopy techniques shed new light on the complex topology of the inner membrane. Cristae indeed are not simply random folds but rather internal compartments formed by profound invaginations originating from very tiny "point-like structures" in the inner membrane [3]. These narrow tubular structures, called cristae junctions, can limit the diffusion of molecule from the intra-cristae space towards the IMS, thus creating a micro-environment where mETC complexes (as well as other proteins) are hosted and protected from random diffusion.

As mentioned before, mitochondria are the main site of ATP production. When glucose is converted to pyruvate by glycolysis, only a small fraction of the available chemical energy has been stored in ATP molecules: mitochondria can "release" the remaining amount of energy with an outstand-

ing efficiency (from a single glucose molecule mitochondria produce 15 times more ATP than glycolysis). The main enzymatic systems involved in this process are the tricarboxylic acid (TCA) cycle and the mETC. Products from glycolysis and fatty acid metabolism are converted to acetyl-CoA, which enters the TCA cycle where it is fully degraded to CO2. More importantly, these enzymatic reactions generate NADH and FADH2, which provide reducing equivalents and trigger the electron transport chain. mETC consists of five different protein complexes: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (ubiquinol cytochrome c reductase), complex IV (cytochrome c oxidase), and complex V which constitutes the F1F0-ATP synthase. Electrons are transferred from NADH and FADH2 through these complexes in a stepwise fashion: as electrons move along the respiratory chain, energy is stored as an electrochemical H+ gradient across the inner membrane, thus creating a negative mitochondrial membrane potential (estimated around -180 mV against the cytosol). H+ are forced to re-enter the matrix mainly through complex V, which couples this proton-driving force to the phosphorylation of ADP into ATP, according to the chemiosmotic principle. ATP is then released to IMS through the electrogenic Adenine Nucleotide Translocase (ANT), which exchange ATP with ADP to provide new substrate for ATP synthesis. Finally, ATP can easily escape the IMS thanks to the mitochondrial porin of the outer membrane, VDAC (voltage dependent anion channel) [4].

Mitochondrial Biogenesis

As mentioned before, mitochondrial proteins are encoded by two distinct genetic systems, the mtDNA and the nuclear DNA (nDNA). Thus, mitochondrial replication must be a highly coordinated process that combines mtDNA duplication with synthesis of gene products of both nuclear and mitochondrial genomes. Recently, new transcription factors that govern mitochondrial biogenesis have been identified, while the signaling pathway that leads to their activation is still debated. The master gene that coordinates mitochondrial biogenesis is peroxisome proliferator activated receptor g coactivator 1a (PGC1a), originally identified as a cold-inducible coactivator in adaptative thermogenesis [5].

PGC1a gene is located on chromosome 4 and encodes a protein containing 798 amino acids placed in the cell nucleus. It is highly expressed in all the tissues where mitochondria are abundant and oxidative metabolism is active, such as brown adipose tissue (BAT), skeletal muscle and heart (but also in brain and kidney). PGC1a acts as a transcription factor which induces the expression of two other transcriptional regulators, NRF-1 and NRF-2 (nuclear respiratory factors 1 and 2), which in turn activate the synthesis

of nuclear encoded mitochondrial-targeted proteins. Moreover, PGC1a induces mtTFA (mitochondrial transcription factor A), a protein transferred to mitochondria where it promotes the expression of mitochondrial encoded proteins and mtDNA replication. By this way, PGC1a can guarantee the critical balance of mitochondrial and nuclear encoded proteins that is necessary for the correct assembling of respiratory complexes [6].

PGC1a regulation seems finely tuned to reflect cellular energy demands, with conditions of increased energy needs, such as cold, physical exercise, or fasting inducing its expression. Fasting induces hepatic PGC1a expression, which increases gluconeogenesis, while physical activity induces its expression in heart and skeletal muscle, thus increasing mitochondrial biogenesis and oxidative phosphorylation. As mentioned before, PGC1a was originally discovered as a cold inducible transcription factor in adaptative thermogenesis, the physiological process through which energy is dissipated as heat in response to environmental conditions such as cold or overfeeding. BAT in rodents and skeletal muscle in humans are the main sites involved in this process. Biochemically, the adaptive thermogenic process needs the stimulation of mitochondria (increasing nutrients catabolism) and the uncoupling of oxidative phosphorylation through the expression of the uncoupling protein UCP1. This protein dissipates the proton gradient created by mETC complexes by increasing mitochondrial inner membrane H+ permeability, thereby inducing energy dissipation as heat. This pivotal role of PGC1a in thermogenesis is lastly demonstrated by observing that knockout mice for this transcription factor are unable to face cold stress due to a continuous drop of the core body temperature [7, 8]. However, the physiological relevance of this protein goes far beyond the simple thermogenic program. Indeed, heart and skeletal muscle are profoundly remodeled upon PGC1a activation. Skeletal muscle fibers are classified in three main categories, showing different metabolic capabilities: slow-twitch type I and fast-twitch type IIa are rich in mitochondria and show a clear oxidative metabolism; fast-twitch type IIb fibers have instead a lower mitochondrial content, being mainly metabolically glycolytic. PGC1a expression is promptly activated by both short-term exercise and endurance training, resulting in the conversion of type IIb fibers into type IIa or type I fibers. Again, transgenic mouse models in which PGC1a is selectively overexpressed in muscle cells show a much higher resistance to fatigue, while null mice show reduced exercise capacities. The upstream signaling that activates PGC1a expression is not yet fully understood, but it seems to be mediated (at least in part) by CaMK pathway. Indeed, the increased contractile activity and neuromuscular stimulation induced by training cause the activation of CaMK and in turn of several transcription factors such as MEF2 that binds to the PGC1a promoter, thus inducing its expression [9, 10].

Mitochondria and Reactive Oxygen Species

The important role of redox signaling in the regulation of physiological responses is underscored by the apparent dysregulation of physiological responses in various disease-related oxidative stress conditions. Excessive levels of reactive oxygen species (ROS) may be generated by mechanisms that produce ROS "accidentally" in an unregulated fashion. This includes the production of ROS by the mitochondrial electron transfer chain, the quantitatively most important source of ROS in higher organisms. These chemical species are characterized by the presence of an unpaired electron on the oxygen atom that can promptly react with virtually any biomolecules. Thus, mitochondrial structures are particularly susceptible to oxidative damage as evidenced by lipid peroxidation, protein oxidation, and mitochondrial DNA mutations [11]. ROS have been implicated in many pathological conditions, in particular in the aging process. Indeed, the "free radical theory" of aging has a long history and it has been originally proposed in the 1950s [12]. This hypothesis was initially hotly debated, at least until the discovery of the first cellular enzyme involved in ROS metabolism, superoxide dismutase [13]. The existence of a protein whose unique function was to scavenge oxygen free radicals represented the first indirect but strong evidence that cells not only produce ROS but they also need systems to protect against them. ROS are generated by many enzymes, such as cyclooxygenases and NADPH oxidases, and in different subcellular compartments (i.e., they are generated by lipid metabolism within peroxisomes). However, the large majority of total ROS are undoubtedly produced by mitochondria, since they are a direct consequence of oxidative phosphorylation [14]. Indeed, at different sites along the mETC (in particular at complex I and III) electrons can "escape" and react directly with molecular oxygen, thus generating superoxide anions. ROS detoxifying enzymes represent the first line of defense against free radicals. Superoxide dismutase (SOD) is today known to exist in two different isoforms: while SOD1 is a copper-containing enzyme present is the cytosol, SOD2 (a manganese-containing protein) is located inside mitochondrial matrix where it converts superoxide anion to H2O2, which can be further degraded to water and oxygen by catalase. The number of ROS detoxifying enzymes grew very fast in last few decades[Q1: Author, can we change this to "the number of *known* ROS detoxifying enzymes"?] and includes the large family of glutathione peroxidases (GPx) and peroxiredoxins (Prx), which was recently reported to exist in mitochondrial matrix (Prx III) [15]. Mitochondria also have another mechanism to protect against ROS. Indeed, the uncoupling of oxidative phosphorilation through the action of UCP proteins and thus the decrease of mitochondrial membrane potential shortens the half-life of the most reac-

tive steps in the electron transport chain, thereby inhibiting ROS production [16]. Thus, given that prevention is better than the cure, mitochondria can "decide" to slow down their metabolism to prevent oxidative damage. This fact is shiningly demonstrated by observing that the above-mentioned PGC1a knockout mice show a much higher sensitivity to oxidative damage, especially in neurons [17]. This means that PGC1a not only promotes mitochondrial biogenesis and oxidative metabolism but it coincidentally takes care of the potentially harmful effect of ROS induction. This is achieved by the two described mechanisms: on one side by increasing ROS scavenging enzymes (SOD1, SOD2, catalase, and GPx) and on the other by decreasing ROS production (through the induction of UCPs).

By the way, cells have always been forced to cohabit with free radicals. Thus, it is not unworthy to wonder whether this harmful chemical species could also be exploited to participate in physiological regulation of normal cellular events. Indeed, one of the most fascinating hypotheses is that ROS, besides their obvious toxic effect, could even participate in signal transduction. This notion is supported by recent works on the role of p66shc, the first mammalian protein whose mutation was demonstrated to increase resistance to oxidative stress and to prolong life span [18]. Intriguingly, upon activation, including phosphorylation by PKCb and Pin1 recognition, p66shc translocates to mitochondria [19] where it exerts its own oxidoreductase activity [20]. Indeed, p66shc directly oxidizes cytochrome c (thus allowing electron to escape mETC) and generates H2O2, leading to mitochondrial permeability transition pore opening (mPTP) and in turn cell death. The existence of a protein that "steals" electrons from the mETC and produces reactive oxygen species represents the first molecular evidence of the role of reactive oxygen species in signal transduction, finally describing the biochemical basis of the free radical theory of aging.

Interestingly, other well-studied proteins such as p53, protein kinase C (PKC), and Apurinic-apyrimidinic endonuclease/Redox effector factor (Ape/Ref-1) play an important role in ROS-mediated pathways and translocate to mitochondria during redox stimulation [21-23].

Mitochondrial Dynamics

Mitochondrial shape appears very heterogeneous within different cell types and, in some cases, even in the same cell. Indeed, they can form either a short, rod-like structure or a continuous, elongated, tubular, highly dynamic and interconnected network. These differences in phenotype are the result of a complex equilibrium among mitochondrial motility, fusion, and fission rates. Since mitochondrial biogenesis occurs predominantly in the perinuclear region, eukaryotes had to evolve efficient systems to transport these

organelles where energy demands are higher or where their peculiar metabolic functions are required. This is of critical relevance in cells with complex topology such as neurons, where mitochondria are abundant in the synaptic region of the axon. This singular distribution most likely reflects the high-energy requirement of the synaptic transmission (ATP-driven release and recycling of vesicles, ATP-dependent pumps that control ions homeostasis, etc.) as well as the specific mitochondrial functions such as Ca2+ signaling regulation. Mitochondria exploit cytoskeletal elements as tracks for their directional movements by using specialized molecular machinery in a way we began to understand only in the last few years. These organelles have been shown to interact with every cytoskeletal element (microfilaments, microtubules, and intermediate filaments) in different species. However, while in budding yeast mitochondria seem to move predominantly through the actin network, in mammals mitochondrial movement is mainly a microtubules-driven process, with actin aiding in short-range mitochondrial positioning in microtubules-poor regions [24]. Recent studies suggest that the main microtubules-associated motors are kinesin-1 (for anterograde transport) and the cytoplasmic dynein (for retrograde movement). These motors are likely present in higher order molecular complexes that bind to mitochondria: for example, it has been demonstrated that the existence of a complex brings together the heavy chain of conventional kinesin-1 with the adaptor protein Milton and the mitochondrial protein Miro (mitochondrial Rho-like GTPase), and this complex is required for mitochondrial axonal transport [25]. Moreover, mitochondrial movement is also influenced by second messengers such as Ca2+, and it actively participates in signaling cascades: for example Ca2+ release from endoplasmic reticulum transiently blocks mitochondrial movements. This inhibition in mitochondrial motility reflects an increased mitochondrial calcium uptake and thus enhances the local Ca2+ buffering capacities of mitochondria, with important consequences in signal transduction [26].

Apart from organelles movement along the cytoskeleton, mitochondria also continuously remodel their shape. In the early 1990s, genetic screens in yeast identified the first proteins involved in mitochondrial morphology and subsequent studies revealed that mitochondrial shape is determined by two dynamically opposed processes, fusion and fission. Indeed, genetic ablation of key regulators of the fusion machinery gives rise to cells with fragmented organelles because of unopposed ongoing fission, while the knockout of genes that mediate fission leads to the formation of an almost unique, deeply interconnected mitochondrial network. Interestingly, in yeast the coinciding ablation of both fusion and fission apparatus produces a wild-type mitochondrial morphology but also shows a high frequency of mtDNA loss, suggesting an essential role of fusion and fission in maintaining mitochondrial genome [27, 28]. Considering the structural complexity of mito-

chondria, it should be immediately clear that the molecular machinery mediating fusion and fission has to be a quite intricate mechanism, requiring the independent but coordinated processing of both outer and inner membranes. Proteins involved in mitochondrial dynamics have been originally identified in yeast but many of these genes have orthologs in mammals, mainly belonging to the large GTPase protein family. The molecular motors playing a pivotal role in outer membrane fusion are mitofusins (Mfn1 and Mfn2): they are characterized by the presence of a highly conserved GTPase domain, two transmembrane regions that enable the anchoring to OMM and two peculiar coiled coil structures, HR1 and HR2 (heptad repeat domain 1 and 2). During organelle fusion, mitofusins mediate the tethering of two adjacent mitochondria by forming trans homotypic (consisting of the same Mfn isotypes) or heterotypic (consisting of Mfn1 and Mfn2) complexes through the interaction of their C-terminal HR2 domains. Whether these two isoforms are functionally different or simply redundant remains to be clarified [29]. After that, mitochondrial inner membrane fusion is achieved through the activity of another protein, OPA1. Surprisingly, this protein has been recently shown to control also IMM ultrastructure: together with the rhomboid protease PARL (presenilin-associated rhomboid like), OPA1 forms oligomers essential for the maintenance of internal cristae structure, thereby controlling their remodeling during apoptotic cell death (see below). On the other hand, the master gene regulating mitochondrial fission is Drp1 (Dynamin-related protein 1). It shares high homology with dynamin, a mechanoenzyme involved in the excision of clathrin-coated endocytic vesicles, and is normally located in the bulk cytosol. Upon induction, Drp1 redistributes into punctuated foci colocalizing with mitochondria where it mediates organelle fission. Conversely, the deciphering of the molecular players mediating remains elusive both in mammals as well as in yeast. It has been proposed that IMM processing could also be a simple mechanical consequence of outer membrane constriction and cleavage induced by DRP1.

Mitochondria and Cell Death

Every multicellular organism has endogenous mechanisms for selectively killing their own cells. This process has a huge physiological relevance since it is necessary to eliminate damaged, superfluous, dangerous, or aged cells and is thus involved in many physiological and pathological processes, such as embryogenesis, development, differentiation, tissue homeostasis, tumorigenesis, neurodegeneration, and viral infections. The term "apoptosis" was originally coined by John Kerr in 1972, describing a peculiar cell death mechanism morphologically characterized by cell shrinkage, chro-

matin condensation, DNA fragmentation, plasma membrane blebbing, and formation of apoptotic bodies [30]. Biochemically, apoptosis is a highly regulated proteolytic event, achieved through the activation of a broad family of evolutionary conserved cysteine aspartate-specific proteases, caspases, which are usually present in cytoplasm as inactive enzymes (zymogens). Caspases, and consequently apoptosis, can be activated by two major pathways. First, the so-called extrinsic pathway triggered by plasmamembrane receptors such as TNFa (tumor necrosis factor a) or Fas (also known as Apo-1 or CD95): the activation of these receptors induces the assembly of a protein complex named DISC (death inducing signaling complex), which recruits and activates caspases cascade. On the other side, the intrinsic pathway relay is activated through the release of several mitochondrial proteins toward the cytosol. The main player in the finely tuned apoptotic activation process is undoubtedly cytochrome c. This protein is encoded by a nuclear gene and synthesized in the cytoplasm as a precursor; after being imported into mitochondria, it is refolded and bound to a hem prosthetic group, localizing in the IMS. The majority of cytochrome c is tightly bound to mitochondrial inner membrane thanks to its electrostatic interactions with acidic phospholipids, but a small fraction probably exists loosely attached to IMM and available for mobilization. This protein is an irreplaceable component of the mETC, shuttling electrons from complex III to complex IV, and it is thus essential to life: the disruption of its only gene is embryonically lethal [31]. Surprisingly, cytochrome c is also one of the pivotal players in the induction of cell death: once released in the cytoplasm, this protein drives the assembly of a caspases activating complex together with Apaf-1 (apoptosis-protease activating factor 1) and caspase 9, the so-called apoptosome. Apaf-1 consists of three functional domains: an N-terminal CARD (caspase-recruitment domain), a central nucleotide-binding domain, and twelve to thirteen WD-40 repeats at the C-terminus of the molecule. This protein is normally present in cytosol as an inactive monomer, where the WD-40 motifs self inhibit the CARD domain from recruiting caspase 9. Cytochrome c, once in the cytosol, induces the rearrangement and hepta-oligomerization of Apaf-1: each of these complexes can recruit up to seven caspase molecules, leading to their proteolytic self-processing and consequent activation [32].

Mitochondria contain many other proapoptotic, IMS-resident proteins, such as Smac/DIABLO, HtrA2/Omi, apoptosis inducing factor (AIF), and EndoG (Endonuclease G). DIABLO (direct inhibitor of apoptosis-binding protein with a low isoelectric point) and HtrA2 (high temperature requirement protein A2) both have an N-terminal domain that can interact and inhibit IAPs (inhibitor of apoptosis proteins). IAPs, such as XIAP, cIAP-1, and cIAP-2, are cytosolic soluble peptides that normally associate and stabilize procaspases, thus preventing their activation. Con-

versely, AIF and EndoG translocate from IMS to the nucleus upon treatment with several apoptotic stimuli where they seem to mediate chromatin condensation and DNA fragmentation [33].

The critical checkpoint in apoptotic intrinsic pathway induction is controlled by the BCL-2 protein family. The founding member, the anti-apoptotic BCL-2 proto-oncogene, was originally identified in human follicular B cell lymphoma and it represents the first oncogene acting as cell death inhibitor rather than as a promoter of cell proliferation. The BCL-2 family can be divided into three main categories according to the presence of the four conserved domains BH1-4 (BCL-2 homology). The anti-apoptotic members (BCL-2, BCL-XL, BCL-W, MCL-1) contain all four conserved domain BH1-4, while the proapoptotic multidomain proteins (BAX, BAK) possess only the BH1-3 amphipathic a-helices. Finally, the so-called BH3-only proteins such as BID or PUMA contain only one of these domains and display proapoptotic function by directly activating BAX and BAK. These two proteins exist as inactive monomers in viable cells with BAX localizing in the cytosol, loosely attached to membranes, and BAK residing in mitochondrial fraction. Upon apoptosis induction, BAX translocates to mitochondria where it homo-oligomerizes and inserts in the outer membrane; similarly, also BAK undergoes a conformational change which induces its oligomerization at the OMM level. Together these events trigger the mitochondrial outer membrane permeabilization (MOMP), the crucial process mediating the release of IMS-resident caspase cofactors into the cytoplasm [34]. Moreover, MOMP requires also the coincident remodeling of IMM structure through the widening of cristae junctions and the consequent mobilization of proteins entrapped in the intra-cristae space. As mentioned before, this process is under the control of the rhomboid protease PARL and the large GTPase OPA1. The latter is normally embedded in the IMM but it can be cleaved by the transmembrane protease PARL, thus originating a soluble pool of OPA1. These two versions of OPA1 (the longer membrane bound and the shorter IMS soluble) help the maintenance of the firm constriction of cristae junctions through their hetero-oligomerization. During apoptosis, the proapoptotic members of BCL-2 family disrupt these oligomers, thus aiding the mobilization of mitochondrial caspase cofactors [35, 36].

Apart from these ultrastructural changes, mitochondria also undergo more "macroscopic" remodeling of their shape during programmed cell death. Indeed, after apoptosis induction, mitochondria become largely fragmented, resulting in small, rounded, and numerous organelles. This process occurs quite early in cell death pathway, soon after BAX/BAK oligomerization, but prior to caspase activation. Interestingly, the perturbation of the equilibrium between fusion and fission rates seems to correlate with cell death sensitivity. In particular, conditions where mitochondrial fission is inhibited, such as

DRP1 downregulation or mitofusins overexpression, strongly delay caspase activation and cell death induced by numerous stimuli. Similarly, stimulation of organelle fission (by DRP1 overexpression or Mfn1/2 and OPA1 inhibition) promotes apoptosis by facilitating cytochrome c release and apoptosome assembly [37]. However, mitochondrial morphology is highly variable among different cell types and so are the signaling events leading to cell death, suggesting that mitochondrial fragmentation is not always a clear symptom of apoptosis. In some conditions, DRP1 overexpression has been reported to protect cells from apoptosis, such as in the case of apoptosis induced by mitochondrial Ca2+ overload [38].

Another hallmark in apoptosis is the loss of mitochondrial membrane potential, caused by the opening of the so-called mitochondrial permeability transition pore (mPTP). The mPTP is a large conductance channel presumably formed through a conformational change of several constituent mitochondrial proteins. Its opening can be triggered by different pathological conditions, such as Ca2+ overload, ATP depletion, oxidative stress, high inorganic phosphate (Pi), or fatty acid. The exact molecular structure of this pore is currently highly debated, but the main players in mPTP assembly seem to include the adenine nucleotide transporter (ANT) in the inner membrane, the voltage-dependent anion channel (VDAC), and the peripheral benzodiazepine receptor (PBR) of the outer membrane and cyclophilin D (CyP-D), a matrix protein [39]. The first obvious consequence of mPTP opening is mitochondrial depolarization followed by organelle swelling, cytochrome c release, caspase activation, and apoptotic cell death. The contribution of mPTP to normal cellular physiology in healthy cells (i.e., transient opening of this high conductance channel) is still a matter of debate. However, the availability of chemical mPTP inhibitors such as CsA (an immunosuppressant drug whose molecular target are cyclophilins) and the development of CyP-D knock-out mouse models clearly underline the huge relevance that permeability transition plays in pathological conditions, such as ischemia-reperfusion injury, liver diseases, neurodegenerative and muscle disorders [40-42].

Conclusions

Energy metabolism and the apoptotic program are the two major determinants of cell fate. Many growth factors increase glucose uptake and induce the translocation of hexokinase (the first limiting step of glycolysis) to mitochondria, thus promoting energy production. At the same time, mitochondria lay at the heart of programmed cell death regulation, representing an authentic “cellular poison cupboard” which takes care of the key components involved in apoptosis. Moreover, this ancient

endosymbiont fully integrated into its host, participating in many aspects of cell signaling (Ca2+ dynamics, steroid biosynthesis, ROS production etc.). All these observations lead to a really complex but fascinating picture, where mitochondria represent a sort of decoding station: they can sense different environmental conditions or receive diverse signals, integrate them all together, finally producing an outcome that can decide the fate of the cell. The understanding of these complex mechanisms is a hard and challenging task, but in the next few years it will provide new chances in the comprehension of numerous still poorly understood human pathologies.

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