Mitochondrial Biogenesis through Activation of Nuclear Signaling Proteins

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The dynamics of mitochondrial biogenesis and function is a complex interplay of cellular and molecular processes that ultimately shape bioenergetics capacity. Mitochondrial mass, by itself, represents the net balance between rates of biogenesis and degradation. Mitochondrial biogenesis is dependent on different signaling cascades and transcriptional complexes that promote the formation and assembly of mitochondria—a process that is heavily dependent on timely and coordinated transcriptional control of genes encoding for mitochondrial proteins. In this article, we discuss the major signals and transcriptional complexes, programming mitochondrial biogenesis, and bioenergetic activity. This regulatory network represents a new therapeutic window into the treatment of the wide spectrum of mitochondrial and neurodegenerative diseases characterized by dysregulation of mitochondrial dynamics and bioenergetic deficiencies.

Mitochondria are dense, double membrane-enclosed organelles that are present in all mammalian cells except mature red blood corpuscles. They uniquely possess their own genome in a circular DNA molecule. Although this genome encodes for only a small fraction of the total genes needed for mitochondrial organelle assembly and function, it is thus necessarily complemented by nuclear-encoded genes. Mitochondria provide a wide variety of biochemical services to the cell. Although it is conventionally accepted that energy production through the aerobic oxidation of carbon substrates and the generation of ATP is the principal function of mitochondria, this narrow view overlooks the many other impressive biosynthetic and regulatory capacities of this versatile

organelle. Mitochondria, for instance, are essential for the synthesis of pyrimidines and purines, contribute to the synthesis of heme, regulate nitrogen balance through the urea cycle, produce ketone bodies, are necessary for sex hormone production, are involved in the processing of xenobiotics, play a critical role in redox balance, and are key regulators of the apoptotic program in mammals. Given the centrality of mitochondria to the maintenance of so many pathways in mammalian cells, it is not surprising that there exist multiple layers of control that enable a cell to coordinate its net mitochondrial activity with fuel sources, biosynthetic demands, proliferation rates, and external stimuli. One important layer in this signaling control is the expression of mitochondrial nuclear-derived genes

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that initiate the cellular program of mitochondrial biogenesis. Defects and failure in the integrity of this signaling system can cause serious perturbations to cellular metabolism and give rise to a broad range of tissue specific as well as systemic pathologies in humans. The same signaling system, on the other hand, also opens many therapeutic opportunities for the correction of mitochondrial defects. In this article, we discuss some of the major signaling factors that serve to regulate mitochondrial biogenesis and their implications for the mitigation of mitochondrial disorders.

REGULATION OF MITOCHONDRIAL BIOGENESIS AND FUNCTION THROUGH NUCLEAR-BASED GENE EXPRESSION

Although mitochondria contain in excess of 1000 proteins, its tiny circular genome encodes for only 13 proteins, 22 tRNAs, and two rRNAs; the balance of this genetic shortage is made up by the nuclear genome (Calvo and Mootha 2010). Because the vast majority of mitochondrial genes are situated in the nucleus, transcription complexes at promoters of these genes control their expression. This affords the cell the opportunity to coordinately regulate the expression of both mitochondrial and non-mitochondrial gene sets to affect a unified specific cellular response. Below we highlight some of the major nuclear transcriptional complexes regulating mitochondrial gene expression and the diverse signaling pathways to which they respond (Fig. 1).

Nuclear Respiratory Factors

Sequence analysis of the electron transport gene cytochrome *c* promoter initially identified nuclear respiratory factor 1 (NRF-1) as an important regulator of gene expression (Evans and Scarpulla 1989). NRF-1 controls the expression of a significant number of the proteins that make up the five respiratory complexes, as well as proteins integral to mitochondrial import and heme biosynthesis (Scarpulla 2008). NRF-1 is also able to integrate nuclear control of the transcriptional and replicative activity of the

mitochondrial genome through the direct modulation of transcription factor A mitochondrial (TFAM) and transcription factor B proteins (TFBs) gene expression; TFAM and TFBs are major regulators of mitochondrial DNA transcription and replication (Gleyzer et al. 2005). NRF-1 may play an integral role in coupling mitochondrial replication with nuclear replication over the course of the cell cycle through regulation of E2F transcription factor targets and mitochondrial genes, indicating a link between cell division and mitochondrial biogenesis (Cam et al. 2004).

Studies involving the promoter region of cytochrome *c* oxidase complex subunit IV (COXIV) revealed the presence of another nuclear respiratory factor, NRF-2, which was able to regulate the expression of proteins in the electron transport chain (Carter et al. 1992). NRF-2 promotes the expression of genes that encode for mitochondrial complex IV cytochrome *c* oxidase (Gugneja et al. 1995). Like NRF1, NRF2 also controls the expression of TFAM and TFBs. The differential regulation of NRF1 and NRF2 is not completely understood but phosphorylation of these factors can alter their transcriptional activities (Scarpulla 2008). In addition, specific coactivators of the PGC-1 family sculpt NRFs-dependent control of mitochondrial gene expression in response to different signaling pathways (Wu et al. 1999).

Nuclear Hormone Receptors

Within the superfamily of nuclear hormone receptors, there are several subclasses that control mitochondrial function and biogenesis. The PPAR group of receptors is one such set. PPAR α , which can be activated by long-chain fatty acids and eicosanoids, can potently promote the expression of genes involved in mitochondrial β -oxidation (Gulick et al. 1994). The activation of PPAR γ activation, on the other hand, triggers mitochondrial biogenesis in white adipose tissue (Wilson-Fritch et al. 2004); PPAR γ activation is also necessary to induce the expression of mitochondrial genes involved in the brown fat-mediated thermogenic program (Puigserver et al. 1998). PPAR δ , in

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Figure 1. An overview of the transcriptional complexes regulating mitochondrial gene expression and the signaling pathways converging upon them. Illustrated are the major transcriptional complexes/chromatin remodeling factors directly associated with changes in mitochondrial gene expression (see text for additional information).

skeletal muscle, is able to up-regulate mitochondrial genes associated with fatty acid oxidation and specify slow twitch fiber type (Narkar et al. 2008; Kleiner et al. 2009).

Thyroid hormone receptors (THR α) can also promote mitochondrial biogenesis and tissue-specific function. This includes mitochondrial-driven thermogenesis that occurs in brown fat during the adaptation to lower temperatures (Silva 1995). In some instances, THR α directly drive the transcription of nuclear-encoded genes, whereas, in others, the effects can occur indirectly through the thyroid hormone-mediated up-regulation of NRF-1 (Venditti et al. 2009). A

truncated form of thyroid hormone receptor α has been reported to be localized to the mitochondrion and may serve to directly activate the transcription of genes in the mitochondrial genome (Casas et al. 1999).

Estrogen-related receptors ERR- α , ERR- β , and ERR- γ are yet another set of nuclear hormone receptors capable of promoting mitochondrial biogenesis. These receptors have no known endogenous ligands and are primary expressed in tissues with high oxidative metabolism capacities (Eichner and Giguere 2011). DNA-binding sites for ERR- α have been mapped in a large number of nuclear-encoded

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mitochondrial genes, including those involved in oxidative phosphorylation, fatty acid oxidation, TCA cycle, and factors regulating mitochondrial fusion/fission. Although ERRs are considered orphan nuclear receptors, their activity can certainly be modulated through recruitment of coactivators/corepressor complexes. Indeed, the transcriptional coactivators PGC-1 α / β are efficacious activators of ERRs and promote the expression of mitochondrial genes (Mootha et al. 2004; Schreiber et al. 2004). The transcriptional activity of this transcription factor/activator module is controlled through deacetylation by sirtuin 1, which allows for an important regulatory mechanism that connects nutrient/hormonal signaling to the control of mitochondrial function (Wilson et al. 2010). From the standpoint of repression of ERR activity, the RIP140 nuclear hormone corepressor seems to be important (Wilson et al. 2010).

Other Transcription Factors: CREB and YY1

CREB, a cAMP activated transcription factor, can promote the expression of several mitochondrial genes, including the proteins which make up complex IV and enzymes involved in the β -oxidation pathway (Gopalakrishnan and Scarpulla 1994). Many mitochondrial genes contain YY1 binding sites within their promoter regions and this transcription factor has been found to work in conjunction with PGC-1 α to regulate their expression (Cunningham et al. 2007). Loss of YY1 expression in skeletal muscle causes exercise intolerance and morphological signs of mitochondrial myopathy in mice (Blattler et al. 2012b).

Transcriptional Coactivators

Transcriptional coactivators do not bind to DNA but can nonetheless promote gene transcription by enhancing the activity of true DNA-binding transcription factors. Biologically, these proteins can set into motion genome-wide transcriptional changes by coactivating many different transcription factors. In mammalian cells, the PGC-1 family of coactivators (PGC-1 α , PGC-1 β , and PRC) potentiate the activity of

several transcription factors involved in the basic functions of the mitochondrion as well as its rate of biogenesis (Puigserver and Spiegelman 2003; Kelly and Scarpulla 2004). Indeed, PGC-1 α and PGC-1 β are sufficient to increase total mitochondrial mass, reactive oxygen species scavenging enzymes, oxidative phosphorylation components, mitochondrial metabolic pathways, protein import complexes, proteins involved in fission and fusion, and the levels of mitochondrial sirtuins (Mootha et al. 2003; Uldry et al. 2006; Cunningham et al. 2007; Rasbach et al. 2010; Handschin and Spiegelman 2011). This process occurs in part through the ability of PGC-1 proteins to potentiate the function of the NRFs, ERRs, and YY1.

In skeletal muscle, the increased mitochondrial biogenesis caused by transgenic overexpression of PGC-1 α can attenuate the development of mitochondrial disease in mouse models of respiratory complex IV/cytochrome *c* oxidase deficiencies (Lin et al. 2002; Wenz et al. 2008; Viscomi et al. 2011). It has also been shown that PGC-1 α / β expression improves mitochondrial respiration capacity in cells from human patients with a complex III or IV deficiency (Srivastava et al. 2009). It should also be noted that age-related declines in mitochondrial function are also mitigated by enhanced activity of PGC-1 α . Skeletal muscle specific PGC-1 α transgenic mice are less susceptible to exercise-induced fatigue and are protected against age-related diseases, such as sarcopenia and metabolic diseases, and as a consequence, these mice show an increased life span (Wenz et al. 2009). Nevertheless, it has yet to be determined whether the beneficial health effects of PGC-1 α in skeletal muscle are attributable to its promotion of mitochondrial function or its effects on nonmitochondrial gene expression.

Sirtuin Proteins

Sirtuins are NAD⁺-dependent protein deacetylases that are homologous to yeast Sir2p, which is one of the genes involved in transcriptional silencing in that organism (Klar et al. 1979). In mammals there are seven sirtuin paralogs. SIRT1, SIRT6, and SIRT7 are nuclear proteins,

SIRT3, SIRT4, and SIRT5 are imported into mitochondria, and SIRT2 is principally cytoplasmic (Finkel et al. 2009; Haigis and Sinclair 2010; Verdin et al. 2010; Guarente 2011). Through their deacylation activities, sirtuins modulate the biological activities of a wide array of target pathways. From the standpoint of mitochondrial biology, SIRT1, SIRT3, SIRT4, and SIRT5 have profound effects on the function of this organelle. SIRT1 deacetylates several key transcription factors that result in the up-regulation of numerous genes involved in mitochondrial respiration (Rodgers et al. 2005; Purushotham et al. 2009) and may (Price et al. 2012) or may not (Park et al. 2012) be necessary for the promitochondrial effects of polyphenolic stilbenoid compounds like resveratrol. In addition, sirtuins deacetylate numerous metabolic enzymes to govern their specific activity. Changes in SIRT3 activity have been shown to be an important determinant in the acetylation state of mitochondrial in response to nutrient availability (Hirschey et al. 2011; Hebert et al. 2013). The acetylation of many mitochondrial proteins, such as isocitrate dehydrogenase 2 (Yu et al. 2012), can alter their catalytic/biological function. As such, loss of SIRT3 activity results in profound aberrations in mitochondrial function and exacerbates the metabolic pathologies associated with chronic nutrient excess. Although no consistent deacylase activities have been shown for SIRT4, it has been shown that SIRT4 antagonizes mitochondrial capacity for fatty acid oxidation in hepatic and skeletal muscle cells (Nasrin et al. 2010). SIRT5, which has been shown to display desuccinylase, demalonylase, and deacetylase activities, targets carbamoyl phosphate synthetase 1, and can regulate mitochondrial urea cycle flux (Nakagawa et al. 2009; Du et al. 2011).

SIGNALING MODULES THAT CONTROL MITOCHONDRIAL BIOGENESIS AND FUNCTION

Cells are constantly engaged in both temporal and spatial control of mitochondrial biogenesis in response to nutrient availability, hormonal cues, and changes in temperature. Integration

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of these variables frequently involves the use of signaling pathways that ultimately converge upon the transcriptional regulators described above. In this section, we discuss the key signaling modules that control mitochondrial genes and lead to increased mitochondrial mass.

AMP-Activated Protein Kinase (AMPK)

AMPK is a phylogenetically ancient cellular sensor that is triggered by high cellular energy demands (Hardie 2007). As such, it is not surprising that AMPK is strongly connected to mitochondrial bioenergetics. When ATP synthesis is impaired or ATP is being consumed and generating AMP at a high rate, AMPK becomes active, which, in turn, phosphorylates several enzymes involved in the stimulation of catabolic pathways, such as glucose transport and fatty acid oxidation and the inhibition of anabolic pathways, such as glycogen synthesis and lipogenesis (Kahn et al. 2005). Chronic activation of AMPK is also able to induce mitochondrial biogenesis through the control of nuclear transcription (Fig. 2). Essential to this process are the AMPK-mediated activation of PGC-1 α and SIRT1. AMPK can directly phosphorylate and activate PGC-1 α (Jager et al. 2007); other pathways that activate AMPK can result in a subsequent increase in PGC-1 α protein expression and mitochondrial biogenesis (Birkenfeld et al. 2011). AMPK through Nampt-dependent and -independent mechanisms is also able to increase NAD⁺ levels, which promotes SIRT1 activity and the deacetylation/activation of PGC-1 α (Canto et al. 2009). Thus, the AMPK/SIRT1/PGC1 α signaling axis is thought to play a key role in the adaptive metabolic programming that occurs during caloric restriction and exercise (Canto and Auwerx 2009). Nevertheless, it has been alternatively suggested that caloric restriction-mediated activation of AMPK serves to maintain mitochondrial protein biosynthesis independently of transcription (Miller et al. 2012).

Calcium Signaling

Intracellular calcium dynamics are affected by a wide array of signaling pathways and calcium, as

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Mitochondrial dynamic process	Nuclear transcription control
• Mitochondrial unfolded protein response	- Zinc finger ZC376.7 (<i>C. elegans</i>) - CHOP/C/EBP β - PGC-1 α /HSF1?
• Mitochondrial fusion/fission	- PGC-1 α /PGC-1 β - ERR α ; YY1
• Mitophagy	- PARIS/PGC-1 α
• Mitochondrial motility	- Unknown
• Mitochondrial protein import	- PGC-1 α /PGC-1 β - ERR α ; NRF-1; YY1
• Mitochondrial transcription	- PGC-1 α /PGC-1 β - ERR α ; NRF-1; YY1

Figure 2. Transcriptional control of processes involved in the regulation of mitochondrial dynamics. Nuclear transcription factors regulate the transcription of specific proteins that are basic components of specific mitochondrial processes associated with mitochondrial dynamics (see text for further information).

a second messenger molecule, and constitutes an important mechanism for initiating changes in mitochondrial gene expression. This phenomenon is most evident in skeletal muscle, a tissue that displays coordinated changes in calcium homeostasis as part of its basic contractile mechanism. In this tissue, calcium-sensitive protein networks, such as calcium/calmodulin-dependent kinase and p38 mitogen-activated kinase, are critical actors in the mobilization of the transcriptional machinery that promotes mitochondrial proliferation and fatty acid oxidation (Wu et al. 2002; Wright et al. 2007).

cAMP Pathway

Yet another well-conserved second messenger system capable of altering mitochondrial function is the cAMP-PKA pathway. The principal transcriptional effector of this pathway is the CREB transcription factor, whose phosphorylation by PKA promotes CREB activity and target gene transcription (Mayr and Montminy 2001). PKA activation can also enhance the expression of PGC-1 α , through the intermediacy of CREB (Herzig et al. 2001), as well as directly activate the catalytic function of SIRT1 (Gerhart-Hines et al. 2011). Biologically, one of the most sa-

lient examples of the power of cAMP-mediated effects on mitochondrial function is found in the adaptive nonshivering thermogenic response to lower temperatures (Cannon and Nedergaard 2004). This heat-generating process is highly dependent on induction of uncoupled mitochondrial respiration in adipose depots—primarily brown fat tissue—through the up-regulation of uncoupling proteins such as UCP1 and the up-regulation of oxidative pathways (Cannon and Nedergaard 2004). Sympathetic stimulation of brown and “beige” fat tissues increases cAMP-PKA tone in these tissues and precipitates the initial uncoupling response. Whereas the specific determination of the brown fat cell lineage is transcriptionally specified through PRDM16 (Seale et al. 2007), the mitochondrial thermogenic response is defined by the PGC-1 coactivators, which account for approximately 30% of the cAMP response in brown fat cells (Uldry et al. 2006).

mTOR Pathway

The mechanistic target of rapamycin (mTOR) pathway is a logic gate for integrating cell growth and cell size with the availability of growth factors, energy levels, and certain nutrients, such

as the branched-chain amino acids. These processes require a net increase in cellular anabolic/biosynthetic capacities, which will ultimately depend on enhanced mitochondrial biogenesis and bioenergetics. The effects of mTOR on mitochondrial biology occur through several mechanisms. One of the mechanisms seems to be independent of transcription through direct effects on mitochondrial organelles and stimulation of respiration (Schieke et al. 2006). In a second mechanism, mTOR modulates mitochondrial gene expression through the regulation of different transcription factors. In liver, mTOR affects genes of fatty acid oxidation and ketone body production through its ability to activate NCoR and inactivate PPAR α (Sengupta et al. 2010). In skeletal muscle, mTOR binds to the transcription factor YY1 and recruits PGC1 α , increasing the ability of YY1 to activate mitochondrial gene expression. mTOR inhibition by using rapamycin, for example, results in a dissociation of PGC1 α and decreases mitochondrial gene expression (Cunningham et al. 2007; Blattler et al. 2012a).

Retrograde Signaling from Mitochondria to the Nucleus

The retrograde transmission of regulatory signals from the mitochondria to the nucleus, from a teleological standpoint, makes sense in that deficits or excesses in mitochondrial activity would need to be ultimately corrected by adjustments in nuclear gene expression. This phenomenon has been actively explored in yeast (Liu and Butow 2006). In mammalian cells, however, the nature of these signals and the mechanisms that they use are less well understood. Nevertheless, evidence of this regulatory phenomenon can be seen in the skeletal muscle of patients with mitochondrial diseases, such as those with genetic mutations of oxidative respiration proteins, which display increased mitochondrial proliferation—perhaps as an effort to compensate for impaired mitochondrial activity (DiMauro and Hirano 2009; Wallace and Fan 2009). The nature of this communication is unknown, but calcium and ROS signaling have been connected to this retrograde control.

Calcium Signaling

Much like the endoplasmic reticulum, mitochondria are quantitatively significant stores of calcium in the cell. As such, they can provide a potential signaling avenue for the regulation of cellular processes—indeed, overloading the mitochondrial calcium pool is a signal for the induction of apoptosis. At this point, although mechanisms of calcium uptake by the mitochondria have been characterized (Baughman et al. 2011; De Stefani et al. 2011), there is no clearly delineated pathway by which mitochondrial calcium can be released to regulate the calcium sensitive transcription/chromatin factor pathways discussed above. Nonetheless, PGC-1 α increases mitochondrial calcium release, which establishes a positive feedback loop for the maintenance of a mutual connection between mitochondrial and nuclear gene expression (Bianchi et al. 2006).

Reactive Oxygen Species (ROS)

ROS are produced at respiratory chain complexes located in the inner mitochondrial membrane. In *Drosophila*, disruption of respiratory complex 1 signals to components of the G1 to S cell cycle machinery through JNK and FoxO (Owusu-Ansah et al. 2008). In mammalian cells, a similar retrograde signaling has been uncovered through a systematic analysis of different mitochondrial dysfunction mutations; this pathway increases *OXPHOS* genes and involves ROS signaling through JNK and the RXR α /PGC1 α pathway (Chae et al. 2013).

CONTROL OF MITOCHONDRIAL PROCESSES THAT CONTRIBUTE TO THE BIOGENESIS OF MITOCHONDRIA

Mitochondrial Unfolded Protein Response and Quality Control

The mitochondria contains an internal protease system that monitors accumulation of unfolded or misfolded proteins (encoded by the nuclear or mitochondrial genomes) in the different compartments, including the outer membrane, intermembrane space, inner membrane, and

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matrix. Thus, during mitochondrial biogenesis or stress, chaperones and quality-control proteases recognize hydrophobic amino acids that are in the interior structure of normal folded proteins (Baker and Haynes 2011). In addition to this organelle's internal quality control, there is a retrograde signaling to the nucleus via translocation of several transcription factors. In *Caenorhabditis elegans*, mitochondrial chaperones such as mtHSP70 proteins bind to unfolded mitochondrial proteins; however, if the amount of unfolded proteins exceeds the chaperone capacity, a protease called ClpXP degrades these proteins into peptides. Mitochondrial efflux of these peptides activates a transcription factor, ZC376.7, which translocates to the nucleus to activate expression of mitochondrial chaperone genes (Haynes et al. 2010). In mammalian cells, accumulation of unfolded proteins in the mitochondrial matrix increases CHOP/C/EBP β transcriptional activities on promoters encoding for mitochondrial chaperone genes, including chaperonins 10 and 60, mtDNAJ, and ClpP (Zhao et al. 2002). In hepatocyte cells, PGC-1 α interacts with HSF1; however, it is unknown whether this pathway controls the mitochondrial unfolded protein response (Charos et al. 2012).

Mitochondrial Fusion/Fission

Mitochondria are dynamic organelles that undergo constant division or fission and fusion (Twig et al. 2008; Westermann 2010; Corrado et al. 2012). The mitochondrial mass undergoes different shapes depending on the cellular state. Fusion of the outer mitochondrial membrane is executed by the activity of two dynamin-related GTPases, MFN1 and MFN2, that form homo- or heterodimers. In addition, OPA1—another dynamin-related GTPase—controls mitochondrial fusion and has also been involved in cytochrome release and apoptosis. The mitochondrial fission machinery is made up of DRP1, FIS1, MFF, and MIEF1. DRP1 is a GTPase protein that is localized in the cytoplasm, but after calcineurin-mediated dephosphorylation is recruited to the mitochondria. Once at the mitochondria DRP1 oligomerizes and forms

ring structures that will constrict and produce fission. DRP1 has adaptor proteins that either activate fission (MFF) or suppress fission (MIEF) modulating the DRP1 GTPase activity. FIS1 is the DRP1 receptor located in the outer mitochondrial membrane and also interacts and sequesters MIEF1. In cells, fusion makeup of interconnected mitochondria is essential to maintain a highly metabolic and energetic state. In contrast, quiescent or resting cells have fragmented mitochondria with small spherical shapes with low metabolic activities (Chen and Chan 2010).

Thus, it seems plausible that the mechanisms that control mitochondrial biogenesis are tightly associated with the fusion machinery. Along these lines, the transcriptional coactivators PGC-1 α and PGC1- β are positive activators of MFN2 (Cartoni et al. 2005; Soriano et al. 2006). Whether there is independent transcriptional control of the fission machinery, or if their regulation is simply bundled with the rest of the nuclear-encoded mitochondrial proteins is currently unknown.

Mitophagy

Mitochondrial mass is defined by the balances of mitochondrial formation and degradation. This latter process largely occurs through a particular type of cellular autophagy, termed mitophagy, that clears defective mitochondria. Mitophagy is a cellular degradation process that maintains “healthy” mitochondrial mass (Ashrafi and Schwarz 2013). Elongated and fused mitochondria are resistant to mitophagy, whereas fragmented mitochondria generated by fission are more accessible to mitophagy. The elimination of damaged mitochondria is mediated by the activities of PINK1 (PTEN-induced putative protein kinase 1) and the E3 ubiquitin ligase, Parkin. PINK1 and Parkin accumulate at the mitochondrial surface of damaged organelles through the loss of mitochondrial membrane potential (Youle and van der Bliek 2012). In turn, this promotes separation from the functional mitochondrial network followed by targeting defective mitochondria for degradation. This latter process requires Parkin-depen-

dent ubiquitination of mitochondrial proteins, but the critical Parkin substrates necessary for mitophagy are unknown. MFN1 and MFN2 are ubiquitinated by Parkin, although they are not required for mitophagy. Mitochondria are selectively removed upon treatment with chemical uncouplers. In this process, opening of the mitochondrial permeability transition pore (PTP) also triggers selective mitophagy. In addition, starvation signals such as glucagon can also induce mitophagy but require opening of the PTP. Similar to mitochondrial fusion/fission, little is known about the transcriptional control of mitophagy. Interestingly, mutations that occur in Parkinson's disease and inactivate the E3 ligase activity of Parkin, lead to the accumulation of Parkin substrates (Narendra et al. 2012). Among them is PARIS, a zinc finger corepressor, which can suppress the transcriptional coactivator PGC1 α (Shin et al. 2011). Consistent with this, PGC-1 α responsive genes are underexpressed in microdissected dopaminergic neurons of Parkinson's disease mouse models, suggesting that this pathway could be causative in this disease (Beal 2009; Zheng et al. 2010). Based on these data, it is possible to speculate that during mitophagy Parkin activation can signal to the nucleus through Paris degradation, resulting in an increase in PGC-1 α and will subsequently promote mitochondrial biogenesis.

Mitochondrial Motility

Depending on the cell type, an increase in mitochondrial mass needs to be coordinated with a precise intracellular localization of mitochondrial organelles to meet local energetic cellular demands. Mitochondrial motility is influenced by the organelle's size, which is in turn controlled by fusion/fission processes. Failure in these processes prevents efficient intracellular movement of mitochondria, as is most evident in neurons, which critically depend on this pathway to localize mitochondria close to synaptic sites (Chen and Chan 2009).

Mitochondrial transport is based on microtubule dynamics. Kinesin-1 type motors are recruited to mitochondria through outer mi-

tochondrial membrane proteins Miro1 and Miro2, involving an adaptor protein termed Milton. This is a calcium-dependent process: High concentrations of calcium halt mitochondria through the EF hands of Miro, anchoring mitochondria to sites of high ATP requirements. It is unclear whether calcium is the only regulator of this movement or there are additional sites of control including establishment of sensing gradients or transcriptional/translational regulation (Stowers et al. 2002; Wang et al. 2011).

Mitochondrial Protein Import

Biogenesis of functional and competent mitochondria requires the import and assembly of proteins synthesized in the cytoplasm. The mitochondria contain more than 1000 proteins that are mostly imported from the cytoplasm through the translocase of the outer mitochondrial membrane (TOM complex) (Endo et al. 2011; Gebert et al. 2011).

Once the mitochondrial protein has passed the TOM channel, it is sorted by interactions with different machineries located in the intermembrane space and inner membrane. Proteins that contain a presequence target signaling to the matrix are imported by the translocase of the inner membrane (TIM23) complex that is associated with the presequence translocase-associated motor (PAM). The TIM23 complex is also involved in laterally releasing proteins to the inner mitochondrial membrane. Several complexes in the intermembrane space participate in sorting mitochondrial proteins. For example, the MIA (mitochondrial intermembrane assembly) complex promotes oxidative folding of intermembrane proteins. The Tim9–Tim10 chaperone complex transfers hydrophobic proteins to the mitochondrial outer membrane through the SAM complex (e.g., β -barrel proteins) or the mitochondrial inner membrane through the TIM22 carrier complex (e.g., inner membrane carrier proteins). Additional import pathways include the oxidase assembly (OXA) machinery that transfers mitochondrial proteins from the matrix ribosomes to the inner membrane, or the mitochondrial import

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1 (Mim1) that imports α -helical outer membrane proteins (Endo and Yamano 2009; Schmidt et al. 2010).

The dynamics of this complex mitochondrial protein import process will depend not only on the number of existing import complexes but also on the amounts of mitochondrial protein translated in the cytoplasm. Little is known about the protein stability or activities of these mitochondrial proteins, for example, control by ubiquitination or other posttranslational modifications (phosphorylation, acetylation, etc.) that control the import processes. However, an important regulatory control is the coordinated transcription of genes encoding for these proteins that will determine the rate of synthesis and cellular concentrations that will promote and contribute to mitochondrial mass. In mammalian cells, this transcriptional control involves the PGC-1 pathway and its interactions with transcription factors including NRFs and YY1 that are bound to promoters of mitochondrial import genes (Blattler et al. 2012b; Scarpulla et al. 2012). Whether there is selectivity or specificity among the different import pathways described here is currently unknown.

Mitochondrial Transcription

Balanced and efficient mitochondrial biogenesis requires the activation of mitochondrial transcription (Peralta et al. 2012). The mammalian mitochondrial DNA is a circular double-stranded genome (~ 16.5 kb) that encodes 37 genes. Transcription of the mitochondrial DNA (mtDNA) is tightly coordinated with nuclear genes (particularly involved in OXPHOS) to achieve competent bioenergetic mitochondrial organelles. The mtDNA contains two strands: the heavy (H) and the light (L) strands. The H strand contains most of the genes. In addition, there are two noncoding regions designated as the D-loop that harbors the promoter of both strands and the origin of replication. Mitochondrial transcription is bidirectional and starts in the D-loop region through the assembly of a complex composed by TFAM, TFB2M, and the RNA polymerase POLRMT. MTERF proteins also bind to promoters and

control mitochondrial transcription (Rebello et al. 2011). How the transcription of mitochondrial-encoded genes is precisely controlled is not completely understood. Several of the proteins that control mitochondrial DNA transcription have posttranslational modifications, including phosphorylation and acetylation. For example, TFAM is specifically phosphorylated (Lu et al. 2013) and acetylated (Dinardo et al. 2003), suggesting that its activity could be modified through cycles of acetylation/deacetylation in response to energetic signals. Another level of control of this mitochondrial transcriptional machinery is by inducing amounts of its components. Along these lines elements essential to mitochondrial transcription, such as TFAM, TFB2M, MTERF, and POLRMT, are regulated through the transcriptional coactivators PGC-1 α and PGC-1 β through their interaction with NRFs, ERRs, and YY1 (Mootha et al. 2004; Blattler et al. 2012b; Scarpulla et al. 2012).

THERAPEUTIC POTENTIAL OF SIGNALING PATHWAYS FOR THE ATTENUATION OF MITOCHONDRIAL-RELATED PATHOLOGIES

Mitochondrial dysfunction, whether it is attributable to deleterious mutations in nuclear or mtDNA genes (DiMauro and Hirano 2009; Wallace and Fan 2009) or to secondary systemic changes caused by factors such as aging/senescence, significantly impairs cell function and can result in a spectrum of pathologies. In certain circumstances, such as aging, the mechanisms for why or how mitochondrial function and dynamics become abnormal are unknown (Petersen et al. 2003; Reznick et al. 2007). Regardless of the mechanism, however, data gleaned from signaling studies suggest that certain elements of these pathways can be leveraged to either prevent or attenuate a wide array of mitochondrial dysfunctions.

Genetically Based Mitochondrial Disorders

Despite the fact that significant advances have been made in understanding the causative molecular mechanisms underlying many genetical-

ly based human mitochondrial disorders, there is a frightening paucity of demonstrable treatments (Hirano et al. 2012). In theory, however, interventions that boost the net mitochondrial activity in these patients to restore energy production and antioxidant capacities to some minimum threshold should be sufficient to attenuate afflicted organ dysfunction and the subsequent pathologies. As such, there are several druggable signaling avenues to pursue.

The first is the PGC-1 α signaling axis. As mentioned earlier, transgenic overexpression of PGC-1 α has been shown to significantly blunt the pathological effects of cytochrome *c* oxidase (Viscomi et al. 2011), COXIV deficiency in mice (Lin et al. 2002; Wenz et al. 2008), and COXIII/IV deficiency in human patient-derived cell lines. Thus, small molecule therapies that potentiate PGC-1 α function or the function of one of its cognate transcription factors could yield tangible benefits. To date, efforts to specifically exploit this pathway have been largely limited to the testing of PPAR panagonists such as the fibrate family of compounds, which are already in clinical use as antihyperlipidemic drugs. Studies with bezofibrate suggest that it is of moderate use in humans suffering from mutations in carnitine palmitoyltransferase II deficiency (Bonnetfont et al. 2009). Data on bezofibrate's ability to correct the phenotypes associated with mutations in respiratory complex proteins, in contrast, have been confined largely to mouse models and have yielded very mixed results (Wenz et al. 2008; Viscomi et al. 2011; Yatsuga and Suomalainen 2012). The major limitation of fibrates is that they target only a single cognate transcription factor of PGC-1 α , namely, PPAR α , which is involved in up-regulation of the mitochondrial proteome. Small molecules that specifically up-regulate PGC-1 α expression or promote its inherent biological activity, such as through deacetylation, are no doubt needed for a full effect.

The second signaling axis is that mediated by AMPK activation. High throughput screenings for compounds that improve the growth and ATP levels of fibroblasts from humans with a complex I deficiency revealed the AMPK activator 5-aminoimidazole-4-carboxamide ribonu-

cleoside (AICAR) as one of the most efficacious molecules (Golubitzky et al. 2011). In several mouse models of cytochrome *c* oxidase deficiency, AICAR treatment was also shown to lead to a partial rescue of COXIV activity and some measure of improvement in motor performance (Viscomi et al. 2011).

The third most therapeutically attractive targets for mitochondrial diseases are the sirtuin family of proteins. Within this family, SIRT1 and SIRT3 offer the strongest connection with improvements in mitochondrial health. Small molecule activation of SIRT1 has been shown in vivo to improve mitochondrial respiratory capacity in mice on a high-fat diet (Minor et al. 2011). In healthy human volunteers, a next-generation SIRT1 activator (SRT2104) has been shown to decrease serum lipid levels and increase the recovery of adenosine diphosphate and phosphocreatine after exercise, which is suggestive of an increase in mitochondrial oxidative phosphorylation (Libri et al. 2012). Although there are no human in vivo studies of the effects of SIRT1 activation on mitochondrial diseases, in vitro studies with the putative SIRT1 activator resveratrol have shown improvements in mitochondrial fatty acid oxidation in fibroblasts derived from patients with carnitine palmitoyltransferase II or very long-chain acyl-CoA dehydrogenase deficiencies (Bastin et al. 2011). In the case of SIRT3, up-regulation of activity has been shown to improve mitochondrial homeostasis in mice (Brown et al. 2013), although no data are available in humans. Ideally, therapies that simultaneously increase SIRT1 and SIRT3 activities, such as supplementation with the precursor nicotinamide riboside to increase the levels of their NAD⁺ cosubstrate (Canto et al. 2012), would be the most desirable for promoting mitochondrial function.

Prevention of Mitochondrial Dysregulation Associated with Aging and Metabolic Disorders

Many of the therapeutic strategies described for the amelioration of genetically based mitochondrial disorders are applicable to the decline in mitochondrial function that accompanies age-

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related senescence or certain metabolic disease states such as Type-2 diabetes. Other therapeutic options are available to the nongenetic mitochondrial disorders, because of their generally reduced phenotypic severity. These options include dietary manipulation and physical exercise. Restriction of caloric intake can result in profound alterations in mitochondrial physiology, including increased proliferation, enhanced bioenergetic efficiency, reduced membrane potential, and reduced production of oxidative species in mice (Lopez-Lluch et al. 2006) and in humans (Civitarese et al. 2007). These mitochondrial changes are thought to be a major contributor to the ability of caloric restriction to prevent the development of diseases linked to aging. Indeed, calorie restriction extends life span in different organisms but the molecular mechanisms are not completely understood (Sohal and Weindruch 1996; Spindler 2001). Proposed mechanisms for the longevity effects include changes in the signaling pathways associated with nutrient availability and insulin levels that converge to regulate mTOR (Kaeberlein et al. 2005; Zoncu et al. 2011) and FoxOs (Salih and Brunet 2008) protein activities. In terms of mitochondrial function, however, other regulatory pathways appear to be connected with the effects of caloric restriction, namely, the AMPK, sirtuins, and PGC1 α regulatory network.

Exercise is an extremely powerful inducer of mitochondrial activity and proliferation—particularly in skeletal muscle (Yan et al. 2011). Coincident with this change, exercise is also able to strongly protect against many types of age-related metabolic disorders. Less clear, however, is whether exercise can mimic the longevity effects of caloric restriction. An important point, however, is whether the beneficial health effects of exercise are to an increase in the mass of mitochondrial and/or the myriad other cellular and systemic changes that occur with exercise.

CONCLUDING REMARKS

Researchers across multiple disciplines have made considerable progress by uncovering many important signaling pathways that con-

trol mitochondrial function at the level of nuclear transcription and discerning how these pathways are targeted through extrinsic signals to ultimately govern mitochondrial activities. Collectively, these signaling pathways can effectively boost the cell's mitochondrial mass, improve the mitochondrial bioenergetics profile, and protect it against oxidative stress. As such, they represent a very fertile ground for the development of therapies for the rectification of diseases characterized by mitochondrial dysfunction.

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