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Mitochondrial Remodeling: Rearranging, Recycling, and Reprogramming

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Abstract

Asthma's a problem of inhaling too much Hyperinflation, air trapping, and such. But physicians find themselves just as aghast When a patient finally exhales his last. So which deserves the frowning pout: Breathing in or breathing out? So it is with the mitochondrion, Which is better, fission or fusion? Both out and in make good respiration Ditto for mitochondrial undulation. *Roberta A. Gottlieb*

Keywords

mitochondria; fission; fusion; mitophagy; biogenesis; metabolism; retrograde signaling; mitochondrial unfolded protein response

I. Introduction

Mitochondria can present many different faces depending on environmental conditions and cellular requirements. They change their structure, their proteome, their metabolism, and even their genome, in response to cellular and environmental cues. As they remodel, they in turn alter cellular programs and capabilities. Mitochondrial remodeling is a component of the pathophysiology of diverse diseases, including neurological, cardiovascular, metabolic and oncologic. In this setting, mitochondrial alterations may be either secondary processes or primary mechanisms due to mutations in genes that regulate mitochondrial remodeling. However, the role of mitochondrial remodeling in normal physiological processes is gaining increased recognition. In this review we will cover structural remodeling, comprising fusion and fission; mitochondrial turnover accomplished by mitophagy and biogenesis; proteomic alterations and posttranslational modifications; metabolic alterations; reactive oxygen species; and retrograde signaling whereby mitochondria modify the nuclear program.

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II. Mitochondrial Structural Remodeling: rearranging and recycling

The dynamic nature of mitochondrial morphology was apparent even to the 19th century scientists who first proposed the name: mitos (thread-like) and chondrion (granule-like) (1, 2). Mitochondrial dynamics are those processes that regulate mitochondrial morphology, including: *(a) Mitochondrial fission:* the process that creates more numerous, smaller and more circular mitochondria; *(b) Mitochondrial fusion:* the process that creates fewer, larger and more elongated mitochondria; *(c) Mitophagy:* the process of removing damaged mitochondria; and *(d) Mitochondrial biogenesis:* the process that increases mitochondrial number and/or volume through increased expression of both mitochondrial and nuclear transcripts. This is illustrated in Fig. 1.

A. Mitochondrial fission is mediated by dynamin-1-like protein (Drp1), a member of the dynamin superfamily, consisting of a GTPase and GTPase effector domain separated by a helical segment. Fission occurs when Drp1 is translocated from the cytosol to the outer mitochondrial membrane (OMM) leading to a complex, and as yet still not fully understood, interaction with three integral OMM proteins, Fis1, Mff, and MIEF1.

1. *Fis-1.* Fis1 is the most evolutionarily conserved pathway, and is a major regulator of fission in yeast. In yeast cells, two adapter proteins (Mdiv1 and Caf4) link Drp1 and Fis1, although similar adapters have not been found in mammalian cells. The role of Fis1 in fission in mammalian cells is still controversial, with some studies supportive (overexpression of Fis1 induces fragmentation; knockdown of Fis1 results in elongation (3–5)), yet others arguing against a role (the absence of bridging proteins; Fis1 is uniformly distributed on the OMM, whereas when Drp1 is translocated, it localizes to punctate structures; altering the level of Fis1 does not affect the distribution of Drp1 on the OMM (5, 6); ablation of Fis1 does not alter mitochondrial morphology or block fission (7)). It is possible, therefore, that Fis1 may play a role in recruitment of Drp1 in certain cell types and under certain stressors, but not others (8, 9). Some of the strongest evidence confirming the role of Fis1 in mammalian cells comes from studies showing that a peptide designed to specifically inhibit the Drp1-Fis1 interaction can block fission in both cardiomyocytes and neuronal cells, attenuating ischemic injury or neurodegeneration, respectively (10–12).

2. *Mff* is an OMM protein not found in yeast. Knockdown of Mff leads to increased mitochondrial fusion whereas overexpression leads to fragmentation. However, Mff and Fis1 are located in different complexes on the OMM (13) and Mff-mediated fission is independent of Fis1 (7, 14), suggesting that these two proteins play different roles or activate fission in response to different stressors.

3. *MIEF1 (mitochondrial elongation factor 1).* Also known as MiD51 and MiD49 (mitochondrial dynamic proteins), MIEF1 is co-localized with Drp1 in punctate structures on the OMM. Overexpression of MIEF1

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recruits Drp1 but, intriguingly, induces mitochondrial elongation rather than fragmentation; similarly, knockdown of MIEF1 leads to fragmentation. As well, the recruitment of Drp1 by MIEF1 is independent of Drp1 phosphorylation or its GTPase activity (15). The current paradigm is that although MIEF1 recruits Drp1, it sequesters it from interaction with Fis1, thus inhibiting its activity. MIEF1 also associates with Fis1 in a Drp1-independent manner (15), competing for MIEF1 binding with Drp1, and thus attenuating the inhibitory effects of MIEF1 on Drp1.

4. *Post-translational modifications (PTMs) are key to the regulation of mitochondrial dynamics.* Drp1 is activated/deactivated via several post-translational modifications (16, 17): Phosphorylation at Ser637 by PKA inhibits fission, whereas phosphorylation at Ser616 by the cell cycle regulator cyclin B1/Cdk1 activates fission (18). Intracellular Ca^{2+} modulates fission at two sites: calcineurin-mediated dephosphorylation (pro-fission) at Ser637 (19, 20) and CaMK phosphorylation (pro-fission) at Ser616 (21). Additional PTMs regulating fission include Drp1 sumoylation (pro-fission) by SUMO1, MAPL and SENP5 (22–24); and S-nitrosylation by nitric oxide (25). The ubiquitin-proteasomal system also plays a role in regulation of fission, with Drp1 ubiquitinated by Parkin (26) and by RING-CH protein MARCH5 (27, 28).

B. Mitochondrial fusion is mediated by the coordinated interaction of mitofusins on the OMM and OPA1 on the inner mitochondrial membrane (IMM).

1. *Mitofusin* exists in two isoforms, Mfn1 and Mfn2, anchored in the OMM by two transmembrane domains and containing an N terminal GTPase domain. Mitofusins form homo- or heterodimers that tether together adjacent mitochondria, promoting fusion, a process requiring activation of the GTPase and the presence of Opa1. The roles of the two isoforms are not identical, as knockout of Mfn1 results in more fragmentation than does Mfn2. Knockout of both results in embryonic lethality (29). Mfn2 is also critical for the tethering of mitochondrial to the sarcoplasmic reticulum in cardiomyocytes, enhancing mitochondrial Ca^{2+} signaling (30).

2. *Opa1 (Mgm1p in yeast)* is a dynamin-related GTPase located in the IMM, containing a mitochondrial targeting sequence (MTS) at its N-terminal which is cleaved after import. There are eight different isoforms, both long and short, with the short form lacking the transmembrane anchor. Processing of OPA1 between long and short forms helps to regulate the balance between fusion and fission. Two IMM peptidases OMA1 and YME1L, convert long OPA1 into short isoforms, with fusion dependent on long OPA1, and fission dependent on the short isoforms (31–33). Opa1 activity requires Mfn1, but not Mfn2. In yeast, Ugo1p acts as an anchor between the Mfn equivalent (Fzo1p) and Opa1 equivalent (Mgm1p), however, no such homologue has been found in mammalian cells.

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Knockout of Opa1 in cultured cells results in fragmentation, whereas knockout in mice is embryonic lethal (33).

3. *MIEF1(mitochondrial elongation factor 1):* In addition to inhibiting Drp1 (above), MIEF1 promotes fusion, even in the absence of Mfn2, and overexpression of MIEF1 can reverse the fragmentation associated with Mfn2 deficiency (34).

C. Mitophagy is a process for clearing damaged mitochondria, and a critical component of normal mitochondrial quality control (35). Traditionally, mitochondrial fragmentation has been associated with pathologic insults such as ischemia (36, 37) or diabetes (38), and thought to be deleterious, due to the propensity for fragmented mitochondria to produce greater reactive oxygen species (ROS) and have lower mitochondrial membrane potential ($\Delta\psi m$) (10, 39). In this setting, fission is asymmetrical, segregating portions of mitochondria with low membrane potential ($\Delta\psi m$) from those with normal $\Delta\psi m$. The low $\Delta\psi m$ daughter mitochondrion is then marked for elimination by mitophagy (40) through accumulation of PINK1 and recruitment of Parkin (41, 42), resulting in autophagic sequestration, lysosomal fusion and degradation.

In addition to the role of PINK1 and Parkin in regulating mitophagy, both proteins play a role in mitochondrial functional regulation by directing the localized translation of nuclear-encoded respiratory chain complex mRNAs on the OMM. This process, mediated by displacement of translational repressors, links dynamic regulation of mitochondrial quality control and oxidative phosphorylation (OXPHOS) (43, 44). Parkin-dependent mitophagy has also been implicated in metabolic programming, e.g. that which occurs in the heart during the transition from fetal (carbohydrate-based) to post-natal (fatty acid-based) metabolism (45).

1. *PINK1 (PTEN-induced putative kinase 1)* is a OMM serine/threonine kinase with an N-terminal mitochondrial targeting sequence (MTS), a transmembrane domain and a cytosolic-facing C-terminal kinase domain. However, PINK1 is also located in the cytoplasm and its recruitment to the OMM is thought to be a mechanism by which cells distinguish functional vs. dysfunctional mitochondria. Healthy mitochondria maintain $\Delta\psi m$, which enhances the import of PINK1 to the IMM, where it is cleaved by PARL and cleared. When mitochondria are stressed or damaged, $\Delta\psi m$ decreases, leading to decreased PINK1 import and accumulation on the OMM, where it can recruit Parkin. PINK1 may also function as a vesicular trafficking pathway, segregating oxidized and damaged proteins for lysosomal degradation, another mechanism by which it regulates mitochondrial quality control (46).

2. *Parkin* is a cytosolic E3 ubiquitin ligase, which translocates to damaged mitochondria when PINK1 accumulates on the OMM, ubiquitinating OMM proteins and inducing engulfment of mitochondria by autophagosomes (mitophagy) through p61 and LC3 (41, 42). Parkin also regulates mitochondrial dynamics by the ubiquitination and degradation of

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other fission and fusion proteins: Mfn1/2, Drp1 and Fis1(26, 47–49). Knockdown of either PINK1 or Parkin leads to mitochondrial fragmentation (50, 51).

3. Other proteins such as Nix and Bnip3 (BH-3 pro-apoptotic proteins), can also function as autophagy receptors by binding to LC3 on the autophagosome and targeting mitochondria to autophagosomes (52, 53).

D. Mitochondrial biogenesis

Mitochondrial biogenesis is controlled by the PPARγ coactivator (PGC) family of transcriptional coactivators, most importantly PGC-1α, PGC-1β, and the PGC-related coactivator PRC. PGC-1α works in tandem with nuclear respiratory factor 2 (NRF-2) to co-activate NRF-1 (54). NRFs direct transcription of nuclear encoded mitochondrial proteins, the mitochondrial protein import machinery, and co-factors required for assembly of the respiratory chain complexes, as well as the regulatory factors required for mitochondrial DNA transcription and translation, most importantly mitochondrial transcription factor A (Tfam), which is also responsible for maintenance of mtDNA copy number (55). Overexpression of PGC-1α is sufficient to drive mitochondrial biogenesis (56). While acetylation of PGC1α suppresses its transcriptional coactivator activity, histone deacetylase sirtuin 1 (Sirt1) can activate PGC1α and mito-biogenesis (57). PGC-1α also responds to a variety of exogenous cues including ROS/RNS, cold exposure, endurance exercise, and intracellular signaling downstream of cAMP.

Mitochondrial biogenesis requires coordinated synthesis and assembly of nuclear-encoded and mitochondrial-encoded subunits of the OXPHOS complexes. Excess or unincorporated subunits are degraded by matrix proteases, and the resulting peptides have been shown (in yeast and *C. elegans*) to trigger the mitochondrial unfolded protein response (see Section VIIA). Nuclear-encoded mRNAs are translated on ribosomes in cytosol or associated with the mitochondrial outer membrane, and in most cases, are co-translationally imported through the TOM (translocase outer membrane) and TIM (translocase inner membrane) complexes. Import of some, but not all, proteins requires adequate mitochondrial membrane potential, ensuring that newly-synthesized proteins are not delivered to senescent mitochondria (58). Information contained in the 3′UTR and in the mitochondria-targeting sequences of the protein-encoding portion of the mRNA is sufficient to target the mRNA or nascent protein to the mitochondria. Additional information, usually in a cleavable pre-sequence, directs the protein to the outer membrane, the intermembrane space, the inner membrane, or the matrix. Peptidases may cleave the signal sequence and additional chaperone machinery assists with protein folding and insertion of prosthetic groups such as FAD, Fe-S clusters, and heme groups (59). Codon usage in mitochondria differs from cytosolic ribosomes, and mitochondria encode their own tRNAs as well as rRNAs. There are 13 mitochondria-encoded proteins that are essential subunits of Complexes I, III, IV, and V. Mitochondrial mRNAs are generated by a mitochondrial RNA polymerase; translation of individual mRNAs is governed by specific and rate-limiting translational activators which may govern several subunits of a Complex (60). The resulting mRNAs are translated on ribosomes associated with the inner mitochondrial membrane Specific assembly factors for OXPHOS complexes are nuclear-encoded and facilitate the assembly of the various nuclear

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and mitochondrial-encoded subunits into functional complexes (61–63). The complexes are then arranged into supercomplexes or respirasomes, for instance I+III_2+IV (64).

There is also post-transcriptional control of mitochondrial biogenesis. Nuclear-encoded mRNAs destined for the mitochondria are associated with ribosomes located in proximity to the mitochondrial outer membrane. Many mitochondrial-targeted mRNAs have a half-life in excess of 24h (65) and may be stored on mitochondria-associated polysomes, in stress granules, or cytosolic processing bodies (P-bodies) (66). Stress granules and P-bodies are known for their role in RNA degradation; the related GW-bodies are important role for mRNA storage (67). Interestingly, P-bodies have been described to interact frequently with mitochondria (68) and more recently, both miRNAs and Ago2, a key protein in P-bodies, have been identified in mitochondria and mitoplasts, where they have been shown to regulate mitochondrial mRNA translation (69). During muscle differentiation, muscle-specific miR-1 enhanced translation of mitochondrial transcripts. Protein levels of ND1 and COX1 increased during differentiation without a significant increase in mRNA levels or mtDNA copy number. In the absence of the P-body scaffold protein GW182, miRNAs interacting with Ago2 can enhance translation by binding to an mRNA target lacking a 5′cap and 3′poly-A tail, features characteristic of mitochondrial transcripts. As GW182 has not been detected in mitochondria, it seems plausible that miRNAs present in mitochondria may enhance rather than suppress translation of mitochondrial transcripts.

E. The balance between fission and fusion in the mitochondrial response to stress

That both fission and fusion are critical to normal cellular function is highlighted by the lethality induced by knockout of Mfn1/2, Opa1 or Drp1 (70–76) and the severe diseases caused by mutations in these proteins (77–79). Mitophagy is also critical for metabolic remodeling (45). The effects of inhibiting fission, however, have been variable, depending on the method used and cell type. Cardiac-specific Drp1 knockouts show preservation of mitochondrial function, but increased mitophagy and eventual heart failure due to opening of the mitochondrial permeability transition pore (MPTP) (71). In contrast, the Drp-1 inhibitor Mdivi decreases mitophagy and rescues heart failure induced by pressure overload (80). Others have shown that disruption of fission decreases mitophagy but results in the accumulation of dysfunctional mitochondria (35, 50, 81). Thus, any disruption of baseline dynamics appears to be deleterious. However, as all of these studies have relied on genetic manipulation of dynamic regulators, the role of fission in normal “healthy” physiologic adaptation is still unknown (82).

Mitochondrial dynamics may also be coupled to respiratory chain function by altering the structure of OXPHOS supercomplexes, controversial protein superstructures that are thought by some to play a role in vivo regulating respiratory function (83). For example, ablation of the IMM fusion regulator Opa1 results in disruption of normal cristae architecture, impairs the assembly of respiratory chain supercomplexes, and alters mitochondrial metabolism (84). The formation of respiratory supercomplexes may themselves alter Opa1 oligomerization, for example during starvation, resulting in narrowing of the cristae, and increasing ATP synthase activity (85).

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F. Mitochondrial dynamics and human disease

Pathologic alterations in mitochondrial dynamics can result in impaired bioenergetics and mitochondrial-mediated cell death, and are associated with a wide spectrum of pathologies, including ischemic cardiomyopathy, diabetes, pulmonary hypertension, Parkinson's and Huntington's diseases and skeletal muscle atrophy (37, 39, 86–92). Diseases where specific mutations of genes encoding mitochondrial dynamic regulators have been found include Mfn2 in Charcot-Marie-Tooth neuropathy (93) and OPA1 in autosomal optic atrophy (94). Alzheimer's disease has been associated with amyloid-β mediated S-nitrosylation of Drp1 (SNO-Drp1) and resultant mitochondrial fragmentation. Neurons are particularly sensitive to changes in mitochondrial function due to their limited capacity for glycolysis and high level of energetic use. Cardiomyocytes, which have one of the highest volume fractions of mitochondria due to the high energy requirements of rhythmic contraction, are also uniquely sensitive to mitochondrial dynamic alterations. The compact organization of the cardiomyocyte sarcomeric structure has led some to suggest that mitochondrial morphologic changes are minimal in the heart, and that dynamic regulators are mostly important for maintaining quality control (95, 96). Although the degree of mitochondrial fragmentation is less in the cardiomyocyte compared to many other cell types, there is still abundance evidence for active mitochondrial structural dynamics in the heart (37, 97–100). In normal physiology, fission plays a role in the post-natal closure of the ductus arteriosus, where redox sensing in the arterial smooth muscle wall is dependent on Drp1 activation (101). Mitochondrial dynamic alterations also play a role in many cancers, where fusion can reduce the susceptibility of cells to apoptotic signaling, and inducing aerobic glycolysis (the Warburg effect) (102, 103). These alterations are thought to be a direct effect of cancer-causing mutations, a secondary effect of tumor hypoxia, or a potentially a mechanism to enhance cancer cell growth by reducing apoptotic cell death.

G. Alterations in mitochondrial dynamics as a normal physiologic response to stress

Studies in skeletal muscle during exercise suggest a role for mitochondrial fragmentation in maintaining energetic homeostasis, evidenced by an increase in Fis1 (104, 105). During recovery, fusion is activated (increased Mfn1/2), and with chronic exercise, mitochondrial biogenesis (PGC-1α) (106–108). However, as none these studies have examined mitochondrial morphology or function, the data remain incomplete. Similar cycles of fission and fusion induce mitochondrial metabolic reprogramming, as seen after starvation and re-feeding (109). Cardiac mitochondrial biogenesis has been demonstrated in chronic exercise conditioning (110, 111). Our preliminary data in the heart suggest that mitochondrial fission may be a component of the normal adaptation to increased energetic demand, such as occurs during exercise, a process we call "physiologic fragmentation" (112). In contrast to pathologic fragmentation, exercise-induced fragmentation is associated with normal or enhanced, rather than degraded, mitochondrial function, maintenance of normal $\Delta\psi m$ and ROS production, and repression, rather than activation, of mitophagy.

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III. Post-translational modification (PTM) of the mitochondrial proteome (redecorating): regulation of both mitochondrial function and dynamics

A. Role of calcium in mediating mitochondrial dynamics and function

Ca^{2+} is a major regulator of both ATP production and mitochondrial dynamics (113, 114). In organs like the heart, where energy needs can rapidly change, β1-adrenergic receptors (ARs) increase $[Ca^{2+}]_i$ in response to increased workload, mediated through activation of L-type Ca^{2+} channels, increasing extracellular Ca^{2+} import and also by post-translational modification of proteins that control exchange of Ca^{2+} across the sarcoplasmic reticulum (e.g. ryanodine receptors [RyR] and the sarcoplasmic reticulum calcium transport ATPase [SERCA]). Changes in $[Ca^{2+}]_i$ are rapidly sensed by mitochondria, the consequence of peri-mitochondrial microdomains where local $[Ca^{2+}]$ can be many-fold higher than the rest of the cytosol, although this relationship varies dramatically between cell types (115, 116). In excitable cells such as cardiomyocytes, Mfn2 tethers mitochondria to the sarcoplasmic reticulum (30), the microdomain where Ca^{2+}-induced Ca^{2+} release is localized. Additional mechanisms for Ca^{2+}-mediated alterations in mitochondrial structure and function include through PKA-AKAP, Ca^{2+}-induced post-translational modifications and the mitochondrial Ca^{2+} uniporter (MCU) (113, 114, 117, 118) (see below).

Mitochondria also serve as a major buffer for intracellular Ca^{2+}, protecting cells against Ca^{2+} overload, and as a mechanism for Ca^{2+} compartmentalization within cellular microdomains (115). In pancreatic acinar cells, for example, mitochondria prevent the cell-wide propagation of Ca^{2+} signaling away from the apical region (119). Mitochondrial Ca^{2+} uptake occurs through the MCU, but given the low affinity of this uniporter, uptake is dependent on localization of mitochondria near high Ca^{2+} microdomains. Uptake through the MCU is also highly dependent on $\Delta\psi m$, which is in turn driven by mitochondrial respiration, and is inhibited by magnesium, and by the OXPHOS uncouplers dinitrophenol and carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) (120). Mitochondrial Ca^{2+} uptake is also mediated by the Letm1 Ca^{2+}/H^+ antiporter, coupling Ca^{2+} uptake to mitochondrial alkalinization, and functioning at much lower (nanomolar) concentrations than the MCU (121).

Mitochondrial release of Ca^{2+} is mediated in excitable (but not in non-excitable) cells, by the mitochondrial Na^+-Ca^{2+} exchanger, NCX (122), although in pathological conditions, such as ischemia, this exchanger may operate in reverse mode, increasing mitochondrial Ca^{2+} overload (123, 124). Ca^{2+} is also extruded via opening of the MPTP (125), which is usually associated with mitochondrial stress and the initiation of apoptotic cell death. Although ablation of the MCU does not alter mitochondrial morphology, it does reduce exercise capacity (114), suggesting that altering mitochondrial Ca^{2+} does affect oxidative function, at least under stress conditions. Increased Ca^{2+} enhances mitochondrial function by activating pyruvate (126), isocitrate and oxoglutarate dehydrogenases (127). As well, increasing Ca^{2+} increases mitochondrial fragmentation (128) through calcineurin (removing an inhibitory phosphate on Drp1) or CaMK (phosphorylating Ser600) (21, 129). This process, although generally associated with pathologic stress, may also play a role in the response to normal physiologic stresses (see below (112)). In contrast, excessive Ca^{2+} opens

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the MPTP and can trigger cell death (36), highlighting the danger of excessive Ca^{2+}, a consequence of chronic β-AR signaling in diseases such as heart failure (130).

B. Post-translational modifications of the mitochondrial proteome

These have been described during both physiologic (fasting) and pathologic (ischemia/reperfusion) stress. As described above, PTMs of Drp1 are a major regulator of the balance between fission and fusion (19, 21, 24, 131). PTM of mitochondrial proteins differs between low vs. high level stresses, e.g. normal levels of ROS act as a signaling second messenger, but excessive ROS leads to oxidative PTMs (oxPTMs) (132). Phosphorylation is also a regulator of mitochondrial function, including complexes I, IV and V, VDAC and ANT, targeted by β-AR-regulated kinases, e.g. PKA, PKC and GSK3β. Modern proteomic techniques, e.g. multiple-reaction monitoring (133–135), have identified >200 different phospho-transitions of proteins in the TCA cycle, and pyruvate dehydrogenase and branched-chain α-keto acid dehydrogenase complexes (118, 133, 135–138). Bioinformatic site analysis suggests the involvement of PKA, PKC, casein kinase II, DNA-dependent kinase, tyrosine kinase, Src kinase, GSK-3, ERK1/2, and EGFR (118). Several of these kinases and phosphatases are either tethered to or are translocated to the OMM where they target membrane proteins such as Bcl-2, BAD, VDACs 1–3 and TOM70 (118). For example, the A kinase anchoring protein (AKAP) family of scaffolding proteins localize cAMP-dependent PKA to the OMM (139). Finally, there are many mitochondrial proteins with oxPTMs, some of which can modulate the phosphorylation status and activity of target proteins, such as ATP synthase (140–143).

PTM of subunits of ATP synthase (complex V) have also been shown to play a role in enhancing mitochondrial respiration. Many of these PTMs are altered during the metabolic remodeling that is associated with diseases such as heart failure, leading to an increase in anaerobic metabolism and a shift from fatty acid utilization to glucose utilization. Whether this shift represents a component of the more global “recapitulation of fetal pathways” (144) or is a mechanism to improve energy economy (145) is still being debated, and there are even contradictory studies showing an increase, not a decrease, in fatty acid oxidation in human heart failure (146, 147). Phosphorylation of the E1 subunit of the pyruvate dehydrogenase complex by pyruvate dehydrogenase kinase (PDK) results in its inactivation, one of the first demonstrations of the role of PTMs in mitochondrial energetics (148). Pyruvate dehydrogenase phosphatase, which mediates activation of this complex, is regulated by intracellular Ca^{2+} (149). Other Ca^{2+}-mediated phosphatases, targeted to mitochondria, include regulators of branched-chain amino acid catabolism (150).

Post-translational control of mitochondrial function may also be mediated directly by intramitochondrial PKA. Activation of soluble adenylyl cyclase directly in the mitochondrial matrix by $[Ca^{2+}]_{mt}$ has been shown to increase mitochondrial cAMP formation, which in turn increases mitochondrial ATP production (60–63). However, the role of direct mitochondrial PKA in these processes is still debated. The identification of true mitochondrial-associated signaling systems is complicated by the difficulty in separating mitochondrial membranes from mitochondrial-associated membranes containing

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endoplasmic reticulum (ER)-associated signaling molecules, and by conflicting results, as has been the case for the putative mitochondrial angiotensin system (64, 151).

Therapeutic maneuvers can reverse many disease-specific deleterious PTMs. In heart failure, cardiac resynchronization therapy (CRT) induces dramatic changes in the mitochondrial proteome, including increases in expression and PTM of most respiratory chain enzymes, and in metabolic pathway enzymes responsible for replenishing Krebs cycle intermediates, including pyruvate carboxylase, pyruvate dehydrogenase, α-keto acid dehydrogenase, ferredoxin and fatty acid-binding protein (142). Phosphorylation of the ATP synthase-β subunit has been shown to be a major regulator of complex V activity. Additional pathways that were upregulated included proteins that mediate mitochondrial protein synthesis and import, mitochondrial membrane channels, and ROS scavenging proteins such as thioredoxin-dependent peroxide reductase (142).

IV. Metabolic alterations in fuel utilization

Mitochondria, as the major site of ATP production, both respond to fuel availability and determine the extent and type of fuel utilization in the cell. Mitochondria can utilize glucose, fatty acids, amino acids, and ketone bodies for fuel, and there are complex inter-regulatory mechanisms that determine fuel choices. These choices will result in global metabolic alterations to the cell, with far-reaching consequences.

A. Glucose utilization

Glucose may be metabolized through anaerobic glycolysis, siphoned off for the hexose monophosphate shunt, or directed into oxidative phosphorylation in the mitochondria. When oxidative phosphorylation is inhibited (e.g., by oligomycin), the cell rapidly switches to anaerobic glycolysis, resulting in the buildup of lactate. When glucose is directed by the rate-controlling enzyme glucose-6-phosphate dehydrogenase (G6PD) into the cytosol-located hexose monophosphate shunt (also known as the pentose phosphate pathway), it generates reducing equivalents in the form of NADPH and ribose 5-phosphate, an essential precursor for nucleotide biosynthesis. G6PD is activated by NADP+ and inhibited by acetyl-CoA. G6PD is also activated by deacetylation mediated by SIRT2. Hexokinase II plays a key role in glucose utilization: when this enzyme is associated with the mitochondrial outer membrane, glucose is utilized for glycolysis; when it is free in the cytosol, glucose is stored as glycogen (152). A key enzyme complex linking glycolysis to mitochondrial oxidative phosphorylation is the pyruvate dehydrogenase complex, responsible for converting pyruvate into acetyl-CoA in the mitochondrial matrix, where it is used in the tricarboxylic acid cycle for oxidative phosphorylation. The pyruvate dehydrogenase complex is regulated by phosphorylation mediated by pyruvate dehydrogenase kinase, which inactivates it. Calcium affects the rate of mitochondrial oxidative phosphorylation through multiple sites including dehydrogenases, the cytochrome chain, the F(1)F(o) ATPase, and substrate delivery to the mitochondrial matrix (153).

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B. Fatty acid oxidation

As mitochondria can generate ATP from a variety of fuels, the ability to dynamically tune fuel utilization will increase cellular adaptability and is known as metabolic flexibility. The pyruvate dehydrogenase complex and its regulatory kinases and phosphatases are key determinants of metabolic flexibility. Phosphorylation of PDC will shift the mitochondria towards fatty acid oxidation, whereas its dephosphorylation will restore glucose oxidation. The generation of malonyl-CoA from acetyl CoA by acetyl CoA carboxylase will prevent fatty acid oxidation through inhibition of carnitine palmitoyltransferase I, the major shuttle for fatty acids into the mitochondria (154). Thus this critical mitochondrial enzyme complex influences glycolysis, glucose oxidation, fatty acid oxidation, lipid storage, and redox poise.

V. Metabolic output

Production of ATP is the prime metabolic output. Additional consequences of glycolysis and oxidative phosphorylation include shifts in pH and the redox poise. Reactive oxygen species, a byproduct of oxidative phosphorylation will be discussed in the next section.

A. ATP production

Approximately 80% of the high-energy phosphates (ATP and creatine phosphate) in the heart are derived from oxidative phosphorylation of fatty acids, with the remainder coming from glucose oxidation, to a small extent, amino acid oxidation. In ischemia, glycolysis may be called into play, utilizing stored glycogen and generating lactic acid (more on this later). Early reports suggested that amino acid supplementation might be beneficial in the ischemic and reperfused heart (155); replenishing amino acid precursors of Krebs' cycle intermediates (e.g., glutamate, aspartate) was felt to be needed to ensure more effective oxidative metabolism to produce energy for cellular repair and subsequent mechanical function (156); subsequent work showed that glutamate could be metabolized anaerobically to contribute up to 20% of the ATP in the ischemic dog heart (157). A subsequent study using NMR phosphate spectroscopy concluded that supplementation with aspartate/glutamate did not improve myocardial bioenergetics (158). However, the aspartate/glutamate-supplemented group had higher intracellular pH at every time point; while they did not find it to be statistically significant in their small sample size (two pig hearts per group), it may hint at a degree of lactate sparing that could have been shown to be beneficial in a larger sample size. Thus the potential benefit of glutamate in ischemic metabolism might be under-appreciated. More pertinent to the present review, cell metabolism that is well-adapted to support anaerobic metabolism of amino acids could demonstrate greater benefit from amino acid availability. Thus metabolic flexibility is important to ischemic tolerance of the myocardium.

B. Consequences of fuel selection on pH and intracellular Ca^{2+}

Under conditions of normal blood flow, fuel utilization carries little consequence and is likely related to the mix of available substrates. In the failing or borderline ischemic heart, fuel utilization is thought to shift to glucose oxidation as the oxygen cost for ATP production is lower than for fatty acids. However, anaerobic glycolysis comes at a high price beyond its inefficiency (2 ATP per molecule of glucose vs. 30 ATP via aerobic metabolism): lactic acid must be eliminated from the cell; the protons are removed via the Na^+/H^+ exchanger, but the

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increased intracellular Na^+ then competes with Ca^{2+} for extrusion via the Na^+/Ca^{2+} exchanger, resulting in a steady rise in intracellular Ca^{2+}. Calcium doesn't have to rise by much in order to wreak havoc, as acidosis increases the calcium sensitivity of some enzymes. For instance, the actin capping protein gelsolin binds phosphatidylinositol 4,5-bisphosphate (PIP2) in the presence of microM Ca^{2+}, but the Ca^{2+} requirement is reduced further as the pH drops (159). Similarly, phospholipase C activity is increased at low pH in the absence of Ca^{2+} (58). An alternate method for extruding H^+ is via the vacuolar proton ATPase, which can pump the protons into autophagolysosomes or across the plasma membrane. Although it consumes ATP, it is Ca^{2+}-sparing. Ischemic preconditioning, which induces autophagy, activates the vacuolar proton ATPase and limits acidification and calcium overload (160, 161). To the extent that mitochondrial dysfunction (.e.g, in the face of hypoxia) forces the cell to rely on glycolysis, there is the likelihood that calcium overload may ensue. Mitochondria play an important role in calcium homeostasis, as discussed above.

C. Redox poise

Mitochondria determine the relative balance of reducing equivalents in the cell through conversion of NADH to NAD^+ in Complex I. The mitochondria govern redox poise (NAD^+/NADH, $NADP^+$/NADPH) and acetyl-CoA levels; in this way they indirectly regulate countless processes through histone acetyltransferases (HATs) and deacetylases (HDACs) that modify cytosolic enzymes to control their activity, transcription factors such as PGC-1α, and histones whose acetylation status determines chromatin structure and access of transcription factors to specific regions (162). Interestingly, there is a close relationship between histone acetylation and intracellular pH: as intracellular pH decreases, histones are globally deacetylated by HDACs, and the acetate anions are exported along with protons through monocarboxylate transporters. As intracellular pH rises, histone acetylation also increases. Interestingly, inhibition of HDACs or monocarboxylate transporters limits acetate export and results in intracellular acidification, a potentially problematic side effect of HDAC inhibitors (163). Availability of acetyl-CoA will determine the extent of histone acetylation; however, the activity of acetyl-CoA carboxylase will determine whether acetyl-CoA is directed into fatty acid biosynthesis versus leaving it available for histone acetylation (164). The HDACs are influenced by both the ratio and absolute levels of NAD^+; therefore its biosynthesis, particularly the activity of the rate-limiting enzyme nicotinamide phosphoribosyl transferase (NAMPT), is an important determinant of HDAC activity. NAMPT expression can increase by 2–3 fold with fasting or caloric restriction (165).

VI. Reactive oxygen species

Byproducts of oxidative phosphorylation are reactive oxygen species (ROS), predominatly superoxide, which is rapidly dismuted to hydrogen peroxide by superoxide dismutase and thence to water by the action of catalase. Hydrogen peroxide represents 1–2% of oxygen consumed in the process of oxidative phosphorylation.

A. Mitochondria generate ROS but have antioxidant defenses

Mitochondria are both a source and a target of ROS, which are generated in the mitochondria at Complex I, Complex II, and Complex III (166–168). Under ordinary

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circumstances, mitochondrial antioxidant defenses (MnSOD, catalase, glutathione peroxidase) convert the superoxide to hydrogen peroxide and then to water, but this may be impaired in certain settings. First, the production of superoxide may be high enough to overwhelm normal defenses. Catecholamine overstimulation increases mitochondrial ROS production due to signaling through protein kinase A (169) or Ca^{2+} (170). Secondly, normal defenses comprising glutathione and glutathione S-transferase P (GSTP) may be attenuated. Polymorphisms of human GSTP are associated with increased susceptibility to doxorubicin cardiomyopathy (171). A variety of stressors can cause depletion of glutathione, or the failure to regenerate reduced glutathione (GSH) from its oxidized counterpart (GSSG). Importantly, there is a dynamic interplay of ROS, GSH, and mitochondrial Ca2+ handling. In the absence of Ca^{2+}-induced stimulation of the tricarboxylic acid cycle, NADH and NADPH become more oxidized and are unable to regenerate the GSH antioxidant system. This leads to an unstable system wherein small increases in hydrogen peroxide result in exaggerated ROS emission with increased workload (172).

B. Oxidative damage to mitochondrial proteins

Mitochondria undergo reversible S-nitrosylation, in which nitric oxide modifies the thiol moiety of cysteine. S-nitrosylation of mitochondrial proteins has been demonstrated in the setting of cardioprotection where it often inhibits OXPHOS components (173, 174). Interestingly, S-nitrosylation increases the activity of parkin, the E3 ubiquitin ligase important for mitophagy (175). Other forms of oxidative protein modifications such as thiol oxidation to sulfonic or sulfinic acid are less reversible. Nitration of tyrosine and carbonylation of cysteine are additional oxidative posttranslational modifications of proteins, many of which are detected in mitochondria. These lesions are repaired through distinct enzymatic systems including denitrosylating enzymes and mitochondrial proteases (AAA proteases including Lon). Alternatively, the entire organelle may also be eliminated via mitophagy, a process discussed above.

C. Lipid peroxidation

Lipid peroxidation is also a common consequence of mitochondrial ROS generation, often resulting in hydroxyl-alkenals such as 4-hydroxy-2-nonenal (HNE), commonly derived from linoleic acid and arachidonic acid, 4-hydroxyhexenal (HHE), and malondialdehyde (MDA). HHE can trigger mitochondrial permeability transition pore opening. These aldehydes can form covalent adducts on proteins by reacting with lysine amino groups, cysteine sulfhydryl groups, and histidine imidazole groups (176). As a result, they can modify protein structure and in many instances can trigger mitochondrial dysfunction. These aldehydes can form adducts on ethanolamine phospholipids, and unsaturated lipids can undergo lipid peroxidation, thereby altering membrane fluidity with myriad effects on cellular processes.

D. Oxidized mitochondrial DNA

Mitochondrial DNA is a common target of oxidative damage, resulting in oxidation of guanine residues (8-oxoG) at levels 2–3 fold higher than nuclear DNA (nDNA) (177). These are recognized and removed by oxoguanine glycosylase (OGG1), one of the few DNA repair enzymes localized to mitochondria (178). Sirt3 is also reported to interact with OGG1. Deacetylation by Sirt3 prevents degradation of OGG1 protein and controls its incision

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activity (179). Thus, oxidative stress elicits a coordinated repair response including both protein folding and DNA base excision. Failure to clear 8-oxoG lesions will result in mispairing with A, resulting in a G:C→A:T transversion (180). When mtDNA was isolated from fibroblasts of humans of various ages, mutations in the control region were found to be more common in older individuals, although the relationship was far from linear (181). It is unknown whether the age-related mtDNA mutations can be attributed to cumulative damage, to age-related deterioration in DNA repair processes, increased production of ROS, or age-related impaired autophagy. If the mtDNA mutations affect mitochondrial function, they would be cleared by mitophagy. Mitochondrial quality control (fission, fusion, mitophagy, and biogenesis) has been suggested to be sufficient for mitochondrial homeostasis even in the presence of ongoing mtDNA damage and limited repair (182). However, autophagy diminishes with age, which could contribute to the increase in mtDNA mutations with age (183, 184). Dysfunctional mitochondria left behind by inadequate mitophagy might also explain the increased ROS production seen with aging. Thus the extent of mtDNA damage seen with increased age might be a consequence of diminished autophagy in the face of a constant burden of DNA damage over a lifespan. There is abundant evidence linking autophagy to longevity (185, 186). Recently CISD2, a protein localized to mitochondria (187) has been linked to longevity through autophagy and regulation of Ca^{2+} homeostasis (188, 189). One also wonders whether the age:mtDNA damage relationship makes sense, since the very oldest individuals have probably survived that long in part because they had effective quality control mechanisms; one might expect the very oldest patients to exhibit less mtDNA damage. A more comprehensive investigation of the age-relatedness of mtDNA mutations, covering both coding and the control regions of the genome, may reveal additional insights.

VII. Nuclear–mitochondrial cross-talk

Nuclear instructions for mitochondrial biogenesis primarily driven by PGC-1α represent the best instance of nucleus-to-mitochondria signaling. Mitochondria “talk back” to the nucleus, a phenomenon called retrograde signaling, in which mitochondrial signals alter nuclear gene expression. This can occur through the mitochondrial unfolded protein response (UPRmt), through mitochondrial-derived peptides, and through metabolic milieu shifts. In addition to nuclear signaling, mitochondria alter the cellular and metabolic milieu in multiple ways as outlined above and diagrammed in Figure 2.

A. The mitochondrial unfolded protein response

This pathway was first characterized in yeast and *C. elegans* and is under active investigation in mammals. Mitochondria possess machinery for responding to an imbalance between imported nuclear-encoded proteins and mitochondrial-encoded proteins. The correct stoichiometry of OXPHOS subunits is essential for assembly of functional respiratory complexes. Excess or unincorporated subunits are degraded to short peptides by matrix proteases of the AAA+ family (ATPases associated with a variety of cellular activities); Lon protease is responsible for the largest share of intramitochondrial protein degradation. In C. elegans, the peptide fragments generated by the related protease ClpP are extruded into the cytosol through the transporter Haf1. The mammalian homolog is thought to be ABCme10.

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The extruded peptides are recognized by transcription factors CHOP, C/EBPβ, and cJun/AP1, which direct expression of mitochondrial chaperone hsp60 and proteases ClpP, Yme1L1, and MPPβ. Upregulation of these factors reduces protein aggregation in the mitochondrial matrix. Additional proteins upregulated in the UPRmt include Tim17A, NDUFB2, and EndoG, but not ER stress proteins Bip, Grp94, calreticulin, or calnexin (190). PKR (double-stranded RNA-activated protein kinase) phosphorylates eIF2α and cJun, with the resulting suppression of translation and import of nuclear-encoded mitochondrial proteins (reviewed in CM Haynes and D Ron)(191). There is much less known about the significance of the UPRmt in mammalian systems or its connection to mitophagy and mitochondrial biogenesis. However, agents such as chloramphenicol and rapamycin, which induce mitophagy/autophagy, are also reported to be potent inducers of UPRmt (192). Chloramphenicol inhibits mitochondrial protein synthesis, which would result in an excess of nuclear-encoded subunits over mitochondrial subunits and would therefore trigger UPRmt. In contrast, rapamycin, which suppresses cytosolic protein synthesis via inhibition of mTOR, would result in an excess of mitochondrial subunits over nuclear-encoded proteins. Thus an imbalance in either direction is sufficient to trigger UPRmt, which may also be linked to activation of mitophagy. Interestingly, over a decade ago we identified mitochondrial elongation factor Tu (mtEF-Tu) as a major target of phosphorylation during ischemic preconditioning (193). In bacteria, phosphorylation of EF-Tu suppresses protein synthesis. Subsequent large-scale proteomic analyses documented phosphorylation of mtEF-Tu at Thr273 and Ser312 (194). Phosphorylation of these residues in the tRNA-binding region is believed to destabilize tRNA binding and thereby inhibit protein synthesis at the elongation step (195, 196). Thus a kinase signaling pathway activated during preconditioning converges on mitochondrial protein synthesis and the UPRmt. mtEF-Tu is also a target of acetylation at K88 and K256 (197). While the significance of acetylation of mtEF-Tu is unknown, mitochondrial SIRT3 deacetylates mitochondrial ribosomal protein L10 (MRPL10) to suppress mitochondrial protein synthesis (197).

B. Apoptotic signaling

Although this topic has been covered by many reviews (188, 189), it is important to mention the release of cytochrome *c* (190) and endonuclease G (191) as two proteins released from the intermembrane space and matrix, respectively, that trigger programmed cell death. Apoptosis Inducing Factor 1 (AIFM1) is an NADH oxidoreductase in the intermembrane space which also triggers DNA fragmentation, activates caspase-7, and inhibits protein synthesis through interaction with EIF3G (192–196). Permeabilization of the outer membrane to release intermembrane space factors (cytochrome *c* and AIFM1) is governed by the balance of pro-and anti-apoptotic members of the Bcl-2 family (reviewed in (197–199)). Calcium overload can activate calpains resulting in proteolytic activation of proapoptotic factors including Bid and AIFM1 (200, 201). Programmed cell death can also be accomplished via the mitochondrial permeability transition pore (MPTP), a large-conductance channel of the inner membrane that can be activated by Ca^{2+} overload or excessive ROS. Recently MPTP opening was shown to involve dimerization of the F_0F_1 ATPase and regulated by cyclophilin D (202). MPTP opening results in matrix swelling, loss of Ca^{2+} homeostasis, depletion of ATP and pyridine nucleotides, and outer membrane rupture with release of proapoptotic factors (203).

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C. Mitochondrial-derived peptides

These short mitochondrial-encoded peptides represent an additional mechanism by which mitochondria communicate with the nucleus. MDPs are short peptide sequences hidden within the rRNA sequence of mtDNA. Two such peptides, humanin and MOTS-c, have been identified to date, and more may exist within the rRNA sequences (187). These peptides can act on intracellular targets including Bax (198) to suppress apoptosis, but can also act as paracrine factors; reports differ as to the specific receptors responsible (199) but in the case of humanin, signaling through STAT3 has been implicated (200). Within the mitochondria, the a humanin analog has been reported to reduce hydrogen peroxide production (201). MOTS-c has been shown to regulate metabolism and mitigate insulin resistance (202, 203). Both mitochondrial derived peptides are associated with longevity (204, 205).

D. Shifts in the metabolic milieu

Metabolic alterations represent a profound and global mechanism by which mitochondria accomplish retrograde signaling. By altering substrate utilization and electron flow, mitochondria can profoundly alter NADH/NAD^+, or acetyl-CoA, thereby activating sirtuins/HDACS or HATS, respectively, with corresponding epigenetic alterations and global transcriptional responses. Mitochondria are responsible for the lion's share of ROS production, and also regulate Ca^{2+} uptake/release, thereby accomplishing global shifts in various signaling pathways.

E. Impact of differing mitochondrial genomes

Differences in mitochondrial genome remodel the cell through altered metabolic milieu eliciting global changes in the nuclear-encoded transcriptome. Recent work exploring mitochondrial haplogroups (ethno-ancestrally distinct patters of SNPs in mtDNA) in an isogenic nuclear background has revealed the profound importance of relatively modest differences in mtDNA. Different mitochondrial haplogroups have been shown to exhibit differences in mitochondrial oxygen consumption, membrane potential, and mitochondrial number (206, 207). These haplogroup differences also result in broad changes in the transcriptome (208, 209). Moreover, mitochondrial haplogroups also modify susceptibility to disease (210–218). This has been elegantly dissected in a mouse model in which the mitochondrial genome of C3H/HeN mice has been placed into the C57Bl/6J nuclear background and vice versa (219). Using these mitochondrial-nuclear exchange (MNX) mice, Ballinger's group was able to show that the mitochondrial genome determined susceptibility to the pathological stress of cardiac volume overload. Thus, mtDNA, which is maternally transmitted, plays an important role in complex multigenic diseases. It seems likely that the future of precision medicine will be required to take mitochondrial function into account in order to optimize patient-specific therapy.

VIII. Conclusions

Mitochondria adapt to a wide range of cellular requirements, adjusting fuel utilization, meeting ATP requirements, regulating ROS output, and titrating the redox poise of the cell. Each metabolic shift results in a corresponding shift in nuclear chromatin, epigenetic marks, the transcriptome, and ultimately, the proteome. Short-term alterations in energy

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requirements may be met by post-translational modifications or reorganization of mitochondrial structure by fission/fusion, while long-term alterations, especially those due to diseases such as myocardial ischemia or diabetes, are accomplished by mitophagy to eliminate mitochondria ill-suited to the new requirements, followed by biogenesis which often involves alterations in the mitochondrial proteome, accomplished by changes in expression of nuclear-encoded genes. These responses are coordinated across genomes to accomplish a synchronized metabolic remodeling. Although not discussed here, this includes circadian alterations as well as developmental changes or those elicited in response to pathologic conditions. While some may argue that the mitochondrial genome is a tiny tail to wag the dog, one should recall there are ~1000 mitochondria per cardiomyocyte that may all be wagging in synchrony.

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Highlights

- Mitochondrial dynamics: fission and fusion
- Mitochondrial turnover: mitophagy and biogenesis
- Metabolism
- Reactive oxygen species and mitochondria
- Mitochondrial unfolded protein response
- Retrograde signaling

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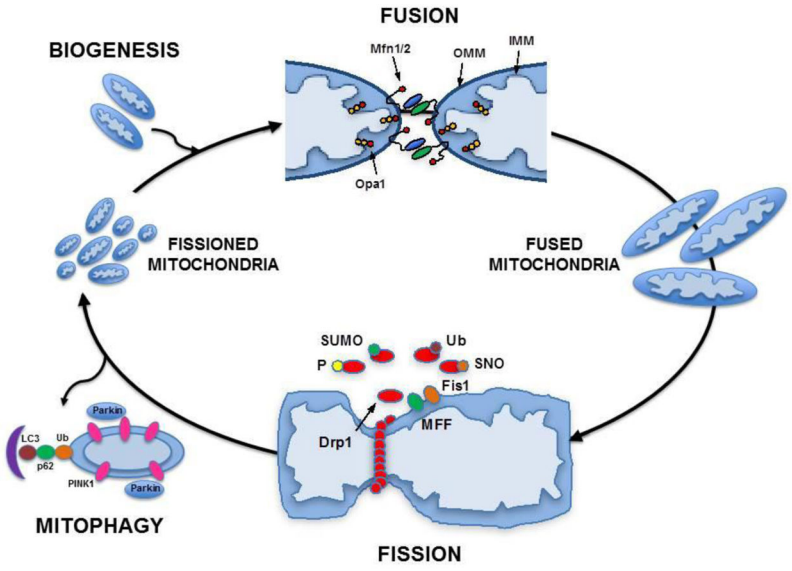

Figure 1. Mitochondrial dynamics

The opposing processes of mitochondrial fission and fusion are both involved mitochondrial quality control and the maintenance of normal cellular homeostasis. **Fission** is regulated by interaction of mitochondrial proteins (Fis1 or MPP) with the cytosolic protein Drp1, after its translocation to the mitochondria fraction. Post-translational modifications (phosphorylation, sumoylation, s-nitrosylation and ubiquitination) regulate this process in response to intracellular cues, including alterations in Ca^{2+}. When fission occurs as a result of pathologic stress, mitochondria with low membrane potential are marked for **mitophagy** by accumulation of Pink1, recruitment of Parkin, and then eliminated through autophagic sequestration, lysosomal fusion and degradation. **Fusion** is regulated by the interaction of mitochondrial inner (Opa1) and outer membrane proteins (Mfn1/2). **Biogenesis** is the process that results in synthesis of new mitochondrial components and is regulated by the PPARγ coactivator (PGC) family of transcriptional coactivators, PGC-1α, PGC-1β, and the PGC-related PRC. (Based in part on Archer et al. NEJM 2013 (91)).

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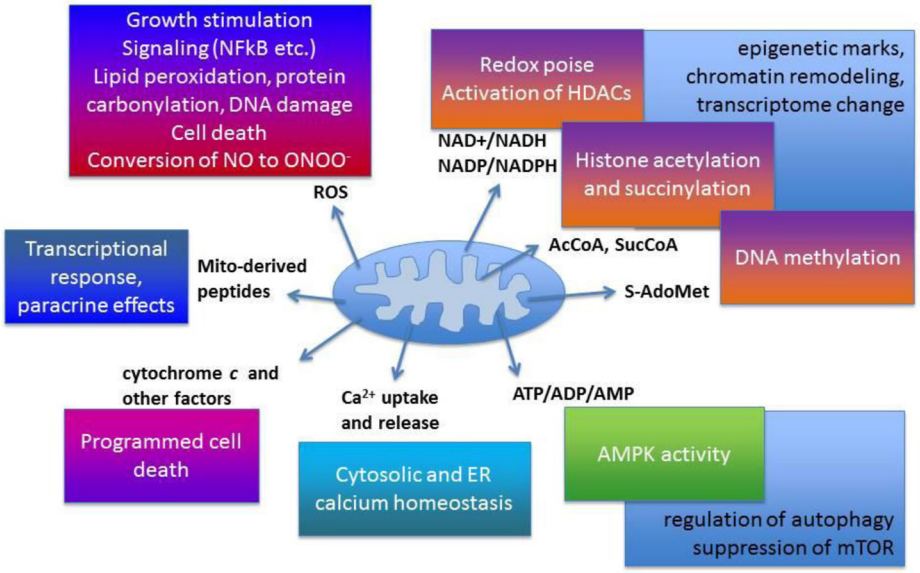

Figure 2. How mitochondria alter the cellular milieu

Mitochondrial ROS production at low levels stimulates proliferation, but at high levels triggers damage to lipids, proteins, and DNA, triggering cell death. Superoxide can interact with NO produced locally or in neighboring cells, generating peroxynitrite and impairing endothelial function. Metabolites including NAD^+, NADP, Acetyl-CoA, and S-adenosyl-methionine affect HDAC activity, histone acetylation and succinylation, and DNA methylation, resulting in chromatin remodeling and transcriptional shifts. The ratio of ATP to AMP regulates AMPK which in turn activates autophagy and suppresses mTOR (when AMP is high). Mitochondria take up Ca^{2+} rapidly and release it more slowly, supporting ER homeostasis and helping to buffer changes in cytosolic Ca^{2+}. Mitochondria can release cytochrome *c* to trigger programmed cell death. Mitochondrial-derived peptides such as humanin elicit transcriptional responses and can have broad paracrine effects. Thus mitochondria impact broad signaling pathways with profound consequences.