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Mitochondrial Dynamics and Heart Failure

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Abstract

Mitochondrial dynamics, fission and fusion, were first identified in yeast with investigation in heart cells beginning only in the last 5–7 years. In the ensuing time it has become evident that these processes are not only required for healthy mitochondria, but also, that derangement of these processes contributes to disease. The fission and fusion proteins have a number of functions beyond the mitochondrial dynamics. Many of these functions are related to their membrane activities, such as apoptosis. However, other functions involve other areas of the mitochondria, such as OPA1's role in maintaining cristae structure and preventing cytochrome c leak, and its essential (at least a 10 kDa fragment of OPA1) role in mtDNA replication. In heart disease, changes in expression of these important proteins can have detrimental effects on mitochondrial and cellular function.

Introduction

Overview, History of Mitochondrial Research

Mitochondria perpetually provide fuel to feed the great energy needs of the heart, which is an elegant, self-repairing pump, which functions nonstop for many, many decades. Altman was the first to note the ubiquitousness of the mitochondria, which after viewing under a rudimentary light microscope, he termed bioblasts. He concluded that the bioblasts performed essential function (s), and even considered that they might be small organisms taken up by other organisms (3). Michaelis was the first to associate redox changes with mitochondria, but did not make the link to energy production (127). The first high resolution electron microcopy images of mitochondria were published in the early 1950's (144). This early work on mitochondria, started from nothing other than intellectual curiosity and a need to understand the natural world, was conducted with rudimentary microscopes in the 1800's, and laid the foundation for subsequent, robust and diverse research on the mitochondrion (51;161).

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Mitochondrial Origins

The endosymbiotic theory was first proposed by biologist Lynn Margulis in the 1960s. Dr. Margulis theorized that mitochondria and their plant counterparts, chloroplasts, arose millions of years ago as a result of the incorporation of prokaryotic organisms by other prokaryotes (116;157). It has been estimated that this event occurred 1.5 giga-years ago (107). Mitochondria have lipid bilayers as membranes and contain circular DNA, like prokaryotes, consistent with this theory. In humans mitochondrial DNA encodes for 13 essential mitochondrial proteins along with tRNAs. Most mitochondrial proteins are encoded by nuclear DNA. It is thought that over time genes migrated to the nuclear genome or were lost. A large amount of energy is required for transcription and translation within the mitochondria, making it inefficient; however, for unclear reasons a subset of genes remain in the mitochondria (107). These genes are primarily involved in oxidative phosphorylation.

Mitochondrial Fission and Fusion Overview

For a long time mitochondria were viewed as important, but static, energy factories, whose main contribution to pathology was over production of ROS. However the mitochondria are now viewed as more dynamic organelles undergoing continuous fission and fusion, which were first described in yeast in the 1990s and found to be essential processes to maintain healthy mitochondria (167). Studies in yeast and cell lines in the 1990's revealed that mitochondria were very active, continuously dividing and fusing (fission and fusion) (136;157). Importantly, mitochondria were found to exist as interconnected networks, rather than isolated energy factories (25;28). Although many studies demonstrated that loss of fission and fusion proteins was detrimental to lethal for the cell, the exact reasons remained obscure (25). Studies on mitochondrial function in biopsies from patients with inherited mutations of fusion proteins, which cause neuropathies, such as Charcot Marie Tooth disease (discussed below), were confusing, as different mutations had different phenotypes, and some seemed to have no phenotype (6). A newly established database of patients with OPA1 mutations should improve our understanding of the associated mitochondrial pathology (54). However, original reports of OPA1 mutations without phenotype cast doubt on the importance of fission and fusion. This issue became moot when Chen et al. demonstrated that mitochondrial fusion was essential for mitochondrial (mt)DNA stability in skeletal muscle cells, providing some of the first information on an essential process impaired by loss of normal fission or fusion (28).

Mitochondrial Fission

Mitochondrial fission and fusion are dynamic events whereby the mitochondria divide and then fuse with other mitochondria. In eukaryotes, a number of proteins have been identified as being involved in fission (fig. 1). Proteins regulating mitochondrial fission in mammalian cells include dynamin related protein (Drp) 1, mitochondrial fission factor (Mff), and fission (Fis)1 (175;201). Fis 1 was originally identified as the homologue of the yeast fission protein, and was thought to be the only protein required for fission in eukaryotes (133). Fis 1 is a tetratricopeptide repeat, which facilitates protein-protein interaction and is important in protein transport and in chaperone and transcription complexes (16). Although Fis1 had been identified as the only protein required for mitochondrial fission, further investigation

revealed that knockout of Fis1 did not impair fission, but knockdown of either Drp1 or Mff compromised mitochondrial fission (143). More recent work suggests that Fis1 has a smaller role in fission, but still participates in this process (111). However, both Fis1 and Mff contribute to the size and number of Drp1 puncta on mitochondria with induction of fission (111). Thus, Fis1 has a lesser role in fission and this may vary by cell type. Fission is more complex in mammalian cells compared to unicellular fungi, and Mff is now recognized as an important mediator of mitochondrial fission.

The 81.9 kDa Drp1 is a member of the GTPase superfamily, and is involved in mitochondrial and peroxisomal division. Drp1 also has a role in apoptosis and in mitochondrial fission for mitosis. Drp1 is a cytoplasmic protein, but at key times it creates complexes at fission sites on the outer mitochondrial mediating fission (175). The 16.9 kDa Fis1's tetratricopeptide repeat motif helps create a scaffold, which facilitates the formation of protein clusters on the outer mitochondrial membrane (181). However, as discussed, Drp1 can complex on the mitochondrial surface in the absence of Fis1, as long as Mff is present. Fis1 has been found to girdle mitochondrial surface, but it does not concentrate at scission sites (181;201). Increased expression of Fis1 increases autophagy, as well as increasing cytochrome c release and apoptosis (64;78;98). Inhibition of Drp1 with P110, which prevents binding of Drp1 to Fis1, but does not effect interactions with Mff or MiD51, ameliorated increased mitochondrial fission after several different toxic stresses (151). Disruption of Fis1 through mutation impairs the removal of damaged mitochondria through autophagy leading to an accumulation of large LC3 aggregates (169). Thus Fis1 has a complex role in maintaining mitochondrial integrity that remains to be fully elucidated.

Mff is a 38.5 kDa protein that has more recently been identified as having a key role in recruiting Drp1 to the mitochondria for fission (58;143). MiD49 and MiD51 also can recruit Drp1 to the mitochondria independent of Mff or Fis1 (145). The increasing number of proteins involved with fission and varying reports of proteins being essential or not essential for fission suggests that there are differences amongst different cell types with regards to the regulation of this important process. It is clear that the regulation of the recruitment and binding of Drp to the mitochondrial surface is complex, and more work will be needed to completely delineate its regulation.

Mitochondrial Fusion

Mitochondrial fusion brings together two different mitochondrion to make a single, larger mitochondrion. In the process, there is mixing of mtDNA, which has been thought to be beneficial (28). Three proteins are essential for fusion (figure 1). Mitofusin (Mfn) 1 and 2 together fuse the outer mitochondrial membrane. Inner mitochondrial membrane fusion depends solely on optic atrophy (OPA)1. Fission and fusion can be impaired under a number of conditions including heart failure and optic neuropathies, including Charcot Marie Tooth Disease and autosomal dominant optic atrophy (ADOA) (76;138;190). Decreased expression of key fusion proteins in ischemic heart failure and the accumulation of small, fragmented mitochondria are consistent with impaired fission and fusion, but no one to date has reported measuring these processes in this setting.

Role of Mitochondrial Fission in Culling Damaged Mitochondria

Once mitochondrial dynamics, with the cycling of fission and fusion events, was identified, many questions arose about its role in the cell (28;104). Although fission and fusion had been shown to be essential processes for cell viability, simple continual mixing of the mitochondrial content would not be sufficient, as dysfunctional mitochondria would be mixed together with normal mitochondria, with no net gain in cellular efficiency and no culling of damaged mitochondria. Significantly, several key studies have delineated how fission can selectively remove impaired mitochondria. Twig et al. were the first to demonstrate that fission events can generate uneven daughter mitochondria with differences in size and membrane potential, $\Delta\psi m$ (187). Others have confirmed these findings, showing that a damaged mitochondrion can undergo asymmetric fission with a smaller, damaged mitochondrion being set aside for removal and the healthier remaining mitochondrion continuing to produce energy (194). Uneven daughter mitochondria have differing fates with one daughter mitochondria with high membrane potential and high probability of fusion and the other with low membrane potential, low probability of fusion, and decreased OPA1. This selective fission leads to removal of dysfunctional daughter mitochondria by autophagy. Twig et al. found a lag time of up to several hours between depolarization and removal of the dysfunctional daughter mitochondria by autophagy (187;188).

It is now established that in the normal cell mitochondria normally undergo fission and fusion, dividing and fusing, and these processes are essential for mitochondrial health (figure 1) (28;33;81;104). Fission and fusion are frequent events in some cells, occurring as often as every two minutes in yeast. Fission and fusion are presumed to take place much less frequently in mammalian cells, taking hours or longer (99;167). There has been skepticism that mitochondria in the densely packed cardiac myocyte would actually experience fission and fusion, but it is widely accepted that fission and fusion are critical processes in all cells (33;45;177). It may be that in complex cells, such as cardiac myocytes, more important than mitodynamics, are the other functions of the fission and fusion proteins, such as in mitophagy, which can remove damaged mitochondria without having ongoing repetitive fission and fusion.

Mitochondria and the Endoplasmic Reticulum (ER)

The ERMES (multi-subunit endoplasmic reticulum-mitochondria encounter structure) tethers the ER and mitochondria together (57). Hamasaki and colleagues demonstrated in mammalian cells that autophagosomes form at contact sites between ER and mitochondria and that ATG14 and ATG5 were present at these sites after starvation (70). ERMES has been localized to sites of mitochondrial division in yeast, but an exact counterpart has not been identified in mammalian cells, although Hamaski's work is an important step (17;70;134). However, inverted formin 2 (INF2, 91), which localizes to the ER, mediates actin accumulation and polymerization between the ER and the mitochondrion at the constriction site, and this is followed by Drp1 recruitment (91). Interestingly, INF2 mutation is associated with the development of Charcot-Marie-Tooth disease accompanied by glomerulosclerosis (190). Mutation of many of the mitochondrial dynamics proteins causes Charcot-Marie-Tooth disease and other related inherited neuropathies (6;190). The

interactions of the mitochondria and the endoplasmic reticulum have not been investigated in the cardiovascular system.

Mitochondrial Biogenesis

Mitochondrial Biogenesis, Fusion and Fission

PGC-1α promotes mitochondrial biogenesis, but PGC-1α's expression was decreased in studies of human heart failure (59;171;193); however others have found PGC-1α to be unchanged (74), or increased (1;85) in heart failure. A moderate increase in PGC1α expression in the heart in mice was associated with increased mortality with TAC surgery and no improvement in mitochondrial function 8 wks. post TAC (82). In fact the PGC1α transgenic mice did worse than non transgenic controls with higher BNP levels, even though expression of PGC1α downstream target genes was improved. Estrogen related receptor (ERR) α, which is not regulated by estrogen, but had some early sequences similarities to estrogen receptors, hence its name, also has a key role in mitochondrial biogenesis. ERRα, which is a downstream target of PGC-1α, is similarly downregulated in heart failure (130;171). mtDNA copy number, a key factor for mitochondrial functional integrity, is decreased in ischemic heart failure (HF, see Table I, (1;83;85). However, one study focusing specifically on dilated, nonischemic cardiomyopathy (DCM) reported higher mtDNA copy numbers in DCM, which evidence suggests is a very different disease from ischemic heart failure (1). Reduced mtDNA copy number corresponded with reduced expression of mtDNA encoded proteins (18;85) Moreover, mtDNA replication was defective in HF (83;85). This is particularly intriguing, given that a 10 kDa fragment of the fusion protein, OPA1, has been identified as being essential for initiation of mtDNA replication, and total OPA1 is decreased in human ischemic cardiomyopathy (ICM or HF, 32;32;47;185).

Mounting evidence supports that ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM), two common types of heart failure, differ significantly from one another at the cell and molecular level, despite the fact that clinically the two diseases are very similar. Investigations of mitochondria in heart failure demonstrate distinct differences between the two. Frequently studies of heart failure combine together DCM and ICM samples for study and variations in the composition of this mix may explain some of the end-point differences noted above. Most convincing is the comprehensive study by Ahuja and colleagues, which identified marked differences between ICM and DCM mitochondria, including greater mtDNA copy number and more mtDNA deletion mutations in DCM compared to ICM (1).

Mitochondrial Structure

Mitochondria constitute 20–40% of the cardiac cellular volume (117). Intact mitochondrial structure is critical for cellular energy production and overall cardiac function. Mitochondria are dynamic subcellular organelles, undergoing fission and fusion events, and constantly move along microtubules to where they are most needed. This is more apparent in neurons, where mitochondria must move down the axons, than in cardiac myocytes. In the 19th century, the morphologists were not sure that they were looking at the same functionally distinct structure in different cells by light microscopy, because of the variability in the

number and shape of mitochondria (3). It was not until the development of high resolution electron microscopy techniques beginning in the 1950's that mitochondrial structure/ morphology could be clearly delineated (79;97).

In adult mammalian cardiac myocytes the mitochondria are intricately organized into three subsets. Most mitochondria are localized between the contractile filaments (interfibrillar), while a minority of mitochondria are localized next to the sarcolemma (subsarcolemmal). A very small amount of mitochondria are perinuclear. In heart failure the interfibrillar mitochondria lose their normal organization (32). Furthermore, size and density of interfibrillar mitochondria decreases in rodent heart failure models (112). OPA1 expression declined in both human and rat ischemic HF, but Mfn1/2, Fis1 and Drp1 are unchanged (32). In contrast, in explanted ischemic failing human hearts, where disease has been present longer due to medical treatment, OPA1 was decreased, Mfn1/2 and Drp1 were increased, and Fis1 unchanged. In dilated, nonischemic cardiomyopathy explanted human hearts had no decrease in OPA1, but an increase in Mfn 1/2 and Drp1(32). Thus, OPA1 expression differs in failing human hearts, depending on type of heart failure. In H9c2 cells, an embryonic cell line, decreasing OPA1 led to more apoptosis at both baseline and after ischemia, through cytochrome c release, consistent with OPA1's role in maintaining cristae tight junctions and preventing cytochrome c release (32;38;56).

Mfn1/2, OPA1 and Mitochondrial Abundance and Structure - Electron microscopic studies revealed more numerous, but smaller mitochondria in a rat model of ischemic HF (32). In contrast, reduced Mfn2, greater Fis1 and reduced OPA1 expression occurred at 18 weeks in a simple rat myocardial infarction model (80). Mfn2 knockout leads to pleiomorphic and somewhat larger mitochondria in cardiac myocytes (146). *In vivo* conditional cardiac Mfn2 knockout mice have a low level of cardiac hypertrophy and minor changes reductions in heart function (146). Overall investigations of Mfn1 and Mfn2 support that each compensate for the loss of the other to a degree, but neither Mfn can compensate for loss of OPA1, knockout of which is embryonic lethal (40;43).

Mitochondrial Structure and Mitochondrial Trafficking

The individual mitochondrion is composed of an inner and an outer membrane. The outer membrane is a selectively permeable membrane, containing integral membrane proteins and pores for transporting molecules. Proteins larger than 5000 Daltons require specific signaling sequences, mitochondria targeting sequence (MTS), to be transported across the outer membrane (2;49;60;192). A consequence of this is that the proteins found in this space differ significantly from the proteins found in the cytosol. Energy produced by the electrons move down the electron transport chain powers the maintenance of the hydrogen ion gradient, which powers the conversion of ADP to ATP. Enzymes needed for the citric acid cycle, along with other essential component including dissolved oxygen, water, carbon dioxide, and the recyclable intermediates that serve as energy shuttles, are part of the mitochondrial matrix.

Cristae

The inner membranes, separating mitochondrial matrix from the intermembrane space, convolute into numerous folds, termed cristae, the site of respiration. The number of cristae is proportionate to the metabolic activity of the cell. Thus very metabolically active cells, like cardiac myocytes, have large numbers of cristae in their mitochondria. Cristae, as they are derived from folding of the inner membrane, expand the surface area and enhance ATP production. As discussed above, a 10 kDa fragment of OPA1 keeps cristae junctions tightly closed, preventing the release of cytochrome c and apoptosis. Dysfunctional mitochondria have been frequently found to have disruption and loss of cristae, such as reported with ischemic heart failure (32;109), with reduction in OPA1 expression (31;152), in Huntington's disease (36), and with reduction in Crif1, as occurs with Alzheimer's disease (9;23).

The Complexes and the ETC

The protein-rich inner mitochondrial membrane remains sufficiently fluid to provide an ideal environment for each distinct, but connected functional complex comprising the electron transport chain (ETC), which provides essential energy to cells via oxidative phosphorylation. The ETC consists of four membrane-bound multi-subunit enzyme complexes (I–IV) and an ATP synthase (complex V). Subunits of complexes I, III, IV, and V are encoded either by the mitochondrial DNA (mtDNA) or, for the majority, by the nuclear DNA (nDNA, 121;150). On the other hand, complex II subunits are encoded by entirely by nuclear genes (174).

Morphologic Changes in HF

Mitochondria can change their overall morphology from elongated interconnected mitochondrial networks to a fragmented disconnected arrangement through fusion and fission (139;139). In ischemic heart failure, mitochondria have increased fragmentation (109;77;176). Others have found a reduction in size and density of interfibrillar mitochondria in rodent models of heart failure (112). Non ischemic heart failure has distinct changes in mitochondria compared to ischemic heart failure (1). Ahuja et al. found distinct differences in mitochondrial morphology and biogenesis and genomic integrity in human ischemic heart failure compared to non ischemic/dilated heart failure. Although mitochondrial dysfunction was present in both types of cardiomyopathy, mitochondria were smaller and increased number in non-ischemic cardiomyopathy vs. both normal hearts and ischemic cardiomyopathy. In contrast dilated cardiomyopathic hearts had a higher mtDNA copy number and mitochondrial density, but a marked increase in mtDNA deletions compared to both normal hearts and ICM hearts (1).

Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 3 (ChChd3) and Cristae Function/ Structure - ChChd3 interfaces with the inner membrane proteins mitofilin and OPA1, which stabilizes cristae morphology, and with the outer membrane protein sorting and assembly machinery (Sam) 50, which is involved in the import and assembly of β-barrel proteins on the outer membrane (38). Knockdown of ChChd3 result in a marked decrease of both mitofilin and Sam50, as well as in several mitochondrial proteins. These results were

interpreted as consistent with ChChd3 being a scaffolding protein, stabilizing protein complexes and retaining cristae conformation and protein import. Thus, ChChd3 would be essential for maintaining mitochondrial structure and function (38). In addition, ChChd3 has been identified as a transcription factor, repressing Bag1 expression (108).

Mitochondrial function

Mitochondria modulate cardiac energetics, reactive radical biology, calcium homeostasis, and apoptosis, which are essential for the function of a normal heart (129;156). The mitochondria are the primary source of the abundant high energy phosphates needed to maintain uninterrupted cardiac contraction, ion flux and membrane potential (197). Protein phosphorylation has been found to be a key element in regulating mitochondrial function and ROS production (41;94).

Energy production

Cellular respiration takes place in the mitochondria, converting biochemical energy from nutrients into adenosine triphosphate (ATP) which powers the cell, followed by the release of waste products. Mitchell in 1961 first proposed that cellular respiration works by chemiosmotic coupling, a chemical reaction that can drive an osmotic gradient (131). The hydrogen atoms that enter the respiratory chain are split into protons and electrons. The electrons pass down the chain via a succession of redox reactions. The energy released by electron flow is used to pump protons across the membrane. The electrical component generates a potential difference in pH, or acidity, with the outside more acid than the inside. The combination of pH and potential difference across the membrane constitutes proton-motive force, which is the force that drives the synthesis of ATP (131;176). Electrons enter the ETC at either complex I: NADH:ubiquinone oxidoreductase or complex II: succinate dehydrogenase, and are passed to complex III: cytochrome bc1 complex by the carrier ubiquinone Coenzyme Q. Cytochrome c carries electrons from complex III to complex IV: cytochrome c oxidase, where they react with protons and oxygen to form water (26;66). In order to function, the heart must rely heavily on oxidative energy produced by the mitochondria. Fatty acids are the dominant energy source for ATP generation in healthy heart muscle. Other energy sources, such as glycolytic metabolism, are only a minor source of ATP in normal cardiac tissue (117;165). Fatty acid β-oxidation and the oxidation of carbohydrates through the TCA cycle produce most of the intramitochondrial NADH and FADH are the primary origin of electrons for the electron transport chain (173). In diseased heart, such as heart failure or acute ischemia, glycolysis is the primary source of energy.

Complex I Dysfunction

Defects of individual ETC complexes or components of the phosphorylation apparatus have been linked to HF. Complex I is crucial for mitochondria energy production. Complex I extracts energy from NADH, produced by the oxidation of sugars and fats. Complex I mutations are a frequent cause of inherited mitochondrial dysfunction, which occur with a frequency of approximately 1 in 10,000 births (186). Complex I abnormalities cause myopathies, encephalomyopathies, and neurodegenerative disorders, including Parkinson's disease and Leigh syndrome (158;189). Complex I defects are most clearly manifest by

neurologic symptoms, as neurologic tissue is highly dependent on energy production, compared to workhorse cell types, such as fibroblasts. Mitochondrial abnormalities are less evident in the heart, likely because significant neurologic abnormalities lead to such global dysfunction, that any cardiac impairments are concealed. A subset of the mitochondrial abnormalities is secondary to mutations in the mitochondrial genome. However, other mitochondrial abnormalities, which are secondary to a decrease in complex I activity or an increase in the production of reactive oxygen species (ROS), are poorly understood. Rats with ischemic heart failure have decreased complex I activity, as well as proteomic remodeling with more than a 50% decrease in protein levels of NADH Dehydrogenase (Ubiquinone) Flavoprotein 1 (NDUFV1) and NADH Dehydrogenase (Ubiquinone) 1 Alpha Subcomplex, 5 (NDUFA5) (109). Complex I dysfunction has been found to lead to increased ROS and protein acetylation, as well as worsening of heart failure (84). In contrast to ischemic heart failure, in a TAC model (mouse) of heart failure based on mitochondrial respiration measurements, complex I activity was not impaired (22). Similarly there was no evidence of complex 1 dysfunction in a rat TAC model of heart failure (168). However, these investigators found a decrease in mitochondrial proteins and respiratory capacity in IFM, but not in SSM mitochondria (168).

Apoptosis

Human heart failure is associated with pathologic cardiac remodeling leading to progressive dilation of the ventricle, increased wall stress and depressed contractility. Apoptosis of cardiac muscle cells is a fundamental process contributing to the progression to heart failure (95;135). It has been shown that mitochondrial dysfunction and apoptosis contribute to the ongoing cell loss in progression of the failing heart (135). OPA1 has a role in protecting cells from apoptosis, at least in part by preventing cytochrome c release from the cristae into the cytosol (32;137). Prohibitins also have a role in regulating cristae structure and OPA1 localization, and thus are indirectly anti-apoptotic (126;142;195).

The mitochondrial death pathway is effectuated by both the intracellular and extracellular death-signals through activation of mitochondrial permeability transition pore (MPTP) formation, which leads to the release of pro-apoptotic proteins, including cytochrome c and apoptosis-inducing factor (AIF), resulting eventually in the disruption of normal mitochondrial physiology (37;61). The Bcl-2 family also controls apoptosis by regulating mitochondrial permeability. The proteins are located on the outer mitochondrial membrane and the anti-apoptotic members of the Bcl-2 family can also inhibit cytochrome c release (21;106).

VDAC and mPTP

The mitochondrial permeability transition pore (mPTP) is a non-selective pore permeable to any molecule < 1.5 kDa between the cytosol and the mitochondrial matrix. Its primary components are the voltage-dependent anion channel (VDAC), the adenine nucleotide translocase (ANT) and cyclophilin D (191). The mPTP is a non-selective pore transporting small molecules (< 1.5 kDa) between the cytosol and the inner membrane. The mPTP opens during conditions of pathologically high $[Ca^{2+}]_m$ (67). An increase in $[Ca^{2+}]_m$ alone is a

relatively inefficacious at triggering pore opening; however, the sensitivity to $[Ca^{2+}]_m$ can be markedly increased by adenine nucleotide depletion, high [Pi] and most significantly, oxidative stress (69). The resulting increased proton permeability leads to a dissipation of the proton motive force as a result of a reduction in both the pH gradient and the membrane potential. Subsequently, mitochondrial ATP synthesis by oxidative phosphorylation drops. Myocytes cannot maintain their ATP levels, therefore undergo necrotic cell death. The mPTP is widely studied in neuronal excitotoxicity, where over stimulation of glutamate receptors leads to excessive calcium entry into the cell (163). It has also been found that decreased NAD+/NADH ratio secondary to complex I deficiency enhances MPTP sensitivity (84). This has important implications for ischemic heart failure, where complex 1 function is impaired.

VDAC

VDAC, provides the aqueous pathway across the outer membrane for the transfer of the substrates generating ATP through oxidative phosphorylation from the cytosol to the inner membrane (100;148). VDAC, with a single pore 2.5–3 nm wide when fully open, allows the flux of metabolites including Pi, ATP/ADP, and Ca^{2+}, across the outer membrane. It is thought that phosphorylation is one factor among many others, that controls the open and closed states of this channel(155). VDAC is reported to be involved in apoptosis of cell lines carrying the mitochondrial A4263G $tRNA^{Ile}$ gene mutation, which is a cause of maternally-inherited hypertension (204). This mutation is thought to lead to mischarging of VDAC secondary to amino acid substitutions. Cell studies of this mutation demonstrated that VDAC associated with Bax and that the mitochondrial membrane potential $\Delta\psi m$ was decreased and apoptosis increased. Cyclosporin A treatment restored $\Delta\psi m$ and decreased apoptosis (204).

Energy production

Mitochondrial Energetics, Respiration and Mfn1 and Mfn2

There is a direct relationship between maintenance of mitochondrial fusion and maintenance of normal mitochondrial function with preserved oxidative phosphorylation (205). Inhibiting mitochondrial fusion results in reduced oxygen consumption (28). Likewise, Mfn2 expression inhibition markedly decreases pyruvate, glucose, and fatty acid oxidation. Intriguingly, skeletal muscle from both obese human and animal models has strikingly less Mfn2 expression (149). In fibroblasts Mfn2 knockdown reduced oxygen consumption and glucose oxidation (14). Compatible with these observations, MEFs with knockout of both Mfn1 and Mfn2 had reduced mitochondrial membrane potential, less respiration, and decreased maximal respiration (29). On the other hand, increased Mfn2 expression results in greater respiratory complex activity, more glycolysis and enhanced mitochondrial biogenesis (149). Similarly, Mfn2 expression increases in settings of intense energy demand, like exercise, and as a response to apoptotic stimuli (29). Hence, Mfn1 and Mfn2 have important effects on mitochondrial respiration and energetics.

Mitochondrial Energetics, Respiration and OPA1

OPA1 has critical role in maintaining cristae structure, and disruption of OPA1 greatly changes cristae morphology (56;124). Furthermore, cristae structure/OPA1 oligomerization varies under different nutritional states (44;56;114). For example, OPA1 is more oligomerized and cristae junctions tighter in states of starvation (124). OPA1 oligomerization also changes with different metabolic substrates.

Complex I and II substrates are associated with much less oligomerization of OPA1 (75). Furthermore, neither rotenone nor the mitochondrial uncoupler, CCCP, affected OPA1 oligomerization, supporting that this is regulated by upstream substrate availability (147). Studies using RNAi to deplete OPA1 in MEFs demonstrated that reduction in OPA1 led to a reduction in basal respiration and loss of the ability to increase oxygen consumption in the presence of the uncoupler 2,4-dinitrophenol (maximal respiration) (29). In studies of the fibroblasts of patients with ADOA, certain OPA1 mutations had impaired complex I driven ATP synthesis, as well as, not unexpectedly, reduced mitochondrial fusion (205). Lodi and colleagues assessed post-exercise phospho-creatinine in the calf muscles of patients with ADOA with OPA1 mutations(heterozygotes for c.2708–2711 deletion TTAG in exon 27, most common OPA1 mutation for ADOA) and controls using phosphor-magnetic resonance spectroscopy (110). With this approach the investigators identified that basal levels of PCr were significantly lower in the OPA1-ADOA patients by about 10%. Furthermore, after exercise the recovery time of PCr levels was markedly prolonged (28 vs. 19 sec, $p<0.01$). Some OPA1 mutations did not alter mitochondrial activity or bioenergetics (122). Thus, there are select OPA1 domains required to maintain normal mitochondrial function. More comprehensive knowledge of post-translational modifications and identification of proteins complexing with OPA1 will lead to greater insight into the mechanism(s) by which select ADOA OPA1 mutations cause disease without impairing measured mitochondrial function.

Mitochondrial Fission Proteins and Mitochondrial Energetics

Mitochondrial fission protein changes can alter mitochondrial metabolism. Drp1 reduction lowered the basal rate of oxygen consumption, reduced coupled respiration, and slowed ATP synthesis (13). Likewise, a dominant negative Drp1 led to a marked reduction in respiratory capacity (187). Hyperglycemia induced fission and cell death, but prevention of this with a dominant negative Drp1 significantly decreased the mitochondria’s ability to increase respiration (202;203). Likewise, Fis1 depletion decreased the maximal respiratory activity, and increasing Fis1 expression restored the phenotype (187). Thus, both DRP1 and Fis1 influence mitochondrial metabolism.

Mitochondrial Protein Trafficking

The outer and inner membrane of mitochondria define the separation from the cytosol and from each other. Ions, nutrient molecules, ATP, ADP, and other small molecules can readily move through the outer membrane. Proteins more than 5000 Daltons in size must have a specific signaling sequence, the mitochondrial targeting sequence (MTS), to be transported across the outer membrane (102). While pores in the outer membrane formed by VDAC make this membrane highly permeable to most small molecules (<4–5 kDa), the inner

mitochondrial membrane is a functional permeability barrier between the cytosol and the mitochondrial matrix. Trafficking of proteins and small solutes in and out of the mitochondria is essential for normal mitochondrial function (figure 2). The traffic of metabolites and ions tightly links mitochondria to the many other cellular activities.

Mitochondrial Protein Importation

The coding capacity of the small mitochondrial genome is quite limited. The mitochondrial genome encodes approximately 1% of mitochondrial proteins. Thus, 99% of mitochondrial proteins are expressed by nuclear genes, synthesized on cytosolic ribosomes and then imported into mitochondria through specialized sorting machineries (164;200). Most synthesized precursor proteins are imported through the translocase of the outer mitochondrial membrane (TOM) complex, and this is facilitated by the chaperones in the aqueous compartments operating along the import pathways (50). Once passed through the TOM channel, the precursor proteins are direct to different sorting machineries by their targeting signals. Almost all proteins imported into mitochondria are targeted to the organelle via a cleavable presequence or mitochondrial transport signal (MTS) (113;159). The MTS is proteolytically removed during import into the mitochondria.

TOM/TIM: the Protein Import Machinery of the Mitochondria

Intensive investigation has led to the identification and structural characterization of the large family of transporters involved in mitochondrial protein trafficking. However work remains to be done, particularly with regards to characterizing the specificity of many of the transporters. TOM/TIM are the major mitochondrial protein import machinery: TOM and TIM complexes is designed to conduct translocation of a polypeptide across both the outer and inner mitochondrial membranes (160). The translocase of the outer membrane (TOM) complex is the main entry gate used by most nucleus-encoded mitochondrial precursor proteins. The translocases of the inner membrane(TIMs) are small proteins localized in the inter membrane space, which form specific aggregates and function as chaperones for unfolded proteins in transit through the intermembrane space (90). The TOM complex and the TIM23 complex in the inner membrane align to form a channel through both the outer and inner mitochondrial membranes to import proteins into the matrix. After entering TOM, the precursor proteins with MTS's are imported by the presequence -TIM23 complex, and then bind to chaperone-like proteins in the intermembrane space, which will remove the MTS.

Role of Heat Shock Proteins in Mitochondrial Protein Trafficking

The HSP70 family members are chaperones for mitochondrial protein import. Macromolecules need to be unfolded for import into the mitochondria and other organelles and the HSP70 group of proteins is responsible for this. HSP75, also known as GRP75, mortalin, mtHSP70, and HSPA9, is an HSP70 family member, which primarily localizes to the mitochondria. HSP75 is also found in the endoplasmic reticulum, the plasma membrane and cytoplasmic vesicles. Schekman originally identified the key role of HSP75 in facilitating the translocation of proteins into the mitochondria (42). Interestingly, HSP75 was significantly decreased in interfibrillar mitochondria in type 1 diabetic heart failure patients (12).

A cytosolic protein is maintained in an unfolded state by association with HSP70 proteins and is thus able to present the MTS to the receptor on the outer mitochondrial surface. When import is initiated, the chaperone proteins are sequentially released as the peptide is transferred into the mitochondrion (162). The dissociation of the chaperone from the imported peptide requires ATP hydrolysis (128). Within the mitochondrial matrix, HSP60 forms a heptameric barrel along with HSP10, which forms the heptameric barrel lid. Within this barrel imported proteins are refolded in a process requiring ATP hydrolysis (119). HSP60 and its prokaryote homologue, GroEL, also protect mitochondrial proteins from denaturation (118;125). In addition, HSP75 has recently been identified as having a key role in assembly of cytochrome c oxidase (complex IV) (19). Thus, heat shock proteins have a critical set of functions with regards to protein import into the mitochondria. First they unfold proteins for transfer, and then other HSPs refold the proteins within the mitochondria. In heart ischemic failure, although mitochondrial levels of HSP60 remain the unchanged, HSP60's overall localization within the cell changes. In the normal heart about 75% of HSP60 is in the mitochondria, with the rest in the cytosol, but in ischemic heart failure, about 8% of HSP60 is located in the plasma membrane fraction (105). At least some of this HSP60 is actually on the surface of the cardiac myocytes, where it may bind toll-like receptor (TLR4), leading to activation of NFκB and production of inflammatory cytokines, including TNFα (87;105;183).

Small Solutes Trafficking in Mitochondria

Ions and small solutes, such as H+,Pi, ADP/ATP, Ca^{+2} are trafficking through mitochondria constantly. The transportation of H+,Pi, ADP/ATP is essential to build the proton gradient and generate energy. Calcium signaling through ion channels is key to cardiac function, regulating the pace and strength of the beat of the heart. In the normal heart mitochondrial calcium concentrations ($[Ca^{+2}]m$) have an essential role in regulating oxidative phosphorylation to keep ATP levels matching the demand for ATP as work load changes. Overall, mitochondrial free calcium is thought to be an important mediator of a range of metabolic activities (154).

Autophagy and Mitochondrial Fission and Fusion

Autophagy, including mitochondrial autophagy (mitophagy), is an essential cellular process for removing irreversibly damaged proteins and organelles (figure 3). There is limited work on autophagy in heart failure, but it does suggest that autophagy is impaired in HF (65;86). The failing heart with increased inflammation and increased ROS would be expected to require a higher rate of autophagy than normal heart. Autophagy, including mitophagy, is an essential cellular process for removing irreversibly damaged proteins and organelles. Autophagy is a highly conserved cellular process responsible for the regulation of cellular degradation. It is referred as a destructive process in which a double membrane envelops cytoplasm and organelles before targeting them to lysosomes for destruction. Ashford and Porter in 1962, first described autophagy as the membrane "shielding the rest of the cell from the general spread of the degradative process" (7). Mitochondria are the center of oxidation and a major source of ROS. Consequently mitochondria are more subject to injury than other organelles, as they are exposed to more ROS than other organelles. Similarly,

mtDNA has greater ROS exposure than nuclear DNA, making it more vulnerable to the threat of mutation than nuclear DNA damage leading to mutation. This may be in part why many genes migrated from the mitochondria to the nucleus, as discussed above. Given the dangerous nature of the damaged mitochondrion, the timely removal of this organelle is vital to maintain normal homeostasis. In recent years, an increasing number of studies have shown that mitophagy is a specific process which involves mitochondrial dynamics, or fission and fusion (196).

Mitophagy is a specialized autophagy governing selective removal of damaged mitochondria by autophagosomes (figure 3). Damaged mitochondria exhibit depolarized membrane potential, triggering accumulation of PINK1, which phosphorylates Mfn2, which then acts as a draw for Parkin (34). Parkin binding Mfn2 triggers mitophagy (figure 3). Others have found a role for mtHSP70 in facilitating the interaction of Parkin and Mfn2 in muscle cells (46). The exact molecular mechanisms involved in the important steps leading to activation of mitophagy remain to be completely defined, and may differ in different cell and tissue types. Following recruitment of Parkin, ubiquitination of OMM proteins, such as Mfn1, Mfn2, and VDAC1 occurs. P62, is recruited and binds the Parkin-ubiquitinated substrates, linking these to LC3 for autophagic degradation. Mitochondria are engulfed after elongation of the isolation membrane. Eventually the autophagosome fuses with lysosomes to form the autolysosomes in which the lysosomal hydrolases degrade the damaged mitochondria.

Mitophagy overlaps with ERMES, discussed above, where tethering of mitochondria to ER leads to formation of autophagosome. Fission is needed to chop up mitochondria to fit in the autophagosome. Twig was the first to show that an asymmetric fission occurred, generating a normal polarized daughter and a smaller depolarized daughter, in which the small depolarized daughter is removed by autophagy (187). Twig et. al observed that fission events often generated uneven daughter units, and one daughter with decreased membrane potential has a lower probability of subsequent fusion. The subpopulation of non-fusing mitochondria generated that were found to have reduced $\Delta\psi m$ and decreased levels of the fusion protein OPA1. Thus fission followed by selective fusion segregates dysfunctional mitochondria and permits their removal by autophagy (187). Twig et. al also found that the probability for fusion is influenced by organelle motility, instead of contact duration and organelle dimensions; a previous fusion event of the individual mitochondrion influenced the likelihood for a subsequent fusion event, as well as the site where the fusion occurred (188).

Cardiomyopathy, Increased ROS, Inflammation and Mitochondria

Heart Failure, cardiomyopathy and ROS/Inflammation

ROS are persistently elevated in both ischemic heart failure and many cardiomyopathies. The mitochondria are considered to be the primary source of the augmented ROS as well as the major target, leading to a concatenation of events with more ROS, more oxidation of mitochondrial proteins, greater mitochondrial dysfunction and the production of even more ROS. Regular fission and fusion are necessary for healthy mitochondria (figure 1) (28;33;81;104). As discussed, a damaged mitochondrion can undergo asymmetric fission with a smaller, damaged mitochondrion being set aside for removal and the healthier remaining mitochondrion continuing to produce energy (194). We have previously shown

that optic atrophy (OPA)1, a critical mitochondrial fusion protein responsible for fusing the inner mitochondrial membrane, is decreased 50% in both rat and human IHF, but not in nonischemic dilated HF (32). Mutations of fusion proteins are associated with inherited optic neuropathies, but have not been previously implicated in heart disease (4;6). This may be because the optic neuropathies associated with fusion protein mutation lead to visual problems and loss of sight. Gradual onset of cardiac impairment is less likely to be noticed as overall activity declines with vision impairment. Knockout of OPA1, modeling one form of ADOA, is lethal. We have shown that the heterozygote mice, hereafter referred to as **OPA1 mice**, at 3 months have increased ROS, decreased mtDNA copy number, and depressed complex IV activity (31). By 12 months they develop a cardiomyopathy with decreased fractional shortening, decreased heart weight/tibia length ratio, depressed LV developed pressure, decreased cardiac output, increased BNP, increased ROS, decreased mtDNA, depressed complex activity (I and IV), decreased state III and FCCP respiration, and loss of contractile reserve (31). These mice had no response to an infusion of isoproterenol. Most striking was decreased expression of a wide range of anti-oxidant genes, all encoded by the nuclear genome. This was seen as early as 3–4 mo and was more pronounced at 12 mo. Protein levels of transcription factors, including Nrf2, involved in regulation of mitochondrial proteins (nuclear encoded) were not changed, but protein levels do not necessarily translate into transcription factor activity.

Mitochondrial Dynamics in Heart Failure

Fission and Fusion in Disease

Mutations involving fusion and fission proteins most often manifest as neurological disease, likely because of the high energy requirements of the nervous system and the high sensitivity of this tissue to any perturbations. Neurologic impairment may mask cardiac abnormalities, as blindness and other neurologic changes reduce activity such that exercise intolerance and other symptoms will not be induced. Charcot-Marie-Tooth (CMT) disease and ADOA, both inherited neuropathies causing blindness, are both associated with mutations in fusion proteins, Mfn2 for CMT and OPA1 for ADOA (6;43), although the list of genes associated with CMT extends beyond fusion proteins. Similarly, Parkinson’s disease, another significant neurologic disease, has been found to have abnormal expression of Mfn2 (104). Parkinson’s disease has also been linked to mutations in OPA1 (24). More unexpected is the decrease in Mfn2, which has been observed in patients with diabetes (11). Mutation in the fusion proteins is associated with cardiac abnormalities, more evident for OPA1 than Mfn1 and 2, each of which can compensate somewhat for the loss of the other. This is discussed further below.

Fission and Fusion in Heart Failure

Since the first report of changes in mitochondrial fusion proteins in ischemic HF, there has been a marked increase in interest in mitochondrial dynamics in heart disease. OPA1 expression was depressed both explanted human hearts and rats with ischemic heart failure (32). In contrast, both Mfn1/2 and Drp1 increased in human dilated cardiomyopathy (32) As discussed above, OPA1 mutation modeling ADOA resulted in a late onset cardiomyopathy, which coincided with the onset of blindness (31) The OPA1 mutant heart has increased

ROS, but decreased anti-oxidant gene expression. Despite increased ROS and impaired mitochondrial function, expression of TFAM, PGC1α, Mfn1/2, Bax, Bak and Nrf2 were unchanged (31). Given that human ischemic HF has a similar decrease in OPA1, these results raise the possibility that human ischemic heart failure is accompanied by similar changes. It is increasingly apparent that abnormalities in fission and fusion contribute to cardiovascular disease (139). Inhibition of mitochondrial fission reduced ischemia/reperfusion injury in the heart (140). As discussed, fission and fusion proteins also have roles in mitophagy and in apoptosis (120). Thus, these proteins have complex functions in the cell beyond mitochondrial fission and fusion.

In neonatal ventricular myocytes exposed to high glucose conditions, mitochondrial fission inhibition using a dominant negative Drp1 mutant (Drp1-K38A), precluded the expected increase in ROS, opening of the mPTP and cell death (202). Cytosolic Ca^{2+} overload, with thapsigargin or potassium chloride treatment, caused a rapid increase in mitochondrial fragmentation in cardiac myocytes (73). Calcium overload occurs frequently in the diseased heart, and this could be expected to lead to increased mitochondrial fission and increased mitochondrial fragmentation. This is consistent with the finding that mitochondria in ischemic HF are small and fragmented. This in itself would suggest loss of the balance between fission and fusion (32), and would be expected to further escalate the energetic abnormalities in HF (81). In the Drosophila heart silencing of OPA1 and mitochondrial assembly regulatory factor (MARF) resulted in increased mitochondrial heterogeneity and dilation of the Drosophila heart tube along with loss of contractility (45). Interestingly, human Mfn1/2 was able to rescue the MARF RNAi induced cardiomyopathy in the Drosophila (45).

Fission Inhibition to Mitigate Injury and HF - Mitochondrial fission has not been studied as a target in ischemic heart failure. In an *in vivo* model of mouse cardiac ischemia, pretreatment with mitochondrial division inhibitor (Mdivi)-1 reduced cardiac injury and preserved mitochondrial elongation and reduced opening of the mPTP (140). Similarly transfection of HL-1 cells with a dominant negative Drp1 reduced mitochondrial fragmentation in HL-1 cells after simulated ischemia (140) In studies of neonatal mouse cardiac myocytes subjected to simulated ischemia, Mdivi-1 treatment cardioprotective with decreased ROS and cell death (166). Parallel studies in a Langendorff rat heart model demonstrated that Mdivi before or after reperfusion was protective, reducing ROS and improving cardiac function, both systolic and diastolic, post-ischemia (166). Interestingly, treatment with the calcineurin inhibitor, FK506, inhibited dephosphorylation at s637, preventing Drp1's mitochondrial translocation and reducing cardiac injury (166).

Despite these promising studies in mitigating cardiac injury associated with ischemia/reperfusion, inhibition of Drp-1 has not been investigated as a treatment for ischemic heart failure. The balance of fission and fusion is thought to be essential for maintenance of healthy mitochondria. Ischemic HF is a chronic disease, and sustained inhibition of either fission or fusion would be expected would be expected to be detrimental. Thus, neither mitochondrial fission or fusion appear to be good therapeutic targets in IHF as this time. Greater understanding of the role of mitochondrial dynamics in the complex cardiac

myocyte may provide new insights into when fission and/or fusion may be dysfunctional in IHF and warrant inhibition.

Cardiomyopathy and Inherited Neuropathies

Fusion protein mutations are a cause of inherited optic neuropathies, which as a rule have a gradual onset. Approximately 20% of Charcot-Tooth-Marie disease type II cases are caused by Mfn2 mutation, and much less often OPA1 mutation leads to CTM (104). In contrast, OPA1 mutations are more often the cause of ADOA, which is characterized by the visual impairment and blindness (40). An OPA1 mutation often associated with ADOA had been found to be homozygous embryonic lethal, and not to cause cardiac abnormalities in the heterozygote, but this was based on basic parameters such as heart weight (40). More intensive investigation with advanced cardiac imaging and functional studies demonstrated that the OPA1 heterozygous mutant mouse develops cardiomyopathy at 12 months (31) Multiple mitochondrial abnormalities were present as discussed above.

Alternate Functions of Fusion/fission Proteins

It is now quite evident that abnormalities in fission and fusion are a factor in cardiovascular disease (139). As detailed above, OPA1 was decreased in ischemic cardiomyopathy, while Mfn1/2 were increased in both ischemic and nonischemic heart failure (32). Deletion of Mfn 2 led to mild cardiac hypertrophy with small functional changes (146). Mfn2 knockout actually slowed opening of the mitochondrial permeability transition pore, protecting cardiac myocytes and leading to better recovery after ischemia/reperfusion (146). Unexpectedly, Mfn2 knockouts had higher levels of the anti-apoptotic Bcl2. Knockout of both cardiac Mfn 1 and Mfn2 was embryonic lethal, while inducible knockdown of the two proteins resulted in greater mitochondrial fragmentation, decreased mitochondrial respiration and a fatal dilated cardiomyopathy (33). Mitochondrial fission inhibition can lessen ischemia/ reperfusion injury in the heart (140). As discussed earlier, both fission and fusion proteins have roles in apoptosis (120). Thus, this group of mitochondrial proteins has cellular functions beyond mitochondrial fission and fusion.

Mfn2

Mfn2 levels are reduced in skeletal muscle both in obesity and in diabetes. Exercise can mitigate some of these changes (10;62). Mfn2 was first identified as the hyperplasia suppressor gene (HSG). Mfn2 inhibited vascular smooth muscle cell (VSMC, from arteries of spontaneously hypertensive rats) proliferation in culture, and also in a rat model of balloon injury (30). Overexpression of Mfn2 results in higher levels of p21 and p27 leading to cell cycle arrest. Thus, increased Mfn2 inhibits VSMC proliferation without increasing the amount of apoptosis (30). Likewise, Mfn2 overexpression inhibited LDL provoked VSMC proliferation and ameliorated atherosclerosis in a rabbit model (68). Hence results of several studies support a significant role for Mfn2 in preventing or reducing vascular disease. Further studies of Mfn2 indicate that this protein has a role in metabolic control, an idea that has been originated by the Zorzano group (206).

Regulation of Expression of Fission/fusion Proteins

Despite great interest in fusion and fission in mammalian mitochondria, work investigating the regulation of expression fission and fusion protein genes has been very limited. The Regulation of Mfn2 expression has been studied, and the Mfn2 promoter has been shown to contain common elements in the human, rat, and mouse Mfn2 promoters. These elements include including the binding elements for NFκB, ERRα, C/EBP and GATA-1 (179). Six Sp1 sites were conserved in the promoters for all three species. Furthermore, the Mfn2 promoter did not contain a TATA box. Finally high levels of CpG islands were present, which would not be expected to be methylated in mammalian cells, and thus would lead to greater activity. Multiple Sp1 sites are present in the Mfn2 promoter, and it has been shown by ChIP assay that Sp1 binds to the Mfn2 promoter in VSMCs (179). Knockdown of Sp1 in VSMCs with shRNA led to much less Mfn2 promoter activity. However, although much is now known about regulation of Mfn2 expression, little is known about regulation of the other proteins involved in mitochondrial dynamics, and they remain to be investigated.

Mfn2 and Atherosclerosis

Mfn2 has been found to possess metabolic properties, as discussed above. In studies of Mfn2 and its possible role in the pre-atherosclerotic artery, using the ApoE-KO mouse on a high fat diet, there was a 50% decline in aortic levels of Sp1 mRNA and a 60% drop in aortic Mfn2 mRNA levels at one week (179). Provocatively, these were transient changes, disappearing after extended treatment. Nonetheless, these results provide intriguing insights into vascular disease and early atherosclerosis.

There is increasing data supporting a link between reduced Mfn2 and vascular disease. Both diabetes and obesity are associated with reduced Mfn2. Sorianello and colleagues identified that a high fat diet led to reduced Mfn2; however this drop in Mfn2 disappeared over time, despite continuing the same high fat diet. Other investigators have demonstrated that both diabetics and the obese have less Mfn2 in their skeletal muscle (10;62). Humans have repeat exposure to high fat meals, instead of a sustained exposure, and this may preclude development of an adaptive response. Exercise has been shown to enhance Mfn2 expression, offering one approach to mitigating the decrease in Mfn2 in diabetes and the obesity (62). Mfn2's positive effects on the vasculature, such as possibly slowing atherosclerosis, make it a strong potential target in diabetes and obesity, both of which greatly enhance the risk of heart failure.

Mitochondria, Free Radicals and Aging

The mitochondrial theory of aging was initially proposed in 1972 by Denham Harman, a pioneer in free radical research. This theory proposes that the rate of aging is a function of the amount mitochondrial free-radical leakage vs. the cell's native ability to repair any resulting damage (71). Aging and aging related diseases can be attributed to mitochondrial free-radical leakage; this is a natural by product of energy production, but greatly increases when there is mitochondrial damage. Free radicals play multiple roles in the cell. Free radicals released from mitochondria can serve to signal respiration, and can cause cellular damage to the nucleus. The deficient respiration signaled by increased free radicals can be

corrected by compensatory changes in the activity of mitochondrial genes. However, if the deficiency is not compensative, the overload of free radicals will oxidize the membrane lipids, and eventually collapse membrane potential (172). The risk of myocardial infarction increases with aging. Although the mortality from myocardial infarction has been reduced through a combination of preventative measures and intervention, heart failure, which can result from coronary disease, continues to have a very high mortality with a 50% five year survival (55;132). Overall there has been an increase in the prevalence of heart failure with better survival from coronary disease (132). It is known that there is an increase in the levels of free radicals, including both superoxide anion and the hydroxyl radical, with cardiac reperfusion, which is a common occurrence in cardiac treatment (5;53;123). During the first minutes of reflow, free radicals increase markedly and remain elevated for a prolonged period after restoration of flow in the coronary (182). Mitochondria have been identified as the major source of free radicals during reperfusion, and a potential site of free radical-mediated dysfunction. During normal cellular metabolism, free radicals are produced at the site of complex I and III (20). Ischemia reduces iron-sulfur and ubiquinone, inhibits complex I (also a source of free radicals in heart failure), and decreases superoxide dismutase activity, which results in increased free radical levels with reoxygenation (182). All of these settings enhance endogenous free radical production, which then compounds any myocardial injury, and this response is increased with aging(92;180).

Conclusions

Mitochondrial fission and fusion are essential processes for maintaining mtDNA and normal mitochondrial function. Changes in the proteins involved in fission and fusion have been reported in a number of diseases, including ischemic heart failure, making them a possible therapeutic target. The fission and fusion protein also have roles in apoptosis and mitophagy. Overall they are essential for maintaining cellular homeostasis. Yet, there are some who are skeptical about the feasibility of repetitive mitochondrial fission and fusion in the densely packed adult cardiac myocyte. However, these processes have been shown essential to maintaining mtDNA and mitochondrial function. Are mitochondrial dynamic processes the only way mtDNA and mitochondrial function are maintained, or are there other mechanisms at work in the heart? Given the high energy needs to the adult heart, the need for these processes would seem greatest in the heart and in the brain. However, processes, such as mitophagy, might substitute for frequent fission and fusion. Much investigation remains to be done to understand the many roles of fission and fusion proteins, some of which extend far beyond the mitochondria. There are many interesting things yet to be explored.

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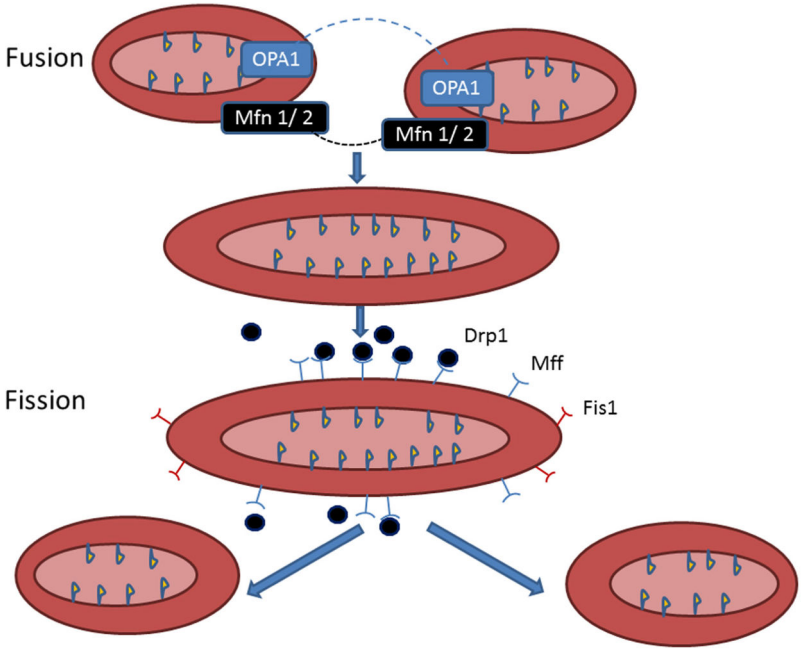

Figure 1.

Mitochondrial Fusion and Fission: Diagram summarizes mitochondrial fusion and fission. Fusion (top panels) - Mitofusin (Mfn) 1 and 2 together fuse the outer mitochondrial membrane. Expression of these two proteins is unchanged in HF. OPA1 fuses the inner mitochondrial membrane, leading to a single, larger mitochondrion, and OPA1 is decreased in HF. Fission (lower panels) - Fission divides one mitochondrion into two smaller mitochondria. Mff recruits Drp1 to the mitochondria for fission. Drp1 is a cytoplasmic protein, but forms complexes at fission sites on the outer mitochondrial mediating fission.Fis1's tetratricopeptide repeat motif helps create a scaffold, which facilitates the formation of protein clusters on the outer mitochondrial membrane, but is not essential for fission, as Drp1 can complex on the mitochondrial surface without Fis1, if Mff is present. Mitochondrial fission and fusion are essential for maintenance of normal mitochondria. A key aspect of fusion in the heart may be asymetric fission, as described by Twig et al. (see text). Asymmetric fission generates a smaller mitochondrion with a decreased $\Delta\psi$ m and a larger mitochondrion with a preserved $\Delta\psi$ m. The small, depolarized mitochondrion is removed by mitophagy. Figure updated from Knowlton and Chen (88).

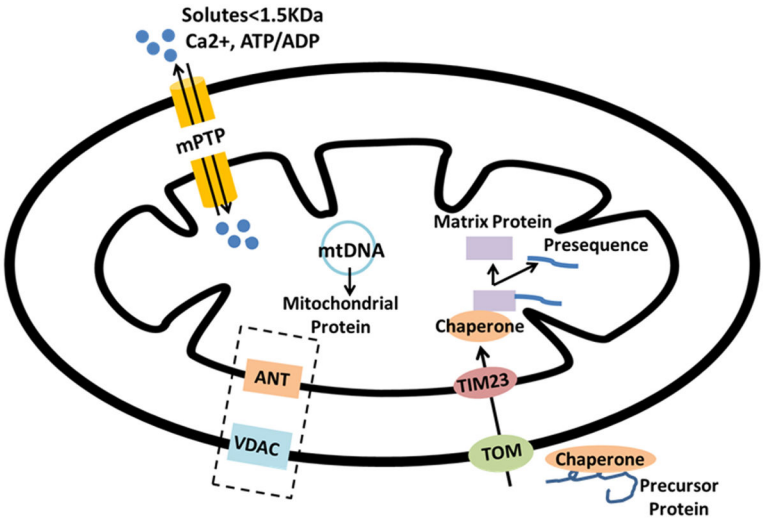

Figure 2.

Mitochondrial Protein Trafficking: Diagram summarizes key pathways for protein and solute import into the mitochondria. 1) The TIM/TOM complex is the major mitochondrial proteins importing machinery. Proteins that are encoded in the nucleus and synthesized in the cytoplasm are transported into the mitochondrion through TIM/TOM assisted by chaperones. 2) VDAC and ANT have roles in small solute trafficking, but in the stressed cell they are part of the mPTP, which opens leading mitochondrial depolarization. 3) mPTP, spanning the IMM and OMM, consists of membranous elements, including VDAC on the OMM, ANT on the IMM. Under conditions like high Ca^{+2} concentration, increased oxidative stress, low ATP, and mitochondrial depolarization, mPTP is allows free diffusion of solutes across the membranes, which ultimately leads to mitochondrial swelling, and apoptosis.

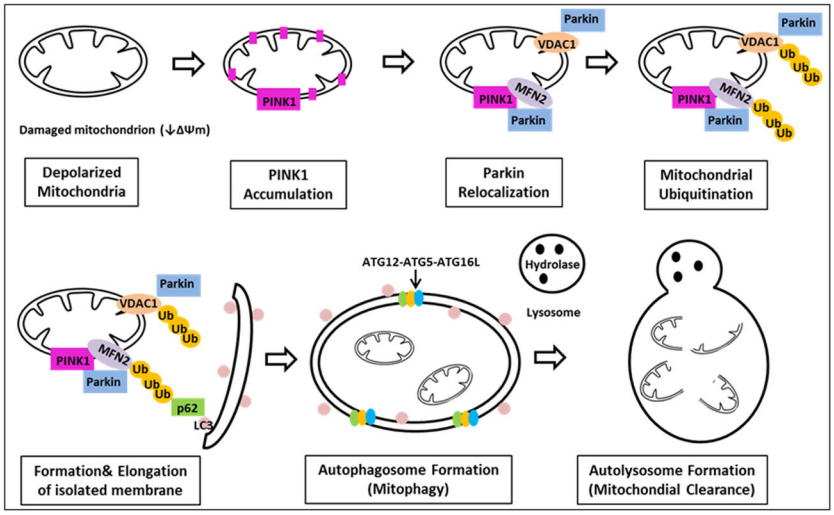

Figure 3.

Mitophagy: Summary of steps involved in mitophagy. The depolarized mitochondrion leads to accumulation of PINK1, which phosphorylates Mfn2, which acts as a lure for Parkin. Parkin binding Mfn2 triggers mitophagy. Outer membrane proteins, including both Mfn's and VDAC, are ubiquitinated. P62 is recruited, binding the ubiquitinated proteins, linking them to LC3. The isolation membrane elongtes and eventually engulfs the mitochondrial pieces destined for autophagy, forming the autophagosome, which eventually fuses with the lysosome, leading to degradation of the enclosed mitochondria.

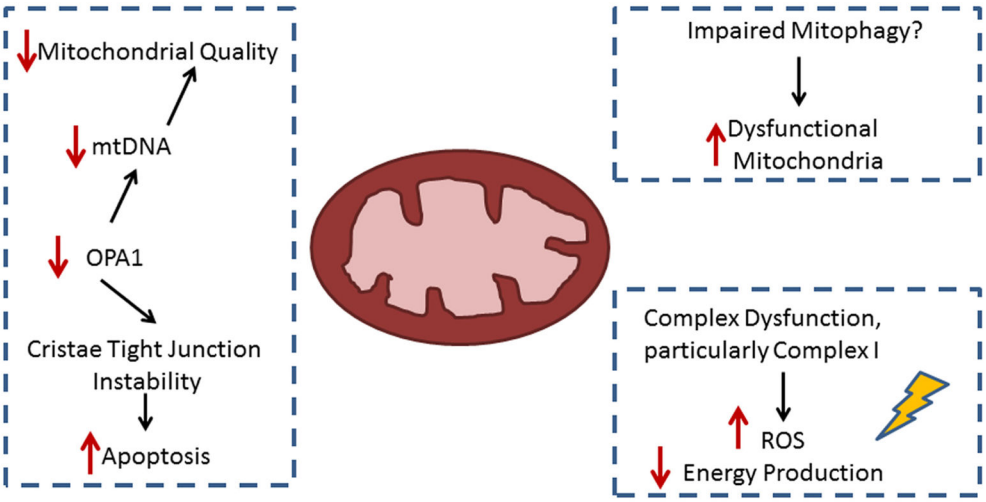

Figure 4.

Summary of Mitochondrial Dynamics and Ischemic Heart Failure. Major findings with multiple studies supporting them are shown. The next few years should lead to identification of more roles for fission and fusion in heart failure.

Table I

Summary of key proteins involved in mitochondrial protein trafficking, mitophagy and dynamics and alterations in their expression/function in heart failure. (Idiopathic) dilated cardiomyopathy (DCM), Ischemic cardiomyopathy (ICM), Hypertrophic cardiomyopathy (HCM), OMM: outer mitochondrial membrane, IMM: inner mitochondrial membrane.

Name	Location	Function	ICM	DCM	TAC and Hypertension Induced HF
Mitochondrial Trafficking					
TOM/TIM	TOM:OMM TIM:IMM	Protein import & sorting		Defect in Pam18 (Tim14) is involved in dilated cardiomyopathy with ataxia (DCMA), by human genome mapping (39).	Tom70 was downregulated in pathological hypertrophic hearts from humans and experimental animals (101).
mtHSP70 (Grp75)	Matrix	Chaperone. Interacts with TIM44 (113) Maintains mtDNA	-	-	-
mtHSP40	Matrix	Chaperone. Maintains mtDNA	-	Mice deficient in Dnaja3 developed DCM and died before 10 weeks of age, at least in part, through its chaperone mtHSP40 activity (72).	-
MPTP	IMM	Non-selective pore for molecules d1.5 kDa	Inhibition of MPTP opening attenuated cardiac ischemic-reperfusion injury in mice (93).	Increased MPTP opening in Drp1 null mitochondria was associated with mitophagy in MEFs and contributed to cardiomyocyte necrosis and dilated cardiomyopathy in mice (178).	Prevention of MPTP opening is protective in patients with obstructive hypertrophic cardiomyopathy (153).
HSP60	Matrix&Cytosol		Increased in IHF in rat and human (89, 105); Abnormal Localization to plasma membrane associated with increased apoptosis (105). Mt HSP60 unchanged in rat IHF(109). Decreased HSP60 in LV cytoplasmic fraction and increased HSP60 in LV mitochondrial fraction in LV (105, 170). Increased in blood in human ICM (115).	Levels of hsp60 doubled in DCM (89). Decrease of HSP60 level in cytoplasmic fraction and increase of HSP60 in LV mitochondrial fractions (170). Increased HSP60 protein level and mRNA level in patients with DCM (95).	
Autophagy/Mitophagy					
Parkin	Cytosol	Protein degradation		Dysfunction of PINK-Parkin interaction leads to dilated cardiomyopathy in Mfn2-deficient mouse embryonic fibroblasts and cardiomyocytes and in Parkin-deficient Drosophila heart tubes (34).	-

Name	Location	Function	ICM	DCM	TAC and Hypertension Induced HF
P53	Matrix&Cytosol	Transcription factor regulated by cell stress, including DNA damage and hypoxia. Regulates genes involved in cell cycle arrest, DNA repair, senescence, apoptosis		Inhibition of p53 leads to dilated cardiomyopathy in adult mice cardiomyocytes (198) Accumulation of P53 leads to DCM through CaMKIIδ in mice (184).	
PINK1	OMM	Mitophagy	-	Dysfunction of PINK-Parkin interaction leads to dilated cardiomyopathy in Mfn2-deficient mouse embryonic fibroblasts and cardiomyocytes and in Parkin-deficient Drosophila heart tubes (34).	$PINK1^{-/-}$ mice develop left ventricular dysfunction and evidence of pathological cardiac hypertrophy as early as 2 mo of age (15)
P62	Cytosol	Mitophagy	Increased in human cardiomyocytes from patients with ICM (35).	Increased in human cardiomyocytes from patients with DCM (35).	Expression of p62 was decreased in Mdivi (mitochondrial division inhibitor) treated TAC mice compared to controls (63). Fission inhibition ameliorates development of HF after TAC (63).
Fission & Fusion					
OPA1	IMM	Fusion	Expression of OPA1, was decreased in in rat and human ICM (32).	No difference in human DCM (32).	-
Mfn1	OMM	Mitofusins, mitophagy	No difference in ICM rat. Increased in human ICM (32).	Increased in human DCM (32).	-
Mfn2	OMM	Mitofusins, mitophagy Mfn2 functions as a mitochondrial receptor for Parkin and is required for quality control of cardiac mitochondria	No difference in IHF rat. Increased in human ICM (32).	Increased in human DCM (32).	Mfn2 was decreased in phenylephrine induced hypertrophy in neonatal rat ventricular myocytes, hypertrophied hearts from spontaneously hypertensive rats, and mice with pressure-overload induced hypertrophy by TAC (1 and 3 weeks). Mfn2 was not decreased in hypertrophied hearts after 15 weeks of TAC, nor in hypertrophied non-infarcted myocardium following MI (52)
Drp1	OMM	Fission	No difference in IHF rat. Increased in human ICM (32).	Increased in human DCM (32). Conditional cardiomyocyte-specific Drp1 ablation evoked mitochondrial enlargement, lethal dilated cardiomyopathy, and cardiomyocyte necrosis (178).	Drp1 was phosphorylated in pressure overload induced hypertrophy in TAC mouse hearts and phenylephrine (PE)-treated rat neonatal cardiomyocytes (27). Mutation of Drp1 leads to myocyte hypertrophy (8).
Fis1	OMM	Fission	No difference in IHF rat. No difference in human ICM (32).	No difference in DCM (32).	
Oxidative Respiration					

Name	Location	Function	ICM	DCM	TAC and Hypertension Induced HF
NDUFA5	IMM	Complex I ETC	Decreased in mitochondria in LV of IHF rat (109).		
NDUFV1	IMM	Complex I ETC	Decreased in mitochondria in LV of IHF rat (109).	Decreased in patients with DCM (141).	Decreased significantly in TAC mice, and restored in TAC after stem cell treatment (199).
Mn-SOD	matrix	Free-radical scavenger		The null mice develop dilated cardiomyopathy and die within 10 days after birth (103).	Zn–SOD was markedly decreased in Copper Deficiency mice which leads to hypertrophic cardiomyopathy (48).