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Mitochondrial Biogenesis and Turnover

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Abstract

Mitochondrial biogenesis is a complex process involving the coordinated expression of mitochondrial and nuclear genes, the import of the products of the latter into the organelle and turnover. The mechanisms associated with these events have been intensively studied in the last twenty years and our understanding of their details is much improved. Mitochondrial biogenesis requires the participation of calcium signaling that activates a series of calcium dependent protein kinases that in turn activate transcription factors and coactivators such as PGC-1 α that regulates the expression of genes coding for mitochondrial components. In addition, mitochondrial biogenesis involves the balance of mitochondrial fission-fusion. Mitochondrial malfunction or defects in any of the many pathways involved in mitochondrial biogenesis can lead to degenerative diseases and possibly play an important part in aging.

Introduction

In eukaryotes, cellular respiration, energy production and other metabolic processes take place in specialized double membrane organelles, containing their own genome, the mitochondria. Mitochondria play a very important role in cell metabolism, not only by energy generation but also by being a major site for production of reactive oxygen species (ROS) and being a key player in apoptosis and calcium homeostasis. Malfunction of mitochondria has been associated with numerous degenerative diseases and aging [1, 2]. Table 1 shows some of the diseases associated with mitochondrial biogenesis that are discussed in this paper.

The origin of this organelle in eukaryotes is explained by the endo-symbiont theory, which proposes that mitochondria originated from bacteria that were incorporated into a host cell and maintained during evolution [3, 4]. This hypothesis is backed by substantial experimental evidence, including the similarities between the bacterial and mitochondrial translational apparatus [5].

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Mitochondrial DNA

Figure 1A illustrates the structure and expression of the human mitochondrial DNA (mtDNA), a double stranded circular molecule of 16,569 bp encoding 2 ribosomal RNAs, 22 transfer RNAs and 13 proteins that form part of the multi subunits complexes of the oxidative phosphorylation system (7 subunits of complex I, 1 subunit of complex III, 3 subunits of complex IV and 2 subunits of complex V).

The mammalian mtDNA contains few non-coding sequences, the largest being the D-loop or displacement-loop, which contains promoters and origins of replication (Figure 1B). Protein coding genes have no intronic regions [6]. Alkaline gradient centrifugation experiments allowed the separation of the mtDNA double strands into a heavy (H-strand) and a light (L-strand) due to their differential content of guanosine and cytidine. The H-strand encodes for the 2 rRNAs, 12 of the polypeptides and 14 of the tRNAs whereas the L-strand encodes for only one of the polypeptides (ND6) and 8 tRNAs. The genetic code of mitochondria differs from the nuclear universal code. The TGA codon codes for tryptophan instead of stop, the AGA and AGG code for stop instead of arginine and the ATA codes for methionine instead of isoleucine [6].

The mtDNA is associated with several proteins packed in structures denominated nucleoids, which are also associated with the inner mitochondrial membrane. In the last few years progress has been made on identifying the composition of these structures. Several studies showed that yeast nucleoids include proteins that bind DNA and are associated with replication and transcription such as the mitochondrial transcription factor A (TFAM), helicase TWINKLE, polymerase γ (Pol γ) and the mitochondrial single-strand binding protein (mtSSB); proteins associated with metabolism such as the E2 subunit of pyruvate dehydrogenase and α -ketoglutarate dehydrogenase, aconitase; and some cytoskeletal proteins [7] Mutations in components of the replication machinery are associated with mitochondrial depletion syndromes (reviewed by [8]).

Mutations in the *PEO1* gene encoding the helicase TWINKLE have been reported in some patients with autosomal dominant progressive external ophthalmoplegia (PEO) and in one patient with sensory ataxic neuropathy dysarthria and ophthalmoparesis (SANDO) [9, 10]. In addition, mutations in the catalytic subunit of polymerase γ (have been associated with a wide range of disease such as PEO, SANDO, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), parkinsonism and Alpers syndrome (review by [8]) whereas mutations in the gene *POL2* encoding one of the subunits of Pol γ , is associated with autosomal dominant PEO [11].

Recently, Wang and Bogenhagen [12] found a novel protein, DHX30 (DEAH-box helicase) to be present in nucleoids purified from HeLa cells [12], In addition, the authors also found ATAD3, a member of the AAA⁺-ATPases, associated with nucleoids. Further studies in rat liver mitochondria by He et al. [13] found that ATAD3 binds mtDNA, preferentially in the D-loop region, and it appears to be involved in nucleoid organization and segregation [13, 14].

Despite these advances, understanding of the composition and structure of mitochondrial nucleoids is far from complete. There are several proteins associated with mtDNA metabolism that are not easily detectable in nucleoids. These include the mitochondrial transcription factors B1 and B2 and regulators of transcription from the mTERF family [12]. The mitochondrial transcription factors B1 and B2 have been found to be required for mtDNA transcription (at least one of them) and may also have a role in methylation of DNA or RNA [15]. TFAM seems to be present at relatively high levels, and by itself is able to organize the mtDNA in “nucleoid-like” structures [16].

Mitochondrial DNA replication and transcription

The mtDNA replication is linked to the transcription of the light strand promoter (LSP) that serves as an RNA primer to initiate DNA replication at the origin of replication of the H-strand (O_H) located in the D-loop (inset on figure 1A) [17]. To form the RNA primer, transcription of LSP is initiated by the mitochondrial RNA polymerase (POLRMT) and then to produce a 3' end of these RNA primer, an endoribonuclease mitochondrial RNA processing (MRP) is required [18, 19]. The mtDNA replication is carried out by POL γ , with 3'–5' exonuclease and 5'-deoxyribose phosphate lyase activities, assisted by the helicase TWINKLE that unwinds the DNA duplex, and by single strand binding proteins (mtSSB) that keep the DNA in a single stranded form.

The exact mechanism for DNA replication is still unknown but two models have been proposed. The first model proposes that the replication of mtDNA is unidirectional and asynchronous (inset in figure 1A) [20, 21]. The synthesis of the leading-strand starts at the O_H and proceeds displacing the parental heavy strand until it reaches and exposes the origin of replication of the other strand (O_L), that is activated and the replication of the lagging-strand starts in the opposite direction. The resulting two new DNA molecules are ligated to form the circular double strand mtDNA [20]. The second model involves the coupled replication of both leading and lagging strands [22].

The mtDNA transcription starts from the promoters present in each strand (HSP and LSP) producing polycistronic RNAs. In the HSP there are two sites of initiation, the H1 site, that produces a short transcript containing the 12S RNA, 16S RNA and the tRNA^{leu}, and the H2 site, that produces a polycistronic transcript of the length of the full genome, whereas in the LSP there is only one initiation site [23–25]. The transcription is carried out by a mitochondrial RNA polymerase (POLRMT) assisted by a mitochondrial transcription factor A (TFAM), that recruits POLRMT to the promoter site, and by mitochondrial transcription factor B1 or B2 (TFB1M and TFB2M; Figure 1A). The termination of the H1 short transcript is dependent on the mitochondrial transcription terminator factor (MTERF) ([26] reviewed in [27, 28]). Recently, Park et al. [29] identified the function of a new transcription terminator factor, MTERF3, as a negative regulator of transcription [29].

Mitochondrial Protein Import

Mitochondrial proteomics in humans has estimated approximately 1500 proteins with a very small portion of these proteins encoded by the mtDNA [30, 31]. Therefore, the majority of the mitochondrial proteins are encoded by the nuclear DNA and imported into the organelle. Progress on the knowledge of the components and the mechanisms of protein transport into the mitochondria have been spearheaded by studies performed in fungi (*Neurospora crassa* and *Saccharomyces cerevisiae*). In many instances, the mammalian counterparts have not been identified yet.

Distribution of mitochondrial proteins into the appropriate compartment in the organelle is a very complex process and the targeting signals for sorting are not obvious. Depending on the mitochondrial location of the protein (matrix, inner mitochondrial membrane, intermembrane space or outer mitochondrial membrane) there are different import pathways. We briefly describe each scenario but for specific details refer to recent reviews [32, 33].

Matrix proteins: these proteins are synthesized in cytoplasmic ribosomes as precursors containing a targeting signal located in the amino terminal end. This signal is about 10–80 amino acids long with no consensus in the primary structure and composed of hydrophobic and positively charged amino acids that can form amphipathic helices. Once the protein is inside the mitochondria this amino terminal sequence is cleaved by mitochondrial

processing peptidases to generate the mature form of the imported protein. There are cases where the targeting sequence is located internally or in the carboxy terminal end of the protein. Protein precursors synthesized in the cytoplasm are associated with cytoplasmic chaperones (e.g. Hsp70 and Hsp90), which keep them in an unfolded state and competent for translocation posttranslationally into the mitochondria, while other proteins seem to be imported cotranslationally [34].

The import of the matrix proteins is mediated by two translocase complexes, one located in the outer mitochondrial membrane (translocase of the outer membrane or TOM) and one in the inner mitochondrial membrane (translocase of the inner membrane or TIM23) that function in a concerted manner. The TOM complex is formed by seven proteins (Tom70, Tom40, Tom22, Tom20, Tom7, Tom6 and Tom5) with specialized functions: 1) surface receptor function (Tom70, Tom22 and Tom20) that recognize the precursor protein and interacts with cytosolic chaperones, and 2) import pore function (Tom40, Tom5, Tom6 and Tom7) that can form up to 3 pores in the holocomplex. Once the protein has crossed the TOM complex it is recognized by the TIM23 complex that is formed by Tim50, Tim23, Tim21, Tim17 and by the motor components that drive the translocation (Tim44, Tim16/Pam16, Tim14/Pam18, Mge1 and mtHsp70). This translocation process is dependent on ATP and a membrane potential. The final step in the matrix import consists in an active mtHSP70 function. Although it is not clear if the pulling of the preprotein into the matrix by mtHSP70 is done by an active conformational change or by a Brownian movement ratchet [35], recent evidence has favored the latter [36].

Inner membrane proteins: there are 3 different ways for inner membrane proteins to be translocated and inserted into the inner membrane. 1) Proteins that contain several transmembrane domains (with negative charges), are synthesized with a presequence and translocated via TOM-TIM23 to the matrix where the presequence is cleaved and they are “exported” into the membrane by Oxa1 [37]. 2) Proteins that contain one transmembrane domain (usually with more charged and hydrophobic residues in the carboxy terminus, than the proteins inserted via Oxa1) are translocated through the TOM complex, pass to the TIM23 complex, get arrested, transferred laterally and integrated into the inner membrane where the targeting sequence is cleaved. 3) Proteins that contain hydrophobic residues and an even number of transmembrane domains are first translocated by the TOM complex and then are intercepted by the small Tim proteins (small polypeptides Tim 9, Tim10 and Tim12, containing Cx₃C motifs that form hexamers) in the inner membrane space [38] and then transferred to another translocase, the TIM22 complex in the inner membrane that inserts the protein into the membranes in a membrane potential dependent manner. The TIM22 complex is formed by 3 membrane proteins Tim54, Tim22 and Tim18 [39].

Import of outer membrane proteins: many of these proteins contain a β -barrel structure and they use the TOM complex to pass through the outer membrane, then are guided by the small Tim proteins to another complex located also in the outer membrane, the translocase of outer membrane β -barrel proteins (TOB) or sorting and assembly machinery (SAM) complex (TOB/SAM), that inserts the protein into the membrane. The TOB/SAM complex is formed by Tom55 (also called Sam50 or Tom50), Tob38 (Sam35 or Tom38) and by Mas37 (Sam37 or Tom37) [40, 41].

Import of intermembrane space proteins: some proteins contain two signal sequences allowing the insertion into the inner membrane via TOM-TIM23 complex and then the mature protein is formed by proteolysis and released into the inner membrane, whereas other proteins are translocated by the TOM complex and then folded in the intermembrane space by the help of Mia40 and Erv1 [42–45]. Figure 2 summarizes the current knowledge of the mitochondrial import machinery.

There are several diseases associated with errors in the import of mitochondrial proteins such as pyruvate dehydrogenase deficiency (mutations in the mitochondrial targeting sequence of subunit E1 α [46]), Alanine/glyoxylate aminotransferase deficiency (peroxisomal protein that is mistargeted into the mitochondria [47]), severe alcoholic liver disease (caused by defects in the import efficiency of the genetic dimorphic manganese superoxide dismutase [48]), Deafness Dystonia Syndrome or Mohr-Tranebjaerg Syndrome (caused by mutations in one of the small Tim proteins, Tim8, that result in an impaired biogenesis of the TIM23 complex [49]), Dilated cardiomyopathy with Ataxia (caused by defects in Pam18, one of the motor proteins of the TIM23 complex [50]) and Spastic Paraplegia-13 (caused by defects in the mitochondrial Hsp60 causing disruption in protein folding in the matrix [51]). For details see review by MacKenzie and Payne [52].

Mitochondrial-Nuclear Communication

Responses to different stimuli such as changes in temperature, nutritional state, energy production, hormonal changes as well as normal homeostasis in mammals, require the action of both mitochondrial and nuclear genomes. These responses involve signals from the nucleus to mitochondria as well as from mitochondria to the nucleus. The nuclear to mitochondrial signal involves the activation of certain transcription factors that regulate the expression of mitochondrial genes leading to mitochondrial biogenesis, whereas the mitochondrial to nucleus signals (also referred to as “retrograde signaling”) sense the mitochondrial functional state (e.g. loss of function by decrease in membrane potential, defects in oxidative phosphorylation, lower ATP levels, uncoupling, mtDNA mutations and hypoxia) [53]. In addition, mitochondrial stress response to heat shock or reduced levels of certain proteins can also trigger a nuclear response [54]. The mechanisms involved in the retrograde signaling are not well understood, although in yeast a number of transcriptional activators mediating this signaling pathway have been identified [55]. As described below, calcium participates in both antero- and retro-grade mitochondrial nuclear signaling in response to stimuli or stress (recently reviewed in [56]).

Mitochondrial Biogenesis

Although the complete pathway controlling mitochondrial biogenesis has not been elucidated, great progress in identifying key players has been obtained in the last few years. The expression of mitochondrial proteins encoded in the nuclear genome participating in oxidative phosphorylation, heme biosynthesis, mitochondrial protein import, and mtDNA transcription and replication, is regulated by transcription factors and transcriptional coactivators. The most prevalent transcription factors activating promoters of mitochondrial genes are the nuclear respiratory factor 1 and 2 (NRF-1 and NRF-2) [57, 58] and the estrogen-related receptor (ERR α) that work in concert with transcriptional coactivators of the peroxisome proliferator-activated receptor γ -coactivator-1 (PGC-1) family, PGC-1 α , PGC-1 β and PRC (PGC-1 related coactivator) [59]. This family of coactivators regulates several metabolic pathways such as cellular respiration, thermogenesis and hepatic glucose metabolism (reviewed in [60–62]). Although these coactivators stimulate mitochondrial biogenesis, PGC-1 α is mainly involved in the regulation of gluconeogenesis and PGC-1 β in the regulation of β -oxidation of fatty acids [63, 64]. Srivastava and Moraes [65] showed that overexpression of PGC-1 α and PGC-1 β was associated with an improvement of respiration in cells with deleterious mtDNA mutations [65].

Physiological mechanisms involved in PGC-1 α regulation have been extensively studied in the last few years. Transgenic mice overexpressing PGC-1 α showed mitochondrial proliferation in skeletal muscle and switch in fiber type composition from the more prominent type II (glycolytic) to type I (oxidative) [66]. In skeletal muscle, endurance

exercise induces an increase in mitochondrial mass that is mediated by the increase in intracellular calcium levels during fiber contraction [67]. This process is regulated by the orchestrated expression of both nuclear and mitochondrial genes and the main pathway involved includes the activation and action of PGC-1 α .

Increased levels of intracellular calcium activates cytoplasmic protein kinases such as calcium/calmodulin-dependent protein kinase (CaMK) or protein kinase C (PKC) that in turn stimulate the expression of several nuclear and mitochondrial genes [68]. Studies *in vitro* in isolated rat epitrochlearis muscle where an intracellular increase of calcium was achieved by incubation with low concentrations of caffeine (concentrations of 3.5 mM caffeine were low enough to not induce muscle contraction but to increase PGC-1 α levels) and using specific Kinase inhibitors showed that the activation of CaMK occurs upstream of the activation of p38 mitogen-activated protein kinase (p38 MAPK) [69]. Moreover, p38 MAPK is responsible for phosphorylating, activating and inducing PGC-1 α expression [69, 70]. In addition, Jager et al. [71] showed that AMP-activated protein kinase (AMPK) directly binds and phosphorylates PGC-1 α [71]. The induction of PGC-1 α expression is also mediated through ATF-2 (activating transcription factor 2) that binds to the PGC-1 α promoter in the CREB (cyclic AMP-response binding protein) element binding site [72, 73]. Figure 3 shows a general diagram of the calcium signaling involved in mitochondrial biogenesis.

The effect of calcium on the expression of mitochondrial proteins is not confined to muscle. Au et al. [74] had shown the increase of COX1, ATPase6, SDH and TFAM (transcript as well as protein levels) in human granulosa cells treated with the calcium ionophore A23187 and this effect was inhibited by EGTA [74]. Likewise, Mercy et al [75] using mtDNA depleted (ρ^0) cell lines, L929 (mouse fibroblast) and 143B (human osteosarcoma) showed that mitochondrial biogenesis is mediated by calcium signaling through CREB [75]. In addition to phosphorylation, another post-translational modification that regulates PGC-1 α activity has been identified. Namely, deacetylation mediated by SIRT1 during fasting promotes the expression of mitochondrial genes involved in lipid oxidation [76].

The control of mitochondrial biogenesis during the cell cycle in mammalian cells remains unknown and controversial. Recently, Lee et al. [77] investigated mitochondria in synchronized cultures of HeLa cells at different points during the cell cycle and showed evidence that mitochondrial morphology varies during the cell cycle. During interphase, the typical mitochondrial tubular network was observed with heterogeneity in mitochondrial size and morphology, but progression into the mitotic phase disrupted this network and the mitochondrial population became more homogeneous [77]. Morphological changes of mitochondria during the cell cycle have been reported previously [78]. The tubular network seen during interphase was associated with microtubules whereas in mitosis, the fragmented mitochondria no longer interacted with microtubules but rather with actin. The mitochondrial mass and membrane potential increased during the progression of G1 to mitosis and after cell division these parameters were reduced again. The levels of mtDNA also increased in G1/S to G2 transition concomitant with increase of NRF-1 levels although the levels of TFAM and PRC were not altered [77].

Mitochondrial biogenesis, can also be stimulated by other pathways like reactive oxygen species [79], nitric oxide [80, 81] and hypoxia [82], which can be caused by a mitochondrial disorder or ischemic insults.

During heat shock or in the case of a respiratory deficiency caused by the decrease of one subunit of one of the respiratory complexes, an accumulation of unassembled subunits may occur, leading mitochondria into stress signals and a cellular unfolded protein response

(UPR) whereby the cell responds by increasing the amount of proteins required for quality control (chaperones or proteases) [54].

Mitochondrial biogenesis is commonly stimulated in mitochondrial disorders, a group of diseases with heterogeneous array of clinical manifestations. These disorders are associated with either mutations in the mtDNA or in the nuclear DNA and often affect tissues with high energetic demands such as brain, heart and skeletal muscle [1, 2]. In addition, alterations of mtDNA (both large deletions and point mutations) have been observed in aging [83, 84], although the functional significance of these is still unclear. One of the hallmarks of oxidative phosphorylation dysfunction is the abnormal mitochondrial proliferation as a compensatory mechanism for the deficiency. This mitochondrial proliferation is commonly described as “ragged red fibers” in muscle [1], but it is also observed in some other tissues [85].

Mitochondrial Fusion and Fission

Mitochondria are dynamic organelles that undergo fusion and fission as part of their normal function. These two events are coordinated and necessary for proper morphology and function and seem to play critical roles during development, cell division and apoptosis. Fusion and fission are interrelated with the distribution of mtDNA within the cell as well as with their inheritance into daughter cells. In yeast, fusion defects cause loss of mtDNA and respiratory function [86]. Most of our knowledge in this field is due to studies performed in *S. cerevisiae*, *Drosophila melanogaster* and *Caenorhabditis elegans*.

The first fusion protein discovered was the fuzzy onion protein (Fzo) in *Drosophila* [87]. Fzo1 is an outer mitochondrial transmembrane GTPase that together with Mgm1 and Ugo1 are involved in mitochondrial fusion. In yeast, defects in Mgm1 lead to loss of mtDNA and respiratory deficiency [88]. In mammals, proteins homologous to Fzo1 and Mgm1 have been identified, and correspond to mitofusins (Mfn1 and Mfn2) and OPA1 respectively [89]. In humans, Charcot-Marie-Tooth 2A and Autosomal Dominant Optic Atrophy, two neurodegenerative diseases involving defects in peripheral and retinal ganglia neurons, are caused by mutations in these two genes responsible for mitochondrial fusion, Mfn2 and OPA1 respectively [90–92].

Although the physiological role of mitochondrial fusion is not known, a conditional knockout mouse lacking Mfn2 in cerebellum showed neuronal degeneration where Purkinje cells lacked proper dendritic extensions, had respiratory function deficiency and abnormal mitochondrial distribution [93].

In yeast, mitochondrial fission involves the action of a dynamin- related protein Dmn1, with GTPase activity, as well as Fis1 and Mdv1. Fis1, an outer mitochondrial membrane protein, in association with Mdv1 recruits Dmn1 to the mitochondrial membrane at the site of division. Dmn1 is able to self assemble, by GTP-dependent conformational changes, into spiral structures that wrap around the mitochondria constricting the membrane resulting in fission (Figure 4) [89]. The human homologues of Dmn1 and Fis1 are Drp1 and hFis1 respectively, but so far no homolog of Mdv1 has been identified. Although the exact mechanism is still unknown, mitochondrial fission seems to be regulated by endophilin B (belonging to a family of self assembling proteins shown to remodel membranes in endocytosis [94] and by sumoylation of Drp1, which protects the protein from degradation [95]. In addition, mitochondrial fusion and fission have been shown to regulate calcium signaling in cells. Overexpression of Drp1, caused a fragmentation of the mitochondrial network that inhibited normal intramitochondrial calcium waves [96]. The general composition of the fusion/fission apparatus is described in figure 4.

Mitochondrial Turnover

Defective mitochondria have to be eliminated to maintain cellular homeostasis. Menzies and Gold [97] estimated that the turnover of mitochondria in liver, heart and brain is 9.3, 17.5 and 24.4 days respectively [97]. Autophagy appears to be the main pathway for mitochondrial turnover (mitophagy) although it has been shown that ubiquitin mediated proteolysis plays a major role in the elimination of the paternal mitochondria during fertilization [98, 99].

Mitophagy consist in the sequestration of the defective organelle into a double membrane phagosome that is later fused with lysosomes forming autolysosomes, where the contents are degraded and can be recycled (reviewed in [100, 101]). In yeast, several genes involved in autophagy (ATG genes) have been described and their mechanism of action requires two novel conjugation systems that are somehow interrelated (reviewed in [102]). In one of the systems, Atg12, a small protein, is conjugated to a lysine on Atg5 by Atg7 and Atg10, in a way similar to the E1 and E2 enzymes of the ubiquitin system, and once Atg5 is tagged with Atg12 it forms a complex with Atg16 [103]. In the other conjugation system, Atg8, also a small protein, is conjugated to phosphatidylethanolamine (PE, membrane phospholipid) by Atg7 and Atg3 [104]. In addition to the conjugated systems, two protein kinase complexes are required for autophagy. The protein kinase complexes are the Atg1 kinase complex and the phosphatidylinositol 3 kinase (PI3K) complex both formed by several proteins. The four systems described above (protein conjugation and kinase complexes) work in concert to produce the autophagosome.

The mitophagy process starts by the recruitment and transient association of the conjugated protein (Atg5-Atg12 conjugated) into a precursor vesicle (PE-Atg8 conjugated) that elongates along the perimeter of the mitochondrion until both ends fuse forming the double membrane of the autophagosome. The PI3K complex is required for the initial process of autophagosome formation and its inhibition stops autophagy [105]. The function of the Atg1 kinase is not very clear but appears to control the autophagy response induced by nutrient deprivation [106].

The process of how an individual mitochondrion is recognized for degradation is not known but appears that there are some proteins such as Uth1 (mitochondrial outer membrane protein) involved in the selectivity of the process [107]. For more details, this topic (calcium and mitochondria autophagy) is covered in another chapter of this special issue of Cell Calcium.

Conclusions

Mitochondrial biogenesis is a very complex cellular process that requires the coordination of several mechanisms involving nuclear-mitochondrial communication, mitochondrial protein expression and import, mtDNA gene expression, assembly of multi subunit enzyme complexes, regulation of mitochondrial fission and fusion as well as mitochondrial turnover in response to various stimuli. Disruption of any of these processes can lead to defective mitochondrial function and therefore to a disease state. Many of the pathways participating in mitochondrial biogenesis require the participation of calcium as a signaling molecule, as discussed in more detail in the subsequent chapters of this book.

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Figure 1. Structure and expression of the human mitochondrial DNA

The 16,569 bp human mtDNA (panel A) showing 13 protein coding genes as well as 2 rRNA- and 22 tRNA-coding genes. Genes coding for subunits of complex I (ND1-ND6), complex III (Cyt b), complex IV (COX I-COX III) and complex V (A8 and A6) are shown by different colors. The insert on panel A illustrates one of the mechanisms proposed for mtDNA replication. It also shows a consensus model of polycyclic transcription, including the approximately binding sites for the mitochondrial RNA polymerase, the mitochondrial transcription factor TFAM, the RNA processing enzyme RNase MRP and the transcription termination factor mTERF. The origins of replication for the H- and L- (OH and OL) strands are also shown. The structure of the regulatory D-loop region is shown in panel B, including the approximate position of the conserved sequence boxes believed to play a role in replication and RNA primer processing. It also shows the location of the two hypervariable regions (HSV1 and HSV2) commonly used for evolutionary studies.

Figure 2. Protein import into mitochondria

Nuclear encoded proteins are imported into mitochondria by different pathways depending on their final destination. Matrix proteins, synthesized as precursors containing a targeting sequence in the amino terminus are translocated through TOM and TIM23-Hsp70/Mge1 complexes and once in the matrix the targeting sequence is cleaved by mitochondrial proteases to render the mature form. Inner membrane proteins are translocated by TOM and TIM23 but get arrested in the latter, transferred laterally into the membrane and have their targeting sequence cleaved. Other inner membrane proteins are imported into the matrix by TOM and TIM23 and inserted into the inner membrane with the help of Oxa1. In addition, very hydrophobic inner membrane proteins with even number of transmembrane domains are translocated through TOM, then passed to TIM22 via small Tim proteins and inserted into the membrane. Intermembrane space (IMS) proteins are transported via TOM-TIM23 and laterally transferred to inner membrane, where the presequence is cleaved and the protein is released into the intermembrane space. Other IMS proteins are translocated through the TOM complex and then folded in the intermembrane space with the help of Mia40 and Erv1 chaperones. Outer membrane proteins are translocated through the TOM complex, guided by the small Tim proteins to the TOB/SAM complex and inserted into the membrane. See text for more details.

Figure 3. Calcium signaling participating in Mitochondrial Biogenesis

Extracellular stimuli such as nutrient deprivation, changes in temperature or exercise increase intracellular concentrations of calcium that stimulates different kinases (depending on the stimuli) that can activate transcription by stimulating specific transcription factors (e.g. CREB). Certain kinases also activate the transcriptional coactivator PGC-1 α by directly phosphorylating it or by activating its transcription. PGC-1 α stimulates transcription factors such as nuclear respiratory factor NRF1 and NRF2 to activate transcription of several mitochondrial genes including proteins necessary for transcription and replication of mtDNA, proteins of the electron transport chain, mitochondrial protein import, TCA cycle and β -oxidation of fatty acids that will lead to mitochondrial biogenesis.

Figure 4. Mitochondrial Fission and Fusion

In yeast, mitochondria fission involves the action of Dmn1, that can self assemble into polymeric spirals and is recruited into the mitochondrial membrane by Fis1 and Mdv1. Dmn1 polymers wrap around the organelle and constrict the membrane until fission occurs. In humans, Drp1 and hFis1 are the homologs of Dmn1 and Fis1 whereas no homolog of Mdv1 has been found yet. Mitochondrial fusion involves the interaction of Fzo1/Ugo1 molecules located in the outer membrane of two mitochondria until outer membrane fuses, and then inner membrane fusion occurs through the interaction of Mgm1 molecules. In mammals, there are two homologs of Fzo1, the mitofusins 1 and 2 (Mfn1 and Mfn2) whereas the homolog of Mgm1 is OPA1. MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane. See text for more details. The cartoon is based on models described in reference [80].

Table 1**Diseases Associated with Mitochondrial Biogenesis**

Mutations	Diseases	References
mtDNA mutations	Multiple respiratory chain defects	Reviewed in [1, 2, 108]
Helicase TWINKLE mutations	Autosomal Dominant progressive external ophthalmoplegia (PEO). Sensory ataxic neuropathy dysarthria and ophthalmoparesis (SANDO)	[9, 10]
Polymerase γ mutations	Catalytic subunit: PEO, SANDO, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Pol2 subunit : PEO	Reviewed in [8]
E1a subunit of pyruvate dehydrogenase (mutations in mitochondrial targeting sequencing)	Pyruvate dehydrogenase deficiency	[46]
Alanine/glyoxylate aminotransferase (mistargeting of peroxisomal protein to mitochondria)	Alanine/glyoxylate aminotransferase deficiency	[47]
Superoxide dismutase (defect in Import efficiency)	Severe alcoholic liver disease	[48]
Mutation in Tim8 (affects biogenesis of inner membrane translocase TIM23)	Deafness dystonia syndrome or Mohr- Tranebjaerg syndrome	[49]
Mutation on Pam18 (motor protein of TIM23 complex)	Dilated cardiomyopathy with Ataxia	[50]
HSP60 mutations	Spastic paraplegia-13	[51]
Mitofusin 2 mutations	Charcot-Marie-Tooth 2A	[90]
Opa1 mutations	Autosomal dominant optic atrophy	[91, 92]