ISSN 0006-2979, Biochemistry (Moscow), 2007, Vol. 72, No. 10, pp. 1115-1126. © Pleiades Publishing, Ltd., 2007.
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REVIEW

The Mitochondrion as Janus Bifrons

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Received June 18, 2007

Abstract—The signaling function of mitochondria is considered with a special emphasis on their role in the regulation of redox status of the cell, possibly determining a number of pathologies including cancer and aging. The review summarizes the transport role of mitochondria in energy supply to all cellular compartments (mitochondria as an electric cable in the cell), the role of mitochondria in plastic metabolism of the cell including synthesis of heme, steroids, iron-sulfur clusters, and reactive oxygen and nitrogen species. Mitochondria also play an important role in the Ca^{2+}-signaling and the regulation of apoptotic cell death. Knowledge of mechanisms responsible for apoptotic cell death is important for the strategy for prevention of unwanted degradation of postmitotic cells such as cardiomyocytes and neurons.

DOI: 10.1134/S0006297907100094

Key words: mitochondria, redox potential, reactive oxygen species, cancer, proliferation, differentiation

Mitochondria are traditionally defined as "power plants of the cell". However, besides such important function these organelles exhibit many other unique functions, which are realized only within mitochondria and which may determine fate of particular cells. In this review, we will consider certain facts that usually do not attract much attention and leave out of detailed analysis the traditional consideration of the role of mitochondria in processes of energy accumulation. Taking into consideration numerous mitochondrial functions, these organelles can be compared with Janus Geminus also known as Junus Bifrons, or Janus Quadrifrons; this mythical hero is usually shown with two or even four faces looking different ways. It is not a senseless analogy: Janus could look into the past and the future (backward and forward) and mitochondria are able to predetermine the development of the cell and its death. By strange coincidence, the first dye used in the first half of XX century in histology for detection of mitochondrial localization within the cell was the Janus Green dye.

Abbreviations: mDNA) mitochondrial DNA; RNS) reactive nitrogen species; ROS) reactive oxygen species; VDAC) voltage dependent anion channel.

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MITOCHONDRIA AS THE "POWER PLANT"

ATP production. In accordance with P. Mitchell's chemiosmotic concept, vectorial transmembrane transfer of electrons and protons is accompanied by generation of electrochemical difference of proton electrochemical potential on the inner mitochondrial membrane; its utilization by ATP synthase induces conformational rearrangements resulting in ATP synthesis from ADP and inorganic phosphate [1]. Details of the mechanism responsible for ATP synthesis are given elsewhere (e.g. [2]).

Generation of membrane potential. Membrane potential ($\Delta\Psi$) generated across the inner mitochondrial membrane is the component of the transmembrane electrochemical potential of H^+ ions ($\Delta\mu H^+$), which provides ATP synthesis together with the concentration component (ΔpH). Maintenance of constant membrane potential is a vitally important precondition for functioning of mitochondria and the cell. Under conditions of limited supply of the cell with oxygen (hypoxia) and inability to carry out aerobic ATP synthesis, mitochondria become ATP consumers (rather than generators) and ATP is hydrolyzed by mitochondrial ATPase, and this is accompanied by generation of membrane potential across the

inner mitochondrial membrane [3]. It is possible that critical importance in generation and maintenance of membrane potential under these conditions is determined by the fact that transport of protein precursors into mitochondria is a potential-dependent process [4].

Involvement in regulation of glycolysis. The existence of interactions between two pathways of ATP generation (anaerobic glycolytic and aerobic oxidative pathways) has been known for a long time. It is also known that aerobic conditions inhibit glycolysis (the Pasteur effect), whereas high content and utilization of glucose inhibit aerobic respiration (the Crabtree effect). Mechanisms of these phenomena are not well understood, but analysis of the corresponding literature is outside the framework of this review. Here we would just like to draw attention to the interaction of these processes (glycolysis and oxidative phosphorylation) at the level of mitochondria. Mitochondria are responsible for oxidative phosphorylation, which occurs at the inner mitochondrial membrane (generation of protonmotive force and ATP synthesis), whereas glycolysis occurs in the cytoplasm. However, one of the key glycolytic enzymes, hexokinase, may be associated with the outer mitochondrial membrane. Four isoforms of hexokinase have been found in mammals: types I, II, III, and IV (glucokinase) [5]; these isoforms exhibit different affinity to glucose and ATP. The N-terminal sequence of hexokinase types I and II has high affinity to the voltage-dependent anion channel (VDAC) or porin of the outer mitochondrial membrane [6, 7], whereas hexokinase types III and IV lack affinity to VDAC. It should be noted that hexokinase type I is tightly bound not just to VDAC, but to VDAC located in the contact site between the inner and outer mitochondrial membranes [8, 9]. Hexokinase type I forming tight contact with the porin channel utilizes ATP only of mitochondrial origin (ATP comes to the channel from the inner membrane); this is a characteristic feature of this isoform. Thus, mitochondria control activity of the key glycolytic enzyme [10]. Hexokinase type II isoenzyme may exist both in mitochondria bound as well as in soluble cytosolic forms. Wilson suggests that this enzyme plays an anabolic role by participating in formation of glucose-6-phosphate required for functioning of the pentose phosphate pathway, supplying lipid synthesis with NADPH [5]. However, in hepatoma this form (as well as the type I isoenzyme) preferentially utilizes mitochondrial ATP.

There is correlation between association of hexokinase with mitochondria and possibility of apoptotic cell death—the higher the association the less the probability of cell death. Some postmitotic cells (myocytes, including cardiomyocytes, neurons, and also tumor cells) possessing a program for apoptosis inhibition are characterized by higher association of mitochondria with hexokinase [11, 12]. These facts suggest that the fate of cells is determined by architecture of the protein complexes of the contact sites rather than isolated enzymes constituting protein complexes.

Supramolecular protein complexes of the mitochondrial contact sites have been intensively studied in D. Brdiczka's laboratory. Using ion-exchange chromatography as the research tool, they recognized two types of complexes: one complex contained hexokinase and mitochondrial porin, whereas the other complex contained creatine kinase and adenine nucleotide translocator [13]. Using native electrophoresis, a Moscow group found supramolecular complex containing mitochondrial porin, adenine nucleotide translocator, creatine kinase, and mitochondrial benzodiazepine receptor [14]. Later collaboration of these two teams culminated in identification of supramolecular complex also containing cyclophilin D, proapoptotic BAX protein, and cytochrome *c* [15, 16]. Detection of a cytochrome *c* pool unrelated to respiratory chain is especially interesting because it is possible that release of cytochrome *c* during apoptotic signal transduction from mitochondria involves this pool. All these arguments suggest the ultimate importance of such protein association not only for energy status of the cell but also its fate as well [17].

It is possible that a clue to elucidation of precise mechanisms responsible for manifestation of the Pasteur and Crabtree effects will be found in the functioning of supramolecular complexes of the contact sites.

MITOCHONDRIA AS REGULATORS OF REDOX STATES OF THE CELL

Redox homeostasis, i.e. the sum of redox components (including proteins, low molecular weight redox components such as NAD/NADH, flavins, coenzymes Q, oxidized and reduced substrates, etc.) is one of important preconditions for normal cell functioning.

Mitochondria generate such potent regulators of redox potential as superoxide anion, hydrogen peroxide, nitric oxide, peroxynitrite, etc. They are actively involved in regulation of cell redox potential and consequently control proteolysis, activation of transcription, changes in mitochondrial DNA (mDNA), cell metabolism, and cell differentiation [18].

Cancer. Oxidative stress causes oxidative damage to cells, which is often accompanied by modification of genetic material [19]. This oxidative modification of DNA is the first step of carcinogenesis as well as aging. Imbalance of the redox state induced by oxidative stress is especially typical for cancer cells. Various cancers are characterized by increased level of oxidant-induced DNA damage; this indicates some links of such damage and cancer etiology [20].

An effective strategy for treatment of cancer emphasizes the existence of links between mitochondria and malignant transformation. In some cases, compounds specifically interacting with mitochondria act as effective antiproliferative agents. Studies carried out in Lan Bo

Chen's laboratory have shown that rhodamine 123, a penetrating cation that is used as a mitochondrial probe for membrane potential, not only selectively accumulates in mitochondria of tumor cells, but in contrast to mitochondria of homologous normal cells it is retained there [21]. Subsequently, the same group also demonstrated the antitumor effect of this compound [22, 23]. Later antitumor activity was also detected for lipophilic cations: thiopyrylium derivative, AA1, dequalinium chloride, and MKT-077 of the rhodocyanine family [24-26]. Interestingly, lipophilic cations, derivatives of ^{99m}Tc, actively accumulating in mitochondria, are widely used for detection of cancer cells [27].

Recently a new group of antitumor drugs known as mitocans due to their high affinity to cancer cell mitochondria has been developed. They destabilize mitochondria and induce release of proapoptotic proteins (cytochrome *c*, AIF, Smac/Diablo) from them. The group of mitocans includes vitamin E derivatives; the most active derivative of vitamin E is α-tocopheryl succinate, a redox neutral substance, which kills cancer cells and does not influence normal cells. It interacts with mitochondrial complex II and generates reactive oxygen species [28]; this compounds acts as a BH3-mimetic [29]. Interestingly, the "mitochondrial" theory of cancer was proposed by a German scientist, the Nobel Prize recipient Otto Warburg. He pointed to equal contribution of glycolysis and oxidative phosphorylation to total energy production of cancer cells (in normal cells oxidative phosphorylation prevailed over glycolysis) and postulated that impairments of mitochondrial oxidative phosphorylation would be the first stage of malignant transformation [30].

Proliferation. Links between cell proliferation and mitochondrial functioning were suggested quite a long time ago because mitochondria produce potent oxidants and reducing equivalents. Usually malignant transformation is accompanied not only by accelerated cell proliferation, but also potent mitochondrial proliferation seen in various malignant tumors, but the most profound in secretory gland tumor. This phenomenon is known as oxyphyllia or oncocytic transformation. In oncocytomas mitochondria occupy up to 90% of the cytoplasm [31].

Some tumors exhibit increased expression of one of the most mysterious mitochondrial proteins, peripheral benzodiazepine receptor, and the level of its expression correlated with the metastases aggressiveness [32]. Some evidence exists that this protein may function as an oxygen sensor [33]. In tumor cells, there was positive correlation between increased binding of a ligand of the mitochondrial benzodiazepine receptor (used in nanomolar range) and the increase in proportion of cells in S-phase [34]. In millimolar concentrations, the same ligand caused cell cycle arrest at G_0/G_1 [35]. These data suggest that the mitochondrial benzodiazepine receptor may be involved in regulation of cell proliferation by regulating the cell cycle.

There is clear evidence that mitochondrial prohibitins (1 and 2) possess various functions, one of them being inhibition of proliferation [36]. Possible regulation of proliferation via interaction with cytoskeleton will be discussed during consideration of transport function of mitochondria.

Cell differentiation. The story on the involvement of mitochondria in regulation of cell differentiation began with the study of Smith et al. [37] demonstrating the dependence of differentiation of rat glial oligodendrocyte precursors on redox status of cells. Shift of redox systems to higher oxidation degree provoked differentiation of these cell precursors into oligodendrocytes or astrocytes, whereas high reduction state maintained cells in the undifferentiated state.

Various regulators such as thyroid and other hormones, growth factors, and some chemical agents drive cell differentiation and simultaneously change cell redox states evidently by influencing mitochondrial functioning. Superoxide anion radical stimulates osteogenic differentiation of mesenchymal stem cells [38, 39]. Proliferative response of smooth muscle cells to PDGF may depend on hydrogen peroxide formation [40].

Mitochondrial functioning may also influence expression of nuclear genes, regulate proliferation, and change phenotype, as demonstrated for myoblasts [41]. Increase in mitochondrial activity as well as increase in number of cell mitochondria result in increased production of reactive oxygen species (ROS), correlating with cell cycle arrest, termination of antigen expression in proliferating cells, and finally to cell hypertrophy. Evidently, such mechanism represents rather frequent response of cells to changes in cell energy supply. For example, significant change in ATP level caused by cytochrome oxidase inactivation resulted in arrest of the cell cycle and differentiation in some tissues of drosophila [42]. These authors have demonstrated that significant decrease in ATP due to cell mutations may be accompanied by cell cycle arrest without significant influence on cell survival, growth, and differentiation. This results in activation of the energy sensor, AMPK, possibly due to the increase in AMP. In turn, AMPK activates the key regulator of the cell cycle, p53, and causes cell cycle blockade due to the decrease in cyclin E.

Involvement of mitochondria in regulation of development of organisms begins in oocytes: the oocyte mitochondria modulate calcium signals during fertilization [43]. Spermatozoon-induced calcium currents on the oocyte membrane are transformed into mitochondrial Ca^{2+}-signals, stimulating mitochondrial respiration and metabolism. Under these conditions, oocyte mitochondria may function as Ca^{2+}-buffer and influence the process of zygote formation.

During subsequent development, mitochondria also play an essential role. It was demonstrated that expression of mDNA transcription factors gives rise to differentia-

tion of embryonic stem cells and provides (at least partially) for their differentiation into cardiomyocytes [44]. Interestingly, a recent study has demonstrated a correlation between directed transport of cardiomyocyte mitochondria into progenitor cells and onset of differentiation of these cells into cardiomyocytes [45].

MITOCHONDRIA AND AGING

There are more than 300 theories of aging known to date [46]. They vary from theory of accumulation of error in the genome, theory of glycosylation or excessive accumulation of cholesterol, damaging cell structures, to theories of programmed hormonal changes considering hypophysial and hypothalamic structures as a "clock mechanism". One of the most popular modern theories is the free radical or mitochondrial theory of aging. According to this theory, mitochondrial ROS formation is critically important for life span [47]. In this connection, there is a most demonstrative correlation between high level of ROS generation in short-lifespan species and low level of ROS generation in long-lifespan species [48]. Age-related oxidative changes, which are significantly higher in mDNA [49], finally result in impairments of synthesis of vitally important proteins, alteration in functioning, and cell death. Some studies have shown that proteins (or their corresponding genes) of mitochondrial electron transport chain are the targets of mitochondrial ROS; this causes further imbalance in ROS generation and impairments of manifestations of oxidative stress [50].

Recently a mDNA mutation has been described in a man who suffered from hypertension, hypercholesterolemia, and hypomagnesemia; such symptoms are typical for a metabolic syndrome [51] that is a risk factor during aging. Moreover, authors of this study have suggested that loss of mitochondrial functions is closely related to aging [52] and may determine appearance of aging-related hypertension and hypercholesterolemia.

Studies of problems of aging focus certain attention on the involvement of histone deacetylases Sir2 (*C. elegans*) and SIRT1-7 (human) into lifespan. These enzymes are known to be responsible for increase in lifespan related to restriction of food calories [53]. In worms and yeasts, Sir2 regulates gene silencing by modifying histones in the telomere regions [54] and also inhibits formation of extrachromosomal cyclic DNA [55]. In humans, its homologs sirtuins (SIRT) determine lifespan of cells exposed to different kinds of stress. Sirtuin-mediated response to stress involves p53, transcription factors FOXO and NF-κB, and DNA repair factor Ku70; this results in suppression of apoptosis and increase in repair process. Deacylation of FOXO proteins may result in increased resistance to oxidative damage [56]. Interestingly, three proteins of the SIRT family (3, 4, and 5) have mitochondrial localization [57]; this also emphasizes the extremely important role of these organelles in aging processes (not only from the viewpoint of the free-radical theory). Since sirtuins are NAD-dependent enzymes that are activated during food calorie restriction [58], mitochondrial localization of some sirtuins may provide a link between processes of energy metabolism and aging. Analysis of the effects of sirtuins on glucose homeostasis also suggests such a relationship [59]. SIRT may increase insulin secretion and increase tolerance to glucose; in *SIRT1* transgenic mice expression of UCP2 decreased; this may promote more effective ATP synthesis. SIRT3 and 4 transported into mitochondria are directly linked to metabolic changes that occur during calorie restriction. SIRT3 regulates involvement of the acetate carbon into the tricarboxylic acid cycle and basic metabolism via deacetylation of acetyl-CoA-synthetase, whereas SIRT4 causes the same effect on involvement of acetate carbon into glutamate metabolism but via ADP-ribosylation of glutamate dehydrogenase.

MITOCHONDRIAL TRANSPORT OF ENERGY AND SUBSTANCES

Transport of energy. Extended mitochondrial structures have been found in various cells. In muscle tissue mitochondria form branched structures that not only cover the whole cellular space, but possibly form supracellular (organ) structure through contacts between mitochondria of one cell with mitochondria of adjacent neighboring cells; such structure is known as the nexus [60].

In the end of the 1960s and beginning of the 1970s, V. P. Skulachev proposed a hypothesis that extended coupling membranes (particularly the inner mitochondrial membrane) may serve for transfer of the electric form of electrochemical transmembrane potential of hydrogen ions [61, 62]. In the 1980s, this hypothesis was experimentally confirmed for two types of organization of extended mitochondria (with common continuous matrix and for the system of mitochondria forming special contacts) [63, 64]. This resulted in a general theory of the mitochondrion as an electric cable transferring membrane potential into all cell compartments. This significantly decreases the value of oxygen gradients in tissues, which was originally suggested to be the main factor responsible for insufficient supply of the whole cell with ATP. The existence of continuous mitochondrial system creates the possibility for ATP synthesis by mitochondrial ATP synthase even within hypoxic sites due to the continuous inner mitochondrial membrane, which is charged at sites favorable for generation of proton-motive force (see details in [64]).

This theory as well as any basic theory had opponents, especially after appearance of a potential-dependent mitochondrial probe, JC-1, distinguishing zones with low and high potential by generation of fluorescence of

various wavelength (green fluorescence in the zones with low membrane potential and red fluorescence in the zones with high membrane potential). Opponents affirmed that alternation of fluorescence of different color can be observed in extended mitochondria [65, 66], which conflicted with the equipotentiality as the basis of the theory considering the mitochondrion as an electric cable. However, heterogeneity of membrane potential appeared after some time of monitoring in the mode of fluorescence excitation; according to the authors of the cable theory, this phenomenon may be attributed to the photodynamic effect and possible separation of mitochondrial volume into several isolated compartments (through mitochondrial partitioning). In this variant, the mitochondrion may represent a set of non-contacting inner membrane vesicles covered with a common outer membrane and therefore looking like a continuous mitochondrion. The final point in this discussion (whether the mitochondrion is a cable or not) was made in 2006 by G. Twig et al.; they investigated the mitochondrial matrix continuum by means of photoactivated GFP and detected that a common equipotential mitochondrial structure is determined by mitochondrial matrix continuum. This means that the mitochondrion is an electric cable [67].

Transport of signal molecules and lipophilic compounds. The mitochondrial continuum may also represent the transport system responsible for transfer of any components and metabolites of mitochondria due to their concentration gradient in the cell. Historically, the first hypothesis was the hypothesis of transport of oxygen and fatty acids along continuous mitochondrial membrane [52], but a significant number of components would be added to this list (e.g. NO, H_2O_2, some lipids such as lipid-soluble antioxidants).

Mitochondrial transport. If cells lack continuous mitochondria (this is usually the case in cells with undefined cytosol structuring) there is certain (but still unclear) movement of these organelles, which sterically compensates loss of lateral transport along continuous mitochondria. The saltatory (from the Latin *salto*, which means jump) movement of mitochondria, particularly axonal transport, which involves mitochondria, is an especially demonstrative example. It should be noted that transfer of genetic material to other cell compartments is also possible if we take into consideration mDNA release from mitochondria [68, 69].

Acute changes in concentrations of sodium, potassium, and calcium ions in cytoplasm of neurons, which represent a basis for appearance and conduction of nerve impulse, require energy supply for functioning of systems responsible for active transport of ions. These circumstances indicate that neuronal activity is highly dependent on mitochondria as the main ATP producers, which also exhibit ability of active accumulation of a large amount of calcium ions. Mitochondria are transported via neuronal dendrites to zones of high metabolism and increased content of intracellular calcium ions, for example, to synapses. Removal of damaged mitochondria, which produce excessive amount of ROS and can induce apoptosis, also involves their transportation to autophagosomes. Thus mitochondrial movement is important for maintenance of normal functioning of neurons and mitochondria themselves and its inhibition causes dysfunction of these organelles and neurons as well [70]. Defects in axonal transport are often associated with various neurological diseases, for example, with Alzheimer's disease. This pathology is characterized by specific formation of β-amyloid plaques in nervous tissue; the β-amyloid can damage mitochondrial transport. In this case, impairments of mitochondrial movement result in neuronal death even with maintenance of mitochondrial membrane potential [71]. Electron microscopy data indicate that Alzheimer's disease is characterized by altered mitochondrial morphology. Mitochondria are characterized by accumulation of osmiophilic material, changes in cristae, and reduction of their size [72]. According to some data, under normal conditions mitochondrial movement in neuronal dendrites does not depend on physiological changes in free cytoplasmic calcium ions ($[Ca^{2+}]_i$) associated with the action potential, synaptic activity, or Ca^{2+} release from intracellular stores [73]. However, in other studies it has been demonstrated that after periods of synaptic activity and membrane depolarization mitochondrial movement terminated, and this correlated with duration of the increase in $[Ca^{2+}]_i$ [74]. It should be noted that mitochondrial swelling followed by their subsequent depolarization caused impairments in mitochondrial motility [75].

Transport of mitochondria in the cell is obviously active and directed. In yeasts, motility within the cell occurs along actin filaments using a protein complex localized at mitochondrial contact sites and providing a link not only between mitochondrial membranes and the actin cable, but also with mDNA [76]. Such a link determines mDNA organization and distribution of mitochondria between maternal and daughter cells; however, reasons underlying such behavior remain unknown. Correspondingly, impairments of such a transport complex are accompanied by pathological changes in mDNA [77]. It is known that cell dynamics of actin is involved in processes of cell aging and cell death; this suggests that mitochondria, realizing cell signal transduction through actin filaments, play an active role in two these processes [78].

In higher eukaryotes, intracellular movement of mitochondria (in those cells where such movement is possible) involves not actin fibers, but microtubules associated with microtubule proteins [79]. It is suggested that cytoskeleton elements, especially tubulin, determine functional links between mitochondria and reticulum, and integrity of the tubulin cytoskeleton determines kinetic parameters of key bioenergetic enzymes [80].

Evident involvement of tubulin in the proliferative response was used for the development of a strategy for anticancer protection based on degradation of the cancer cell tubulin skeleton [81] and similar antisclerotic therapy directed to prevention of unwanted proliferation at the locus of angioplastic operation [82]. This suggests that the mitochondrion is a putative element of the proliferative response of the cell, but this link still requires additional investigation. A certain link exists between mitochondria and intermediate filaments; it has been demonstrated that such link is important for determination of mDNA structure and possibility of its repair after oxidative damage [83].

Mitochondrial fragmentation. The evident advantage of organization of mitochondrial reticulum may become a disadvantage under pathological conditions. Certain factors may cause fragmentation of the mitochondrial reticulum in some cells normally possessing such reticulum. One can suggest that the increase in number of mitochondria and consequently the increase in isolation of mDNA copies in them may protect at least some proportion of mDNA against modification.

Mitochondrial fragmentation was originally found in a cell culture exposed to the high concentrations of diazepam, and then the same phenomenon was also demonstrated in the presence of many mitochondrial inhibitors [84-86]. It is clear that mitochondrial fragmentation involves ROS [87], which probably play an indirect role as activators of elements of the signal cascade finally causing fragmentation of the inner and then the outer mitochondrial membranes. Details of the cycle fragmentation (fission)/fusion of mitochondria remain unclear, but novel proteins responsible for changes in mitochondrial morphology are constantly reported [88]. Apoptosis is also involved in this process; it has been demonstrated that the mitochondrial dynamics determines not only mitochondrial morphology but also mitochondrial functions [89]. Mitochondrial fragmentation in mammals is mainly determined by a protein similar to dynamin, Drp1 [90]; it exhibits GTPase activity contributing to membrane fragmentation using GTP hydrolysis [91]. Comparison of Drp1 with its yeast analog Dnm1 [92] resulted in the hypothesis that Drp1 exhibiting mosaic distribution is responsible for fission of both (inner and outer) membranes at the sites of its localization. Considering involvement of mitochondria in neuronal functioning (see above), we have already indicated that defects of mitochondrial dynamics may determine the incidence of neurological diseases.

SYNTHETIC ACTIVITY OF MITOCHONDRIA

Mitochondria are responsible for synthesis of various compounds, and most of them are now recognized as signal molecules.

Steroid biosynthesis. Synthesis of steroids occurs in both mitochondria of steroidogenic tissues and in cytosol; during the initial stage cholesterol is transported into mitochondria, where it undergoes cytochrome P450scc-dependent conversion into pregnenolone. The latter leaves the mitochondrion and undergoes subsequent conversions in cytosol. These include conversion of pregnenolone into progesterone and then into 17α-hydroprogesterone and finally into 11-deoxycortisol. 11-Deoxycortisol enters mitochondria again, where it is converted into cortisol. Compartmentalization of steroid biosynthesis (cytosol–mitochondrion–cytosol–mitochondrion) obviously provides fine control of biosynthesis by these two participants (see details in [93]).

Heme biosynthesis. Similar compartmentalization has been observed in heme biosynthesis, which begins in mitochondrial matrix as a branch of the tricarboxylic acid cycle: succinyl-CoA condenses with glycine followed by formation of δ-aminolevulinate. The latter leaves mitochondria for cytosol, where it is converted into porphobilinogen and then into uroporphyrinogen III and coproporphyrinogen IX, which enters mitochondria and is sequentially converted into protoporphyrinogen IX and protoporphyrin IX. At the final stage, the enzyme ferrochelatase catalyzes incorporation of an iron atom into protoporphyrin IX and this yields protoheme IX (see details in [93]).

Synthesis of iron-sulfur clusters. Frataxin is a protein encoded in the nucleus but localized in mitochondria. It plays a marked role in storage of iron ions in human cells. It is the source of iron for oxidatively damaged iron-sulfur proteins; frataxin converts inactive 3Fe-4S cluster into the active cluster 4Fe-4S [94]. Frataxin also supplies ferrochelatase with a bivalent iron [95] and contributes to the first stage of synthesis of iron-sulfur clusters, which occurs in mitochondria [96]. Insufficient synthesis of frataxin has been noted in a neurodegenerative disorder known as Friedreich ataxia [97].

Formation of ROS and reactive nitrogen species (RNS). Mitochondria are one of the main cell sources of ROS and also make substantial contribution to cell generation of RNS. Mitochondria are not only the source but also important targets for both ROS and RNS. The increase in ROS and RNS above the level required for normal cell homeostasis causes appearance of oxidative and nitrosyl stresses, respectively. Since ROS and RNS are signal molecules required for normal functioning of cells and the whole body, it is senseless to "fight" with them [98]. A reasonable strategy should consider maintenance of normal values of these cell messengers. A comprehensive review by Andreev et al. [99] summarizes current knowledge on ROS generation in mitochondria. There are nine sites of ROS generation in mitochondria. In the outer mitochondrial membrane, they include monoamine oxidase and cytochrome b_5 reductase; on the outer surface of the inner mitochondrial membrane,

these are dihydroorotate dehydrogenase and α-glycerophosphate dehydrogenase. On the inner surface of the inner mitochondrial membrane ROS are formed by α-oxoglutarate dehydrogenase and succinate dehydrogenase, whereas in mitochondrial matrix aconitase contributes to ROS formation; it is generally accepted that in the inner mitochondrial membrane complexes I and III of the respiratory chain are the main ROS generators.

Although there is *in vitro* evidence that complexes I and III are the most productive ROS generators [100], there is real suspicion that under *in situ* conditions these complexes make little contribution to gross ROS production. In submitochondrial particles generation of superoxide anion by complex I was not observed in the presence of physiological concentrations of NADH (either in forward or in reversed electron transport) [101].

Similar doubts also exist about the putative role of complex III as one of the main ROS generators. The major argument is that ROS generation was noted only after blockade of the electron transport chain with antimycin A [102], which causes strong structural rearrangements of this complex [103]. Natural ligands that would mimic the action of antimycin in mammals remain unknown, and therefore the problem of ROS generation by complex III under physiological conditions still requires convincing experimental evidence.

Recently it has been found that induction of nonspecific permeability in cardiomyocyte mitochondria is accompanied by ROS generation (ROS-induced ROS release) [104, 105]; however, the nature of mitochondrial origin of this phenomenon remains unclear. It was imitated *in vitro* after addition of physiological concentrations of NADH to isolated mitochondria; the authors suggested significant conformational changes in complex I [106]. Thus, one can make the general conclusion that ROS generation by mitochondria may significantly depend on conformational rearrangements in mitochondria.

The interaction of mitochondria with nitric oxide and other RNS represents another aspect of this problem. As in the case of ROS [87], mitochondria may represent the source and the target of RNS. This creates a possibility for fine regulation of mitochondrial functions and viability of the whole cell. This became especially evident after discovery of mitochondrial NO synthase (mtNOS) [107, 108]. The rate of NO production by intact coupled mitochondria is about 1.5-2 nmol/min per mg of protein, but total rate of NO generation may significantly differ in various tissues and in various animal species [109]. Studies of mtNOS distribution have shown that in mitochondria it is mainly localized in the inner mitochondrial membrane [110, 111], contact sites, and possibly in matrix.

Enzymatic activity of mtNOS clearly depends on Ca^{2+} concentration [112]. Since mitochondria are one of the Ca^{2+} buffers in cells and many signaling pathways (including oxidative stress) involve Ca^{2+} oscillations, Ca^{2+} dependence of mtNOS may represent the main mechanism of regulation of mitochondrial functions by NO.

Besides mtNOS-dependent NO synthesis, it is suggested that under certain conditions mitochondria can reduce nitrite to NO [113] (this may occur in ischemia [114]). Mitochondrial NO is located near components of the respiratory chain and may possibly interact with them. The main effect of mitochondrial nitric oxide consists of inhibition of the respiratory chain due to inhibition of cytochrome oxidase or nitrosylation of other respiratory chain complexes. NO binds to the heme and copper-containing sites of cytochrome oxidase and exhibits both competitive and O_2-independent inhibition [115, 116]. The influence of NO on activity of cytochrome oxidase may be related to: i) regulation of oxygen consumption by cells and tissues; ii) mitochondrial ROS formation and regulation of oxidative stress; iii) cytotoxic activity of macrophage NO. The regulation of oxygen consumption may be the main function of mtNOS under normal physiological conditions. The main mechanism responsible for energy supply of compartments located far away from oxygen sources is attributed to mitochondria as electric cable of the cell [64]. Mitochondrial NO synthase may serve as an additional mechanism of equilibration of oxygen concentration both in the whole tissue and particular cells [110]. Cells located close to capillaries have maximal oxygen supply; they exhibit high mtNOS activity and lower cytochrome oxidase activity, and this promotes diffusion of larger amounts of oxygen inside tissues [117].

Certain evidence suggests that mtNOS activity may protect cells against oxidative stress. For example, nitric oxide production increased cell resistance to oxidative stress induced by exogenous hydrogen peroxide [118]. The (small and transient) increase in RNS accompanied by suppression of mitochondrial respiration and increased generation of ROS may represent a basis for mechanisms of ischemic preconditioning. This suggestion is supported by the fact of increased activity of mtNOS under hypoxic conditions [119].

In addition to inhibiting cytochrome oxidase, NO irreversibly inhibits other respiratory chain components (complexes I and II) and inactivates aconitase. The NO derivative peroxynitrite, formed by nitric oxide condensation with superoxide anion, is a potent oxidizing and nitrosylating agent; its formation in mitochondria may damage complexes I and II, ATP synthase, creatine kinase, aconitase, and superoxide dismutase [120, 121].

Inhibition of complex I may originate from modification of Fe-S centers, S-nitrosylation, N-nitrosylation, and Fe-nitrosylation [122, 123]. Nitrosylation of various mitochondrial proteins, including components of the respiratory chain, has been found under conditions of ischemia/reperfusion [124]. Actively respiring mitochondria also exhibited denitrosylation processes; the latter suggests that this reversible process may have some regu-

latory role. Interestingly, S-nitrosylation of complex I was accompanied by cardiomyocyte protection against ischemia/reperfusion, and this emphasizes ambiguous role of RNS.

Thus, mitochondrial NO production is an additional mechanism for regulation of oxygen consumption by tissues and cells. On the other hand, the mitochondrion employs this mechanism for transduction of various external signals modulating membrane potential, respiration, and ATP synthesis. Finally, under conditions of stress, targets and sources of NO give the cell additional chances to avoid fatal damage and death, but in the case of inadequate protection they are involved in augmentation of the destructive signal and accelerated cell death.

Urea cycle. The urea cycle (also known as the ornithine cycle) provides detoxification of potentially hazardous ammonia followed by stepwise formation of the nontoxic product, urea, which is excreted in urine [125]. This process occurs in hepatocytes, and two of five reactions of this cycle occur in mitochondria (and three reactions occur in cytoplasm). As in the case of synthesis of heme and steroids, the bi-compartmental mode of urea synthesis [126] obviously provides more accurate control at the level of amino acid transport through mitochondrial membranes.

MITOCHONDRIA AS MODULATORS OF CALCIUM SIGNAL

Intracellular Ca^{2+} signaling is mainly determined by influx and efflux of calcium ions into and out of the cell, as well as buffer capacity of intracellular systems, which can accumulate these ions in an energy-dependent manner. Rapid binding of intracellular Ca^{2+} is mediated by calcium-binding proteins, whereas slow redistribution of Ca^{2+} occurs due to calcium pumps and exchangers. Calcium ion fluxes into the cell involve voltage-dependent ligand-gated channels, which mainly determine the amplitude and duration of the calcium signal. The systems responsible for Ca^{2+} efflux from the cell through the plasma membrane (Ca^{2+}-ATPase and Na^{+}-Ca^{2+} exchanger), sarco/endoplasmic reticulum, and mitochondria also contribute to these two parameters [127].

In the reticular membrane, there are sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and two channels for Ca^{2+} efflux—1,4,5-inositol triphosphate and ryanodine receptors. Concerted and synchronized interaction between calcium channels and receptors is the basis for normal functioning of each cell; it is especially pronounced in cardiomyocytes, which carry out periodic and controlled alternation of muscle contraction/relaxation determined by oscillations of intracellular concentrations of free Ca^{2+}.

Three systems of Ca^{2+} transport have been described in mitochondria. The first carries out electrogenic, ruthenium-sensitive cation transfer by its gradient. This system is responsible for both mitochondrial Ca^{2+} influx and efflux [128]. The second system is responsible for Na^{+}-dependent and Na^{+}-independent Ca^{2+} exchange [129, 130]. The third system is involved in the efflux of mitochondrial calcium through induction of the mitochondrial megachannel also known as the phenomenon of Ca^{2+}-dependent nonspecific mitochondrial permeability [131-133].

Recently, the existence of two characteristic reticular Ca^{2+}-transporting systems, SERCA and the ryanodine receptor, has been recognized in mitochondria [134, 135]; this suggests not only close functional but also structural interrelationship between mitochondria and reticulum [104, 136]. However, this interrelationship is still a matter for discussion: particularly it remains unclear whether mitochondria can totally "monitor" rapid changes in intracellular Ca^{2+} content in cardiomyocytes [137]. Some researchers assume the existence of Ca^{2+} oscillations in mitochondrial matrix of contracting cardiomyocytes (e.g. [138, 139]), others rule out such possibility [140, 141].

MITOCHONDRIA AND CELL DEATH

Current knowledge on the involvement of mitochondria in programmed cell death (apoptosis) has been recently summarized in many excellent reviews (e.g. [142]). The pioneering studies by the laboratories of Kroemer and Wang demonstrated that release of an apoptosis-inducing factor (AIF) [143] and cytochrome *c* [144] from mitochondria is an obligatory event for triggering terminal stages of apoptosis; according to Kroemer's definition "point of no return" [145]. The mitochondrial pathway for apoptotic degradation of the cell "collects" signals from various processes and elements including growth factor withdrawal, damage of cytoskeleton, DNA damage, inhibition of synthesis of macromolecules, reticular stress (causing release of calcium ions), etc. [146]. Amplification of an extramitochondrial upstream signal may be achieved by release of numerous signaling molecules located in the mitochondrial intermembrane space; this requires high (possibly selective) permeability of the outer mitochondrial membrane. Damage of the outer mitochondrial membrane causes release of cytochrome *c* normally localized in the intermembrane space. In cytosol, cytochrome *c* binds to the apoptotic protease activating factor (APAF-1) and induces formation of the apoptosome, activating terminal caspases 9 and 3.

There are two viewpoints on the mechanism responsible for high permeability of the outer mitochondrial membrane. The first considers the mitochondrion as an "osmometer" that swells after removal of all ion gradients but preserves high protein content in the mitochondrial matrix. Since the surface of the inner mitochondrial

membrane is significantly greater than that of the outer mitochondrial membrane, swelling of mitochondrial matrix causes cristae stretching and breaks in the outer membrane [147]. According to the second viewpoint, specific release of apoptotic factors from the intermembrane space does not involve breaks in the outer mitochondrial membrane. It should be noted that the outer mitochondrial membrane has potentially high permeability due to VDAC; opened VDAC is permeable to molecules with molecular mass up to 1.5 kD. The suggestion that VDAC can form oligomers responsible for transport of proteins (e.g. cytochrome *c* [148]) is skeptically accepted by classical researchers of mitochondrial porin [149]. Oligomerization of proapoptotic BAX (and/or BAK) protein, which can form the giant pores permeable for cytochrome *c* seems to be more probable [150]. Permeabilization of the outer membrane is a highly ordered process involving proteins of the bcl-2 family, characterized by the presence of up to four homologous domains defined as BH1-4. Proteins possessing all these domains exhibit antiapoptotic properties, whereas proteins possessing less than the four domains demonstrate proapoptotic properties [151]. The group of proapoptotic proteins can be further subdivided into two subgroups, one of which contains proteins with many BH domains (BH1,2,3) (e.g. BAX and BAK) and also proteins with just BH3 domain (BID, BIM, PUMA). It should be noted that only multidomain proapoptotic proteins BAX and BAK increase permeability of the outer mitochondrial membrane; this suggests that in resting (non-apoptotic) cell these proteins are inactive [152]. The latter is supported by the fact that in mitochondrial contact sites there are some amount of proapoptotic BAX and cytochrome *c*; the former does not exhibit proapoptotic properties and the latter is not linked to the respiratory chain [11, 16]. It is possible that the mitochondrial contact sites contain the same fraction of cytochrome *c*, which is then released from mitochondria in response to an apoptotic signal.

Some authors believe that nonspecific permeability of the inner membrane is an obligatory factor of the mitochondrial part of the apoptotic cascade [153, 154]. Moreover, the protein complex of the contact sites responsible for induction of nonspecific permeability [155] is the key element underlying involvement of mitochondria in apoptosis. Experiments *in situ* have shown that cell death induced by change in hypoxia for normoxia (and accompanied by oxidative stress) involves obligatory induction of nonspecific mitochondrial permeability [156, 157]. It becomes clear that in the systems where apoptotic death is an unwanted event (e.g. postmitotic cells such as neurons or cardiomyocytes) strategy for prevention of cell death should be based on prevention of induction of nonspecific mitochondrial permeability [158]. A recently discovered mitochondrial protein, connexin-43 typical for cell–cell contacts, protects cardiomyocytes against death induced by oxidative stress [159]; however, the mechanism of this protective effect remains unclear.

Very often it is difficult to discriminate apoptotic and necrotic systems of cell death; however, recent data indicate that apoptosis is accompanied by fragmentation of mitochondria and changes in inner mitochondrial membrane topology [142], which have not been seen in necrosis.

Besides involvement in regulation of programmed cell death, mitochondria are suggested to be involved in programmed control of their own destruction [160, 161]; this has been proposed within concepts of mitoptosis [162] and phenoptosis (death of an organism) [162].

Recently, TG2 isoform of transglutaminase has been found in mitochondria [163]; this enzyme containing only the BH3 domain may cross-link various proteins including BAX [164]; this further strengthens the viewpoint that the mitochondrion is a potent regulator of cell death.

This work was supported by the Russian Foundation for Basic Research (grant Nos. 05-04-48411, 05-04-48412, and 05-04-49697).

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