Review: Mitochondrial Medicine – Cardiomyopathy Caused by Defective Oxidative Phosphorylation

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Abstract. During experimental hypertensive cardiac hypertrophy, the heart energy metabolism reverts from the normal adult type that obtains the majority of its requirement for adenosine triphosphate (ATP) from metabolism of fatty acids and oxidative phosphorylation (OXPHOS), to the fetal form, which metabolizes glucose and lactate. Mitochondrial synthesis and function require an estimated 1000 polypeptides, 37 of which are encoded by mitochondrial (mt) DNA, the rest by nuclear (n) DNA. Inherited or acquired aberrations of either mtDNA or nDNA mitochondrial genes cause mitochondrial dysfunction. Tissue expression of OXPHOS enzyme defects is often heterogeneous. As a result, cardiomyopathy and cardiac failure are frequent but unpredictable complications of mitochondrial encephalopathy, neuropathy, and myopathy. Several nuclear genes that encode mitochondrial proteins have been sequenced and specific defects associated with nuclear genes that affect mitochondrial structure and function have been linked to hypertrophic and dilated cardiomyopathies and to cardiac conduction defects. Thyroid hormone and exercise stimulate expression of a nuclear respiratory factor (NRF) that induces the nuclear gene TFAM, which encodes the mitochondrial transcription factor A that controls mitochondrial replication and transcription. TFAM-null mouse embryos lack mitochondria and fail to develop a heart. Mitochondrial dysfunction enhances the generation of radical oxygen species (ROS), which damage mtDNA, nDNA, proteins, and lipid membranes. Mice lacking the mitochondrial antioxidant enzyme manganese-superoxide dismutase (SOD) develop dilated cardiomyopathy. Palliative mitochondrial therapy with L-acetyl-carnitine and coenzyme Q10 improves cardiac function in patients with cardiomyopathy. Cure is only achievable by mitochondrial gene therapy. Experimental direct gene therapy uses vectors or targeting signal sequences to insert genes into mtDNA; indirect gene therapy employs viral or non-viral vectors to introduce genes into nDNA. Clinical repair of damaged somatic and germline genes that encode mitochondrial proteins may soon be within reach. (received 15 June 2003; accepted 23 June 2003)

Keywords: cardiomyopathy, mitochondria, mtDNA, nDNA, OXPHOS, statins, Q10, gene therapy.

I. Introduction

Cardiomyopathies are characterized by a decrease in cardiac function [1,2]. Hypertrophic cardiomyopathy is often caused by mutations in cardiac myosin genes and dilated cardiomyopathy is associated with aberrations of the dystrophin gene [3]. The fact that mitochondrial defects can cause cardiomyopathy has been known for several decades [4], but until recently, except for involvement of mutations of the maternally inherited mitochondrial genome, knowledge of the molecular pathology of mitochondrial dysfunction as a cause of cardiomyopathy was incomplete [5].

The first patient with mitochondrial disease caused by a mutated nuclear-encoded mitochondrial gene was reported in 1995 [6]. The patient had Leigh syndrome, which was caused by defective oxidative phosphorylation (OXPHOS) [6]. Since then, several nuclear genes that code for mitochondrial proteins have been sequenced [7] and specific defects associated with nuclear genes that affect mitochondrial structure and function have
been linked to hypertrophic and dilated cardiomyopathies and cardiac conduction defects [8-10]. Historically, when declining cardiac function was caused by a mutation of mtDNA, it was referred to as mitochondrial cardiomyopathy [11]. However, the nuclear genome encodes most proteins required for mitochondrial synthesis and function, and mutation of a nuclear gene encoding a mitochondrial protein can also cause cardiomyopathy. This has been clearly shown in mouse gene knockout models and by studies of human tissues [12].

Mitochondrial dysfunction due to nuclear or mitochondrial gene mutations or toxic inhibition of their products enhances oxidative stress, which interferes with mitochondrial and nuclear genomes and mitochondrial biosynthesis [13-15]. The importance of oxidative stress in the etiology of cardiomyopathy is illustrated by a knockout model of the gene that encodes an intra-mitochondrial free-radical scavenging enzyme, manganese-superoxide dismutase (Mn-SOD). The null mice develop dilated cardiomyopathy and die within 10 days after birth [16].

The objective of this review is to elucidate the role of mitochondrial dysfunction in cardiomyopathy and cardiac failure. Evidence of dysregulation of mitochondrial function in cardiomyopathies has been derived from embryology, studies on cardiac tissues of patients with cardiomyopathy, clinical and autopsy findings, animal models, and in vitro findings. Specifically, this paper focuses on the roles of nuclear and mitochondrial genes in cardiomyopathy induced by dysfunction of mitochondrial oxidative phosphorylation (OXPHOS).

II. Cardiac energy metabolism

Disorders of mitochondrial OXPHOS are clinically and biochemically heterogeneous and lead to neuropathy, myopathy, or cardiomyopathy. The latter may be the only clinical presentation; however, it is often part of a wider spectrum of clinical manifestations. Cardiomyopathy should be especially suspected in patients who present with myopathy [17].

The high energy demand of the myocardium requires an ample and secure supply of adenosine triphosphate (ATP), which is mainly obtainable from mitochondrial OXPHOS [18]. As a result, cardiac mitochondrial dysfunction leading to reduced ATP generation causes cardiac contractile dysfunction and if severe, cardiac failure and death [8]. The fetal heart derives its energy mainly from glucose and lactate. By comparison, the postnatal myocardial contractile system depends primarily upon energy provided by fatty acid metabolism [2]. Over one third of the adult myocardial mass consists of mitochondria, which supply most of the cardiac requirement for ATP. Cardiac mitochondria of the adult heart preprocess fatty acids through β-oxidation and the Krebs cycle to provide high-energy metabolites as fuel for OXPHOS.

A. Oxidative phosphorylation

Mitochondria developed from archebacteria. The OXPHOS energy conversion system of mitochondria therefore resembles that of bacteria. During evolution most of the bacterial genes were transferred to the nucleus of the host cell or were lost. As a result, an intracellular dual genome symbiont was formed, which developed into the present-day mitochondrion. Its double membrane structure, which consists of two bilipid membranes, is unique (as reviewed in [19]).

The mitochondrial OXPHOS system consists of a fuel cell deriving energy from food metabolites, means for temporary storage of the harvested energy as a trans-membrane potential, and means for conversion of the stored energy into ATP or heat, or to energize trans-membrane transport, which is required for importation of nuclear-encoded mitochondrial proteins. Two fuel cell inputs are provided, one for electrons from NADH, and the other for electrons from the Krebs cycle via flavin adenine dinucleotide (FAD$_2$).

Energy is released from the fuel by electrons that flow through the electron transport chain (ETC), consisting of 4 enzyme complexes and 2 electron carriers to oxygen as the electron acceptor. The ETC pumps protons from the mitochondrial matrix to the inter-membrane space, establishing a proton gradient across the inner mitochondrial membrane. Electron flux is regulated to maintain a charge across the membrane of about 150-160 mV (Fig. 1).
Enzyme complexes I, III, and IV, which contain proton pumps subunits encoded by mtDNA, are completely or partially embedded in the inner membrane. In contrast, complex II lacks a proton pump. Electrons from NADH or from the Krebs cycle via FADH₂ enter through complex I (NADH dehydrogenase, ND) and II respectively; they flow through ubquinone (coenzyme Q₁₀, Q₁₀) to complex III, then via cytochrome c to complex IV, the terminal enzyme of the electron transport chain, and finally to oxygen as the electron acceptor. Complex I, III, and IV associate to form supercomplexes, also called “respirasomes,” which improve enzyme stabilities and reduce electron diffusion distance [20].

Most of the energy that is dynamically stored in the membrane potential is coupled to phosphorylation of adenosine diphosphate (ADP) to triphosphate (ATP) by F₀F₁-ATPase (Complex V). Its transmembrane F₀ moiety channels protons back to the matrix where they join molecular oxygen to form water. F₀ is connected by a stalk to the F₁ moiety, which protrudes into the matrix space. The membrane energy released by F₀ is utilized by F₁ for phosphorylation of ADP to ATP, most of which is exported to the cytoplasm of the host cell.

Uncoupling may be due to membrane leakage or to heat production by uncoupling proteins (UCP1, UCP2, and UCP3), which participate in the regulation of the membrane potential and prevent hyperpolarization of the membrane by channeling protons back into the matrix. Lowering the membrane potential lowers ETC oxygen radical formation. The membrane potential is continuously consumed and must be constantly replenished to prevent energy expenditure beyond supply and depletion of the proton gradient. Dynamic maintenance of the membrane potential requires vigilant regulation of the electron transport chain flux to replenish lost energy, so as to keep the potential within its physiological operating range.

III. Mitochondrial genetics

A. Dual genome symbiont

Mitochondrial replication is controlled by the nucleus, but is not synchronized with nuclear division in dividing cells. Even mitochondria of postmitotic cells (eg, the cardiomyocyte) are continuously degraded and must be constantly replaced independent of nuclear division, so-called “relaxed replication.” Enhanced mitochondrial synthesis can offset a certain amount of mitochondrial dysfunction, for instance induced by ethanol consumption [21-23].

Remarkably, mitochondrial replication and function requires the synthesis of an estimated 1000 polypeptides, all but 37 of which are coded by nDNA (Fig. 2). The electron transport chain comprises almost 100 polypeptides including 13 mtDNA-encoded structural subunits, which provide hydrophobic proton pumping subunits, and 2 subunits of complex V. Normally, a few thousand small, identical, circular, maternally inherited mitochondrial genomes are present in each cell, so-called “homoplasmy.” By contrast, heteroplasmy exists when mitochondrial genomes with different sequences simultaneously inhabit a cell, for example due to inherited or acquired mutation. Slow generation of antioxidant enzymes in cardiac
myocytes exposes the heart to a high risk of free radical injury and development of mutations or deletions [24]. Reduced synthesis of antioxidant enzymes during aging or exposure to environmental toxins or therapeutic agents that inhibit mitochondrial energy conversion may exacerbate the risk of damage [25].

**B. Mitochondrial transcription factor**

The nuclear-encoded mitochondrial transcription factor A (Tfam, mtTFA) is imported into mitochondria where it regulates the rate of transcription of the mitochondrial genome [26]. It co-localizes with nuclear-encoded mtDNA polymerase-γ (POLG), and phage T-7 gene 4-like protein with intramitochondrial nucleoside location (Twinkle), forming dynamic nucleoid assemblies that are able to divide, suggesting that they are the units of mitochondrial inheritance [27].

The gene for Tfam, TFAM, is located on chromosome 10q21 [28]. A locus CMDIC at chromosome 10q21-q23 has been linked to autosomal dominant familial dilated cardiomyopathy [29], but no report that CMDIC is TFAM or of involvement of TFAM mutation in a patient with cardiomyopathy has been forthcoming. On the other hand, animal models show that faulty TFAM expression causes cardiomyopathy. To prevent the development of cardiomyopathy, TFAM must be upregulated during blastocyst implantation.
Evidence for transcription control by Tfam was obtained by transfecting a monkey kidney cell line [30] with an expression vector harboring the TFAM gene, inserted in the sense- or antisense orientation. Transfection with the antisense plasmid caused reduced expression of TFAM and inhibited transcription of mtDNA [31]. Additional evidence of a regulatory role by Tfam was obtained using heterozygous and homozygous TFAM knockout mice. The heterozygous knockout reduces mtDNA copy number and lessens cardiac mitochondrial electron transport capacity. Homozygous knockout mice die prior to embryonic day 10.5 after normal implantation and gastrulation [32,33], showing that adequate TFAM expression is essential for mtDNA replication and transcription to ensure normal post-implantation development.

Such experiments have demonstrated that the timing of onset of TFAM expression during development is critical. This insight was gained when the time of knockout was manipulated by coupling its initiation to promoters that are activated at different times during development. For example, muscle creatinine kinase, which is activated from embryonic day 13, can serve to activate conditional knockout. Such transgenic mice have normal cardiac mitochondrial electron transport function at birth, but they develop postnatal cardiomyopathy after the knockout is activated [34].

Transgenic mice with TFAM knockout regulated by the α-myosin heavy chain, which is expressed from embryonic day 8, show mitochondrial cardiomyopathy during embryogenesis. Nevertheless, about 25% of TFAM heterozygous knockout mice survive the neonatal period but die from dilated cardiomyopathy, within several months, suggesting the presence of modifying genes. Studies of mice intercrossed between long-lived knockouts confirm that modifying genes affect their life-span [34]. TFAMloxP-mice with conditional knockout of cardiac TFAM have symptoms that resemble Kearns-Sayre syndrome. They show cardiac-specific OXPHOS deficiency, conduction block, dilated cardiomyopathy, and die at 2-4 weeks of age [35].

Cases of mitochondrial disease caused by TFAM mutations have not been reported. However, abnormal Tfam levels have been found in a few patients with mitochondrial disease caused by mtDNA mutations [36,28]. One patient presented with exercise intolerance, muscle wasting, and ocular myopathy. Muscle biopsies showed ragged-red fibers indicating accumulation of a large number of abnormal mitochondria. Heteroplasmy with the presence of an mtDNA deletion between nucleotide positions 8,300 and 12,400 was detected in muscle cells [36]. The increased Tfam level in this patient may have been a compensatory response to mtDNA deletion, causing reduced ATP generation.

C. Nuclear respiratory factors

Nuclear respiratory factors (NRF) -1 and -2 regulate Tfam expression. NRF-1 expression is essential for implantation and growth of the implanted blastocyst. Complete lack of NRF-1 is lethal. The NRF-1(-/-) blastocyst shows very low mtDNA levels and lacks the usual enhancement of mtDNA replication required to support rapid growth at the time of implantation. NRF-1 null blastocysts die within 3.5-6.5 days post-conception. In comparison, the NRF-1(+/-) blastocyst has mitochondria that show reduced rhodamine staining, which indicates reduced mitochondrial membrane potential compared with mitochondria in the wild-type blastocyst. Nevertheless, when it is allowed to progress, the NRF-1(+/-) blastocyst develops into a surviving mouse [37].

Muscle contractile activity or thyroid hormone (T₃) enhances the rate of mitochondrial protein import. T₃ stimulates expression of NRF-1 and the transcriptional coactivator peroxisome proliferators-activated receptor-γ coactivator (PGC)-1α [38]. The heart has high constitutive tissue levels of PGC-1α and exercise causes a transient increase in its transcription [39,40]. T₃ increases mitochondrial import of the nuclear-encoded mitochondrial heat shock protein (mtHsp)-70, and the increased matrix level of this chaperon enhances import of other mitochondrial proteins from the cytosol [41].

PCG-1α and cytochrome c oxidase subunit VII heart isoform promoter regions contain binding sites for myocyte enhancer factor-2 (MEF2). In the mouse, MEF2 is activated by embryonic day 8.5 [42]. In the rat model of cardiac myocyte response to pressure overload, MEF2 is an important signaling
molecule in gene activation leading to cardiac hypertrophy [43]. Cardiac-specific conditional knockout of MEF2 is usually lethal, demonstrating the importance of MEF2 expression for myocyte differentiation and the maintenance of cardiac mitochondrial function [44,45].

The promoter region of the cardiac α-myosin heavy-chain gene contains binding sites for MEF2 and thyroid hormone receptor, which synergistically induce transcription of the gene [46]. Moreover, the UCP3 gene promoter region contains binding sites for MEF2 and thyroid receptor [47]. The gene for MEF2A, the isoform expressed predominantly in cardiac tissue, is located on chromosome 15q26 [48]. No aberration in this gene with linkage to either dilated or hypertrophic cardiomyopathy has been identified [42]. However, a disease gene at region 15q24-q26 has been linked to 5 cases of congenital ataxia, optic atrophy, and mental retardation in a consanguineous family [49].

Human genes that contain binding sites for NRF-1, NRF-2, or both, include TFAM at chromosome 10q21, and OXPHOS genes such as ATPase β and γ subunits, and complex IV subunits Vb, Vic, and VIIa [44]. Furthermore, the gene for the low-density lipoprotein related receptor protein (LRP), which is important for cellular uptake of fatty-acid-rich lipoproteins, harbors NRF-1-binding sites in its promoter region [50]. In the mouse, the gene for vascular endothelial cadherin, which is essential for angiogenesis, contains binding sites for NRF-1 [51]. Such findings suggest that NFR-1 and NRF-2 coordinate mitochondrial replication, energy generation, embryonal vasculogenesis, and angiogenesis to supply oxygen and nutrients, and provide for the removal of cellular waste products. The presence of such binding sites for NRF-1 in the LRP promoter region and in other genes involved in processing high energy lipids suggests that NRF-1 also regulates the supply of fuels for mitochondrial energy conversion.

D. Mitochondrial import

Nuclear-encoded mitochondrial proteins are synthesized with mitochondrial targeting sequences. Cytosolic chaperon molecules guide such proteins to translocases of the mitochondrial outer membrane (Tom complexes), where they are unfolded [52,53]. Translocases of the inner membrane (Tim complexes) sort proteins to the various mitochondrial sub-compartments. Translocate Tim22 inserts proteins that lack a matrix targeting signal peptide into the inner membrane for assembly into multimeric OXPHOS enzyme complexes or carrier proteins such as mitochondrial nucleotide translocator (ANT) -1 [54]. The membrane potential-driven Tim23 presents proteins that carry a positively charged N-terminal mitochondrial-matrix signaling peptide to mitochondrial matrix mtHsp70, which pulls proteins into the matrix. Next, a mitochondrial processing peptidase cleaves the targeting sequence, whereupon mtHsp60 and matrix chaperons refold the shortened sequences into functional proteins or protein subunits [55,53].

IV. Molecular pathology

A. Defective OXPHOS enzyme activity

OXPHOS enzyme complexes have been analyzed using simple enzyme complex antibodies or specific subunit antibodies for immunohistochemistry on explanted cardiac tissues or endocardial biopsies, or immunoblot assay [56]. Enzyme subunits have been separated by two-dimensional electrophoresis (2-DE) or 2-DE followed by high-resolution analysis using mass spectrometry [57,58]. The following selected findings exemplify the diversity of OXPHOS enzyme defects in patients with cardiomyopathy and illustrate the great variety of clinical symptoms and syndromes with which cardiomyopathy may be associated.

Defective complex I activity causes about one third of cases of OXPHOS disease in humans [59]. Cases with isolated complex I disease with cardiomyopathy are illustrated in Fig. 3. The enzyme defects were diagnosed using cultured skin fibroblasts. All patients died within 2 years of age. 4 of the patients were diagnosed at birth. The shortest time between diagnosis and death occurred in a patient whose complex I deficiency was traced to a mutation in nuclear mitochondrial gene NDUFS8, located at chromosome 11q13 [60].

Of 101 children diagnosed to have OXPHOS disease, 17 had abnormal cardiac enzyme activities
and suffered from cardiomyopathy. The enzyme complex that was defective varied greatly, as did the clinical diagnoses, which included Alpers, Sengers, and Kearns-Sayre syndromes. Moreover, the age of the patients at the onset of cardiomyopathy and at the time of death varied greatly (Fig. 3) [61,62].

A 14-year-old boy with MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms [63]) had cardiomyopathy and markedly decreased complex I and IV enzyme activities, but the activities of complex III and V were only moderately lower than normal [64]. No defect was found in complex II activity in this patient, but rare cases of cardiomyopathy due to reduced activity of complex II have been reported. Thus, complex II activity was significantly reduced in cardiac biopsies from 2 brothers with hypertrophic cardiomyopathy and gait abnormalities [65]. Muscle biopsy from a 25-year-old female with Kearns-Sayre syndrome (KSS) revealed complex II deficiency, cardiac conduction defects, ragged-red fibers, and mitochondrial inclusions. KSS patients typically have progressive degeneration of the retina, short stature, dementia, and heart block [66]. By

Fig. 3. Illustration shows patients with cardiomyopathy due to mitochondrial oxidative phosphorylation system (OXPHOS) enzyme deficiency. Bar graph shows the age at the time of diagnosis and the age at the time of death. Age is shown in years and in days at the top and bottom of the graph respectively. A, top shaded area: Cardiomyopathy due to isolated complex I deficiency. In one case the defect was caused by a mutation of the nuclear mitochondrial gene NDUFS8, which codes for a nuclear-encoded subunit of complex I. [Data from Loeffen JL et al. Hum Mutat 2000;15:123-134]. B, lower section: Patients with cardiomyopathy caused by a variety of OXPHOS enzyme deficiencies and presenting with diverse clinical syndromes. Enzyme complexes indicated by roman numerals. In this study the time of onset and the time of death varied greatly. KSS: Kearns-Sayre syndrome; MERF: myoclonus epilepsy with ragged red fibers, IMM: infantile mitochondrial myopathy with complex IV deficiency; MEM: mitochondrial encephalomyopathy; Alpers: Alpers syndrome; Sengers: Sengers syndrome. [Data from Holmgren D et al. Eur Heart J 2003;24:280-288]. For further details see text.
comparison, a mycotoxin, neurotoxin, and food contaminant, 3-nitropropionic acid (3-NPA), a suicide inhibitor of complex II [67], reduces ATP generation and induces bradycardia in the isolated beating heart [68]. In the mouse model, 3-NPA reduces cardiac complex II activity to 20-30% of normal [69].

Often, mitochondrial enzyme defects show remarkable tissue differences in their expression. As examples, examination of autopsy tissues from a case of fatal infantile cardiomyopathy revealed severe reduction in complex I and IV activities in the heart as well as the skeletal muscle, but activities of complex II and III were significantly lower in the heart compared to skeletal muscle [70]. Marked decrease of either complex I, IV, or both, was found in endocardial biopsies in 15 of 32 infants diagnosed with idiopathic hypertrophic cardiomyopathy [71].

In 8 children with cardiomyopathy, examination of OXPHOS enzyme activities revealed defects of complexes I, III, IV, V in 2, 5, 3, and 4 cases respectively, but no defect was detected in complex

![Diagram of mitochondrial DNA point mutations and deletions detected in mitochondrial cardiomyopathies.](image-url)
II activity [72]. In contrast, a study involving 157 cases of mitochondrial disorders in childhood determined that enzyme defects were most common in complex I and complexes I + IV, and were often associated with parental consanguinity [73].

Low activities of cardiac complex III activities were found in studies of patients with idiopathic dilated cardiomyopathy. For example, in 10 heart transplant patients, who had been diagnosed with dilated cardiomyopathy, myocardial activities of complex III and IV, both associated with cytochrome c, were reduced, but activities of complex I and II were unaffected [74]. These results probably exclude the presence of an mtDNA deletion reaching from the cytochrome b gene (complex III) to the COIII gene (complex IV), because such a deletion would remove several ND-subunits of complex I as well (Fig. 4). The findings are more consistent with a loss of cytochrome c, because it interacts with both complex III and IV. Loss of cytochrome c suggests that apoptosis had a significant role in the pathogenesis of cardiomyopathy in these patients.

Another study of patients with dilated cardiomyopathy reported that 37 of 55 patients had reduced enzyme activity of at least one OXPHOS enzyme. Complex III was most frequently affected, followed in declining frequency by complex V, IV, and I; again, complex II was unaffected. Patients less than 18 years old were more likely to have single defects. Patients 19-64 years old had more multiple enzyme activity defects [75].

A third study involved 3 patients with low cardiac complex III activity compared to donor hearts. Comparison of cardiac mtDNA cytochrome b gene sequences in these patients with the Cambridge mtDNA sequence [76] revealed only neutral polymorphism. The investigators concluded that decreased complex III activity was most likely due to secondary causes such as oxidative stress [77].

Defective complex IV activity in the left ventricular wall was detected in explanted hearts from 6 patients with ischemic and 8 patients with non-ischemic dilated cardiomyopathy. The activity decrease correlated with diminished ejection fraction. It was suggested that defective complex IV activity resulted in reduced cardiac ATP generation, which contributed to the cardiomyopathy [78]. In a group of 16 patients with end-stage cardiomyopathy, 10 children and 2 neonates showed decreased cardiac mitochondrial activity of enzyme complex I, III, IV, and V. Sequence analysis revealed heteroplasmic in 4 patients with mutations in the ND5 gene, in cytochrome b, and mitochondrial transfer RNA (tRNA<sub>Arg</sub>). Because mutation of the latter affects only mitochondrial transcription, lack of deficiency in complex II, which only contains nDNA-encoded subunits, is not surprising [79].

By comparison, whereas activity of complex I was significantly decreased in terminally failing myocardium in a series of 43 explanted failing hearts compared with 10 donor hearts, no general mitochondrial genetic abnormality could be detected. The amounts of mtDNA, mtRNA and Tifam were unaltered compared with donor hearts [80]. These findings suggest the presence of either a mutation of a nuclear mitochondrial gene or inhibition of complex I by a toxin or other agent. For example, many insecticides and neurotoxins inhibit complex I either at the PSST or ND1 subunit of complex I, which are involved in electron transfer from complex I to Q10 [81-84].

B. Abnormal expression of mitochondrial genome

1. Inherited mtDNA point mutation. Many different types of abnormalities in mtDNA that cause defective OXPHOS enzymes have been detected in patients with hypertrophic and dilated cardiomyopathy [85]. Mutations of the mitochondrial genome frequently affect several organs. Accordingly, patients suffer from various disorders such as neurological disease, diabetes, and cardiomyopathy [86] or combinations of these afflictions. Cardio-myopathy is a relatively frequent companion of mitochondrial neuromuscular disorders. Thus, in 8 patients diagnosed with spinal muscular dystrophy, abnormal electrocardiograms and thickening of the myocardium were common findings [87].

Occasional cases of cardiomyopathy as a result of homoplasmic inherited mutation with the presence of only mutated mtDNA in all cells have been reported [88, 89], but most patients with mitochondrial disease caused by inherited
mitochondrial DNA mutations exhibit heteroplasmy. Typically, mitochondria in affected cells contain a mixture of wild-type mtDNA and over 20% mutated mtDNA, but the level of mutated mtDNA is occasionally much higher. For example, an infant son of a mother who carried a T8993G mutation of the MTATP6 gene in her leukocytes presented with Leigh syndrome and hypertrophic cardiomyopathy. MTATP6 encodes the ATP synthase6 subunit of complex V [90]. The heteroplasmy in the infant reached 90% in his fibroblasts and skeletal muscle. This case illustrates the variability of tissue expression of mtDNA gene defects. The mutation was present in the patient’s heart, but not in his liver [91]. A brother had died at age 2 from sudden infant death syndrome [92].

Different mutations may be expressed as the same clinical syndrome. For instance, Leber’s hereditary optic neuropathy (LHON) may be caused by single point mutation of ND4 or ND6, or by mutations in other parts of the mitochondrial genome [93,94]. Because complex I is the largest OXPHOS enzyme complex with the most subunits, 7 encoded by mtDNA and 36 encoded by nDNA, patients who present with Leigh syndrome or exhibit other clinical symptoms of OXPHOS disease should be screened for complex I gene defects, even if enzyme activities are normal in cultured skin fibroblast. This recommendation is based upon the finding of mutated NDUFV1 in 6 patients with complex I disease [95].

Investigation of a series of 28 patients with idiopathic dilated cardiomyopathy and marked reduction of OXPHOS enzymes in cardiac tissues revealed point mutations involving the ND5 subunit gene, the COII and COIII genes for subunits of complex IV, and the mtDNA gene for cytochrome b [96]. In a sequencing study of the mtDNA D-loop control region, which includes nucleotides 110-570, critical mutations were found in 8 of 47 patients with cardiomyopathy. These mutations were unrelated to aging, based on a comparison with 40 age-matched control subjects who had no history of heart disease [97].

The importance of defective mitochondrial genome translation is illustrated by the A8344G mutation, which results in abnormal tRNA\(^{Lys}\) and faulty translation, and causes myoclonus epilepsy with ragged red fibers (MERRF). Comparative proteomic studies showed that this mutation lowers the synthesis of all mitochondrial proteins that normally harbor lysine in their structure [58]. It is therefore not surprising that the A8344G mutation may cause a variety of additional symptoms such as ataxia, mental and motor retardation, ophthalmoplegia, and cardiomyopathy [98]. Another such mutation, A8296G, which affected the tRNA\(^{Lys}\) gene, was detected in an 8-day-old patient who was diagnosed with hypertrophic cardiomyopathy by echocardiography. The infant died of cardiac failure. The mutation was present in all tissues examined after autopsy [99].

A variable phenotype is also noted in maternally-inherited myopathy with cardiac involvement (MIMyCa syndrome), which can be caused by heteroplasmic A3260G\(^{Leu}\) mutation, which causes a significant decrease in transcription of mtDNA encoded leucine-containing subunits for complex I and IV [71].

2. Somatic point mutations. Humans accumulate somatic mutations during aging, but they are usually present in a much lower proportion (0.01%-1%) than in the inherited mitochondrial diseases [86,63]. It has been proposed that by relaxed replication, a low level of mutated mtDNA or mtDNA with deletion may gradually reach a threshold concentration beyond which the cellular energy metabolism is insufficient for maintenance of normal cellular function and disease or death ensues [100,86].

Another suggestion is that mitochondria form cellular genetic networks and exchange gene sequences. When this assumption was included in a computer model of single post-mitotic cells it showed that, even in cases of an initially rare somatic mutation, relaxed replication could lead to random drift with increased proportion of the accumulated mutant mtDNA within the cell [101]. However, in vitro findings in cell lines harboring full-length mitochondrial genomes with point mutations, indicate that mutated mtDNA does not repopulate mitochondria at a faster rate than the wild type genome [102].
3. Deletions. The number of cardiac mtDNA deletions increases with normal aging and deletions emerge at earlier ages in patients with cardiomyopathy. Significant increase of a 7.4 kilobase (kb) deletion (deleting nucleotides 8637–16084) but not of a 5kb deletion (deleting nucleotides 8469–13447) was found in myocardium of patients 41 years and older with idiopathic dilated cardiomyopathy compared with controls. In this study the 5kb deletion was also detected in 3 of 7 controls [103]. By comparison, the 7.4 kb deletion was present in all 15 and the 5kb deletion in 7 endocardial biopsies from 15 patients with idiopathic dilated cardiomyopathy. But similar deletions were found in control subjects, suggesting that the deletions were not the cause of the cardiomyopathy [104]. On the other hand, presence of inherited deletions has been reported in cases of dilated cardiomyopathy [105]. Whether or not such deletions are generally primary and pathogenic or occur as secondary events remains a controversial question [106].

In any case, oxidative stress is the main etiologic candidate as the cause of the deletions. Normal mitochondrial respiration produces a small amount (1-3%) of oxygen radicals that are normally neutralized by anti-oxidant enzymes. During mitochondrialOXPHOS dysfunction due to enzyme defects or enzyme inhibition, oxidative stress is significantly increased. Besides, during human aging, the levels of antioxidant enzymes decline. Oxygen radicals that are not neutralized by antioxidant enzymes damage nucleic acids and proteins, and cause lipid peroxidation [107]. Thus oxidative stress may induce mtDNA mutations, fragmentation, and deletion of part of the mitochondrial genomic sequence, resulting in smaller genomes (deleted mtDNA, δmtDNA). In vitro, δmtDNA replicates faster and repopulates mitochondria more efficiently. It has been proposed that the increased rate of replication is due to their smaller size compared with wild-type mtDNA [102].

Experimental evidence that oxidative stress induces mtDNA damage in vivo was obtained using mice lacking the heart and skeletal muscle-specific isoform of adenine nucleotide translocator-1 (ANT1). Lack of ANT1 uncouplesOXPHOS by abolishing exchange of ADP and ATP across mitochondrial membranes. In the ANT1-null mouse, the electron transport chain responded with a striking increase in hydrogen peroxide generation. This was compensated by a marked increase in the antioxidant enzyme manganese-superoxide dismutase (Mn-SOD) in skeletal muscle but much less so in the heart, which resulted in a major upsurge of cardiac mtDNA rearrangements [108].

Because the level of mutated mtDNA in aging cardiac tissue is relatively low, alternative etiologies have been proposed to explain aging-related cardiomyopathy due to declining mitochondrial function. It has been reported that thiol proteases, which are pivotal in mitochondrial degradation, are particularly sensitive to damage by radical oxygen species generated by the electron transport chain [109]. Besides, perinuclear lipofuscin or “aging pigment,” which indicates abnormal mitochondrial degradation, is frequently observed in aging cardiac myocytes [110]. These findings corroborate the notion that faulty mitochondrial degradation may play a role in the development of cardiomyopathy in older patients.

C. Abnormal expression of nuclear OXPHOS genes

The nuclear genes that code for mitochondrial proteins are distributed over several chromosomes (Table 1). At first glance, the distribution may appear random. However, in a few cases, a clustering of these genes with other disease-related genes is evident at chromosome locations 1q21, 7q32, 10q24, and 15q25, but most conspicuously at chromosome region 11q13. Interestingly, several cancer suppressor genes or oncogenes have been mapped to the same chromosome regions as the genes for nuclear-encoded mitochondrial proteins, suggesting an association between abnormal mitochondrial synthesis and function and neoplasia. Also, some clusters are located at or close to chromosomal viral integration points or fragile sites.

Mutations of two nuclear genes, NDUFV2 and NDUFS2, encoding complex I subunits were strongly linked with early onset hypertrophic cardiomyopathy in 3 siblings in a consanguineous family. The mutation caused a marked decrease in the amount of nuclear encoded subunit and complex I activity [111]. Mutation of NDUFV2 has also...
Table 1. Selected nuclear mitochondrial genes involved in the synthesis and function of the mitochondrial oxidative phosphorylation system (OXPHOS). The genes, which are located on many different chromosomes, have either been shown to cause cardiomyopathy, or are located at or close to other genes critical for mitochondrial synthesis and maintenance. Sources of further gene data are available from OMIM™ (On-line Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=OMIM). For further detail see text.

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been linked to Parkinson’s disease [112], an association that may illustrate the large variation of clinical expression of mitochondrial disorders. Others have noted mis-sense mutations of NDUFS2, which is located on chromosome 1q23, a breakpoint found in Ebstein-Barr virus (EBV)-related neoplasia [113,114]. Transgenic mice expressing EBV nuclear-antigen-leader peptide consistently develop dilated cardiomyopathy [115]. The product of the NDUFS4 gene is necessary for complex I assembly as evidenced by non-sense mutations of the gene, which prevents assembly of complex I in the inner mitochondrial membrane [116]. Clinically, NDUFS4 mutations are associated with Leigh-like syndrome or fatal neurological syndrome [117,118].

An interesting complex I gene, NDUFA2 is located at 5q31 [119]. A study of 7 pesticide sprayers (average age 36 years) with long exposure to pesticides revealed an aphidicolin-sensitive fragile site at chromosome region 5q31. This region is also a breakpoint found in cancer [120]. NDUFA6, NDUFA7, and NDUFB8 are located at chromosome regions 22q13.1, 19p13.2, and 10q23.2-23.33, respectively. NDUFV1 and NDUFS8 are located at chromosome 11q13. NDFUV1 encodes a complex I subunit that provides a binding site for iron cluster N-2 [121,122].

Chromosome locations of these nuclear mitochondrial genes are not only of interest to elucidate the pathogenesis of cardiomyopathy, but also because of findings of mutations and breakpoints occurring at these locations in benign and malignant tumors. For example, the tumor suppressor PTEN is located within the regions to which NDUFB8 has been mapped. It has been associated with prostate, endometrial, and thyroid cancers, and many other tumors. The cluster at chromosome 11q13 includes UCP2, UCP3, and MEN1 (menin-1); the latter is associated with endocrine neoplasia. Why are several genes involved in energy metabolism located at or near tumor suppressor genes?

A partial answer may be provided by observations of a direct link between OXPHOS disease and neoplasia in complex II defects. Complex II (succinate dehydrogenase, EC 1.3.99.1), consists of 4 protein subunits, all encoded by nuclear DNA. The two subunits encoded by genes SDHC and SDHD span the inner membrane. They are connected on the matrix side to flavoprotein and iron-sulfur protein subunits encoded by genes SDHB and SDHA respectively. SDHB, SDHC, and SDHD encoded subunits are involved in the assembly of the holoenzyme, and may partake in oxygen sensing and signaling. Mutations of these genes are associated with formation of hereditary paraganglioma and pheochromocytoma. The flavoprotein is the catalytic subunit that participates in the Krebs cycle converting succinate to fumarate. It carries electrons from FADH to Q10. Mutation of SDHA reduces Krebs cycle activity and OXPHOS energy conversion [123,124]. The first cases of mitochondrial disease caused by a mutated nuclear-encoded gene involved the mutation of SDHA in 2 first cousins who presented clinically with Leigh syndrome [6].

Complex III (ubiquinol cytochrome c reductase, E.C. 1.10.2.2) consist of cytochrome b, a mtDNA encoded subunit, and nDNA-encoded subunits cytochrome c1, 2 core proteins, an iron-sulfur protein, a hinge protein, 3 low molecular weight proteins, and QP-C, the ubiquinone cytochrome c reductase binding subunit encoded by the nuclear gene UQCRB, which must be imported from the cytoplasm.

Isolated complex III defects are uncommon, but a small number of cases have been reported. In a consanguineous patient, a deletion was found in UQCRB. The parents were heterozygous for the deletion, but none of 55 control subjects carried the deletion [125]. Patients with cardiomyopathy associated with isolated complex III deficiency illustrate the importance of normal cytochrome b expression for the activity of the complex. Five such patients had less than half the normal enzyme activity owing to a C15452G transition of the cytochrome b gene [126]. A different patient who developed exercise intolerance carried a G15242A mutation of the cytochrome b gene, but mutations of any of the 10 nDNA encoded subunits were absent [127].

Deficiency of complex IV (cytochrome c oxidase, EC 1.9.3.1) is often caused by a defect in
its synthesis [128]. The holoenzyme consists of 3 mtDNA-encoded and 10 nDNA-encoded subunit proteins. It carries electrons from cytochrome c to molecular oxygen, which is reduced to water. Energy from the electron flux through the 3 prokaryotic catalytic subunits powers the built-in proton pump, which helps to maintain the inner membrane potential as it pumps protons from the matrix to the intermembrane space [129,130]. Two copper-containing prosthetic groups, cytochrome a, and cytochrome a-3, are essential for the enzymatic function of the holoenzyme. Lack of copper hinders heme insertion into the complex and results in complex IV deficiency [131]. Copper deprivation leads to complex IV defect and increased oxidative stress in cultured cells [132].

The eukaryotic proteins SCO1 and SCO2 are required for assembly of the complex. Experimental mutations of either protein further demonstrate the requirement for copper in proper complex IV function. For example, mutation of yeast SCO1 (Sco1) hampers inclusion of a mitochondrial cytochrome c oxidase subunit and foils integration of copper into the enzyme complex. Significantly, copper supplementation rescues complex IV activity in yeast and bacteria that harbor mutated Sco1 [128]. Also, patients with mutation of the copper-binding assembly protein SCO2 suffer from loss of integration of mtDNA-encoded subunits and lack of inclusion of copper into complex IV. They have severe complex IV deficiency, affecting especially cardiac and skeletal muscle, and die of fatal infantile cardiomyopathy [133]. Importantly, it has recently been demonstrated that copper supplementation of myoblasts and fibroblasts from patients with SCO2 mutations restores nearly normal complex IV activity. This suggests that copper supplementation could conceivably be used to treat patients with such mutated mitochondrial assembly protein genes [134].

The variable clinical presentation caused by abnormal complex IV assembly is illustrated by how different SCO2 mutations affect the clinical presentation and pathology of affected patients. A case of a patient with a SCO2 mutation that resulted in a truncated protein showed the overlap between clinical symptoms often observed in mitochondrial diseases. The patient had lactic acidosis, hypotonia, generalized weakness, and hypertrophic cardiomyopathy. The clinical presentation resembled spinal muscular atrophy, known as Werdnig-Hoffmann disease, which, next to cystic fibrosis, is the most lethal autosomal recessive disease in Caucasians. At autopsy, loss of motor neurons and astrocytosis were noted in the ventral horns of the spinal cord. Spinal muscular atrophy is caused by a mutation of 1 of 3 SMA genes located at or near 5q12-q13. However, no mutation of the SMA gene could be detected in this patient [135].

Loss-of-function mutations of SURF1, another complex IV nuclear-encoded assembly protein, presents clinically as Leigh syndrome. In the mouse model, nearly all Surf1 knockout animals die post implantation. Mice that survive show complex IV deficiency in liver and heart, lack of motor strength, and early-onset post-natal mortality [136]. A homozygous missense mutation of COX10 leading to complex IV deficiency was detected in a consanguineous family. The gene, which maps to chromosome 17p12-p11.2, close to the SCO1 gene, encodes an enzyme required for the synthesis of the heme A prosthetic group of complex IV [137].

D. Electron carriers, ANT, and frataxin

The electron carrier coenzyme Q10 is an antioxidant. Q10 deficiency has been associated with congestive heart failure and cardiomyopathy [138]. But no depletion of Q10 and no increased rate of cell death were observed in cultured fibroblast from patients harboring a mutation of the fibroblast ATPase6 gene or in the nuclear gene SDHA that, as noted above, encodes the catalytic Krebs-cycle flavoprotein of complex II [139]. On the other hand, idebenone treatment restored OXPHOS function and dramatically improved the clinical picture of patients with deficiencies of complex I-III, all of which interact with the Q10 pool [140]. By comparison, the electron carrier cytochrome c receives electrons from the heme group of cytochrome b of complex III to its own heme group and then delivers the electrons to cytochrome c oxidase, the terminal enzyme of the electron transport chain (for graphics, refer to www.rcsb.org/pdb/molecules/pdb36_1.html).
Sengers syndrome is characterized by hypertrophic cardiomyopathy, myopathy, cataracts, and lactic acidosis [141]. It is associated with marked reduction in the protein content of ANT1. However, no genetic defect has been identified [142]. Of 12 patients with Sengers syndrome, 3 died as neonates and 6 died in early adulthood [143]. The FRDA gene on chromosome region 9q13 encodes the mitochondrial protein frataxin. A gene located in this region is associated with familial dilated cardiomyopathy suggesting that this gene may be FRDA.

Mutation of FRDA leads to a deficit in frataxin, which causes mitochondrial iron accumulation and Friedreich’s ataxia, an autosomal recessive degenerative disorder with failure of cardiac mitochondrial energy metabolism. Treatment using Q10 or idebenone plus vitamin E considerably ameliorates the symptoms of the disease [144].

E. Abnormal uncoupling

In rodent postnatal myocardium, UCP-2 is induced by fatty acids and UCP-3 by peroxisome proliferator-activated receptor-α (PPARα) [145]. The latter serves as a “lipostat” that regulates expression of several genes, which are required for cardiac mitochondrial fatty oxidation as evidenced by myocyte fat accumulation in the PPARα(-/-) mouse [146]. The non-steroidal anti-inflammatory drug (NSAID) sulindac sulfide, which has anti-cancer properties, induces PPARα [147-149].

Uncoupling can occur during opening of the mitochondrial permeability transition (MPT) pores. For example, reperfusion after cardiac ischemia opens the pores, depolarizes the membrane, and induces apoptosis [150]. Several NSAIDs or their metabolites induce MPT pore opening. Salicylate, a metabolite of aspirin, opens MPT pores in vitro. The induced membrane depolarization may contribute to the pathogenesis of in vivo aspirin-induced myocardial injury in Reye's syndrome [151].

F. Defective protein import

Defects of the mitochondrial protein import system can lead to defective oxidative phosphorylation. The genes for the human pre-protein translocase subunit of the outer membrane complex (TOM22) and inner membrane subunit (TIM10) have been mapped to 22q12-q13 and 11q12.1-q12.3, respectively. In yeast, the Tim22 complex harbors Tim9, Tim10, and Tim12; these proteins structurally resemble the deafness/dystonia syndrome peptide (DDP) whose gene TIMM8A has been mapped to chromosome Xp22. Mutations of TIMM8A are associated with Jensen syndrome and Mohr-Tranebjærg syndrome (MTS), two rare neurodegenerative diseases. MTS affects post-mitotic tissues leading to cortical blindness, dystonia, and mental deficiency. Its clinical presentation resembles syndromes caused by defects in mitochondrial oxidative phosphorylation [152,153] and illustrates that abnormal import of nuclear-encoded mitochondrial proteins affect OXPHOS function.

Metaxin, another karyotic protein that is associated with mitochondrial protein import, is essential for embryogenesis. MTX, the gene for metaxin, is located at chromosome 1q21, a non-random human cytomegalovirus integration locus [154-156]. This chromosome region harbors the glucocerebrosidase gene involved in Gaucher's disease, a storage disease which can affect the heart [157]. However, no case of cardiomyopathy caused by MTX mutation has been reported.

G. Dual genome disease

Mutation of Twinkle [158], ANT1 [159], or the nuclear gene for the mitochondrial DNA polymerase-γ subunit (POLG) [160], result in an unusual form of inheritance, exhibiting Mendelian inheritance combined with clinical expression of maternally inherited mitochondriopathies. Proteins encoded by these genes support mtDNA stability; mutation of any of the genes causes multiple mtDNA mutations and loss of mtDNA with formation of multiple deletions that lead to significant pathology [161]. For example, mutation of the Twinkle gene causes progressive external ophthalmoplegia (PEO) of the autosomal dominant type [158]. Twinkle has been mapped to chromosome 10q24, and clusters with the COX15 gene, mutation of which is associated with hypertrophic cardiomyopathy [162]. Moreover, 10q24 is an integration site for human papilloma
virus type 6 (HPV6AI1) [163], which has been linked to the development of benign neoplasms.

Childhood onset of PEO, autosomal recessive type, is associated with multiple mtDNA deletions and with severe cardiomyopathy that requires heart transplantation [164]. Besides Twinkle, PEO can be caused by mutations of POLG and ANT1 [159]. Mutation of POLG is implicated in male infertility [165], illustrating the unpredictable clinical consequences of mutations that affect mitochondrial function. Noteworthy, but of unknown significance, is that fact that POLG clusters with the COX5A gene at chromosome 15q25 [166].

V. Therapy

Patients with cardiomyopathy caused by mitochondrial defects, especially those with cardiac-specific defects, can benefit from cardiac transplantation [167]. By comparison, gene therapy for mitochondrial disorders was, until a few years ago, considered highly speculative and theoretical [168]. However, several novel approaches have been reported to restore normal mitochondrial function in vitro. Direct mitochondrial gene therapy uses a vector or targeting signal sequences to insert mitochondrial genes into mitochondria. Indirect mitochondrial gene therapy uses viral or non-viral vectors to introduce genes into the nucleus for nuclear coding of mitochondrial proteins [169].

Synthetic biotinylated polynucleobase molecules are taken up by cells in vitro and enter mitochondria when attached to the mitochondrial target sequence of the nuclear-encoded COX7 subunit [170]. Repair of defective ATP generation in LHON mitochondria harboring a G11778A transition was achieved in vitro by importing a synthetic ND4-subunit into mitochondria in LHON cybrids. The synthetic subunit, which included a mitochondrial targeting sequence, entered the defective mitochondria, successfully restored complex I function, and normalized ATP generation [171]. In vitro, direct microinjection of restorative mtDNA sequences into the mouse oocyte can correct mitochondrial defects [172]. Repair of mitochondria in the human germline or even insertion of mitochondrial sequences that have been found to be associated with longevity now appear scientifically feasible. However, religious and ethical considerations may influence the decision whether or not to implement these approaches to alter the human germline [173].

Cardiac oxidative damage increases with age in rodent models of aging. However, dietary restriction reduces oxidative damage in both the rat and mouse models [174]. The pineal sleep hormone and antioxidant, melatonin, may have anti-aging properties and other beneficial effects [175]. It dose-dependently increases the activities of complexes I and IV of mitochondria from rat liver and brain and enhances ATP synthesis [176].

Exercise can significantly increase SOD activity in young rats; however, in old rats, vitamin E supplementation is required to achieve similar benefits. Exercise training in young rats enhances the activity of tissue SOD of both the left and right ventricle, compared with sedentary controls. By contrast, old rats fail to show this response to exercise. Administration of oral vitamin E to old rats is required to remedy their lack of SOD activity enhancement and to reduce lipid peroxidation. Levels of cardiac catalase are also affected by exercise and vitamin E supplementation. In the left ventricle of young rats’ hearts, exercise enhances catalase activity compared with non-supplemented sedentary rats. A remarkable decrease in cardiac catalase activity was observed in 22-month-old sedentary rats compared to 4-month-old sedentary rats. In the young rats, vitamin E supplementation alone increased left ventricular catalase activity by about 80% compared to non-supplemented controls, but only by about 4% in old rats. In the latter, exercise enhanced catalase activity by 17% and when combined with oral vitamin E by 23%, compared to sedentary age-matched controls [24].

Some drugs induce adverse mitochondrial side effects. For example, drugs such as acidic NSAIDs inhibit mitochondrial function [177]. Azidothymidine (AZT) reduces ATP and glutathione levels in cultured cells [178]. Patients receiving AZT therapy may develop mitochondrial myopathy; there is one reported case of LHON associated with AZT therapy [179].
The rate of substrate production by the mevalonate pathway can enhance or decrease the velocity of Q10 synthesis [180] (Fig. 5). Iatrogenic inhibition may occur with therapeutic use of Adriamycin, gemfibrozil, or statins. The latter inhibit β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase, [181]. Importantly, inhibition of Q10 synthesis may be tissue-specific, affecting high-energy consuming organs more than other organs. In a rat model of the effects of inhibition by a statin drug, synthesis of ATP in the heart was decreased, but ATP production in liver was unaffected [182]. One possible reason for the different effects of statins on different organs may be variations in the uptake of Q10 by different tissues. After iv Q10 administration in the guinea pigs, Q10 rapidly accumulates in the liver from which it is redistributed by blood lipoproteins to kidney, heart, brain, and adrenals, with the organ uptake peaking at 8, 24, and 168 hr respectively. The adrenal exhibits the highest level of Q10 uptake, compared to the other organs [183]. Q10 treatment can improve cardiac function in patients with cardiomyopathy. When statins that inhibit Q10 synthesis are administered to patients, Q10 supplementation is recommended. Furthermore, oral Q10 therapy should be supplemented with agents that are essential for endogenous synthesis of the Q10 aromatic ring structure from tyrosine (Fig. 5).

As noted above, thyroid hormone plays an important role in the regulation of mitochondrial synthesis. However, a surplus of thyroid hormone reduces cardiac ATP levels and induces cardiac dysfunctions. A study of 20 hyperthyroid patients demonstrated an inverse correlation between serum levels of thyroid hormones and Q10. In hyperthyroid patients with cardiac dysfunction, oral Q10 supplementation (120 mg/day) reversed the cardiac
Further evidence of beneficial effects of Q10 in hyperthyroidism was obtained in a rabbit model. Sustained administration of L-thyroxine (T₄) to 29 animals induced cardiac dysfunction. Examination of the rabbit hearts showed reduced levels of ATP. By contrast, co-treatment of a comparison-group of animals with Q10 protected ATP levels and prevented T₄-induced cardiac dysfunction [185].

Dietary lipids affect the lipid composition of the mitochondrial membrane and the function of oxidative phosphorylation enzymes. Dietary lipids have a relatively short half-life in the membranes. In the rat model they are rapidly altered by changes in the composition of dietary fatty-acids. In an 11-day crossover study, rats were fed either only soybean oil or rapeseed oil. The group of rats that were changed from soybean oil to rapeseed oil rapidly developed a membrane lipid composition resembling that found in rats fed only rapeseed oil [186]. Rapeseed oil contains erucic acid, which lowers the respiratory capacity of heart mitochondria, presumably by an effect on the mitochondrial membrane [187].

Erucic acid is normally not detectable in human tissues. However, when present in vegetable oil in food or animal feed it accumulates in human or animal tissues, such as the heart, where it can induce lipid accumulation and cardiac fibrosis [188,189]. Another vegetable oil acid, linoleic acid, an 18:2 acid and a constituent of margosa oil, has been implicated in the etiology of Reye's syndrome. Peroxidized linoleic acid reduces rat liver mitochondrial ADP-stimulated (state 3) respiration, and uncouples respiration [190].

Of relevance to therapeutic options is the recent demonstration of mitochondrial DNA sequences in plasma or serum of cancer patients [191]. This observation suggests that sensitive analytical methods might be devised for early detection of mitochondrial dysfunction due to nDNA-encoded mitochondrial gene abnormalities or mtDNA mutations and deletions. Such an approach might provide opportunities for early intervention and prevention of cardiomyopathy.

VI. Conclusions

The tissue expression of OXPHOS enzymes in patients with cardiomyopathy caused by mitochondrial defects is often heterogeneous. Initial diagnosis is based on finding abnormalities in metabolites in body fluids or biopsies, followed by analysis of OXPHOS enzyme activities or molecular analysis of inherited or acquired gene defects [192]. Many findings of altered mitochondrial function in cardiomyopathies have been based upon studies of mitochondrial enzyme activities only, and the exact nature of genetic causes of defective enzyme function may have been only partially investigated. Central to the pathogenesis of cardiomyopathies is a disturbance of the mitochondrial membrane potential, which is normally strictly regulated to provide close tracking between depletion of the proton gradient and its restoration.

Whereas many nuclear genes for mitochondrial proteins have now been sequenced, in many cases their structure, promoter regions, and regulation have not yet been completely clarified. Dividing cells loose daughter cells with inefficient, deleted mtDNA, but post-mitotic cardiac myocytes lack this option.

Enhanced oxidative stress in the heart is a high risk factor for development of increasing heteroplasmy, particularly during the later decades of human life. When heteroplasmy reaches the cardiac tissue threshold level, mitochondrial energy generation becomes insufficient for normal physiological myocardial functions, and cardiomyopathy develops.

Several palliative therapeutic approaches are currently available for patients with cardiomyopathy, including heart transplantation, use of regimens or drugs that prevent mitochondrial damage (especially damage caused by oxidative stress), supplementats that protect or restore the mitochondrial oxidative phosphorylation enzymes, and the avoidance of toxic foods and environmental agents (such as certain pesticides) that inhibit mitochondrial function.

The cure of cardiomyopathies is only feasible through gene therapy. Methods for the repair of damaged mitochondrial genes or nuclear genes that encode mitochondrial proteins are almost within
reach. Whereas modification of the human germline to repair mitochondrial defects now appears to be scientifically possible, clinical applications of such gene therapy may be limited to some extent by religious and ethical considerations.

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