

# Mitochondrial centrality in heart failure

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**Abstract** A number of observations have shown that mitochondria are at the center of the pathophysiology of the failing heart and mitochondrial-based oxidative stress (OS), myocardial apoptosis, and cardiac bioenergetic dysfunction are implicated in the progression of heart failure (HF), as shown by both clinical studies and animal models. In this manuscript, we review the body of evidence that multiple defects in mitochondria are central and primary to HF progression. In addition, novel approaches to therapeutic targeting of mitochondrial bioenergetic, biogenic, and signaling abnormalities that can impact HF are discussed.

**Keywords** Mitochondria · Heart failure · Apoptosis · Bioenergetics · Molecular biology

## Introduction

The failing heart encompasses a complex phenotype that includes reduced myocardial contractility, diminished capacity to respond to specific hypoxic and oxidative stresses (OSs) resulting from myocardial ischemia and diverse neurohormonal stimuli, changes in ion channels and electrophysiological function, increased myocardial fibrosis, cellular and subcellular remodeling with increased myocyte loss, and marked changes in myocardial bioenergetic reserves and substrate utilization. These alterations generally are present at both the tissue and cellular level.

Observations from animal models of heart failure (HF), as well as from clinical studies, suggest that mitochondrial bioenergetic defects may underlie several of the aforementioned aspects of the HF phenotypes. In addition to their contribution to bioenergetic function, mitochondria are integrally involved in regulating intracellular  $\text{Ca}^{2+}$  flux, myocyte cell death and remodeling events, in ROS generation and antioxidant response, and in furnishing cardioprotective responses to physiological insults.

The overall centrality of the mitochondrial organelle in HF (depicted in Fig. 1) with its manifold roles in bioenergetic production, sensor and transport, as well as its effect on ROS generation and signaling, cell death,  $\text{Ca}^{2+}$  levels, and contractility has often been missed and is the focus of this review. Here, we will discuss the mitochondrial changes that occur in HF with emphasis on mitochondrial bioenergetics and biogenesis, ROS and OS, and their downstream effects on myocyte function (i.e., contractility and stimuli response) and cell death (i.e., apoptosis and necrosis). Such a discussion requires a larger view of mitochondria within the cellular context including its interactions and cross-talk with other organelles in the development of HF. In addition, we will present current approaches to assess the role(s) that mitochondria play in the failing heart including observations from experimental animal models, the use of transgenic models, gene profiling analysis, *in vitro* studies with isolated cardiomyocytes to identify pathway components in HF and to pinpoint potential targets for therapeutic intervention.

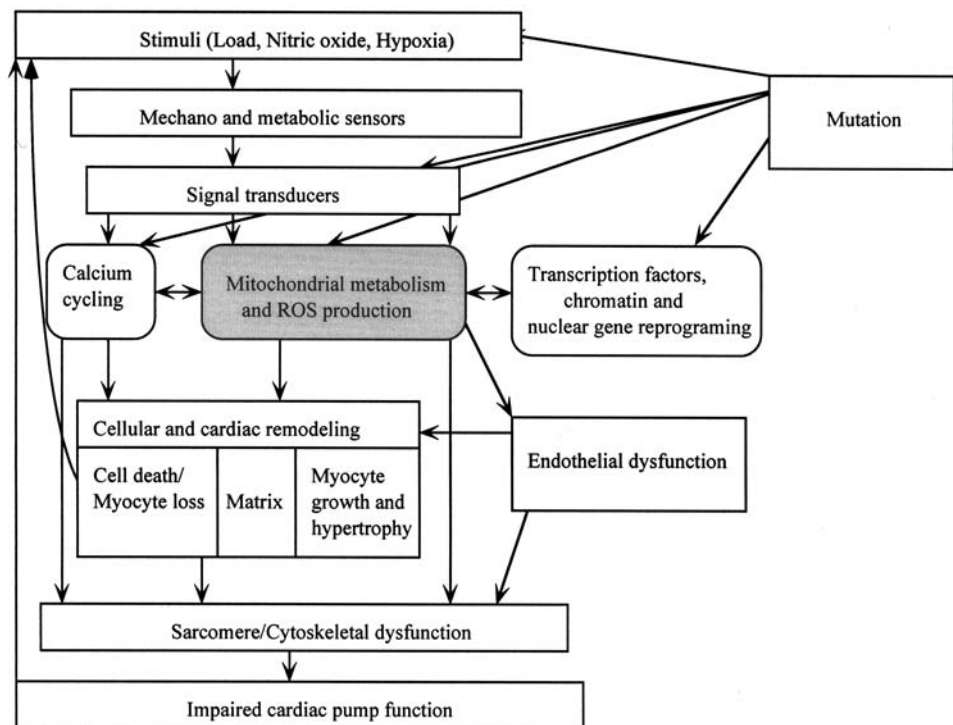
## Bioenergetics

The role of fuel supply, and in particular ATP levels are critical for myocardial contractility and electrophysiology

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**Fig. 1** Centrality and interactions of mitochondrial energy metabolism with molecular triggers and cellular pathways leading to heart failure



[1–5]. The primary ATP-utilizing reactions in the myocyte involve actomyosin ATPase in the myofibril, the  $\text{Ca}^{2+}$ -ATPase in the sarcoplasmic reticulum (SERCA), and the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase in the sarcolemma. ATP produced by mitochondrial oxidative phosphorylation (OXPHOS) is used preferentially to support myocyte contractile activity [6]. Indeed, mitochondria appear to be clustered at sites of high ATP demand and are organized into highly ordered elongated bundles, regularly spaced between rows of myofilaments and in contact with the SR. Moreover, structural contacts between the SR and mitochondria have been revealed by electron microscopy, and there is compelling evidence of coordination between these organelles at the level of  $\text{Ca}^{2+}$  homeostasis and regulation of ATP production [7].

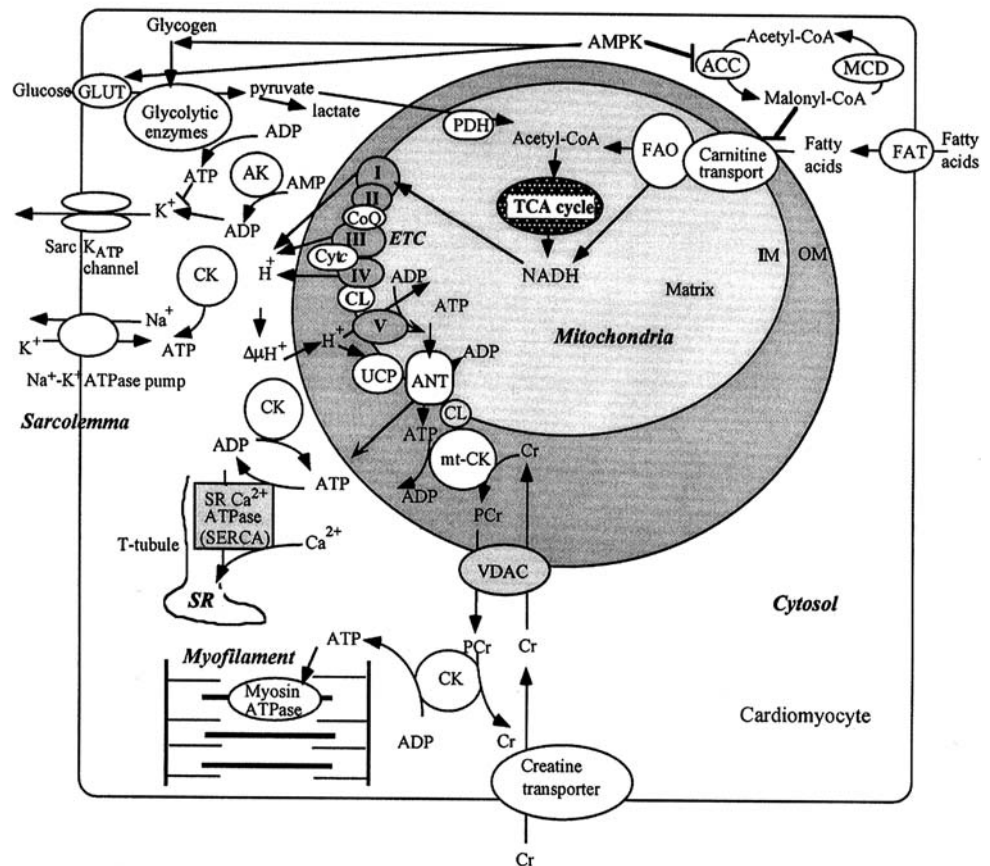
Fatty acids are the primary energy substrate for heart muscle ATP generation by mitochondrial OXPHOS and the respiratory chain, the most important energetic pathway providing over 90% of cardiac energy. The supply of ATP from other sources such as cytosolic glycolytic metabolism is limited in normal cardiac tissue. In addition, mitochondrial-localized fatty acid  $\beta$ -oxidation (FAO) and the oxidation of carbohydrates through the matrix-localized TCA cycle generate the majority of intramitochondrial NADH and FADH which are the direct source of electrons for the electron transport chain (ETC) and also produce a portion of the ATP supply (Fig. 2). In addition, the heart maintains stored pools of high-energy phosphates including ATP and phosphocreatine (PCr). The enzyme creatine

kinase (CK), which has both mitochondrial and cytosolic isoforms, transfers the phosphoryl group between ATP and PCr at a rate estimated to be 10-times greater than the rate of ATP synthesis by OXPHOS [8]. Under conditions which increase ATP demand in excess of ATP supply such as in acute pump failure in ischemia, utilization of PCr via the CK reaction is an important mechanism that maintains steady myocardial ATP levels. Another relevant enzyme-mediated transfer system dedicated to maintaining ATP levels involves adenylate kinase (AK) transferring phosphoryl groups among adenine nucleotides. In addition, the adenine nucleotide translocators (ANT) are a family of inner membrane proteins that exchange mitochondrial ATP for cytosolic ADP, providing new ADP to the mitochondria while delivering ATP to the cytoplasm for cellular work.

The concept of an energy-starved or deficient myocardial phenotype has been buttressed by the use of powerful analytical technologies such as nuclear magnetic resonance (NMR) spectroscopy and positron emission tomography (PET) [1–5]. Moreover, this concept has significant clinical implications in the management of patients with HF since pharmacological interventions that reduce metabolic demand, such as ACE inhibitors, angiotensin blockers, and  $\beta$ -blockers improve clinical outcomes, and conversely agents that increase metabolic demand, such as positive inotropic drugs, are less effective in improving outcomes and often increase mortality [2, 3].

Observations from patients in HF have shown reduced activity levels of mitochondrial bioenergetic enzymes

**Fig. 2** Mitochondrial bioenergetic pathways and their interplay with critical cellular sites of myocardial ATP utilization



including selected respiratory enzymes and mt-CK [9–12]. Moreover, the failing human heart has a 25–30% decline in ATP levels as gauged from observations with human biopsy specimens [13], and by  $^{31}\text{P}$  NMR spectroscopy [14]. Data from animal models of HF have shown that the loss of ATP associated with a loss in the total adenine nucleotide pool in the failing myocardium is slow and progressive suggesting that a decline in ATP content might only be detectable in the severely failing heart [15].

In animal models of HF, the total pool of cardiac creatine, phosphorylated by CK to form PCr, is reduced by as much as 60% [16, 17] and the magnitude of creatine depletion correlated with the severity of HF in patients [18]. Similarly, PCr levels as measured by  $^{31}\text{P}$  NMR and PCr/ATP ratios were significantly lower in subjects with dilated cardiomyopathy (DCM) and HF [19]. Even at moderate workloads, a decrease in PCr/ATP ratio has been consistently reported in the failing human heart and in experimental HF, and is a strong predictor of cardiovascular mortality in patients with DCM (even better than LV ejection fraction) [20]. While PCr levels or PCr/ATP are not specific markers of HF (since they also decline in compensated left ventricular hypertrophy), they are markers of mismatch between ATP supply and ATP demand for utilization. Potential mechanisms responsible for the

decline in PCr include reduced number or activity of the creatine transporter [21], and altered CK expression/function [22].

In transgenic mice, targeting of genes associated with mitochondrial bioenergetic function can also lead to cardiomyopathy and HF. For instance, mutational inactivation of the heart/muscle isoform of *Ant1* gene in transgenic mice will result in the development of skeletal myopathy and cardiomyopathy leading to HF [23]. The *Ant1* gene deficient mice exhibit mitochondrial abnormalities including a partial deficit in ADP-stimulated respiration, consistent with impaired translocation of ADP into mitochondria in both skeletal muscle and heart. *Ant1*<sup>-/-</sup> mice also exhibit a progressive cardiac hypertrophic phenotype coincident with the proliferation of mitochondria [24]. This mitochondrial biogenic response may be a compensatory mechanism to correct the energy deficit, but could also be contributory to cardiac remodeling. Interestingly, null mutations in either the mitochondrial or cytosolic CK gene in mice also lead to increased LV dilation and hypertrophy [25, 26]. Recently, Palmieri et al. have reported the presence of a recessive mutation in the heart/muscle specific isoform of ANT1 in a patient with HCM and mild myopathy with exercise intolerance and lactic acidosis [27]. This mutation resulted in complete loss of adenine nucleotide

**Table 1** Specific nuclear gene defects in mitochondrial metabolism leading to clinical cardiomyopathy and HF

Gene loci	Protein function	Cardiac phenotype
SCO2	Cytochrome <i>c</i> oxidase (COX) assembly	HCM
NDUFV2	Respiratory complex I subunit	HCM
NDUFS2	Respiratory complex I subunit	HCM
ANT	Adenine nucleotide transporter/mtDNA maintenance/PT pore component	HCM
ACADVL	VLCAD activity (FAO)	HCM/DCM
FRDA	Mitochondrial iron import	HCM/FA
COX10	Cytochrome <i>c</i> oxidase (COX) assembly	HCM
COX15	Cytochrome <i>c</i> oxidase (COX) assembly	HCM
TAZ (G4.5)	Tafazzin (FAO)	DCM/Barth syndrome
HADHA/HADHB	Mitochondrial trifunctional protein (FAO)	DCM

FAO: Fatty acid oxidation; FA: Friedreich ataxia; HCM: Hypertrophic cardiomyopathy; DCM: Dilated cardiomyopathy

transport function and was associated with increased levels of muscle mtDNA deletions.

Furthermore, clinical evidence indicating that mitochondrial bioenergetic metabolism may be critical and primary in the development of HF has come from the identification in individuals with a variety of cardiomyopathies of specific nuclear gene defects in mitochondrial/metabolic proteins including ANT, respiratory complex enzyme subunits, molecules involved in complex IV assembly and FAO enzymes (Table 1). Specific mtDNA gene defects associated with clinical CM/HF (often with associated neuropathy) have also been identified in tRNA genes [28], although mutations in structural genes such as ATP6/ATP8 and *cytb* (mitochondrial genes encoding subunits of complexes V and III, respectively) can also lead to CM/HF [29–31].

Hence mitochondrial bioenergetic pathways including ETC, OXPHOS, the TCA cycle, and FAO are crucial for the myocardial intracellular ATP-requiring pumps that control sarcomeric contractile functioning, calcium cycling, and membrane ion transport. While increasing evidence suggests that multiple deficits in these pathways likely contribute to the bioenergetic decline in observed human HF, indicative of a programmatic shift in bioenergetic production and utilization, it remains unclear when and how this occurs.

#### ROS generation and antioxidant response

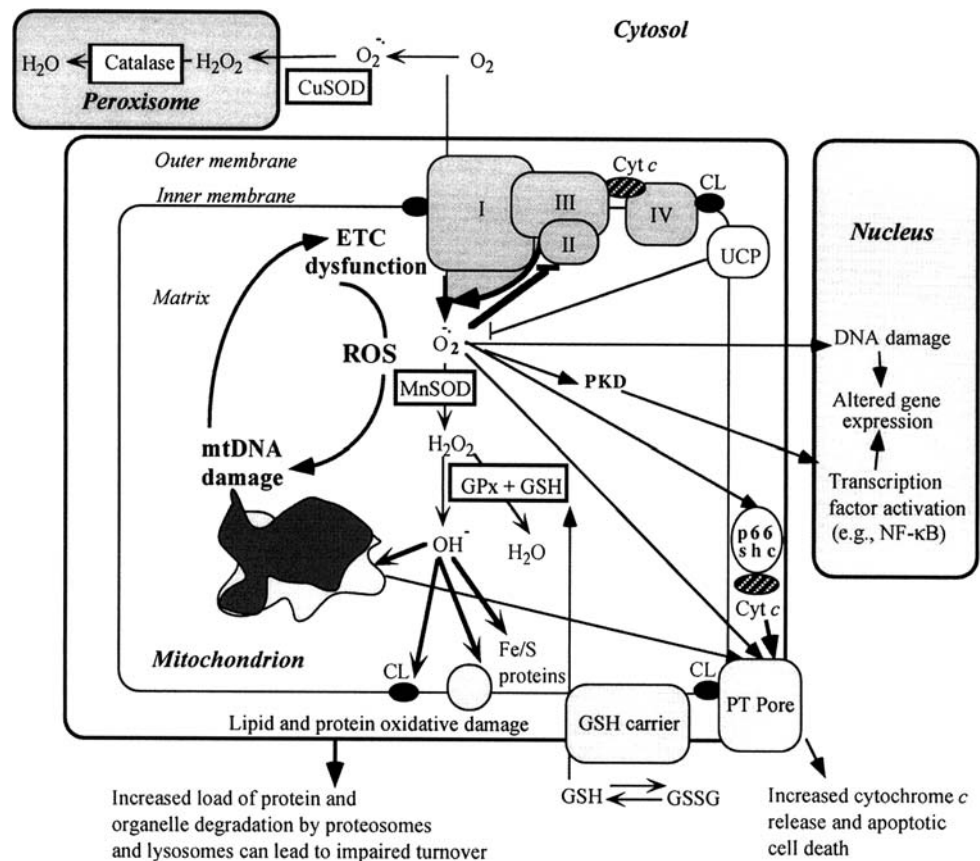
One attractive hypothesis for the mitochondrial contribution to HF relates to its role in ROS production. The generation of a majority of intracellular ROS including superoxide and hydroxyl radicals, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a by-product of normal mitochondrial metabolism and bioenergetic activities (Fig. 3). Side reactions of mitochondrial respiratory enzymes (primarily complexes I and III) with oxygen directly generate the superoxide anion radical; either excessive or diminished electron flux at

these sites can stimulate the auto-oxidation of flavins and quinones (including coenzyme Q) producing superoxide radicals. The superoxide radicals can react with NO to form peroxynitrite, which is a highly reactive and deleterious free radical species or can be converted by superoxide dismutase (SOD) to H<sub>2</sub>O<sub>2</sub> that can further react to form highly reactive hydroxyl radicals. The high reactivity of the hydroxyl radical and its extremely short physiological half-life of 10<sup>-9</sup> s restrict its damage to a small radius from its origin since it is too short-lived to diffuse a considerable distance. Mitochondrial-generated ROS can lead to extensive oxidative damage to macromolecules such as proteins, DNA, and lipids particularly targeting proximal mitochondrial components including mitochondrial respiratory enzymes, matrix enzymes (e.g., aconitase), and membrane phospholipids such as cardiolipin. There is also evidence that mitochondrial ROS damage affects a wide spectrum of cardiomyocyte functions including contractility, ion transport, and calcium cycling. Mitochondrial ROS also plays a role in cell signaling (e.g., in triggering cardioprotective pathways) and in the transcriptional activation of select nuclear genes eliciting a novel transcriptional programming (Fig. 3).

Limited data from animal models of HF have shown an increase in hydroxyl and superoxide radicals. With murine HF created by ligation of the left anterior descending coronary artery for 4 weeks, Ide et al. [32] found that LV dilatation and decreased contractility were accompanied by significant increases in levels of hydroxyl radicals and lipid peroxides. Moreover, the infarcted LV from mice exhibited diminished activity of respiratory complexes I, III, and IV (enzymes each containing subunits encoded by mtDNA) while the mitochondrial enzymes encoded only by nuclear DNA (e.g., citrate synthase and complex II) were unaffected. Ide et al. [33, 34] using electron spin resonance (ESR) spectroscopy to directly assess ROS levels in the canine model of pacing-induced HF, demonstrated a significant increase in superoxide anion and hydroxyl radical levels in paced myocardial submitochondrial fractions.



**Fig. 3** Molecular and subcellular events leading to ROS and oxidative stress. Damage to proteins, lipids and mtDNA caused by mitochondrial-generated ROS including  $H_2O_2$ , superoxide ( $O_2^-$ ), and hydroxyl radicals ( $OH^-$ ), leads to PT pore opening and apoptotic cell death, mitochondrial enzyme dysfunction, and nuclear DNA damage as well as modulated DNA repair and gene expression in the nucleus. Also shown are antioxidant factors in peroxisomes (catalase), mitochondria (GPx, GSH and MnSOD) and in the cytosol (CuSOD) that function to scavenge ROS and reduce their impact



These studies suggested that the elevated myocardial ROS production was secondary to the functional block of electron transport, resulting from a marked decrease in mitochondrial respiratory complex activities (primarily complex I) and also documented a significant positive correlation between myocardial ROS levels and abnormal LV contractility.

However, direct measurement of short-lived ROS is extremely difficult and several laboratories (including our own) have reported increased levels of ROS-mediated damage (e.g., lipid peroxidation, DNA and protein oxidation) as an indirect index of ROS/OS in several animal models including pacing-induced HF [35–37]. Left ventricles from paced animals exhibited increased aldehyde levels and marked reductions in the activity of respiratory complexes III and V together with increased levels of large-scale mtDNA deletions [37].

While elevated ROS activation in the failing heart has been shown to arise from both mitochondrial and extra-mitochondrial sources, the role of endogenous antioxidants in ameliorating myocardial OS and the dynamic balance between these counteracting forces should be considered. It is well known that both cytosolic antioxidant enzymes (e.g., catalase, SOD1/CuSOD) and mitochondrial-localized antioxidants including SOD2/MnSOD, thioredoxin, and glutathione peroxidase can reduce ROS levels. Moreover,

thioredoxin and thioredoxin reductase form an enzymatic antioxidant and redox regulatory system implicated in the regeneration of many antioxidant molecules, including ubiquinone, selenium-containing substances, lipoic acid, and ascorbic acid [38].

Among the most compelling evidence supporting a primary role for mitochondrial ROS and OS in cardiomyopathy and HF are findings with antioxidant genes in transgenic mice. Strains harboring null mutations in either MnSOD or in TrxR2 encoding mitochondrial thioredoxin reductase exhibit DCM and HF [39–42]. Li et al. [42] found that mice homozygous for MnSOD deficiency resulted in early neonatal death from severe DCM, and metabolic acidosis. Moreover, these strains displayed severe reduction in myocardial succinate dehydrogenase (complex II) and aconitase (a TCA cycle enzyme) suggesting that MnSOD is required for maintaining the integrity of mitochondrial enzymes susceptible to direct inactivation by superoxide.

Mice in which MnSOD-deficiency was targeted to skeletal muscle and heart (i.e., H/M-*Sod2*<sup>-/-</sup> strains), displayed progressive congestive HF with depressed cardiac contractility by 8 weeks, cardiac enlargement by 16 weeks, and death from HF by 22 weeks [42]. Cardiac pathology was associated with specific defects in mitochondrial respiration (i.e., severely reduced respiratory complex II

and moderately reduced complexes I and III activities) and in myocardial mitochondrial ultrastructure. Immunoblot analyses showed significant expression of the SDHA and SDHB subunits from myocardial complex II H/M-*Sod2*<sup>-/-</sup> mice, with moderate suppression of complex I  $\alpha$  9, Rieske iron–sulfur protein and Core I subunit of complex III, and  $\alpha$  and  $\beta$  subunits of complex V. Mitochondrial superoxide production was also significantly higher in these mice as was mitochondrial (but not cytosolic) lipid peroxidation suggesting that oxidative damage was specifically localized in mitochondria. In addition, myocardial ATP production and content were significantly diminished, which may account for the absence of energy-dependent apoptosis in H/M-*Sod2*<sup>-/-</sup> mice. This study also offered further evidence that ROS and OS are intimately linked to the progression of cardiomyopathy/HF, since the administration at 8 weeks of age of the antioxidant MnSOD mimetic (MnTBAP) significantly improved cardiac contractility and ameliorated the overall phenotype.

Overexpression of the antioxidant glutathione peroxidase in transgenic mice inhibited the development of LV remodeling and failure after myocardial infarct (MI), and was associated with the attenuation of myocyte hypertrophy, apoptosis, and interstitial fibrosis [43]. Moreover, Schriener et al. [44] have demonstrated that overexpression of catalase (primarily a peroxisomal-localized enzyme) targeted to the mitochondria increased overall mouse longevity, diminished OS and ROS-mediated mitochondrial protein and mtDNA damage, and delayed the onset of aging-mediated cardiac pathology including subendocardial interstitial fibrosis, vacuolization of cytoplasm, variable myofiber size, hypercellularity, collapse of sarcomeres, mineralization, and arteriosclerosis, changes commonly observed in elderly human hearts, and often found in association with congestive HF. Another important finding was that it mattered in which subcellular compartment the over-expressed catalase was localized with little evidence of benefits from nuclear or peroxisomal-localized as compared to striking benefits of mitochondrial-localized catalase activity.

In clinical cases, a clear link between OS/ROS and chronic ventricular dysfunction has only been established in anthracycline-mediated and alcoholic cardiomyopathies. In contrast, it remains unclear whether ROS or OS have a pathophysiologic role in the vast majority of patients with congestive HF or cardiomyopathy due to ischemic, hypertensive, valvular, or idiopathic causes [45]. Recently, superoxide anions as assessed by EPR with an O<sub>2</sub><sup>-</sup> spin trap were reported to increase more than 2-fold in the failing ventricular myocardium from patients with end-stage HF undergoing transplant [46]. Moreover, despite increased MnSOD mRNA levels, a marked decline in mitochondrial-localized MnSOD protein and activity was detected. Both

increased ROS levels and decreased antioxidant response would be expected to lead to enhanced OS in the failing heart, which in turn may result in increased transcription of antioxidant enzymes. That excessive ROS/OS in HF may serve as a potent trigger for changes in specific nuclear gene expression may also underlie the programmatic shift in mitochondrial bioenergetic function previously discussed, as well as acting as a catalyst in myocyte remodeling.

#### Apoptosis and necrosis

The overall role that apoptosis play in HF has not been definitively established. Currently, there is only limited morphological evidence that significant cardiomyocyte apoptosis occurs in MI or at any stage of HF. Apoptotic rates are higher after human MI (ranging from 2% to 12%) than in end-stage (NYHA class III–IV) human HF (range 0.1–0.7%) [47, 48]. While the rate of apoptosis is quite low when viewed in absolute terms, when the relatively low rates are viewed in the context of months or years, the actual chronic cell loss attributable to apoptosis (particularly among non-dividing myocytes) could be substantial. Moreover, apoptotic measurements represent only the number of cells undergoing apoptosis at a single point in time and the accurate assessment of true rates and their consequences remain to be established.

The mitochondrial-mediated intrinsic apoptotic pathway features an extensive dialog between the mitochondria, the nucleus, and other subcellular organelles as depicted in Fig. 4. The release of several mitochondrial-specific proteins from the intermembrane space including cytochrome *c*, endonuclease G (EndoG), apoptosis inducing factor (AIF), and Smac are central to the early triggering events in the apoptotic pathway including downstream caspase activation, nuclear DNA fragmentation, and cell death [49]. The release of EndoG and AIF, and their translocation to the nucleus, specifically promote nuclear DNA degradation, even in the absence of caspase activation [50, 51]. The release of both Smac and cytochrome *c*, which required modification in the mitochondrial organelle to become apoptotically active, are involved in cytosolic caspase activation, i.e., Smac binds and inhibits endogenous cytosolic signaling complexes (e.g., IAPs) that modulate apoptosis, thereby promoting caspase activity. Interestingly, Smac is highly expressed in the heart. In the cytosol, cytochrome *c* binds Apaf-1 along with dATP and promotes procaspase-9 recruitment into the apoptosome, a multi-protein complex resulting in caspase activation [52].

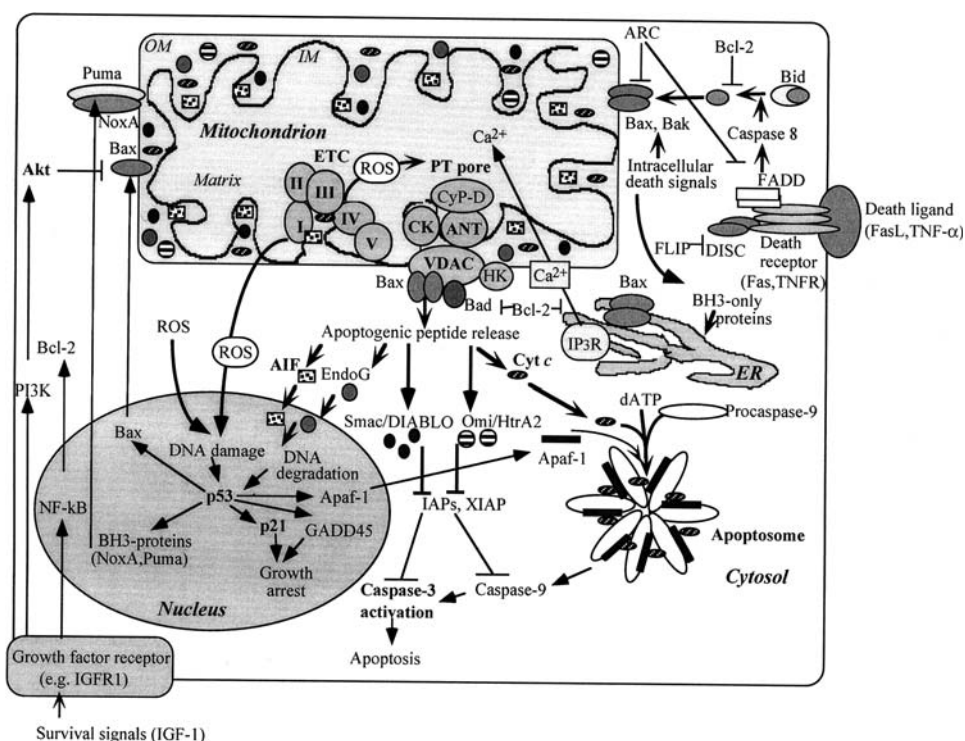
The release of these mitochondrial peptides involves the permeabilization of the outer membrane mediated by proapoptotic cytosolic factors Bax, Bak, and tBID. In

response to both external pro-death signals largely provided by the extrinsic apoptosis pathway (e.g., ischemia/hypoxia, TNF- $\alpha$ , and Fas ligand) and nuclear signals (e.g., p53), these factors translocate to mitochondria where they bind outer membrane proteins (e.g., VDAC) and can initiate outer membrane channel formation [49].

Protein release from the intermembrane space and cristae, where the majority of cytochrome *c* is localized, also is associated with opening of the voltage-sensitive PT pore located at the contact sites between inner and outer membranes. Opening of this non-specific pore is responsive to mitochondrial membrane potential ( $\Delta\psi/M$ ), mitochondrial ROS and  $Ca^{2+}$  overload, pro-oxidant accumulation and NO [53]. The peptide composition of the PT pore while still controversial is thought to involve several key players in mitochondrial bioenergetic metabolism, including ANT, mt-CK, the outer membrane porin

(VDAC), and inner membrane cyclophilin-D. Opening of the PT pore promotes significant changes in mitochondrial structure and metabolism, including increased mitochondrial matrix volume leading to mitochondrial swelling, release of matrix  $Ca^{2+}$ , altered cristae, and cessation of ATP production secondary to the uncoupling of ETC and dissipation of  $\Delta\psi/M$  [54]. Therefore, cytochrome *c* efflux appears to be coordinated with activation of a mitochondrial remodeling pathway characterized by changes in inner membrane morphology and organization, ensuring complete release of cytochrome *c* and the onset of mitochondrial dysfunction, which might further contribute to the HF phenotype [55].

Opposing the progression of this apoptotic pathway, antiapoptotic proteins (e.g., Bcl-2), localized to the outer mitochondrial membrane, either directly compete with or impede proapoptotic factor activity, stabilizing



**Fig. 4** The intrinsic and extrinsic pathway of apoptosis. An array of extracellular and intracellular signals triggers the intrinsic apoptotic pathway, which is regulated by proapoptotic proteins (e.g., Bax, Bid, and Bak) binding to the outer mitochondrial membrane leading to outer-membrane permeabilization and PT pore opening. Elevated levels of mitochondrial  $Ca^{2+}$  as well as ETC-generated ROS also promote PT pore opening. This is followed by the release of cytochrome *c* (Cyt *c*), Smac, EndoG, and AIF from the mitochondria intermembrane space to the cytosol and apoptosome formation (with Cyt *c*) leading to caspase 9 activation, DNA fragmentation (with nuclear translocation of AIF and EndoG), and inhibition of IAP (by Smac), further stimulating activation of caspases 9 and 3. Bax- and Bid-mediated mitochondrial membrane permeabilization and apoptogen release

are prevented by antiapoptotic proteins (e.g., Bcl-2). Also shown are the major proteins comprising the PT pore, including adenine nucleotide translocator (ANT), creatine kinase (CK), cyclophilin D (CyP-D), and porin (VDAC). The extrinsic pathway is initiated by ligand binding to death receptors leading to recruitment of FADD and DISC which stimulates the activation of caspase 8 resulting in caspase 3 activation and Bid cleavage (a C-terminal fragment of Bid targets mitochondria). FLIP and ARC can stem this pathway's progression at specific points. Intracellular stimuli trigger ER release of  $Ca^{2+}$  through both Bax and BH3-protein interactions. Also depicted is the survival pathway triggered by diverse survival stimuli mediated by growth factor receptors, transcription factor activation (e.g., NF- $\kappa$ B) and enhanced expression of IAPs and Bcl-2

mitochondrial membranes and their channels thereby preventing mitochondrial disruption and inhibiting PT pore opening. Similar to their roles in transducing upstream signals to the mitochondria, proapoptotic proteins appear to relay upstream death signals to the ER triggering  $\text{Ca}^{2+}$  release which in turn can rapidly accumulate in mitochondria promoting PT pore opening [49]. Moreover, as shown in Fig. 4 endogenous myocardial factors including ARC whose regulation during HF is of great interest can target discrete loci impacting mitochondrial-based apoptotic progression and preserving mitochondrial function [56]. In addition, prosurvival factors from growth factor signaling pathways (e.g., IGF-1) can inhibit the progression of the apoptotic pathway as well as hypoxia-mediated cardiomyocyte mitochondrial dysfunction albeit their precise mechanism of action is unknown [57].

Recently, it has been suggested that myocardial apoptosis is not a feature of end-stage disease. Akyurek et al. [58] have reported the presence of higher Bax levels in mild HF compared with moderate or severe HF suggesting that cardiomyocytes are more prone to apoptosis in earlier stages of the disease. This susceptibility to apoptosis showed a close correlation with echocardiographic and hemodynamic data indicative that apoptosis may have a role in the transition from mild to end-stage HF.

While myocardial apoptotic induction is frequently associated with abnormal mitochondrial respiratory function, the basis of this relationship remains unclear. Targeting apoptotic factors has been shown to reverse the cardiomyopathy/HF phenotype and associated mitochondrial dysfunction underscoring the interconnected roles of apoptosis and mitochondrial dysfunction in HF. Bcl-2 proteins attenuate p53-mediated apoptosis in cardiomyocytes [59], increase the  $\text{Ca}^{2+}$  threshold for PT pore opening, decrease mitochondrial  $\text{Ca}^{2+}$  efflux due to  $\text{Na}^{+}$ -dependent  $\text{Ca}^{2+}$  exchanger in mouse heart mitochondria [60], and inhibit hypoxia-induced apoptosis in isolated adult cardiomyocytes [61]. In desmin-null transgenic mice, which develop cardiomyopathy and HF as well as defective mitochondrial function, there is extensive cardiomyocyte loss in focal areas, and decreased cytochrome *c* levels in heart mitochondria [62, 63]. Bcl-2 overexpression in these desmin deficient mice dramatically ameliorated the cardiomyopathic phenotype, restored ETC function and changed the mitochondrial sensitivity to  $\text{Ca}^{2+}$  exposure [64]. Furthermore, Bcl-2 family proteins are potential regulators of mitochondrial energetics [65]. During ischemia, when mitochondrial ETC and ATP generation are inhibited because of lack of oxygen, the mitochondrial F1F0-ATPase (complex V) runs in reverse and pumps protons out of the matrix while glycolytic ATP is consumed in an attempt to restore  $\Delta\psi/\text{M}$ . Bcl-2 has been demonstrated to reduce the rate of ATP consumption

during ischemia by inhibiting the F1F0-ATPase. Imahashi et al. have reported that transgenic mice overexpressing Bcl-2 in the heart displayed a decreased rate of ATP decline during ischemia as well as reduced acidification, suggesting that Bcl-2 may provide myocardial protection by inhibiting consumption of glycolysis-generated ATP by the F1F0-ATPase [65].

Another link between apoptosis and mitochondrial respiratory function was established with the demonstration that mice containing a cardiac-specific deletion of AIF developed severe DCM, HF and decreased ETC activities, particularly of complex I [66, 67]. In addition, isolated hearts of AIF-deficient animals developed poor contractile performance in response to ETC-dependent energy substrates, but not in response to glucose consistent with the concept that impaired heart function in AIF-deficient mice results from abnormal ETC function. These results also suggest that AIF similar to cytochrome *c* serves an essential (albeit largely uncharacterized) role in OXPHOS. With apoptosis, mitochondrial loss of both AIF and cytochrome *c* impacts ETC and promotes AIF nuclear translocation and DNA degradation, revealing another side of nuclear-mitochondrial cross-talk.

Other types of cell death including necrosis are evident in the failing heart at similar (and in some cases greater) numbers [68–70]. In contrast to apoptosis, necrotic cell death is not energy-requiring and exhibits characteristic features that include swelling of the cell and its organelles, extensive mitochondrial disruption, blebbing and ultimately irreversible disintegration of the plasma membrane. Necrotic disruption of the plasma membrane leads to release of cellular content into the extracellular space (e.g., release of CK from necrotic myocardial cells), which promotes further inflammatory reaction and subsequent damage or death of neighboring cells. Many of the morphological differences between apoptotic and necrotic processes appear to result from caspase action that is unique to apoptosis, although there is a rather fine line between apoptosis and necrosis that complicates their differentiation. Honda et al. have shown that in the absence of phagocytic cells (to remove damaged apoptotic cells), plasma membrane disruption of apoptotic cells can occur leading to secondary necrosis [71]. Furthermore, shared signaling pathway elements between these different modes of cell death include mitochondrial-based events. For instance, a common event leading to both apoptosis and necrosis is mitochondrial permeabilization and dysfunction (i.e., both involve mitochondrial PT pore opening), although the mechanistic basis of mitochondrial injury appears to vary in different settings [72]. ATP level is a critical factor determining which type of cell death will proceed [73–75]. If ATP levels fall profoundly, plasma membrane permeabilization and cell rupture ensue leading



to necrosis. If ATP levels are partially maintained, apoptosis (which requires ATP for its progression) follows PT pore opening. In transgenic mice with enhanced sarcolemmal L-type  $\text{Ca}^{2+}$  channel activity with progressive myocyte necrosis that led to pump failure and premature death, necrosis in association with dysregulated  $\text{Ca}^{2+}$  handling and  $\beta$ -adrenergic receptor signaling and subsequent HF could be prevented by the targeted loss of cyclophilin-D, a regulator of the mitochondrial PT pore [76].

A third type of cell death associated with autophagy has recently been described in HF [70, 77]. In contrast with necrosis, autophagic cell death similar to apoptosis is characterized by the absence of tissue inflammatory response. However, in contrast to apoptosis, which features early collapse of cytoskeletal elements with preservation of organelles until late in the process, autophagic cell death exhibits early degradation of organelles and preservation of cytoskeletal elements until later stages. Also, unlike apoptosis, caspase activation and DNA fragmentation occur very late (if at all) in autophagic cell death [78]. Although limited information is presently available concerning its signaling pathway and interaction with mitochondria, autophagy appears to be triggered by mitochondrial ROS and the role(s) of defective mitochondria in autophagosome biogenesis and in selective cell removal in aging are being actively explored [79].

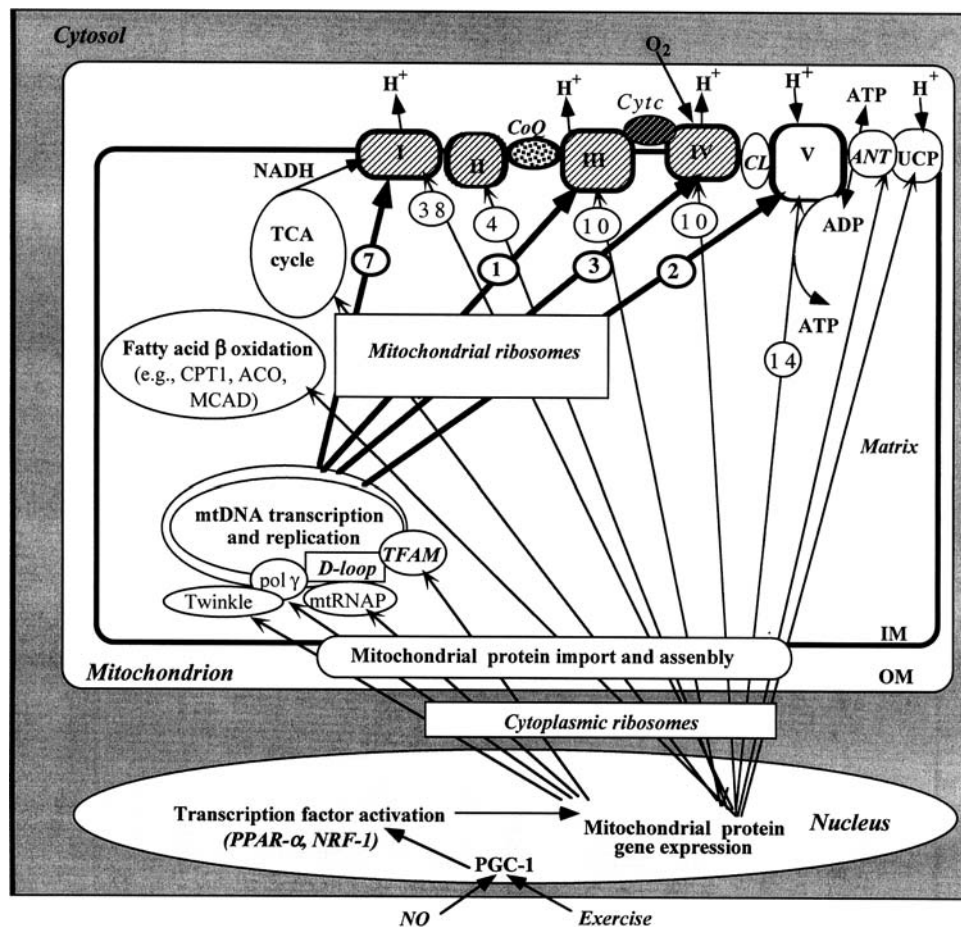
#### Nuclear-mitochondrial communication

Given that mitochondria with their pivotal role in determining bioenergetic capacity, OS levels and cell-death progression are important contributory factors in HF, the regulation of the organelle's biogenesis, which is influenced by factors such as nutrients, hormones, exercise, hypoxia, and aging, has become of increasing interest to clinical cardiologists and researchers as well. While mitochondria have their own DNA, which encodes 13 proteins, 22 tRNAs, and 2 rRNAs, more than 98% of their protein complement (over 1,500 different proteins) is encoded by the nuclear genome (see Fig. 5). Hence, mitochondrial biogenesis requires the coordinated expression of both nuclear DNA and mtDNA. Moreover, there is substantial bidirectional cross-talk between the nucleus and mitochondria to maintain both mitochondrial function and cellular homeostasis, and to mediate responses to specific stresses [80, 81]. Communication from nucleus to mitochondria (direct signaling) makes use of cytosol-translated proteins that upon mitochondrial import impact this organelle's bioenergetic function, biogenesis, and induction of cell-death. Conversely, metabolic signals (e.g., mitochondrial-generated ROS as well as ETC dysfunction) sent from

mitochondria to the cytosol and nucleus (retrograde signaling) are critical to the activation of signaling pathways and specific transcriptional factors that will affect nuclear gene expression leading to programmatic changes in the cellular phenotype. Perturbation and deficiencies in these interorganelle-signaling pathways can result in abnormal phenotypes leading to HF.

Over the last decade, several nuclear factors have been identified that regulate the biosynthesis of the mtDNA-encoded proteins and the nuclear-encoded, cytoplasmic-translated mitochondrial proteins. These nuclear factors include the nuclear respiratory factors (NRF-1 and NRF-2), mitochondrial transcription factor A (TFAM), a pivotal factor in mtDNA transcription, replication and maintenance and nuclear receptor proteins including peroxisome proliferator-activated receptors (PPAR) [82–85]. Upstream of these factors, is the transcriptional co-activator of peroxisome proliferator activated receptor  $\gamma$  (PPAR $\gamma$ ), known as PGC-1, a key regulator of mitochondrial function capacity and mitochondrial biogenesis, which participates in the transduction of physiological stimuli to myocardial energy production and lipid metabolism. In response to a variety of stimuli, PGC-1 can activate NRF expression and activity that in turn upregulates the expression of nuclear-encoded mitochondrial proteins and of the nuclear-encoded TFAM [84–86]. Thus, global transcriptional regulators including PPARs and PGC-1, which are responsive to a variety of physiological stimuli as well as stressors, modulate nuclear-gene expression of mitochondrial proteins [84]. In addition to TFAM, other nuclear-encoded proteins play important roles in mtDNA replication, repair, and gene expression including mitochondrial DNA polymerase  $\gamma$  (encoded by POLG), the helicase Twinkle, the SSB protein, and an additional transcription factor (TFB).

Studies with transgenic mice have demonstrated that modifications in several of these nuclear-encoded mitochondrial proteins can promote cardiomyopathy and HF suggesting that defects in nuclear-mitochondrial cross-talk can lead to HF. For instance, transgenic mice containing a cardiac-targeted Y955C *POLG* allele displayed cardiomyopathy, mtDNA depletion, mitochondrial OS, and premature death [87]. Transgenic mice heterozygous for a null allele of *TFAM* exhibited reduced myocardial mtDNA copy number and ETC defects, whereas homozygous *TFAM* knockout strains showed severe mtDNA depletion with decreased OXPHOS function and died in embryonic development [88]. Wang et al. reported that mouse strains containing conditional cardiac and muscle-specific null *TFAM* alleles developed in the postnatal heart a mosaic pattern of progressive and severe ETC defects (respiratory complexes I and IV), with reduced mtDNA levels and gene expression, resulting in DCM, atrioventricular conduction block, early HF, and death between 2 and 4 weeks [89].



**Fig. 5** Heart mitochondrial biogenesis and bioenergetic pathways are signal driven. Shown are the major mitochondrial bioenergetic pathways (fatty acid oxidation (FAO), TCA cycle and the respiratory complexes (I–V)) involved in ETC and OXPHOS; the subunits comprising the majority of mitochondrial proteins including the enzymes of the FAO and TCA cycles, proteins involved in ATP production/distribution, and electron transport coupling (e.g., ANT and UCP) and all but 13 proteins involved in respiratory complexes I, III, IV and V are encoded by nuclear genes translated on cytoplasmic ribosomes and incorporated in the mitochondria by a dedicated mitochondrial import apparatus comprised of inner and outer membrane proteins and molecular

chaperones. These genes are under the regulation of specific nuclear transcription factors and co-activators including NRF-1, PGC-1, and PPAR- $\alpha$  that can be modulated by physiological stimuli including TH, NO, electrical stimulation and exercise. Expression of the mtDNA genes generates 2 rRNA, 22 tRNA and the 13 proteins encoding respiratory complex subunits (translated on mitochondrial ribosomes) dependent on nuclear-encoded proteins regulating both the transcription and replication of mtDNA (e.g., mitochondrial DNA polymerase (pol  $\gamma$ ), the helicase Twinkle, mtRNA polymerase, and TFAM, the latter which is modulated by the nuclear transcription factors (e.g., PGC-1, NRF-1) in response to physiological stimuli

Along with the development of severe cardiomyopathy, the tissue-specific *TFAM* knock-out mice exhibited increased myocardial apoptosis in vivo which is consistent with the finding of massive apoptosis in *TFAM* knockout embryos and suggests that defects in ETC may predispose cells to apoptosis [90]. In addition, global gene profiling analyses in tissue-specific *TFAM* knockout mice revealed a metabolic switch early in the progression of cardiac mitochondrial dysfunction akin to the activation of a fetal gene expression program in which a number of genes encoding critical enzymes in FAO showed decreased expression, while several genes encoding glycolytic enzymes showed increased expression [91]. In more advanced disease, the

metabolic switch was followed by increased mitochondrial biomass which did not result in a rate increase in myocardial ATP production. Based on these findings, the observed switch in metabolism appeared unlikely to benefit energy homeostasis in the ETC-deficient hearts and may actually promote further cardiac dysfunction.

In contrast, *TFAM* overexpression in mice can ameliorate the mitochondrial deficiencies and the HF resulting from MI [92]. *TFAM* overexpression attenuated the decline in mtDNA level and respiratory activities in post-MI hearts, as well as significantly reduced LV dilatation and dysfunction accompanied by a decline in LV remodeling (i.e., decreased myocyte hypertrophy and interstitial

fibrosis). Interestingly, while survival of the infarcted animals was affected, the infarct size was not.

Since the majority of the components of mitochondrial bioenergetic, biogenesis, and signaling pathways are encoded by nuclear DNA, the regulation of the programmed changes in mitochondria detected in HF largely arise in the nucleus. Even direct damage to mitochondrial-specific molecules such as mtDNA mutations and large-scale mtDNA deletions and mtDNA depletion can arise from defective nuclear gene functions leading to cardiomyopathy and/or HF [93–95]. For instance, mice that express a proofreading-deficient mitochondrial DNA polymerase  $\gamma$  targeted to the heart exhibit elevated levels of cardiac mtDNA mutations (average two per mitochondrial genome) and myocardial apoptosis, and eventually (over several weeks) develop severe DCM and interstitial fibrosis, often leading to HF [96]. This and subsequent studies have suggested that mtDNA damage may be a triggering event in apoptotic induction and critical to HF progression, interestingly without the intermediary action of ROS [97, 98]. This suggests that while ROS/OS may be contributory to HF, it may not be necessary.

### Conclusion: the road ahead

The critical involvement of mitochondrial pathways in myocardial bioenergetic regulation, the balance of oxidant and antioxidants, and the progression of cell-death are contributory factors to the cardiac dysfunction and remodeling found in the failing heart. The identification of nuclear and mitochondrial components of these pathways and delineation of their cross-talk has suggested a number of potential targets for the clinical treatment of HF.

Among the novel intervention strategies being developed include the targeting of apoptosis which appears to be reversible, at least in part; such an approach will have to be finely targeted since apoptotic inhibition or pro-survival pathway stimulation could result in unwanted proliferative growth. A second approach involves the targeting of metabolic remodeling. Several observations suggest that increasing the capacity for glucose utilization may delay HF progression; however, it remains unclear whether upregulating FAO will be helpful. Findings from rodent models that this metabolic modulation can be effected by targeting global nuclear receptors/transcriptional regulators (i.e., PPARs and PGC-1) with exogenous agonists or by nutritional interventions (e.g., caloric restriction) may eventually be clinically applied in treating HF. This approach of targeting transcriptional regulation addresses the programmatic shift in mitochondrial bioenergetic function indicated by the broad array of bioenergetic loci

affected in HF and by transcriptional profiling studies in both animal models and patients [99–101].

Novel approaches to increase ATP or PCr levels and ATP synthesis in the failing heart are critically needed; while pharmacological approaches are more likely in the more immediate future, down the road, both genetic and cell-mediated therapies (e.g., stem-cells) which have shown promise in preclinical models may be utilized for clinical treatment. Along these lines, strategies aimed at activating mitochondrial responses against OS and targeting the signaling pathways identified in pharmacological and ischemic conditioning (which feature mitochondrial elements) may also offer the potential to provide clinical cardioprotection in the treatment of human HF.

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