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REVIEW ARTICLE

Mitochondrial-nuclear Cross-talk in the Aging and Failing Heart

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Abstract
Hypothesis Damage to heart mitochondrial structure and function occur with aging, and in heart failure (HF). However, the extent of mitochondrial dysfunction, the expression of mitochondrial and nuclear genes, and their cross-talk is not known.
Observations Several observations have suggested that somatic mutations in mitochondrial DNA (mtDNA), induced by reactive oxygen species (ROS), appear to be the primary cause of energy decline, and that the generation of ROS is mainly the product of the mitochondrial respiratory chain. The free radical theory of aging, that could also be applied to HF, and in particular the targeting of mtDNA is supported by a plurality of observations from both animal and clinical studies showing decreased mitochondrial function, increased ROS levels and mtDNA mutations in the aging heart.
Discussion Aging and HF with their increased ROS-induced defects in mtDNA, including base modifications and frequency of mtDNA deletions, might be expected to cause increased errors or mutations in mtDNA-encoded enzyme subunits, resulting in impaired oxidative phosphorylation and defective electron transport chain (ETC) activity which in turn creates more ROS. These events in both the aging and failing heart involve substantial nuclear–mitochondrial interaction, which is further illustrated in the progression of myocardial apoptosis. In this review the cross-talk between the nucleus and the mitochondrial organelle will be examined based on a number of animal and clinical studies, including our own.

Key words aging · heart failure · mitochondria · gene expression · nuclear genes

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Introduction

Phenotypic similarities have been found in both the failing and the aging heart. These include reduced myocardial contractility, diminished capacity to respond to specific stresses such as hypoxic and oxidative stresses, which result 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, marked changes in myocardial bioenergetic reserves, capacity and substrate utilization. These alterations generally occurred at both tissue and cellular level.

Structural and functional changes in myocyte mitochondria are present in both aging and heart failure (HF), including defects in the electron transport chain (ETC), oxidative phosphorylation (OXPHOS), Krebs cycle and fatty acid oxidation (FAO), increased generation of reactive oxygen species (ROS), oxidative stress and damage, decreased mitochondrial biogenesis, increased mtDNA damage, alterations in the permeability transition (PT) pore as well as in

mitochondrial membrane potential. Observations from both animal models of aging and HF, as well as from humans, suggest that these mitochondrial defects may underlie several of the aforementioned shared aspects of the aging and HF phenotypes. Furthermore, the role that mitochondria play in aging and HF extend well beyond their classic function in bioenergetics, which is underscored by the organelle's integral involvement in nitric oxide (NO) signaling, intracellular Ca^{++} flux, myocyte apoptosis and remodeling events, as well as providing antioxidant and cardioprotective responses to physiological insults. The centrality of mitochondria in pathways leading to HF is shown in Fig. 1 [7].

Since the majority of the components of mitochondrial bioenergetic, biogenesis and signaling pathways are encoded by nuclear DNA, the mitochondrial programmed changes detected in aging and HF are largely derived from the nucleus. Even direct damage to mitochondrial-specific molecules such as large-scale deletions in mtDNA appear to arise from defective nuclear gene function (e.g., DNA polymerase γ [polγ]) which can lead to premature aging as well as to cardiomyopathy and/or HF [119, 137]. Alternatively mitochondria can influence nuclear transcriptional events by virtue of ROS generation and signaling, suggesting the presence of substantial cross-talk between the nucleus and the organelle that likely participate in shaping the aging and failing heart phenotypes.

In this review we will examine mitochondrial changes that occur with aging and HF with emphasis on mitochondrial bioenergetics and biogenesis, ROS and apoptosis, as well as on nuclear–mitochondrial interactions with a particular effort to critically gauge their significance. In addition we will discuss current approaches to evaluate the role that mitochondria play in the aging and failing heart, including transgenic models, microarray and proteomic analysis, in vitro studies with isolated cardiomyocytes, and the identification of targets for potential therapeutic intervention.

Bioenergetics and gene expression

The role of fuel supply, and in particular levels of ATP, is critical for myocardial contractility and electrophysiology [66]. The function and structure of the mitochondria, in which the oxidation of fatty acids (the primary energy substrate of the adult heart), accompanied by aerobic respiration, including the Krebs or TCA cycle, ETC and OXPHOS pathways proceed, are crucial factors determining the function of the normal heart during aging, and if perturbed might result in HF.

The importance of bioenergetic function to the heart is underscored by genetic disorders in which defective components of the metabolic pathways lead

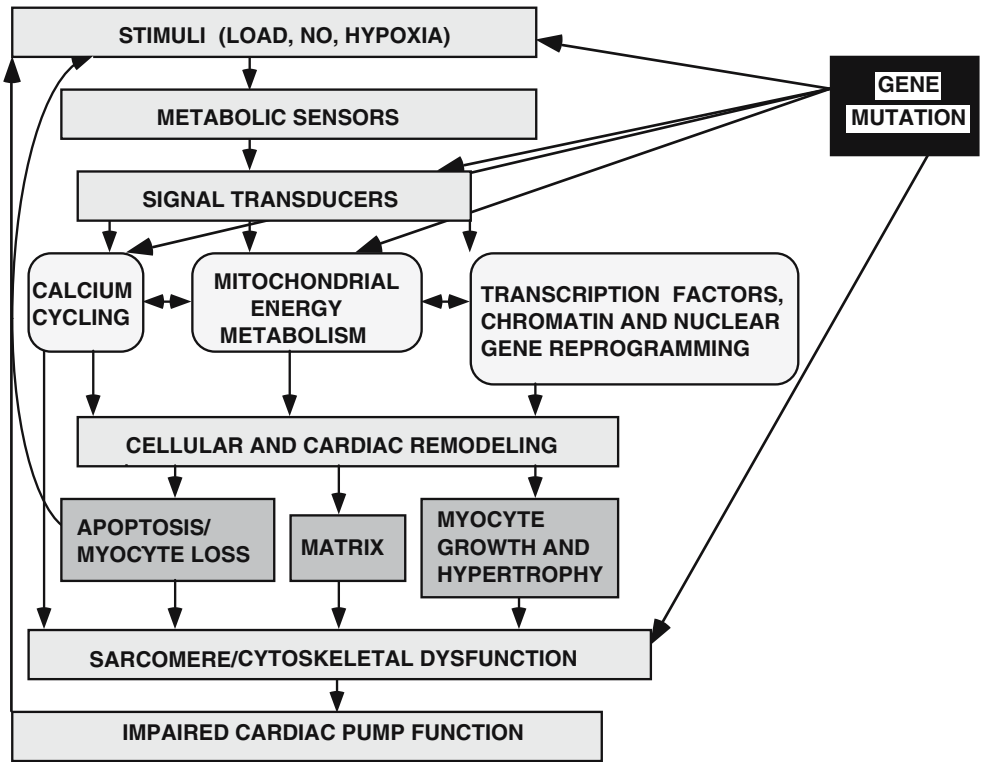

Fig. 1 Model of molecular triggers and cellular pathways leading to heart failure showing the centrality and interaction of mitochondrial energy metabolism

to cardiac pathologies, including cardiomyopathy, hypertrophic remodeling, and HF. Interestingly, these mutations may be localized in nuclear and/or mitochondrial genes.

Aging

Previously, gathered observations from both animal models of aging and human have suggested that there are significant alterations in myocardial TCA cycle, ETC and OXPHOS [33, 96, 130]. However, these observations have generated some controversy among investigators with some suggesting that the aging heart has either a modest, nonsignificant reduction or no overt effect on either the ETC or OXPHOS function [72, 75, 86, 118] while others found significant alterations in ETC, in which single respiratory complexes were more affected than others, i.e., complex I [30, 65], complex IV [88, 98] or complex V [19, 32]. In our laboratory we have found a modest decrease in complex I and IV activities, and a more severe decline in complex V activity in the hearts of 30-month Fischer 344 rats (unpublished data).

Heart mitochondria are composed of two distinct subpopulations: one beneath the sarcolemma (subsarcolemmal mitochondria [SSM]), and another along the myofilaments (interfibrillar mitochondria [IFM]). A preferential loss of IFM function with age has been reported, including a significant reduction of complex III [41, 67] and complex IV [24] activities.

Age-associated mitochondrial membrane changes include increased membrane rigidity, elevated levels of cholesterol, phosphatidylcholine, omega-6 polyunsaturated fatty acids (PUFA) and 4-hydroxy-2-nonenal with decreases, and oxidative modifications, in omega-3 PUFA and cardiolipin. These changes are potentially responsible for the increased susceptibility of the aging heart to the damaging effects of ischemia/reperfusion, including mitochondrial Ca^{++} overload, opening of the permeability transition (PT) pore and cell death. The aging myocardium (under the appropriate conditions) may exhibit increased permeability of the inner mitochondrial membrane to solutes causing mitochondrial swelling, 'proton leak,' reduced respiratory chain efficiency and uncoupling of OXPHOS [34, 36]. Furthermore, ATP production in the aging myocardium might be limited by increased uncoupling of OXPHOS from mitochondrial respiration. Unpublished studies from our laboratory have confirmed increased PT pore sensitivity to Ca^{++} in the senescent rat myocardial mitochondria, and these findings are consistent with other observations of enhanced calcium vulnerability and calcium-induced damage in mitochondria from senescent animals hearts [20, 50].

Data from animals and human studies have shown that myocardial fatty acid metabolism decreases and glucose metabolism increases with aging [55, 66]. In addition, carnitine acetyltransferase and 3-hydroxyacyl-Co-A dehydrogenase activities are significantly decreased in the aging rat heart [33, 64], as well as levels of myocardial carnitine and mitochondrial carnitine-acylcarnitine translocase activity [97, 116].

Several global transcriptional nuclear regulators of genes, including the fatty acid activated-peroxisomal proliferating activating receptor (PPAR) and its coactivator (PGC1) are involved in the modulation of key myocardial energy metabolic pathways, such as the mitochondrial and peroxisomal FAO [43]. Furthermore, these factors have also been critically implicated in the control of mitochondrial biogenesis. Compared with sedentary young rats (4 months), sedentary aged rats (23 months) have lower myocardial expression of PPARα, which was significantly higher in exercise-trained aged rats compared with sedentary aged rats. Ietmitsu et al. [48] have reported that in association with changes in myocardial PPARα mRNA and protein levels, myocardial activity of PPARα DNA binding to the transcriptional regulatory elements on FAO metabolic enzyme genes is altered together with mRNA expression and enzyme activity of 3-hydroxyacyl CoA dehydrogenase (*HAD*) and carnitine palmitoyl transferase-I (*CPT1*), which are PPARα target genes. On the other hand, Lemoine et al. [64] found no significant changes in relative transcript levels of nuclear transcriptional regulators, including PPARα, PPARβ, PPARγ or PGC1 in very old Fischer 344 rats (35 months).

The regulation of mitochondrial gene expression in the aging heart is variable. Whereas some investigators have found a decline in the levels of mtDNA-encoded transcripts in senescent rat heart [2, 27, 42], others have found either no significant changes [5, 64] or increased levels of mtDNA-encoded mRNAs [31] in aging cardiac myocytes. In regard to this, it is worth noting that no changes in levels or activity of the mitochondrial transcription factor mtTFA (or TFAM), implicated in mtDNA replication and transcription, were detected in the aging rat heart compared to age-related increases reported in liver and brain [21]. Also, heart mtTFA mRNA levels have been found unchanged with aging [80]. On the other hand, global nuclear regulators of mitochondrial transcription such as the nuclear respiratory factors NRF1 and NRF2 were found to be up-regulated in the aging heart [64]. From these observations it is evident that further

research is needed to elucidate the role that nuclear regulatory factors and nuclear-encoded enzymes (Table 1) which regulate mitochondrial biogenesis, play in the aging heart.

Similar to the transcription studies, there is significant variability in regard to the available data on mtDNA levels or copy number in the aging heart. While some investigators reported increased mtDNA levels in the aging rat heart [28, 80], a majority have found that aging did not affect mtDNA levels in both rat and human heart [5, 21, 25, 83].

Myocardial gene expression profiling in young and old mice demonstrated that aging is associated with transcriptional alterations that are consistent with a metabolic shift from fatty acid to carbohydrate metabolism [63]. With aging, genes involved in fatty acid uptake and oxidation, including CD36, hormone sensitive lipase, CPT1 and CPT2, carnitine acyltranferase, acyl-CoA denhydrogenase and UCP (which encodes an uncoupling protein), were significantly down-regulated. On the other hand, genes involved in carbohydrate metabolism such as Glut4, phosphofructokinase and enolase were up-regulated. Furthermore, caloric restriction (CR) initiated in middle-aged rats, retarded or completely prevented the age-enhanced transcriptional changes together with several age-dependent physiological and biochemical alterations, including increased steady-state levels of oxidative damage to lipids, DNA, and proteins. Moreover, CR resulted in modification of myocardial gene expression consistent with preserved fatty acid metabolism, reduced endogenous DNA damage, and apoptosis modulation. Gene profiling studies of ventricular cardiomyocytes from aging mice have shown decreased levels of mtDNA-encoded cyt*b* and COXIII transcripts [8]. However, interpretation and comparison of profiling studies is complicated because of the use of different methodologies and animal strains together with variability in the employed starting materials (whole heart, with its heterogeneous cell-types, versus ventricular cardiomyocytes). Moreover, myocardial mitochondrial biogenesis can be promoted by exercise, electrical and hormonal stimuli as well as by xenobiotics [4], which may also constitute confounding variables. Other factors such as gender may also play a role in myocardial gene expression patterns. Yan et al. [133] carried out proteomic analysis of the aging heart in a primate model (*Macaca fascicularis*) and found gender differences in the decreased expression and function of mitochondrial proteins responsible for ETC, TCA and OXPHOS and also in cytosolic proteins involved in glycolysis and glucose oxidation, all affected primarily in the ventricles from old male monkeys.

Heart failure

Under pathologic conditions, the heart relies more on glucose, as seen in cardiac hypertrophy, or may rely almost solely on fatty acids, as observed in cardiac tissue of animal models of diabetes [43]. Initially this switch in metabolic substrate provides adequate energy to maintain normal cardiac function, however over time diastolic dysfunction and HF develop together with depletion in high-energy phosphates.

As demonstrated by proteomic analysis, many of the cardiac proteins altered in a canine model of pacing-induced HF are involved in energy metabolism [40]. Observations with a canine model of pacing-induced HF have also documented a significant reduction in cardiac respiratory complex III and V activities together with a similar pattern of specific respiratory complex dysfunction in skeletal muscle [76]. A murine model of HF following myocardial infarction showed decreased levels of complex I, III

Table 1 Nuclear-encoded factors involve in the regulation of myocardial mitochondrial biogenesis

Factor	Function	Principle target
PPARα	Global transcription factor	FAO genes
PGC1	Transcription factor/coactivator	FAO genes/NRF1/NRF2/mtTFA
NRF1	Transcription factor	Nuclear ETC genes
NRF2	Transcription factor	Nuclear ETC genes
mtTFA (TFAM)	Mitochondrial transcription factor, mtDNA replication and maintenance	mtDNA-ETC genes
DNA polymerase γ	mtDNA replication	mtDNA
TWINKLE	mtDNA replication	mtDNA
TR	Thyroid hormone receptor, transcription factor	mtDNA and nuclear genes
ANT	Adenine nucleotide translocator, DNA maintenance, PT pore component	mtDNA,
AIF	Apoptosis inducing factor, OXPHOS regulator	ETC
eNOS	Nitric oxide synthase, signaling factor, mitochondrial isoform	ETC

NRF Nuclear respiratory factor, *eNOS* endothelial nitric oxide synthase

and IV activities and a parallel decrease in mtDNA-encoded transcripts whereas entirely nuclear encoded enzymes, complex II and citrate synthase were not affected [47].

Marked reduction in levels of mitochondrial transcription has also been demonstrated in other animal models of HF. Using an experimental rat model in which HF was induced by aortic banding, decreased levels of myocardial oxidative capacity and mitochondrial enzyme activities (citrate synthase and complex IV), together with a parallel decrease in mRNA levels of COX I and IV, but no change in mtDNA content, were detected [29]. Furthermore, marked down-regulated expression of nuclear regulatory factors, including mtTFA, NRF2 and PGC1, which are involved in both nuclear and mitochondrial bioenergetic gene expression, was detected in this model. Moreover, mitochondrial enzymatic dysfunction, down-regulation of nuclear regulatory factors and COX gene expression have been also found in skeletal muscle of animals in HF. The effect that experimental HF has on skeletal muscle metabolic function is consistent with gathered observations of early muscular fatigue and exercise intolerance in patients with HF. Besides the myocardial changes occurring in HF, analysis of biopsied skeletal muscle has also shown a 20% reduction in mitochondrial volume (irrespective of age), and decreased oxidative capacity with significantly reduced complex IV activity [22].

Observations from transgenic mice have also highlighted the importance of both an operative respiratory system and the nuclear regulatory factors needed to keep it running. Mouse strains with null mtTFA alleles display decreased mtDNA expression, enzymatic dysfunction and early progression of cardiomyopathy and HF [128]. Even mouse strains null for genes encoding cytosolic proteins, such as desmin and calcineurin, develop HF associated with significant mitochondrial dysfunction [11, 84, 105]. Studies with desmin-deficient strains implicated a role for desmin-associated cytoskeletal intermediate filaments in myocardial mitochondrial function [11]. While the precise mechanism by which desmin affects mitochondrial function is not known, proteomic analysis of desmin-null mice detected significant changes in peptide levels of several respiratory enzymes suggesting altered activity of regulatory factors, underscoring and probably expanding, the role of nuclear-cytosol-mitochondrial cross-talk in HF [26]. In calcineurin-null mice, loss of this signaling molecule also results in decreased mitochondrial respiratory subunit peptide levels leading to impaired ETC. This was associated with high levels of superoxide production, which might contribute to HF development [105].

Clinical studies of HF have also shown marked cardiac mitochondrial respiratory enzyme dysfunction, albeit variable in the extent of dysfunction and the specific enzymes affected, motivating some opposing views among investigators concerning these findings. While some studies reported increased incidence of complex III deficiency in DCM [51, 73], others reported increased complex IV deficiency [93]. Decreased complex I activity (30%) has been also found while complex III and IV activities were unchanged. Furthermore, no changes were found in myocardial mtDNA or mtTFA levels, nor in mtDNA-encoded transcripts excluding a generalized defect in mitochondrial gene expression or mtDNA damage as a reason for the enzyme deficiency noted in these patients [106]. This suggests that a post-transcriptional modification of complex I activity was the result of oxidative injury.

Interestingly, several gene-profiling studies in patients with end-stage HF identified an increase of OXPHOS transcripts [44, 134]. However, whether this reflects a compensatory adaptation to mitochondrial dysfunction or damage similar to that found in our studies of aging rat (unpublished data) remains to be seen.

Inherited FAO disorders caused by mutations in nuclear genes, encoding enzymes in the mitochondrial FAO pathways, may lead to impaired mitochondrial catabolism of fatty acids and may manifest a cardiomyopathic phenotype leading to HF [56]. Cardiomyopathy in these patients usually appears during childhood and often presents with sudden onset HF or ventricular dysrhythmias induced by stress. Mitochondrial dysfunction involving FAO has also been reported in acquired cardiomyopathies. Furthermore, in end-stage HF and in pressure overload hypertrophy, FAO and OXPHOS pathways are impaired [124, 126].

Abnormal regulation of global transcription factors PPARα and its co-activator PGC1α as well as their control of cardiac fatty acid utilization, have been found in human cardiomyopathy and in experimental animal models [43]. In animal models of HF induced by pressure overload or ischemia, the expression of PPARα, PGC1α, and downstream target genes encoding cardiac FAO enzymes is diminished [6]. This observation has been confirmed in human HF and suggests that deactivation of the PPARα and/or PGC1α is involved in the metabolic switch from fatty acid catabolism [101]. While abnormal regulation of the PPARα/PGC1 factors is likely involved in the metabolic remodeling that occurs in HF, the exact role of these metabolic "switches" in the development of HF is not yet known.

Defects in fatty acid accumulation can result in either lipotoxicity or in myocardial dysrhythmias [37, 77, 103]. Furthermore, several observations have suggested that targeting specific enzymes (that otherwise can lead to accumulation of toxic metabolites), regulatory factors (to effect metabolite switches) or bypassing specific deficiencies (such as ETC defects) might be effective adjuncts in the treatment of HF.

ROS generation and damage

Aging

ROS, a by-product of normal metabolic processes are generated from electrons produced (or leaked) from the ETC at complexes I, III and IV, although non-mitochondrial sources of ROS generation are both active and physiologically relevant in the heart. The inefficiency of electron transfer through the mitochondrial ETC and the overall level of antioxidant defenses are the primary source of ROS and oxidative stress in the aging heart [82]. Moreover, bioenergetic dysfunction occurring with aging further increases the accumulation of ROS. Mitochondrial ROS generation is increased in cells with abnormal ETC function as well as under physiological and pathological conditions where oxygen consumption is increased. Furthermore, complex I plays a major role in the formation of superoxide radicals [65], and a decline in complex I activity (with increased state 4 respiration) elevates ROS production during aging. This promotes the generation of prooxidant compounds, leading to modulation of PT pore opening, abnormal mitochondrial membrane potential, and finally induction of apoptosis.

A contributory role of ROS in aging is the underlying premise of the free radical theory of aging, which suggests that increased accumulation of ROS and the attendant oxidative damage occurring with age leads to a decline in the function of organ systems that eventually will result in failure and death [35]. Tissues with high oxidative activity, such as the heart, suffer the most damage. Among its many targets in the cardiomyocyte ROS causes extensive damage in particular to mitochondria (proximal to its source). ROS can reduce fluidity in the mitochondrial inner-membrane by attacking polyunsaturated fatty acids and the anionic phospholipid cardiolipin, which may affect mitochondrial protein import and ETC function. Protein modifications, such as carbonylation, nitration, and the formation of lipid peroxidation adducts such as 4-hydroxynonenal (HNE), are by-products of oxidative damage secondary to ROS [113]. While modifications in the protein subunits of respiratory complexes I to V secondary to ROS-mediated nitration, carbonylation, and HNE adduct formation, and an associated decline in enzymatic activity in vitro have been reported, a study of bovine heart submitochondrial particles found that proteins sustaining oxidative damage generated from in vivo basal level of ROS were primarily localized in the mitochondrial matrix [14]. Superoxide is also especially damaging to the Fe-S centers of metabolic enzymes (e.g., complex I, aconitase, and succinate dehydrogenase). In addition, inactivation of mitochondrial aconitase by superoxide, which generates Fe (II) and H_2O_2, also increases hydroxyl radical formation through the Fenton reaction [125].

Interestingly, the highly reactive peroxynitrite irreversibly impairs mitochondrial respiration [10] since it inhibits complex I activity. This is largely mediated by tyrosine nitration of several targeted subunits [89, 102]. Peroxynitrite also modifies cytochrome *c* structure and function, affects cytochrome *c* oxidase activity [12], inhibits mitochondrial aconitase [13], and promotes induction of PT pore opening [94]. Increased calcium levels potentiate some effects of peroxynitrite on its mitochondrial targets, for example the PT pore [9]. Significantly, the effects of peroxynitrites on mitochondria can be distinguished from the effects of NO that often are reversible [10].

Another central premise of the mitochondrial theory of aging suggests that somatic mutations in mtDNA, induced by oxygen free radicals, are a primary cause of energy decline. Oxidative damage affects nucleic acids, and in particular mtDNA, by the induction of single- and double-strand breaks, base damage, and modification (including 8-oxoguanosine formation) resulting in the generation of point mutations and deletions [62, 110, 132].

Data collected from a number of animal studies demonstrated that with aging there is progressive accumulation of mtDNA damage, including large-scale mtDNA deletions in the heart. While several types of myocardial mtDNA deletions increase with aging, their relative incidence appears to be significantly lower in rats as compared to humans [135, 136]. Significant accumulation of a 4.2 kb mtDNA deletion has been found in the myocardium of senescent mice compared to young or middle-aged animals [90]. Similarly, in Fischer 344 × Brown Norway F1 hybrid rats of ages 5, 18 and 36 months, specific mtDNA deletions of 8–9 kb have been detected in the right and left ventricle and their abundance increased with age [127]. Interestingly, recent observations have shown that over 40% of cardiac mtDNA deletions in the

aging rat heart contain unique point mutations located near the deletion breakpoints [95]. On the other hand, no significant accumulation of specific mutations in the mtDNA control region has been found in the aging mouse heart [111].

In senescent Fischer rats (30 months), we have found significant myocardial mtDNA damage in association with decreased mitochondrial respiratory enzyme activities and increased mtDNA transcription (unpublished data). These findings suggest the presence of an adaptive mechanism that promotes mitochondrial transcription in the presence of mitochondrial dysfunction and mtDNA damage, which involves considerable mitochondrial-nuclear cross-talk. A similar adaptive or compensatory mechanism with increased mtDNA gene expression and extensive mtDNA damage has been described in patients with ischemic heart disease [16], as well as in patients with mitochondrial cytopathies [39].

During the last decade several investigators have reported increased levels of mtDNA point mutations in aging humans. Increased levels of mutations at specific sites within the mtDNA D-loop control region were initially reported in skin fibroblasts and skeletal muscle of aging individuals [15]. However, no evidence was found of an age-mediated accumulation of homoplasmic or heteroplasmic mutations in the myocardial D-loop from either patients with cardiomyopathy or normal controls [78]. On the other hand, an age-dependent increase in specific large-scale mtDNA deletions has been demonstrated in aging patients with or without cardiac disease [3, 16, 17, 38, 74, 81]. Furthermore, there is evidence that a significant fraction of cardiomyocytes containing clonally expanded mtDNA deletions, are present in the heart of very old individuals [58]. However, the role that these somatic mtDNA deletions play in determining the cardiac phenotype or contributing to cardiac dysfunction has not been clearly established. Recent research in human subjects has been focused on the role that pathogenic mtDNA mutations may play in programmed cell death, even though mtDNA damage and defective mitochondrial respiration appear not to be essential factors for the process of apoptosis to occur. Nevertheless, during aging mtDNA integrity may influence the rate of apoptosis possibly by regulating ROS production [60].

The role of point mutations and deletions in mtDNA in aging has been addressed experimentally by creating homozygous knock-in mice that express a proofreading-deficient version of Polγ, the nuclear-encoded catalytic subunit of mtDNA polymerase [119]. The knock-in mice developed a three- to five-fold increase in the levels of mtDNA point mutations, as well as increased amount of deleted mtDNA. The increase in somatic mtDNA mutations was associated with a reduced life span and premature onset of aging-related phenotypes, including cardiomegaly, thus providing a causative link between mtDNA mutations and aging. Further studies with mtDNA mutator mice unexpectedly did not find significant accumulation of ROS, changes of antioxidant enzymes nor markers of oxidative stress. This occurs despite severe respiratory deficiency, which in these strains may be the primary inducers of aging, rather than ROS [120]. Furthermore, in post-mitotic tissues significant accumulation of age-dependent mtDNA point mutations and deletions has been found in individuals bearing either mutation in the mitochondrial helicase Twinkle or in Polγ which suggests that the activity of proteins involved in mtDNA replication may be selectively targeted in aging [129].

Neutralization of ROS by mitochondrial antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) becomes of increased significance in the aging heart. Recent observations in the aging rat heart indicated that interfibrillar (but not subsarcolemmal) mitochondria have a significantly higher rate of oxidant production and a decline in mitochondrial ascorbate levels and GSH redox status [114]. Significant age-related increases in both cardiac MnSOD and GPx activities were found in both subpopulations of mitochondria, possibly as an adaptive mechanism to cope with increased ROS generation [53].

Mimetics of the antioxidant enzyme SOD are currently been considered as a method of reversing the accumulation of ROS, and therefore aging. However, caution is required since this modality of therapy may also override the beneficial effects provided by ROS within the cytosol as signaling molecules. Synthetic peptide antioxidants containing dimethyltyrosine, which are cell-permeable and concentrate 1,000-fold in the mitochondria, have been used in a cell model to target and effectively reduce mitochondrial ROS and cell death. In ischemic hearts, these peptides effectively improved contractile force in an ex vivo heart model [139]. Other bioactive molecules with antioxidant activity have been selectively delivered to the mitochondria to decrease lipid peroxidation and oxidative protein damage. A synthetic ubiquinone analog (termed *mitoQ*) has been targeted to mitochondria by the addition of a lipophilic triphenylphosphate cation [57]; these positively charged lipophilic molecules rapidly permeate the lipid bilayers and accumulate at high levels within nega-

tively charged energized mitochondria from heart and brain. Another modified antioxidant, a synthetic analog of vitamin E (MitoVitE), after oral administration at therapeutic concentrations was successfully incorporated into the heart mitochondrial matrix attenuating oxidative damage [112].

Mitochondrial ROS can be an important contributory factor in determining mammalian longevity. Support for the free radical theory of aging recently came from the work of Schriner et al. [107]. They constructed a transgenic mouse strain (MCAT) harboring a 50-fold increase in its expression of the antioxidant catalase in cardiac and skeletal muscle mitochondria. The MCAT strain with elevated catalase activity displayed reduced severity in age-dependent arteriosclerosis, reduced oxidative stress (e.g., H_2O_2 production and oxidative damage) and increased genomic stability as indicated by reduced levels of mtDNA deletions in heart and skeletal muscle. Both median and maximum lifespan were increased about 20% compared to wild-type controls. The relatively large increase in lifespan resulting from the up-regulation of a single gene involved in boosting antioxidant defenses, and its targeting to the mitochondria, reinforces the notion that mitochondrial ROS and oxidative stress play a critical role in determining lifespan.

Also, ROS function as signal transduction intermediates to induce transcription factor activation (e.g., NF-κB), gene expression, cell growth, and apoptosis. It is worth mentioning that upstream and downstream components of ROS-mediated signaling pathways in the myocardium include the mitogen-activated protein kinase (MAPK) family, the Rho family of small GTP binding proteins, the Src family of tyrosine kinases, Ras, and cytokines.

Heart failure

Research from several animal models has shown that ROS is involved and gradually increased in HF. Using electron spin resonance (ESR) spectroscopy in a canine model of pacing-induced HF, Ide et al. [45] have demonstrated a significant increase in superoxide anion levels in myocardial submitochondrial fractions. The elevated ROS production was attributed to the functional block of electron transport, resulting from a marked decrease in mitochondrial complex I activity. Further research with this model of HF revealed increased levels of the highly reactive hydroxyl radical, a particularly potent ROS in the failing myocardium, and demonstrated a significant positive correlation between myocardial ROS level and left ventricle dysfunction [46]. Furthermore, decreased levels of mtDNA copy number, mitochondrial transcripts and activity of respiratory complexes with mtDNA-encoded subunits (e.g., I, III and IV) were demonstrated [47].

In a mouse model of HF secondary to coronary artery ligation, ROS generation, as measured by ESR spectroscopy, significantly increased as did the formation of thiobarbituric acid (TBA), and reactive substances in mitochondria [121]. Similarly, increased ROS levels were found in limb skeletal muscles of HF mice in association with both increased ROS-mediated lipid peroxidation and reduced levels of mitochondrial complex I and III activities [122].

The chronic release of ROS, largely derived from myocyte mitochondria and from nonphagocytic NAD(P)H oxidase, has been linked to HF progression and to the development of left ventricular hypertrophy. Besides its direct deleterious effects on cellular enzymatic and protein function in the aging heart, ROS have been implicated in the development of agonist-induced cardiac hypertrophy, cardiomyocyte apoptosis and in the remodeling of the failing myocardium.

Since levels of short-lived ROS are difficult to directly gauge, demonstration of increased ROS levels in patients with HF has been limited. Recently, in ventricular myocardium from patients with end-stage HF undergoing transplant, superoxide anion was found to increase more than two-fold in the failing myocardium, as assessed by EPR with an O_2- spin trap [104]. Moreover, marked decline in mitochondrial-localized MnSOD protein and activity, despite increased MnSOD mRNA levels, was detected. Both increased ROS levels and decreased antioxidant response would be expected to lead to enhanced oxidative stress in the failing heart, which in turn may result in increased transcription of antioxidant enzymes.

Cumulative damage to mtDNA, which can result in either point mutations, large-scale deletions or changes in mtDNA copy number in relation to aging-mediated ROS production has also been implicated in the progression of HF. Mutational inactivation of the mouse *Ant1* gene encoding the mitochondrial adenine nucleotide translocator (ANT) resulted in multiple mtDNA deletions associated with elevated ROS production and the development of skeletal myopathy and cardiomyopathy leading to HF [23]. Using a conditional cardiac-specific disrupted allele of the mitochondrial transcription factor A (mtTFA or TFAM), involved in both mtDNA transcription, replication and maintenance, mutant animals develop a progressive mosaic of cardiac-specific ETC defects and DCM, resulting in early HF [128]. Mice heterozy-

gous for a null allele of mtTFA exhibit reduced mtDNA copy number and ETC defects in heart, whereas homozygous mtTFA knockout strains exhibit severe mtDNA depletion with decreased OXPHOS function [61]. Overexpression of mtTFA in transgenic mice ameliorated defects in mtDNA copy number and respiratory activities, and improved cardiac function in strains subjected to coronary artery ligation [49].

Mice that express a proofreading-deficient mitochondrial DNA polymerase in the heart generated cardiac mtDNA mutations and eventually (over several weeks) developed severe DCM, often leading to HF [137]. In these strains mitochondrial respiratory function, mitochondrial ultrastructure and number remained normal, although cytochrome *c* was released from mitochondria indicating that the elevated frequency of mtDNA mutations might trigger the initiation of apoptosis. Interestingly, the activation of myocardial programmed cell death pathway precedes (and may itself trigger) a vigorous prosurvival response including the up-regulation of antiapoptotic proteins such as Bcl-2, Bcl-xl, Bfl1, and heat shock protein 27 [138].

Apoptosis

The mitochondrial-mediated intrinsic apoptotic pathway, which has been documented in the aging and failing heart, may play a significant role in the development of cardiac dysfunction and pathogenesis. This pathway also features an extensive dialogue

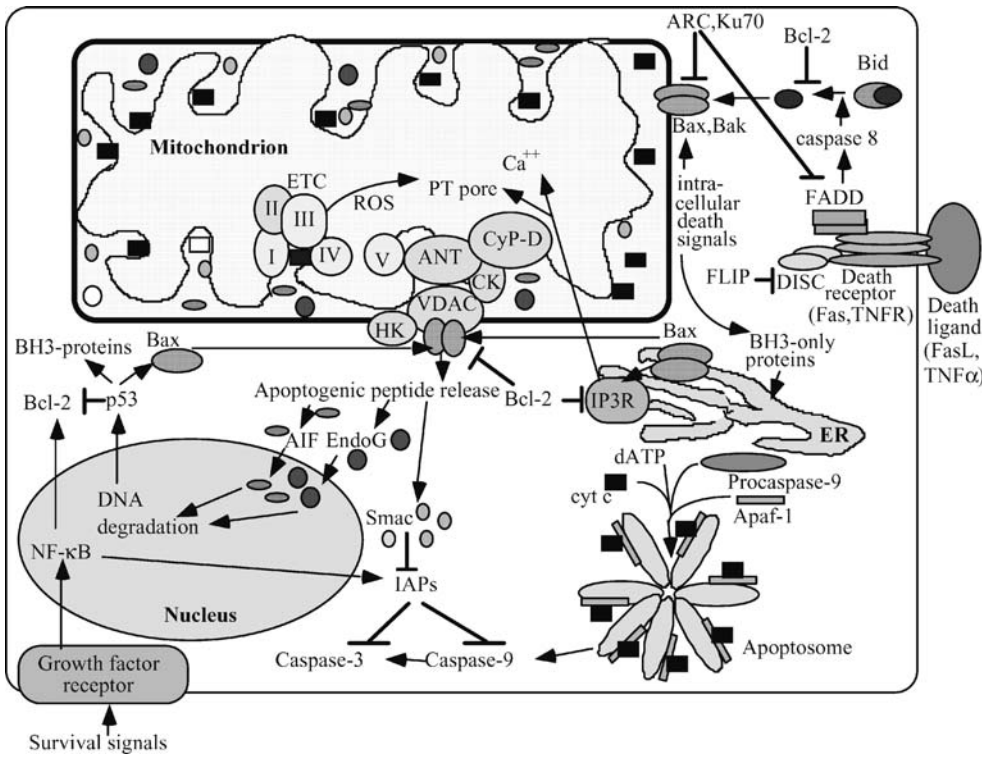

Fig. 2 The intrinsic and extrinsic pathway of apoptosis. An array of extracellular and intracellular signals triggers the intrinsic apoptotic pathway, which is regulated by proapoptotic proteins (e.g., Bax, Bid and Bak) binding to the outer mitochondrial membrane leading to mitochondrial outer-membrane permeabilization and PT pore opening. Elevated levels of mitochondrial Ca^{++} as well as ETC-generated ROS also promote PT pore opening. This is followed by the release of cytochrome *c* (Cyt *c*), Smac, endonuclease G (Endo G), and apoptosis-inducing factor (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 antiapoptogenic proteins (e.g., Bcl-2). Also shown are major proteins comprising the PT pore including hexokinase (Hx), 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, ARC and Ku70 can stem this pathway's progression at specific points. Intracellular stimuli trigger ER release of Ca^{++} through both Bax and BH3-protein interactions. Also depicted is the survival pathway triggered by survival stimuli mediated by growth factor receptors, transcription factor activation (e.g., NF-κB) and enhanced expression of IAPs and Bcl-2

between the mitochondria, the nucleus and other subcellular organelles as depicted in Fig. 2. The release of a number of mitochondrial-specific proteins from the mitochondrial intermembrane space including cytochrome *c*, Endonuclease G (Endo G), AIF (apoptosis inducing factor) and Smac/Diablo are central to the early triggering events in the apoptotic pathway, leading to downstream caspase activation, nuclear DNA fragmentation and cell death [18]. The release of Endo G and AIF, and their translocation to the nucleus, specifically affect degradation of nuclear DNA, even in the absence of caspase activation [68, 115]. Both released 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 cytosolic signaling complexes (e.g., IAPs) that modulate apoptosis. Once in the cytoplasm, cytochrome *c* binds Apaf-1 along with dATP and promotes recruitment of procaspase-9 into the apoptosome, a multiprotein complex resulting in caspase activation [1, 71]. The release of these mitochondrial peptides may primarily involve an outer membrane permeabilization mediated by propapototic cytosolic factors Bax, Bak and tBID. In response to both external pro-death signals largely provided by the extrinisic apoptosis pathway (e.g., ischemia/hypoxia, cytotoxic cytokine TNF-α 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 form channels in the outer membrane [18].

Opposing the progression of this pathway, antiapoptic proteins (e.g., Bcl-2), localized to the outer mitochondrial membrane, either directly compete with or impede the proapoptotic factors activity, fortify and stabilize, or remodel the mitochondrial membranes and their channels preventing mitochondrial disruption and inhibiting PT pore opening. Recently, the endoplasmic reticulum (ER) has been recognized as an important organelle in the intrinsic pathway, mediating the cell death elicited by a subset of stimuli such as oxidative stress [109]. Similar to their roles in transducing upstream signals to the mitochondria, proapoptotic proteins appear to relay upstream death signals to the ER triggering the release of Ca^{++}, which in turn can rapidly accumulate in mitochondria promoting PT pore opening. Pro-survival factors from the growth factor signaling pathways (e.g., IGF-1) also can inhibit the progression of the apoptotic pathway.

Protein release from the intermembrane space and cristae, where the majority of cytochrome *c* is located, also appears to be associated with the opening of the voltage-sensitive PT pore located at the contact sites between inner and outer membranes, which is responsive to membrane potential changes, mitochondrial ROS and Ca^{++} overload, pro-oxidant accumulation and NO [59]. The composition of the PT pore while still controversial is thought to involve several key players in mitochondrial bioenergetic metabolism, including ANT, mitochondrial creatine kinase, the outer membrane porin molecule (VDAC) and inner membrane cyclophilin D (Fig. 2). 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 calcium, altered cristae, and cessation of ATP production secondary to the uncoupling of ETC and dissipation of the mitochondrial membrane potential [79]. Therefore, the efflux of cytochrome *c* is coordinated with the activation of a mitochondrial remodeling pathway characterized by changes in inner mitochondrial membrane morphology and organization, ensuring the complete release of cytochrome *c* and the onset of mitochondrial dysfunction, which might further contribute to the aging and/or failing heart phenotype [108].

Aging

An attractive hypothesis driving considerable research interest is that during aging mitochondrial dysfunction and ROS generation may trigger increased apoptosis, with resultant cell loss. Mitochondrial oxidative stress and declining mitochondrial bioenergetic production can lead to apoptotic pathway activation in vitro; whether this also occurs in the in vivo aging heart remains unknown. Also unknown is the role and extent that apoptosis plays in normal myocardial aging, although there is ample evidence of cardiomyocyte apoptosis as supported by studies demonstrating the release of cytochrome *c* from the aging rat heart mitochondria, decreased levels of Bcl-2 and unchanged Bax levels [99, 100]. Furthermore, compared to myocytes from younger animals, myocytes derived from the heart of old mice displayed increased levels of markers of cell death and senescence [117].

As suggested by experimental models, mitochondria-related apoptosis is contributory to the mechanisms of aging. The accumulation of mtDNA mutations was not associated with increased markers of oxidative stress or defective cellular proliferation, but it was correlated with the induction of apoptotic markers, particularly in tissues characterized by rapid cellular turnover. The levels of apoptotic markers which increase during aging in normal mice, increased further in mouse strains expressing the aforemen-

tioned proofreading-deficient version of mitochondrial DNA polγ, and correlated with the accumulation of mtDNA mutations in the heart [60]. Mice containing an IGF-1 transgene had attenuated levels of senescence-associated gene products (e.g., p27Kip1, p53, p16INK4a, and p19ARF), Akt phosphorylation in myocytes and compared to wild-type mice exhibited decreased levels of myocyte DNA damage and cell death. Unfortunately, neither myocyte mitochondrial structure nor function were evaluated [117].

Heart failure

The overall role of apoptosis in HF has not been definitely established, and there is little morphological evidence of significant cardiomyocyte apoptosis during any stage of myocardial infarction and HF. However, apoptotic rates are higher after human myocardial infarct (ranging from 2 to 12%) than in end-stage (in NYHA class III–IV) human HF (range 0.1–0.7%) [91, 92]. When viewed in absolute terms, the rate of apoptosis is quite low, but when the relatively low rates are viewed in the context of months or years, it is plausible that the actual burden of chronic cell loss attributable to apoptosis could be rather substantial. Unfortunately, the timing of the apoptotic process is not well defined, and the accurate assessment of true rates and their consequences is not clearly established.

As it happens with aging, there is indirect evidence that DNA fragmentation and apoptosis-related factors are elevated in HF [54]. Furthermore, findings from in vitro systems and animal models showed that myocardial apoptosis rates can be elevated in response to a plurality of insults, including ischemia–reperfusion, myocardial infarction, atrial pacing, mechanical stretch, as well as by pressure overload secondary to constriction of the aorta. Moreover, apoptotic induction has also been found in canine pacing-induced HF [70, 87]. Parenthetically, it is noteworthy that nearly all cases of myocardial apoptotic induction are associated with altered mitochondrial respiratory function.

To further support the role of apoptosis and mitochondrial dysfunction in several experimental models of HF, the targeting of apoptotic factors has been shown to reverse the cardiomyopathy/HF phenotypes and associated mitochondrial dysfunction. In the previously described desmin-null transgenic mice, which develop cardiomyopathy and HF as well as attenuated mitochondrial function, there is extensive cardiomyocyte cell loss in focal areas, and decreased levels of cytochrome *c* in heart mitochondria [69, 85]. Bcl-2 overexpression in these desmin deficient mice dramatically ameliorated the cardiomyopathic phenotype, restored ETC function and changed the mitochondrial sensitivity to calcium exposure [131]. Moreover, mice containing a cardiac-specific deletion of the AIF protein developed severe DCM, HF and decreased mitochondrial respiratory activity, particularly affecting complex I [52, 123]. Furthermore, the finding that isolated hearts derived from mutant animals displayed poor contractile performance in response to a ETC-dependent energy substrates, but not in response to glucose, brings further support to the concept that impaired heart function in AIF-deficient animals results from abnormal mitochondrial ETC function. Following coronary artery ligation, mice harboring overexpressed mtTFA developed cardioprotection against cardiac remodeling, mtDNA defects, decreased respiratory enzyme activities, contractile dysfunction and HF [49].

Conclusion

The critical involvement of mitochondrial pathways in myocardial bioenergetic regulation, the balance of oxidant and antioxidants and the progression of apoptosis, is being increasingly reported as contributory to the cardiac dysfunction and remodeling found both in the aging and in the failing heart. These pathways involve considerable cross-talk between both nuclear and mitochondrial components that represent potential targets for the treatment of HF and for reversing the cardiac dysfunction occurring with aging. Furthermore, the development of novel strategies, by either targeting these factors directly (e.g., apoptotic factors), promoting or redirecting bioenergetic resources, or activating mitochondrial responses against apoptosis and/or oxidative stress, holds great promise for providing cardioprotection in HF and in the aging heart.

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