Mitochondrial-Nuclear Communications

Michael T. Ryan and Nicholas J. Hoogenraad

Department of Biochemistry, La Trobe University, Melbourne 3086, Australia; email: m.ryan@latrobe.edu.au, n.hoogenraad@latrobe.edu.au

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Abstract

Mitochondria cannot be made de novo but replicate by a mechanism of recruitment of new proteins, which are added to preexisting subcompartments. Although mitochondria have their own DNA, more than 98% of the total protein complement of the organelle is encoded by the nuclear genome. Mitochondrial biogenesis requires a coordination of expression of two genomes and therefore cross talk between the nucleus and mitochondria. In mammals, regulation of mitochondrial biogenesis and proliferation is influenced by external factors, such as nutrients, hormones, temperature, exercise, hypoxia, and aging. This complexity points to the existence of a coordinated and tightly regulated network connecting different pathways. Communications are also required for eliciting mitochondrial responses to specific stress pathways. This review covers the mechanisms of mitochondrial biogenesis and the way cells respond to external signals to maintain mitochondrial function and cellular homeostasis.

Contents

INTRODUCTION

Mitochondria serve a critical function in (*a*) the maintenance of cellular energy supplies, i.e., thermoregulation and synthesis of essential molecules (such as phospholipids and heme); (*b*) apoptosis; and (*c*) mediating multiple cellular signaling pathways. Alterations in mitochondrial function are responsible for a range of inherited and acquired human diseases and are implicated in the aging process. These conditions manifest themselves in pathologies associated with tissues strictly dependent on proper mitochondrial function, such as nerve and muscle tissues (1–3). There is a direct correlation between energy demand and mitochondrial abundance, pointing to sophisticated regulatory mechanisms that control mitochondrial biogenesis (4).

The mitochondrion is an unusual organelle. It cannot be made de novo, but it divides by a process that recruits new proteins, which are added to preexisting subcompartments and protein complexes to a point whereby the organelle grows and divides by a process of fission. Mitochondria are also unusual in that they undergo constant fusion and fission events. The formation of reticular networks is an essential process in the normal function of mitochondria, and thus, the morphology of mitochondria is associated with the functions of cells (1, 5, 6).

The process of mitochondrial biogenesis is complicated by the fact that the organelle has its own genome, although most proteins are encoded by nuclear genes. The expression of two sets of genes has to be accurately coordinated during mitochondrial biogenesis, making mitochondrial-nuclear communication a central part of this problem. In addition, mitochondria need to respond to changes in the physiological milieu of the cell and to control damage caused by mutations of mitochondrial (mt) DNA, which could potentially produce damaged oxidative phosphorylation (OXPHOS) subunits that cannot fold properly, thereby leading to aggregation (7, 8).

OXPHOS: oxidative phosphorylation

THE PROTEIN COMPLEMENT OF MITOCHONDRIA

Mitochondria contain both an inner and outer membrane, leading to the formation of two aqueous compartments, the matrix and intermembrane space. Using integrative analyses of proteomic and biochemical data with mitochondrial targeting programs, Calvo et al. (9) recently estimated that some 1500 different proteins are found in mitochondria, and they identified 1040 of these. The matrix harbors the majority of proteins and may reach a concentration of up to 560 mg/ml (10). This

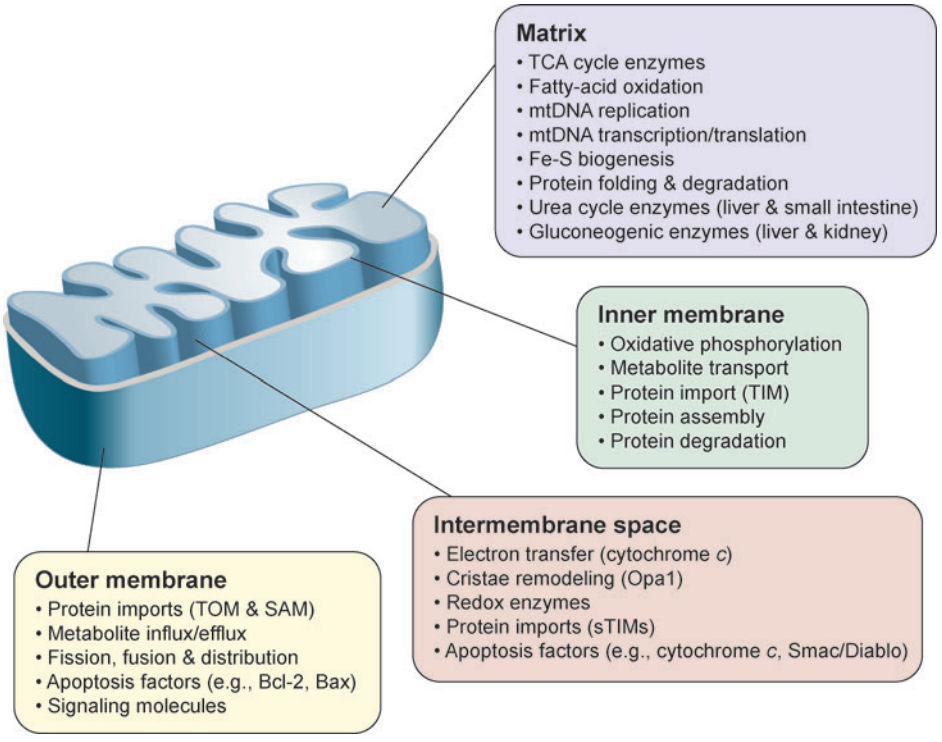

Figure 1

The mitochondrial subcompartments. Examples of compartment-specific processes and proteins are depicted. Abbreviations: Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma protein 2; Opa1, Optic atrophy 1; SAM, sorting and assembly machinery; sTIMs, small TIM proteins; TIM, translocase of the mitochondrial inner membrane; TOM, translocase of the mitochondrial outer membrane.

compartment contains various enzymes involved in metabolic processes, such as the tricholoroacetic acid (TCA) cycle, fatty-acid oxidation, Fe-S biogenesis, and heme synthesis (**Figure 1**). The matrix also harbors a number of copies of mtDNA and the protein machinery involved in its maintenance and replication as well as components involved in transcription/translation. The inner membrane is protein rich and harbors the abundant respiratory complexes involved in oxidative phosphorylation and ATP production, plus a large number of gated channel-forming proteins, including some 49 different metabolite transporter proteins (11). In mammals, five respiratory complexes are found. Complexes I–IV are involved in substrate oxidation with electron transfer reactions pumping protons into the intermembrane space, and proton transport back into the matrix through complex V (F_1F_o-ATPase) generates ATP.

The intermembrane space can be separated into two regions: the intermembrane boundary, which is a narrow stretch of space between the outer and inner membranes and includes areas of protein-mediated contact, and the cristae that form relatively long tubules and/or folds that project into the matrix (12). The protein content is much less than in the matrix, and its most prominent member is cytochrome *c*, which is involved in respiration in normal cells and apoptotic induction upon its release into the cytosol. In addition to cytochrome *c*, other potential apoptotic inducers are present as well as a variety of small proteins that contain cofactors or are disulfide bound (13, 14).

In humans, mtDNA is a circular molecule of 16,569 bp and is packaged with many proteins into punctate nucleoid structures adjacent to the inner membrane (15). Human mtDNA contains 37 genes and codes for 13 encoding polypeptides, 22 tRNAs, and 2 rRNAs. All 13 polypeptides are subunits of the OXPHOS machinery of the inner membrane. The remaining 76 subunits of

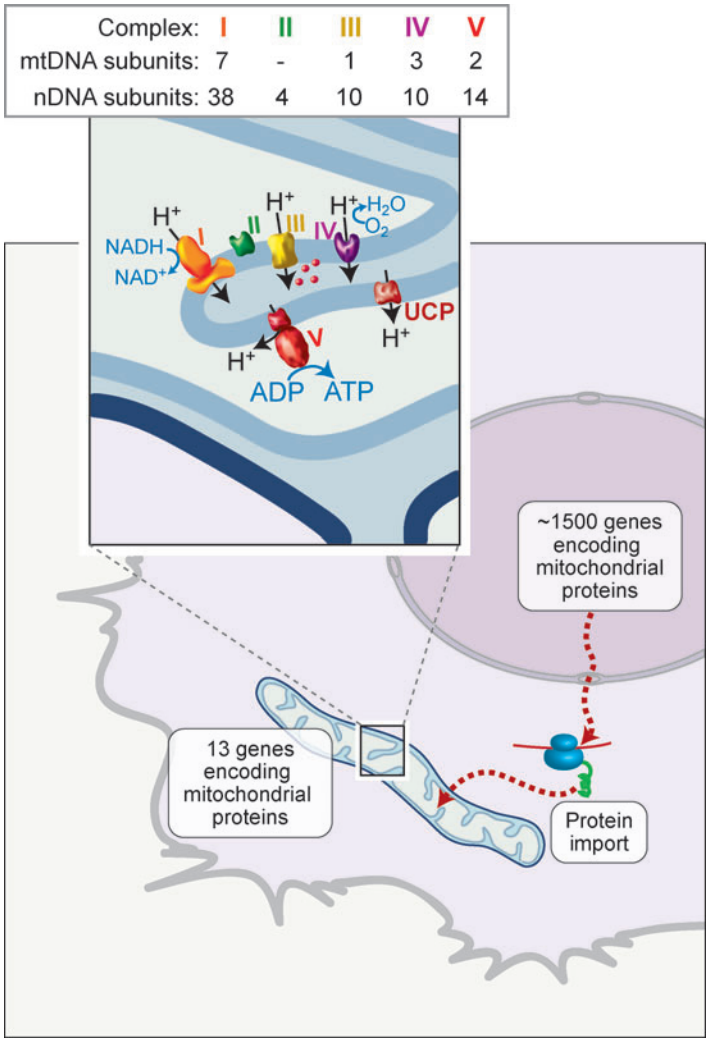

Figure 2

Mitochondrial biogenesis and the OXPHOS machinery. The protein complement of mitochondria consists of 13 subunits, encoded by mtDNA, which are synthesized in the organelle. The remaining proteins are encoded by nuclear genes and are made in the cytosol and imported into mitochondria. Inset: the OXPHOS machinery of the inner membrane consists of complexes I–IV, involved in electron transfer and proton export to the intermembrane space, whereas complex V uses the proton gradient to generate ATP. Uncoupling protein (UCP) uses proton flow to generate heat. Cytochrome *c* (*red*) is found in the intermembrane space. The total number of subunits encoded by nuclear and mtDNA are shown for each of the OXPHOS complexes (3).

the respiratory chain are encoded by nuclear genes and must be imported into the mitochondria (**Figure 2**). In addition, all of the protein machinery involved in mtDNA replication, transcription, and translation (including all of the ribosomal protein subunits) are also encoded by nuclear genes. Thus, respiratory complexes are assembled through the interactions between mtDNA and nuclear-encoded subunits. This process requires the

action of a large number of assembly factors and chaperones involved in subunit folding and maturation as well as providing structural support (16).

Protein translocases exist in both the outer and inner membranes to enable precursor protein translocation and/or integration into membranes. The translocase of the outer membrane (TOM) acts as a universal entry gate for all cytosolically synthesized mitochondrial precursor proteins (17–18a). TOM receptors bind to precursor proteins and/or the molecular chaperones that aid in precursor delivery to the outer membrane (19). The precursors then transit through the channel formed by Tom40 (20). Precursors that contain typical N-terminal leader sequences engage with the TIM23 (translocase of the mitochondrial inner membrane 23) complex of the inner membrane, whereas more hydrophobic precursors, which contain internal targeting signals and are directed to the inner membrane (e.g., metabolite transporters), instead engage with the TIM22 complex. This latter group of precursors are chaperoned across the intermembrane space through the action of the hexameric small Tim family (14). The recent finding that the small Tim family contains intramolecular disulfide bonds (14) correlates with other evidence for the presence of a redox protein folding system in the intermembrane space, analogous to the bacterial periplasm (13).

A prerequisite for protein import into the matrix is that proteins must be unfolded in order to transit the protein translocase channels (18, 21). Following import, the leader sequence is most often proteolytically cleaved, and the protein must be then folded. In many cases, protein folding is facilitated through the action of molecular chaperones resident within the matrix. The main players in folding are the evolutionarily conserved Hsp70 and Hsp60/10 (Chaperonin 60/10) molecular chaperones (22, 23), which function in a similar manner to their bacterial counterparts, DnaK and GroEL/GroES, respectively. Other molecular chaperones and proteases involved in protein maturation are also found in the matrix and inner membrane and include ClpP, Lon, Yme1, DnaJ, and Hsp78. Not only are these proteins involved in folding of newly imported proteins, but they are also critical components in mitochondrial quality control. For example, many are increased in level following increased cellular temperatures ("heat-shock") or other global stresses. Some are also induced specifically in response to mitochondrial stresses (see below). In all cases, their induction is tuned to impaired protein folding and/or aggregation within the mitochondrion.

Mitochondrial morphology: the physical form of mitochondria, resulting from organellar fission-fusion homeostasis and distribution

MITOCHONDRIAL NETWORKS

Mitochondria should not be considered as individual organelles of defined size and nature that are floating within a cell. They are in fact a dynamic network and undergo directed movements as well as fission and fusion via specific processes. This begs the question: "Does the morphology of mitochondria affect its function and how does mitochondrial morphology contribute to the function of the cell?"

Mitochondrial fission is achieved through the actions of a set of proteins, including the dynamin-related protein Drp1 and an outer-membrane receptor-like protein termed Fis1 (5, 6, 24, 24a). Drp1 is proposed to polymerize around the mitochondrion and utilizes GTP hydrolysis in constriction, leading to eventual scission. The levels of Fis1 appear to regulate mitochondrial fission (25), perhaps by recruiting and assembling Drp1 at the mitochondrial surface. Because mitochondrial fission occurs on the cytosolic face of the organelle, additional proteins within mitochondria may not be required. A premise, however, is that mtDNA should also be transmitted to the daughter progeny. Whether this takes place at random or is regulated by additional factors is not yet known; however, it has been observed that mutations in a number of proteins involved in the maintenance of mtDNA causes abnormalities in

Mfn: mitofusin

mitochondrial morphology (1), whereas some proteins involved in mtDNA maintenance appear to connect to other proteins in the mitochondrial outer membrane (26).

Although mitochondrial fission makes biological sense (as the organelle accumulates protein and lipid, it triggers division), mitochondrial fusion is not so simple to interpret. In mammals, the proteins involved in fusion include the homologous mitofusins (Mfns) 1 and 2 of the outer membrane and the intermembrane space/inner membrane protein Opa1 (5). Mfns contain GTPase and coiled-coiled domains and are related to *Drosophila melanogaster* fuzzy-onion (fzo) protein. Male flies defective in fzo protein are sterile because of defects in fusion and reorganization of mitochondria around the midpiece of the sperm (27). Mitochondrial fusion seems to occur in all cell types, and the importance of this process is demonstrated because both Mfns are essential for mouse embryonic development (28) and because mutations in Mfn2 cause neuronal disease (1). Fusion is a means of mixing individual compartments and complementing any defects that may otherwise occur in a single organelle (29).

The state of mitochondrial morphology appears to influence a variety of other cellular functions. Mitochondrial fusion has been suggested to play an important role in mitochondrial proliferation (30) and in the propagation of signals through the organellar population (31). The mitochondrial fission and fusion machineries as well as those proteins involved in mitochondrial distribution have also been shown to act in cell differentiation, such as in neuronal development (32) and in pancreatic β-cell function (33). Both mitochondrial fission and fusion also appear important in regulating apoptotic events by influencing cytochrome *c* release (31, 34).

CALCIUM AND MITOCHONDRIA

Mitochondria also act as calcium sinks, sequestering these ions upon their release from endoplasmic reticulum (ER) stores or following increased Ca^{2+} uptake across the plasma membrane (35). An example of this is observed in cardiomyocytes wherein mitochondrial Ca^{2+} levels oscillate in response to the cellular Ca^{2+} spikes required for contraction (36). The mitochondrial network can also perform a buffering role for the cell. For example, different populations of mitochondria are found in pancreatic acinar cells. Influx of Ca^{2+} from the plasma membrane causes localized increases in mitochondrial Ca^{2+} around this area and enables signaling of insulin release to occur in a concerted fashion. A separate population of mitochondria around the nucleus protects the cell from potential global effects owing to rapid uptake in Ca^{2+} levels (33). Ca^{2+} uptake also appears to play a number of roles within mitochondria. Some metabolic enzymes and metabolite carriers within mitochondria require Ca^{2+} for their activity (35, 37), whereas mitochondrial Ca^{2+} signaling can induce apoptosis (38). The relay of Ca^{2+} can be attenuated through mitochondrial fusion events because increased mitochondrial fission impairs the propagation of calcium waves and can interfere with apoptotic induction (39). Mitochondria can also release their calcium to increase the local concentrations in the cytosol or in subcellular regions to activate different processes, including mitochondrial proliferation (40) and retrograde regulation in response to accumulation of reactive oxygen species in mitochondria (41).

MITOCHONDRIAL PROLIFERATION SIGNALS

One of the best documented examples of mitochondrial signaling is proliferation. For example, a diagnostic phenotype often observed in patients with mitochondrial disease is so-called ragged-red fibers in skeletal muscle, which corresponds to increased mitochondrial staining owing to proliferation of the organelle. For example, ragged-red fibers are seen in MERRF (myoclonic epilepsy

associated with ragged-red fibers) syndrome patients who have mutations in mitochondrial $tRNA^{Lys}$, which leads to decreased translation of proteins within mitochondria and to OXPHOS defects. The defects in the respiratory chain somehow signal a response back to the nucleus to increase mitochondrial mass, presumably in an attempt to correct the reduction in ATP levels (3). Likewise, it is well established that mitochondria proliferate in exercise-conditioned skeletal muscle (42) or in adipose tissue in response to cold (43). Thus, mitochondria have the capacity to communicate with the rest of the cell. The question is: How is this achieved? Mitochondria are bound by a dual membrane system, and the inner membrane is impermeable, with metabolite and protein import occurring through specific and gated protein channels. However, this tight gating means that any perturbations may result in an efficient amplification in signaling back to the nucleus. Signaling may therefore be mediated by changes in metabolite and ion flow (e.g., Ca^{2+}) or in structural changes to the organelle itself (e.g., by changes to fission-fusion homeostasis).

The loss of respiratory activity has the potential to affect the rest of the cell, and this can result in an increase in the transcription of nuclear-encoded genes, which restores ATP synthesis. There could be a reduction in the membrane potential ($\Delta\psi$) caused by decreased electron transport and hence proton export, as well as the accumulation of NADH and reduced flavin adenine dinucleotide. The cytosolic ADP/ATP ratio would also increase, potentially leading to the activation of signaling pathways. The decreased local ATP concentrations may therefore be sufficient to activate the synthesis of nuclear-encoded respiratory subunits and to induce mitochondrial-encoded ones by triggering factors involved in mtDNA transcription/translation and replication (see below).

A number of investigations have pointed to a role of mitochondrial morphology in modulating respiratory activity. For example, ectopic upregulation of Mfn2 levels has been found to increase expression of OXPHOS subunits, increase glucose oxidation, and increase $\Delta\psi$. Conversely, downregulation of Mfn2 showed the opposite effect and induced increased cellular glucose uptake for glycolysis (44). This has also been shown in Mfn double mutant mouse cells (45). Knockdown of Opa1 also induced increased mitochondrial fragmentation and a loss in mitochondrial respiration. The effect was not due to loss of mtDNA because the respiration defect can be circumvented by restoration of fusion protein levels (45). More recently, Opa1 has been found to play an important role in the formation of cristae (46). Opa1 has two forms. One is anchored in the inner membrane and binds to a soluble form that is thought to oligomerize and scaffold the membrane to create cristal junctions (46). Moreover, the cristal tubules were enriched with cytochrome *c* and harbored the respiratory complexes (47). The cristal tubules may also act in stabilizing the respiratory complexes in their supercomplex forms for substrate channeling, and defects in these tubules may impair respiratory activity (48, 49). Related to this, Mfn2 was reduced in skeletal muscle from obese individuals as well as from obese rat models (44, 50). Conversely, Mfn2 levels increase in brown adipose tissue in response to cold exposure (51). Paradoxically, Yu et al. (52) found that when cells were treated with high concentrations of glucose, their mitochondria fragmented, and the $\Delta\psi$ became hyperpolarized with a concomitant increase in reactive oxygen species production. When mitochondrial fission was blocked, the respiration rate was not induced upon glucose stimulation. The effect of mitochondrial fragmentation may be to increase the rate of uptake of pyruvate, produced from glycolysis (52). Although the mechanisms of mitochondrial fission-fusion are not yet well understood, it appears that the morphology of mitochondria is a key element in mitochondrial-nuclear communication.

$\Delta\psi$: membrane potential

Coactivator: a protein that stimulates transcription by indirect association with DNA through a DNA-binding protein

MSR: mitochondrial stress response

Tfam: mitochondrial transcription factor A

NRF: nuclear respiratory factor

LEVELS OF MITOCHONDRIAL-NUCLEAR COMMUNICATIONS

Mitochondrial-nuclear communications operate broadly at two levels. One mechanism involves a set of transcription factors, or coactivators, that regulate both nuclear and mitochondrial gene expression as occurs in response to changes in environmental temperatures (53), external stimuli such as changes in caloric intake (54, 55) or exercise (56, 57), or changes in the levels of certain hormones such as thyroxine (58, 59). In this mechanism, there is a change in the program of gene activation that results in the ability of mitochondria to undergo the synthesis and recruitment of mitochondrial and nonmitochondrial proteins.

The second mechanism involves cellular responses to changes in the functional state of the mitochondria itself, a process also called "retrograde regulation" (60). Examples of this response may be the loss of mitochondrial function caused by a loss of electrochemical potential (uncoupling), of OXPHOS (61), or by the accumulation of unfolded proteins in the organelle (62), processes broadly described as mitochondrial stress responses (MSRs). These responses enable mitochondria to recover from stress.

REGULATION OF MITOCHONDRIAL BIOGENESIS

A single transcriptional element was recently discovered in *Drosophila* (63), which was conserved in genes involved in ATP generation and in genes encoding protein import components as well as in genes encoding the mitochondrial protein synthesis machinery. However, the search for a similar element in species other than insects suggests that such an element does not exist in vertebrates or fungal species. Several factors have been identified in mammals that coordinately control the expression of a subset of genes in mitochondria and the nucleus, such as sequence-specific transcription factors, coactivators, and hormones acting upstream of these transcriptional activators (reviewed in References 64 and 65). For example, mitochondrial transcription factor A (Tfam), which stimulates the transcription of mtDNA into a polygenic transcript (66) and is processed into 14 tRNAs, 12 mRNAs, and two rRNAs, is itself activated by the transcription factor nuclear respiratory factor 1 (NRF1) (65). Aside from Tfam, NRF1 controls many nuclear genes involved in mitochondrial function and biogenesis, suggestive of an integrative role. Analysis of nuclear-encoded cytochrome *c* and cytochrome oxidase genes has led to the identification of a number of additional transcription factors, such as SP1, YY1, CREB, and NRF2 (reviewed in Reference 65). However, regulation of most nuclear genes encoding mitochondrial proteins cannot be accounted for through the action of these factors. Thus, the mitochondrial biosynthetic program in vertebrates appears to involve the integration of multiple transcriptional regulatory pathways, controlling expression of both nuclear and mitochondrial genes in a tissue- and stimulus-specific way.

A Coactivator That Integrates Mitochondrial Biogenesis

A number of these transcriptional regulatory pathways appear to be regulated by a class of related coactivators that bind to a wide range of nuclear receptors and transcription factors, creating a regulatory cascade that results in the transcriptional activation of nuclear and mitochondrial genes encoding mitochondrial proteins as well as nonmitochondrial proteins involved in energy metabolism (reviewed in References 67–69). The peroxisome-proliferator-activated receptor coactivator-1 (PGC-1) comes closest to a universal regulatory system for mitochondrial biogenesis in vertebrates (**Figure 3**).

The transcriptional coactivator PGC-1α (PPARγ coactivator 1) was discovered as an interacting partner of the nuclear receptor PPARγ in brown adipose tissue (53). PGC-1α

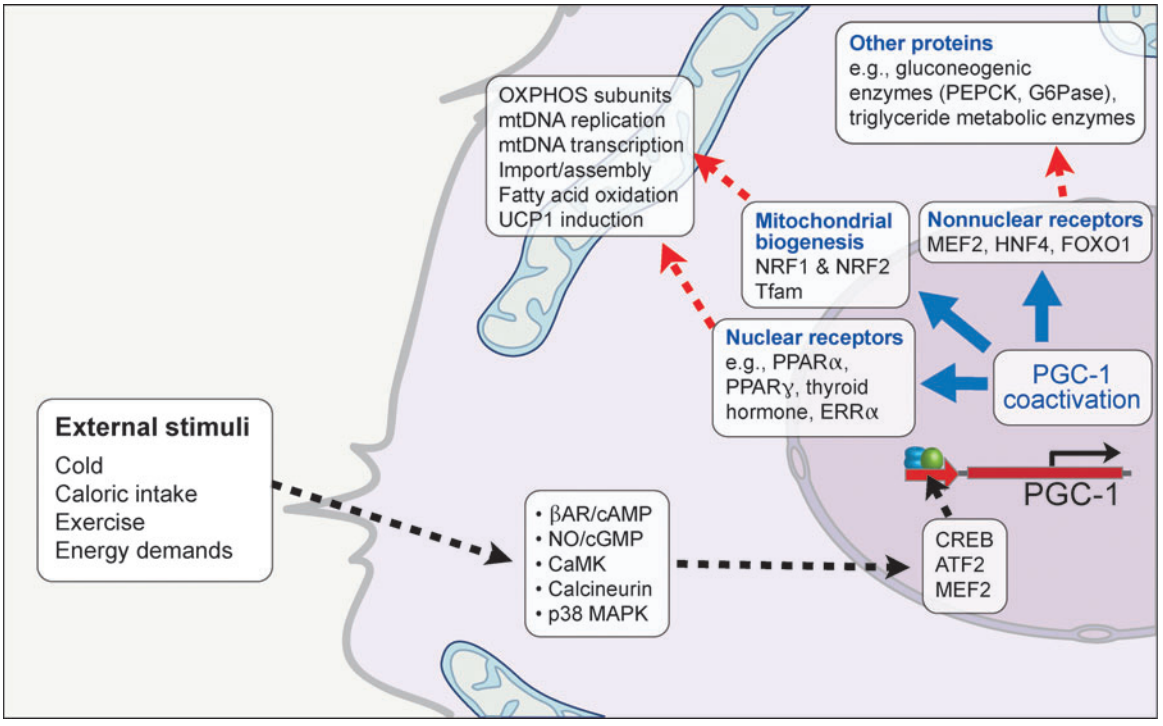

Figure 3

Induction of mitochondrial biogenesis through peroxisome-proliferator-activated receptor-gamma coactivator-1 (PGC-1). External stimuli affect the transcriptional activation of PGC-1α in a tissue-specific manner via cyclic AMP (cAMP), cGMP, or Ca^{2+} signaling pathways, which results in the activation of transcription factors such as CREB, MEF2, and ATF2. PGC-1α coactivates expression of estrogen-related receptor alpha (ERRα), which activates expression of both NRF1 and NRF2 and through these, Tfam, leading to mtDNA replication and transcription. PGC-1α also coactivates nuclear receptors such as PPAR-γ and PPAR-α, which regulate the expression of nuclear genes encoding mitochondrial proteins. PGC-1α also activates transcription of nonmitochondrial proteins through nonnuclear receptors, such as MEF2, HNF4, and FOXO1, to induce gluconeogenic enzymes and lipoprotein enzymes (68). Other abbreviations: βAR, β-adrenergic receptor; CaMK, calcium/calmodulin-dependent protein kinase; G6Pase, glucose-6 phosphatase; NO, nitric oxide; PEPCK, phosphoenoyl pyruvate carboxy kinase; p38 MAPK, microtubule-associated protein kinase; UCP1, uncoupling protein 1.

homologs PGC-1β and PGC-related coactivator (PRC) have also been found (70–72). Although the PGC-1 coactivators are present in a wide range of chordates, they have not been found in lower eukaryotes (68). The PGC-1 coactivators contain a conserved N-terminal domain that interacts with proteins capable of remodeling chromatin, including histone acetyl transferases, such as CREB-binding protein, p300, and steroid receptor coactivator 1 (73). This chromatin remodeling allows access to additional factors that enhance transcription. The C-terminal domain of PGC-1 binds a second activating complex, consisting of the steroid hormone receptor-associated protein, TRAP/DRIP (74), and containing an RNA-binding domain that facilitates pre-mRNA splicing (75).

PGC-1α is strongly induced in specific tissues in response to environmental cues. These include induction in brown adipose tissue, as a result of cold-exposure (53), and skeletal

AMPK: AMP-activated protein kinase

CaMK: calcium/calmodulin-dependent protein kinase

muscle following exercise (56, 57). The level of PGC-1α in cells is closely correlated with the number of mitochondria (65), and ectopic overexpression of this coactivator in cells in culture results in the activation of many genes involved in respiration, oxidative metabolism, and uptake and utilization of energy substrates (76). Using a microarrray approach involving the analysis of gene sets involved in certain metabolic processes, Mootha et al. (76) found that patients with type 2 diabetes displayed drastic decreases in the expression of OXPHOS genes in skeletal muscle, heart, and brown fat. The levels of PGC-1α were also reduced by ~20%.

These pleiotropic effects of PGC-1α on energy modifying processes are due to a wide variety of interacting partners (**Figure 3**), which include nuclear receptors and other transcription factors, such as NRF1 and 2, which in turn regulate Tfam with the concomitant outcome of stimulating mtDNA replication and gene expression (59). NRF1 and 2 further regulate transcription of nuclear genes encoding respiratory complex subunits and other mitochondrial proteins (65). However, PGC-1α has been shown to lead to the transcriptional activation of a very large number of genes that encode mitochondrial proteins, including those required for the import of nuclear-encoded proteins into mitochondria (76). This is achieved through the activation of many of the nuclear receptor class of transcription regulators, such as PPARα, which stimulate mitochondrial fatty-acid oxidation, oxidative phosphorylation (77), and PPARγ, which induces, among other genes, uncoupling protein 1 (UCP1), an inner membrane protein transporter involved in thermogenesis in brown adipose tissue (53). PGC-1 also coactivates a large number of nonnuclear receptor transcription factors that are involved in the expression of nonmitochondrial proteins, involved in a range of functions [such as gluconeogenesis, e.g., HNF-4 and FOXO1 (78, 79); glucose transport (MEF-2) (80); lipogenesis (SREBP1) (81); and chondrogenesis (SOX9) (82)]. These complex interactions between a family of coregulators and a large collection of target genes allow for very distinct patterns of regulation in different tissue settings and at different stages of development. For example, respiration stimulated by PGC-1α overexpression in differentiated C2C12 myocytes is less tightly coupled than that produced by PGC-1β (83).

The levels of Mfn are also induced in cells undergoing increased energy expenditure and mitochondrial proliferation, such as in skeletal muscle of individuals after exercise (84), and following cold exposure (51), which occurs in a PGC-1α- and ERRα-responsive manner.

Transcriptional Regulation of PGC-1

The external stimuli, which influence mitochondrial function, are sensed in various ways by different tissues. This leads to the activation of signal transduction pathways that allow tissue-specific activation of PGC-1 transcription. For example, cold exposure leads to the activation of β-adrenergic receptors in brown adipose tissue cells, activating the cAMP pathway to transcriptional activation of PGC-1α and downstream expression of UCP1, thereby leading to uncoupled thermogenesis (53). Long-term exercise in mice, by contrast, leads to chronic energy deficits, which are sensed by AMP-activated protein kinase (AMPK), which in turn leads to mitochondrial biogenesis via calcium/calmodulin-dependent protein kinase (CaMK) and PGC-1α (85). Thus, transgenic mice overexpressing a dominant negative mutant of AMPK in skeletal muscle had decreased AMPK and did not induce PGC-1α or mitochondrial biogenesis. AMPK was also involved in PGC-1α induction and mitochondrial biogenesis in hyperglycemia-induced intracellular and mitochondrial reactive oxygen species production in endothelial cells (86).

The role of perturbations in calcium levels and calcium signaling pathways in PGC induction is illustrated by experiments in transgenic mice, which constitutively overexpress

CaMK in skeletal muscle, and overexpressed CaMK results in increased levels of PGC-1α and mitochondrial biogenesis (87). Similarly, CaMK and calcineurin have been shown to activate PGC-1α transcription in myogenic cell lines (88). The effect of calcineurin was through a MEF2 response element in the PGC-1α promoter (80), whereas the effect of CaMK is through CREB binding to the PGC-1α promoter (73). Intriguingly, CREB is also localized to the mitochondrial matrix of neurons (89), where it binds to the cAMP response element in the mitochondrial *ND5* gene, thereby regulating complex I activity (90). The action of CREB in the nucleus and in the mitochondrion provides an additional mechanism for the integration of mitochondrial and nuclear coordination in mitochondrial biogenesis.

Nitric oxide was also implicated as a signaling molecule in PGC-1α induction via a cGMP-dependent mechanism in a wide range of cell types (91, 92). Transgenic mice with deficient epithelial nitric oxide synthase were deficient in mitochondrial biogenesis (92). Likewise, the transcription of PGC-1α was regulated through the release of repression by p160 through p38 MAP kinase (93).

Regulation of PGC-1 Activity

PGC-1α is also regulated posttranscriptionally. It has a very short half-life of about 2–3 hours (68), and its phosphorylation by the p38 MAP kinase stabilizes the protein and thereby increases its steady-state concentration (93, 94). Phosphorylation further increases the transcriptional activation of PGC-1α by causing the dissociation of the transcriptional repressor, p160 myb-binding protein (95). PGC-1α is also strongly inhibited by the pregnane X receptor and small heterodimer partner, which compete with binding partners, thereby releasing the inhibition of the coactivator (96, 97). PGC-1α is directly activated by the lysine deacetylase SIRT-1 (98). During fasting, accumulation of pyruvate induces SIRT-1, which induces PGC-1α and deacetylates specific lysine residues in a reaction requiring NAD^+. Through this mechanism, PGC-1α specifically induces genes required for hepatic glucose production without affecting the transcription of mitochondrial genes (98). Thus, two covalently modified forms (acetylated/deacetylated) of PGC-1α can independently regulate mitochondrial biogenesis and glucose homeostasis.

PGC-1β, like PGC-1α, is also highly expressed in cells with a high respiratory activity (71, 72), and although there is some overlap in function, as seen in PGC-1α-deficient mice (99, 100), it is unable to completely compensate for the loss of PGC-1α. Instead, PGC-1β appears to play a more specific role in brown fat differentiation (100) and is a key regulator of hepatic lipogenesis and lipoprotein secretion (81). This involves the induction of PGC-1β by saturated fatty acids in the diet and leads to coactivation of the SREBP and LXR families of transcription factors. These effects of PGC-1β do not include the induction of transcription of other mitochondrial genes, although it shares a strong effect on the expression of genes involved in fatty-acid oxidation and ketogenesis with PGC-1α (54). Overall, on the basis of the noninducibility of PGC-1β in conditions that strongly induce PGC-1α, it has been suggested that PGC-1β may be responsible for the basal respiratory and energy requirements of tissues (101).

The third PGC-1 member, PRC, is a ubiquitously expressed coactivator whose function in currently poorly understood, but it plays a role in mitochondrial biogenesis in dividing cells (70).

An additional factor has recently been found that regulates the total mitochondrial mass, but it does not appear to do this through PGC-1. This protein, termed MIDAS (mitochondrial DNA absence sensitive factor) was discovered in experiments with HeLa cells totally depleted of mtDNA (102). MIDAS is colocalized to the Golgi and the mitochondrial intermembrane space, and it regulates mitochondrial mass by stimulating the

synthesis of cardiolipin and total mitochondrial lipids, but not through a generalized enhancement of transcription of genes encoding mitochondrial proteins. Although discovered by comparing cells lacking mtDNA with those with normal mtDNA, MIDAS levels were also elevated in muscle cells from patients lacking cytochrome oxidase activity (102). Induction of MIDAS may be driven by CREB/ATF-1 transcription factors because the MIDAS promoter has a putative ATF-1 site.

Mitochondrial retrograde signaling: signaling from mitochondria to the nucleus, resulting in the expression of nuclear genes to salvage mitochondrial function

UPR: unfolded protein response

MITOCHONDRIAL-NUCLEAR SIGNALING DURING MITOCHONDRIAL STRESS

Metabolic cues or other damage that occurs within mitochondria can culminate in wide-ranging changes in nuclear gene expression via retrograde signaling from the mitochondria to the nucleus (**Figure 4**). These responses are widely referred to as MSRs (61, 103) and are broadly defined as a response to altered mitochondria membrane potential, induced either by treating cells with ethidium bromide to deplete mtDNA content (ρ^0 cells) or with uncouplers of OXPHOS. These treatments result in the elevation of cytosolic Ca^{2+} and activation of CaMK and calcineurin-responsive genes (65). Such responses appear to be mediated through PGC-1α (87). Thus, overexpression of CaMK in muscle of transgenic mice resulted in generalized increases in mitochondrial biogenesis, fatty-acid oxidation, and PGC-1. In cultured myocytes, CaMK was capable of inducing PGC-1 transcription through a direct effect on the gene promoter (87). This activation is dependent on MEF2-responsive elements in the case of calcineurin A and on the CREB-binding site in the case of CaMK (88). The genes activated by these changes in Ca^{2+} levels include a number of genes involved in Ca^{2+} transport and storage (103) as well as a large number of transcription factors (59). The general net effect of activation of this gene network is to facilitate recovery of physiological function.

MITOCHONDRIAL UNFOLDED PROTEIN RESPONSE

Cells respond to the accumulation of unfolded proteins by increasing the level of proteins, including molecular chaperones and proteases, involved in protein quality control. The best-studied example of this is the heat-shock response wherein protein unfolding caused by heat stress leads to the transcriptional upregulation and activation of a wide variety of genes that contain a heat-shock element in their promoters (104). The ultimate outcome of this response is the reestablishment of normal cellular function. Cells also have organelle-specific responses to the accumulation of unfolded proteins in the organelle, such as the ER unfolded protein response (UPR). This is a well-documented example, wherein a large collection of genes involved in the maintenance of ER function are upregulated (105–108). In mammalian cells, UPR gene expression largely relies on the activation of transcription factors PERK and ATF-6 (105, 107). The UPR also results in the upregulation of CHOP (C/EBP homologous protein), which dimerizes with members of the C/EBP (CAAT/enhancer-binding protein) family of transcription factors, through which it can either attenuate the transcription of genes containing a C/EBP element or stimulate transcription of genes containing a CHOP element (109). The role of CHOP on the ER stress response is enigmatic, although CHOP may play a role in apoptosis induced by UPR (110).

Like that of the ER, mitochondrial function is highly dependent on molecular chaperones and proteases. The targeting of many mitochondrial proteins synthesized on cytosolic ribosomes requires the participation of molecular chaperones such as cytosolic Hsp70 and Hsp90 (19, 111). Likewise within the mitochondrial matrix, mtHsp70 is required to drive import of unfolded proteins across the inner membrane and into the matrix, and with its cofactors (matrix DnaJ and GrpE homologs) as well as the chaperonins

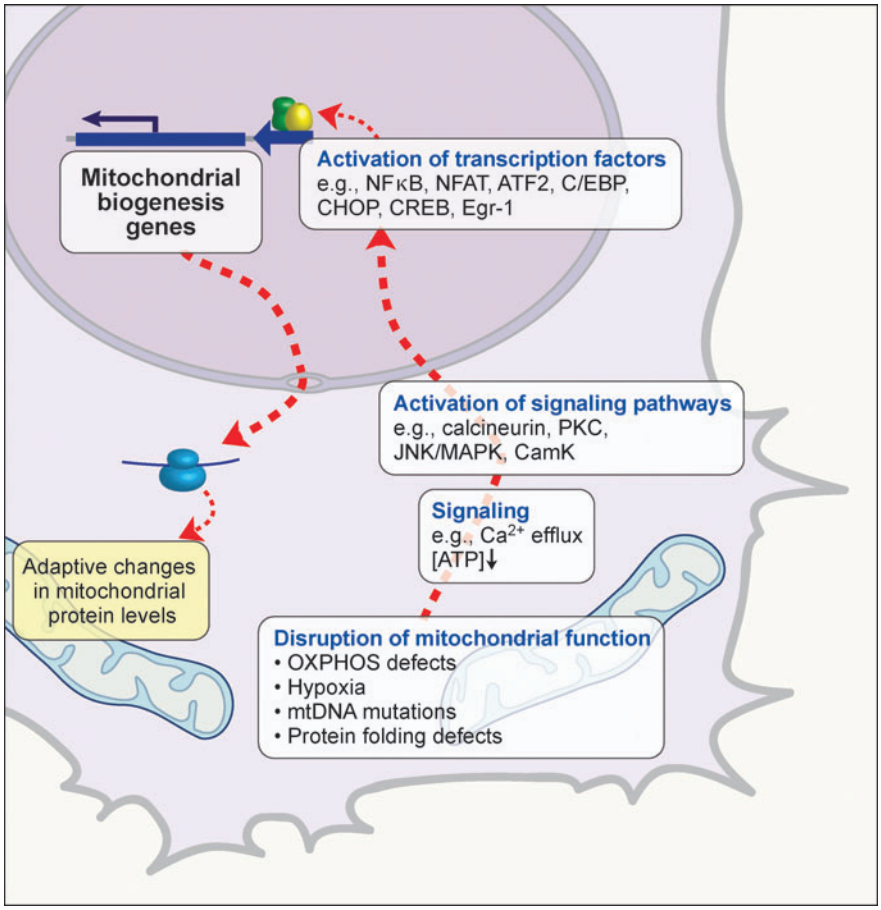

Figure 4

Model for retrograde signaling in mitochondrial stress pathways. Disruption of mitochondrial function is due to the action of inhibitors of the respiratory chain; hypoxia or mutation/deletions in mtDNA leads to a loss of $\Delta\psi$ and a loss of ATP generation capacity. These causes of stress result in signaling to the cytosol via the release of Ca^{2+} from the mitochondrion. The increase in cytosolic Ca^{2+} activates calcium-sensitive signaling proteins, such as calcineurin, calcium/calmodulin-dependent protein kinase (CaMK), and protein kinase C (PKC) as well as the JNK/MAPK pathway. This leads to the activation of transcription factors and nuclear gene transcription that produce adaptive changes to rescue the mitochondria from stress or that cause the release of proapoptotic proteins from the mitochondria.

Hsp60/10, protein folding is accomplished (112).

Early work suggested that a MSR could be observed in cells depleted of mtDNA (113). Rat hepatoma cells depleted of mtDNA through ethidium bromide treatment showed an increase in the transcript and protein levels of Hsp60 and Hsp10, presumably owing to an impairment of the respiratory chain and accumulation of nuclear-encoded subunits in the mitochondrial matrix. More recently, this work has been supported by studies of *Caenorhabditis elegans* wherein Yoneda et al. (114) showed that the genes encoding both mitochondrial Hsp70 and Hsp60 were upregulated in worms treated with ethidium bromide. This response was not due to a loss in $\Delta\psi$ because the addition of the uncoupler

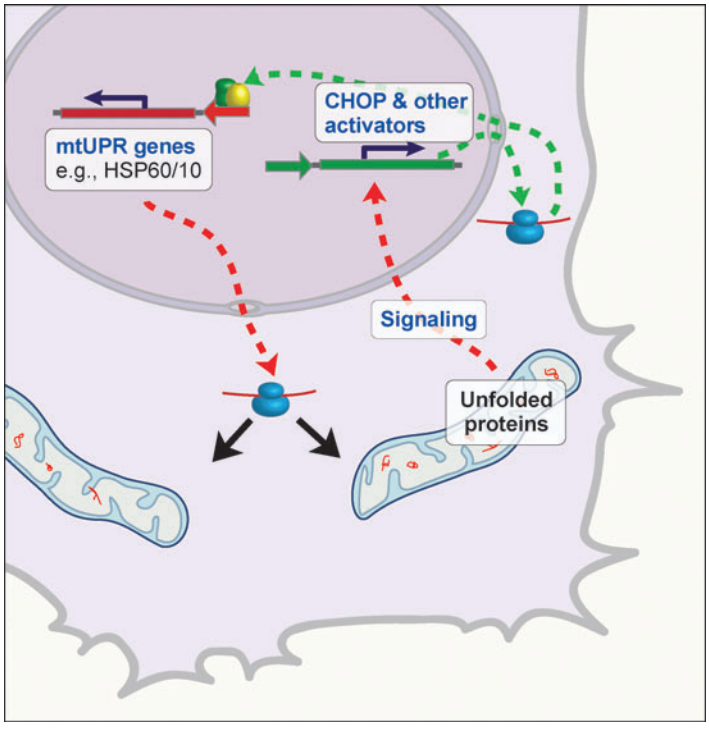

Figure 5

The mitochondrial unfolded protein response (mtUPR). The mtUPR is a form of mitochondrial stress caused by the accumulation of unfolded proteins in the mitochondrial matrix. This process, in mammalian cells, is a two-stage process, during which the sensing of the accumulation of unfolded proteins leads initially to the activation of transcription of the *CHOP* gene, via a signaling pathway not yet delineated. This activation is through an mtUPR element adjacent to, but separate from, a ER-UPR element. In a second stage, CHOP dimerizes with C/EBPβ and binds to the promoters of mtUPR-responsive genes containing a CHOP-C/EBPβ element and adjacent conserved regions, which are essential for transcription activation of genes encoding mitochondrial quality control proteins, such as molecular chaperones and proteases (62).

dinitrophenol did not induce such an effect. The response was also independent of reactive oxygen species. Using an RNA interference screen of 1160 genes, 32 genes were identified that specifically activate mitochondrial Hsp70 and Hsp60 promoter reporter constructs (114). Thirty of these genes were mitochondrial, and most were subunits of multimeric protein complexes. Reduced levels of a single subunit within a complex are likely to increase the levels of unassembled partner proteins, which may act as substrates for molecular chaperones, thereby triggering a mitochondrial UPR. Moreover, cultured cells engineered to express mutant proteins that aggregate in the mitochondrial matrix show an upregulation of the molecular chaperones Hsp60 and Hsp10 and proteases such as ClpP that are specific to mitochondria (62). The activation of the nuclear genes as a result of the mitochondrial UPR (**Figure 5**) was, at least in part, through a CHOP-C/EBPβ element, and transcriptional activation required the heterodimerization of CHOP and C/EBPβ. However, 3899 nuclear genes in the human genome are predicted to contain the 7-bp CHOP-C/EBPβ element (J. Aldridge & N.J. Hoogenraad, unpublished results), so

additional regulatory mechanisms must be involved in activation of the mitochondrial UPR.

Promoter deletion analysis showed that the mitochondrial stress response element, which includes the CHOP-C/EBPβ element, lies within 168 bp of the *HSP60* transcription start site (62). This region is highly conserved in the promoters of other responsive genes, and 15 nuclear genes, encoding mitochondrial proteins, contain this region. Although CHOP appears to be a required transcription factor for the mitochondrial UPR, the *CHOP* gene is itself upregulated by the accumulation of unfolded proteins in mitochondria (62). This suggests that the mitochondrial UPR is a two-stage regulatory process. First, the sensing of unfolded proteins in mitochondria leads to retrograde signaling to the nucleus and subsequent activation of the *CHOP* gene (and possibly other genes). Second, CHOP, in conjunction with C/EBPβ, then binds to target promoters and activates the transcription of mitochondrial-responsive genes. Preliminary deletion analysis of the *CHOP* promoter reveals adjacent but separate mitochondrial and ER UPR elements (T. Horibe & N.J. Hoogenraad, unpublished results). This provides a mechanism for the separate induction of the *CHOP* gene in response to either stress response pathway.

Unlike the ER UPR, the mitochondrial UPR has not been reported in lower eukaryotes other than *C. elegans* (114). Although a mechanism for sensing and signaling from the mitochondria is yet to be found, it has been suggested that the efflux of peptides resulting from the activity of proteases, such as those induced by the mitochondria UPR, may carry out this function. Thus, Yme1, an i-AAA protease of the mitochondrial inner membrane, was shown responsible for the generation and efflux of peptides, which regulate nuclear gene expression in yeast (115).

Although the ER UPR and mitochondrial UPR responses appear subject to separate regulatory mechanisms, the regulation of certain nuclear genes encoding mitochondrial proteins also appears to be influenced by ER stress (116). This mechanism requires the suppression of cytosolic protein synthesis by PERK (117) and leads to the upregulation of the mitochondrial matrix proteases Lon, mtHsp70, and Yme1 (116), most likely in a mechanism that does not involve CHOP. This mitochondrial response to ER stress has the outcome of reducing the steady-state levels of nuclear-encoded subunits of OXPHOS complexes.

MITOCHONDRIA AS INTEGRATORS OF INTRACELLULAR SIGNALING

It is now widely accepted that the complexity of organisms is determined by factors well beyond the number of protein-coding genes in the genome of a species. Many proteins have more than one distinct function, such as cytochrome *c*, which functions within the mitochondrion in oxidative generation of ATP and in the cytosol, after release from the organelle, in apoptosis. Similarly, there are many reports of proteins being localized to multiple compartments in the cell. Although some of these may represent artifacts because of (*a*) the difficulty of accurately fractionating some cellular compartments, or (*b*) the mistargeting of heterologously expressed proteins with epitope or fluorescent tags, or (*c*) simply overexpressing a protein, many of these examples of multiple localization are well supported and are the result of specific physiological stimuli. For example, mutations in the *PARKIN* gene are associated with autosomal recessive juvenile Parkinsonism (118), and in differentiated neurons, parkin is localized to the cytosol, where it functions as a ubiquitin-protein ligase (119). However, transition to a proliferative state results in the translocation of parkin to the mitochondria, where it acts as a coactivator of Tfam and through this stimulates mitochondrial DNA replication and transcription (120).

A large number of observations of this kind lead from the concept that the functions of mitochondria go beyond the generation of ATP and the regulation of energy metabolism to

one of playing a major role as an integrator of intrinsic and extrinsic signals, which can affect the health and survival of the cell (6). Thus, mitochondria house proteins that can be released in response to specific signals to initiate apoptosis and thereby protect healthy cells from fortuitous activation of apoptosis. Mitochondria have also evolved a role in regulating innate immunity through a mechanism whereby viral infection induces the production of type I interferon (121). In this process, intracellular dsRNA, an intermediate of viral replication, activates the RNA helicases, RIG-1 and Mda-5 (122). The RIG-1 pathway leads to the activation of a suite of transcription factors that assemble into a multiprotein enhancer complex upstream of the type I interferon promoter (123). The involvement of the mitochondrion in this process is through a protein that interacts with RIG-1 (121, 124) and mediates the signal transduction pathway for interferon expression. This protein was called MAVS (for mitochondrial antiviral signaling) by Seth et al. (121) and is tail anchored in the mitochondrial outer membrane. In response to viral infection, MAVS moves to a detergent-resistant domain in the outer membrane, a process that is required for its function in signaling.

Ian4 (immune-associated nucleotide-binding protein) is a GTP-binding protein of the mitochondrial outer membrane protein required for the maintenance of mitochondrial integrity in lymphocytes (125). Removal of Ian4 from T cells caused mitochondrial dysfunction and led to T-cell apoptosis, a process that was prevented by the activation of T cells (125). Although the role of Ian4 in immune regulation is still unclear, a natural knockout of Ian4 in diabetes-prone rats (126) results in the development of spontaneous autoimmune diabetes. This has been suggested as the result of a loss in regulatory T cells owing to apoptosis combined with a rescue of activated T cells that survive the apoptotic effects of the deletion of Ian4 (127).

It has also been argued that mitochondria have evolved as a signaling platform by having a unique membrane environment, potentially with microdomains into which molecules can move for activation, as has been suggested for MAVS (6). The partitioning of proteins into membrane microdomains, such as lipid rafts, has been shown for proteins associated with the plasma membrane. For example, the signaling GTPases H- and K-ras interact with raft proteins in the inner leaflet of the plasma membrane to form nanoclusters (128), and H-, K-, and N-ras have been shown to associate with mitochondria in interleukin-2-supplemented or -deprived cells to regulate apoptosis (129). It has recently been shown that the phosphorylation of K-ras by protein kinase C causes movement of K-ras from the plasma membrane to the mitochondrial outer membrane, where it associates with Bcl-XL to induce apoptosis (130).

Mitochondria have evolved mechanisms to prevent cell division under conditions of nutrient deficiencies through the regulation of the cell cycle. For example, the energy status of the cell can be sensed by AMPK. A high AMP:ATP ratio can activate AMPK and initiate a signaling pathway, which leads to alterations in the ATP status in the cell (see "transcriptional activation of PGC-1," above). One of the AMPK target genes is the tumor suppressor p53, which upon phosphorylation arrests the cell cycle (131). Deletion of p53 rendered cells insensitive to nutrient-dependent cell cycle arrest and led to the eventual loss of cell viability, confirming the role of p53 in the nutrient-dependent cell cycle regulation. Moreover, Matoba et al. (132) recently found that p53 regulates OXPHOS by activating the expression of at least one factor involved in the assembly of cytochrome *c* oxidase (complex III). Thus, loss of p53 function in cancerous cells acts to downregulate OXPHOS and switches cells to become glycolytic. This finding is consistent with the downregulation of mitochondrial respiration in cancer cells and the utilization of glycolysis to produce the bulk of ATP needed for cellular activity in tumors that grow under hypoxic conditions (the Warburg effect).

Recent analysis of the mitochondrial phosphoproteome shows that mitochondria have a surprisingly large complement of phosphorylated proteins (133). This phosphorylation is significantly influenced by extramitochondrial Ca^{2+} levels, pointing again to the importance of Ca^{2+} in mediating mitochondrial signaling. These findings also suggest that the covalent modification of mitochondrial proteins is likely to play a major role in the regulation of mitochondrial function, an area that has received little attention so far. This indicates that there should be a number of mitochondrial protein kinases and protein phosphatases. Some of these are known and include pyruvate dehydrogenase kinase, which inhibits enzyme activity in response to low substrate levels such as occurs in starvation and diabetes (134); Akt, a cytosolic protein kinase, which translocates to mitochondria in response to insulin stimulation (135, 136); and PTEN-induced kinase 1, a mitochondrial matrix protein associated with sporadic forms of Parkinson's disease, whose substrates are not yet identified (137).

SUMMARY POINTS

1. Mitochondrial-nuclear communications are at the center of regulating mitochondrial function because the mitochondrion is made up of proteins encoded by two genomes, the mitochondrial and nuclear genomes, whose expression needs to be coordinated.
2. Mitochondrial biogenesis in humans is driven by the energy demands and metabolic requirements of tissues and is largely coordinated by the PGC-1 family of coactivators. These activators integrate the expression of genes in the nucleus and mitochondria through a regulatory cascade, involving the sequential activation of transcriptional regulatory proteins such as transcription factors.
3. Mitochondria have evolved to perform disparate functions in cells, such as generation of ATP, integration of intermediary metabolism, storage of toxic proteins (which, upon release, activate apoptosis), and provision of a mobile platform for the distribution of signaling molecules to various parts of the cell through the association of mitochondria with the cytoskeleton of the cell.
4. Because mitochondrial function is critical to cellular survival, the cell has evolved mechanisms of regulated gene expression to maintain mitochondrial function in the face of a variety of mitochondrial stresses. Mitochondrial to nuclear signaling is a key component of these responses.

FUTURE ISSUES

The past decade has produced a remarkable expansion in our understanding of mitochondrial function. The organelle has long been known for its role in the provision of oxidative energy in the eukaryotic cell and as the cellular compartment, which houses the major pathways for the catabolic degradation of fuel molecules, such as sugars, fatty acids, and amino acids. It has also been known as a vehicle for the compartmentation of key intermediates in different metabolic pathways, such as in gluconeogenesis and glycolysis and the biosynthesis of urea and pyrimidine nucleotides through the compartmentation of carbamyl phosphate. However, the discovery of PGC-1α in 1998 (53)

set the scene for a substantial development in understanding how the mammalian cell integrates gene expression in the nucleus with that in the mitochondrion for mitochondrial biogenesis, because PGC-1 has proven to have all of the characteristics of a master integrator of gene expression for this process. But, more than this, PGC-1 has also been found to have a broader integrative role in energy metabolism, as it also plays a role in gluconeogenesis and lipid biogenesis and transport, and inevitably in medical conditions such as diabetes and obesity.

In parallel with these developments, there has been a steady stream of observations on the use of mitochondria as a vehicle for more specialized functions, with the discovery of the transfer of many proteins important in signaling and regulation into or onto the mitochondria. This has placed mitochondria at the center of many diverse cellular functions as a master integrator of signals between the organelle and the nucleus, including functions as diverse as a protector in viral infection to a mediator of selected cell removal through apoptosis.

As the roles played by mitochondria are broad, there are still major gaps in our knowledge of how specificity is determined. It is likely that subtle covalent changes in key proteins will be at the center of these processes by determining selectivity of protein-protein interactions allowing different partners to provide specificity in gene expression. Thus, the role of covalent modification of mitochondrial proteins is a likely area of increased investigation in the future.

Given the importance of mitochondria in key cellular functions, it is not surprising that the organelle has evolved a suite of procedures for maintaining its function in the face of stress. These processes require the expression of a specific collection of nuclear genes whose products need to be targeted to the organelle, a process that involves sensing of perturbations in mitochondrial functions and retrograde signaling to the nucleus. The mechanisms by which this occurs for different forms of stress and the physiological anlagen of stress, which initiates these stress response pathways, is also likely to be an area of active investigation. This is particularly so because loss of mitochondrial function is central to many key diseases, ranging from the major metabolic disorders of obesity and diabetes to defects in apoptosis, which leads to cancer and neurodegenerative diseases associated with the aging process (3). A more detailed understanding of the role of mitochondria in these processes holds out the prospect of medical intervention such as the production of specific inhibitors of key components in these processes.

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Errata

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