Regulation of mitochondrial proliferation in the heart: power-plant failure contributes to cardiac failure in hypertrophy

Steffi Goffart*a, Jürgen-Christoph von Kleist-Retzow*a,b, Rudolf J. Wiesner*a,*

aInstitute of Vegetative Physiology, University of Köln, Robert-Koch-Str. 39, 50931 Köln, Germany
bDepartment of Pediatrics, University of Köln, Joseph-Stelzmann-Str., 50924 Köln, Germany

Received 12 March 2004; received in revised form 22 June 2004; accepted 28 June 2004
Available online 20 August 2004
Time for primary review 19 days

We would like to dedicate this review to Radovan Zak (1931–1999), Professor at The University of Chicago Medical School, Associate Editor of Circulation Research and Journal of Molecular and Cellular Cardiology, who contributed much to our understanding of the basal mechanisms of cardiac hypertrophy

Abstract

During hypertrophy, proliferation of mitochondria does not keep pace with the increasing energy demand of the heart. This probably contributes importantly to cardiac failure, together with other phenotypic changes occurring during the growth process. The problem may be even aggravated if defects of mitochondrial function itself and not external factors cause the hypertrophic process. Here we review the basic mechanisms controlling mitochondrial biogenesis, especially the pathways coordinating expression of nuclear encoded mitochondrial genes and the small mitochondrial genome, and how these mechanisms may be connected to the cardiomyocyte differentiation program during development as well as under physiological and pathological circumstances.

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1. Why are mitochondria important for heart function and especially interesting in heart hypertrophy?

The heart is the organ with the highest oxygen uptake rate in the human body, consuming about 0.1 ml O2/g min at basal rates [1]. In the smallest mammal, the etruscan shrew weighing around 2 g, myocardial oxygen consumption is around 0.7 ml O2/g min (resting heart rate 800 beats/min: [2] and K.D. Jürgens, personal communication), and in the whole animal kingdom, only insect muscle consuming up to 5 ml O2/g min during flight surpasses the mammalian heart [3]. Due to this extraordinary demand for continuous synthesis of ATP by oxidative metabolism, cardiac myocytes are the cells with the highest volume density of mitochondria in the body. The heart also has an impressive reserve capacity and is able to increase its output upon demand following sympathetic and/or adrenergic stimulation, and consequently, myocardial oxygen consumption can rise about fourfold in humans [1]. In such situations, the increased ATP turnover is perfectly matched by an adequate increase in oxidative ATP synthesis, i.e. no oxygen debt accumulates as in working skeletal muscle, and the heart even oxidizes lactate derived from the periphery as a fuel [1]. It is unclear whether the maximal myocardial oxygen consumption is limited in vivo (i) by supply, which includes its flow from the capillaries to cytochrome c oxidase (CYTOX), the oxygen-consuming enzyme of the mitochondrial respiratory chain (RC), (ii) by the maximal turnover rate of this enzyme, or (iii) whether ATP-consuming reactions of the contraction–relaxation cycle, such as actomyosin-ATPase or Ca2+-ATPases, are the rate-limiting steps [4]. Especially in such situations, any imbalance between

Abbreviations: CYTOX, cytochrome c oxidase; FAO, fatty acid oxidation; HCM, hypertrophy cardiomyopathy; mtDNA, mitochondrial DNA; NEM, nuclear encoded mitochondrial; RC, respiratory chain

* Corresponding author. Tel.: +49 221 478 3610; fax: +49 221 478 6965.
E-mail address: rudolf.wiesner@uni-koeln.de (R.J. Wiesner).
rates of ATP synthesis and consumption would lead to a deleterious drop in energy-rich phosphate compounds.

Cardiac hypertrophy is primarily a compensatory reaction to match an increased workload imposed upon the ventricle, maintaining a constant relationship between systolic pressure and the ratio of wall thickness to ventricular radius, irrespective of ventricular size [5]. Heart muscle cells increase either in diameter, so that the ventricle can produce higher pressure against an increased afterload, or increase in diameter and/or length when pumping more volume due to an enhanced preload. An increased preload may be due to exercise (as with an athlete’s heart) or constant volume overload (e.g. following insufficiency of the valves), and an increased afterload is mostly due to arterial hypertension or outflow tract obstruction. In addition, mutations of genes encoding proteins of the contractile apparatus, the Ca2+-handling system, or other equipment with a new ion channel repertoire (molecular remodelling), an insufficient rise of RC capacity has long been noticed and contributes importantly to the development of heart failure as well [11]. It was shown long ago by morphometric analysis of electron microscopic pictures that the mitochondrial content per myocyte decreases in hypertrophic hearts in late stages, both in experimental animal models as well as in patients (reviewed in detail by Ref. [8]). Simultaneously, the hypertrophic heart reduces oxidation of fatty acids and uses glucose again as an important substrate, as it does during the fetal period, where mitochondrial content is also rather low (see below, Ref. [12]). It is tempting to speculate that the switch to glucose utilization, which needs less oxygen per mol of ATP produced, is an adaptive mechanism, since oxygen consumption per beat is enhanced due to an exaggerated increase of systolic pressure in hypertrophic hearts, especially when pumping against an increased afterload or during exercise [13]. However, the stimulation of oxidative metabolism is not sufficient to maintain normal levels of high-energy phosphates under such conditions, and many studies have shown that this is not caused by inadequate perfusion, as might have been expected [14]. In fact, the ratio of phosphocreatine to ATP in patients hearts in vivo is a good predictor of mortality due to cardiomyopathy [15]. In conclusion, an inadequate proliferation of mitochondria in cardiac myocytes facing an exaggerated energy demand, especially during high work load, and a simultaneous switch from fatty acid oxidation to glucose metabolism seem to be hallmarks of cardiac hypertrophy and important factors for compensation, ischemic vulnerability, and heart failure (summarized in Refs. [4,16]).

2. Proliferation of mitochondria does not match the energy demand of the hypertrophic heart

Important defects that limit the performance of the hypertrophic heart are localised at the level of the cardiac myocyte itself [9], although other factors like interstitial fibrosis, which is often associated with hypertrophy (organ remodelling), also contribute to cardiac failure [10]. And, while many factors are involved in the contractile failure of the cardiomyocytes, e.g. inappropriate Ca2+ handling, replacement of sarcomeric proteins by other isoforms, and equipment with a new ion channel repertoire (molecular remodelling), an insufficient rise of RC capacity has long been noticed and contributes importantly to the development of heart failure as well [11]. It was shown long ago by morphometric analysis of electron microscopic pictures that the mitochondrial content per myocyte decreases in hypertrophic hearts in late stages, both in experimental animal models as well as in patients (reviewed in detail by Ref. [8]). Simultaneously, the hypertrophic heart reduces oxidation of fatty acids and uses glucose again as an important substrate, as it does during the fetal period, where mitochondrial content is also rather low (see below, Ref. [12]). It is tempting to speculate that the switch to glucose utilization, which needs less oxygen per mol of ATP produced, is an adaptive mechanism, since oxygen consumption per beat is enhanced due to an exaggerated increase of systolic pressure in hypertrophic hearts, especially when pumping against an increased afterload or during exercise [13]. However, the stimulation of oxidative metabolism is not sufficient to maintain normal levels of high-energy phosphates under such conditions, and many studies have shown that this is not caused by inadequate perfusion, as might have been expected [14]. In fact, the ratio of phosphocreatine to ATP in patients hearts in vivo is a good predictor of mortality due to cardiomyopathy [15]. In conclusion, an inadequate proliferation of mitochondria in cardiac myocytes facing an exaggerated energy demand, especially during high work load, and a simultaneous switch from fatty acid oxidation to glucose metabolism seem to be hallmarks of cardiac hypertrophy and important factors for compensation, ischemic vulnerability, and heart failure (summarized in Refs. [4,16]).

3. Two genetic compartments are regulated mainly at the transcriptional level during mitochondrial proliferation

The mitochondrial matrix, containing the enzymes of the citric acid cycle, fatty acid β-oxidation (FAO), and other essential metabolic pathways, is surrounded by two membranes. The inner membrane is folded into cristae containing the large multisubunit complexes of the RC and all the carriers needed for exchange of metabolites with the cytosol, while the outer membrane containing porins is rather permeable to most solutes [17]. Mitochondria are generated by division or budding from existing organelles, although it is a matter of hot debate whether individual organelles exist at all or whether they form a large tubular network in vivo [18]. The organelle seems to turn over as a unit, since inner membrane proteins like cytochrome aa3, b and c, inner membrane lipids like cardiolipin as well as mitochondrial DNA (mtDNA) in the matrix all have the same half life, around six days in rat heart and liver [8]. Mitochondria are probably degraded in toto by autophagosomes. Therefore, proliferation of mitochondria needs an increased supply with proteins, nucleotides, and membranes. Very little is known about mitochondrial membrane...
Mitochondrial proteins are encoded on two separate genomes: mtDNA encodes only 13 essential subunits of the RC complexes, while—based on extrapolation from the yeast genome—up to 1000 nuclear encoded mitochondrial (NEM) genes lie on chromosomes (Fig. 1). These proteins include subunits and assembly factors of the RC complexes, membrane carriers, matrix enzymes, but also a large number of proteins necessary for transcription, replication and repair of mtDNA as well as structural proteins of mitochondrial ribosomes including translation factors. Considering the huge efforts eukaryotic cells undertake to produce a few mtDNA encoded proteins, it is still an enigma why they kept the small mitochondrial genome during evolution. Import of nuclear encoded proteins into the matrix, the inner membrane, and the intermembrane space has been investigated thoroughly in lower eukaryotes [19], but also in mammalian cells [20]. After import, nuclear encoded subunits are assembled with the mtDNA encoded subunits with the help of accessory factors to yield functional RC complexes like CYTOX.

CYTOX activity correlates well with levels of mRNAs for subunits derived from the two genetic compartments when several rat tissues covering a wide range of RC content are compared [21]. Also, in many experimental models of mitochondrial proliferation, transcripts for mitochondrial proteins were shown to increase: thus, mitochondrial biogenesis seems to be mainly regulated on the gene expression level [22]. To become significant, such correlations have to take into account the very different amounts of total RNA that different cell types in the body contain, varying by a factor of at least 7 [21]. Also, in some situations in which mitochondria proliferate, e.g. skeletal muscle training [23], cold adaptation of brown fat [24], or hyperthyroidism [25], total cellular RNA, which is mainly ribosomal RNA, increases together with mitochondrial transcripts. As a consequence, especially in animal models of cardiac hypertrophy [26], no specific increase of mitochondrial mRNAs (per tissue RNA) can be measured, although their cellular content actually does rise. An increased content of total RNA seems to be necessary to keep the content of CYTOX per gram of tissue constant, at least during the early growth phase. The molecular basis of this rise in total tissue RNA is an increased activity of RNA polymerases, which has been known for decades and lies probably at the basis of the whole hypertrophy process [27].

4. Regulation of mitochondrial DNA transcription and replication

Human mtDNA is a closed circular molecule of approximately 16.6 kb (Fig. 1). Each cell contains, depending on its size and mitochondrial content, between a few thousand and several tens of thousands of molecules, although exact estimates are available only for a few cell types. Mammalian mtDNA contains 37 genes encoding 13 mRNAs for polypeptides, which are all subunits of five RC complexes, two ribosomal RNAs as structural components of the mitochondrial ribosomes, and 22 tRNAs needed for translation. The genome has no introns, and only two non-coding regions exist, containing two promoter regions for transcription as well as the origin of replication. Both strands are completely transcribed in opposite directions.

Fig. 1. Involvement of two genetic systems in mitochondrial biogenesis. The simplified scheme illustrates two major classes of NEM genes regulated by different DNA-binding transcription factors, probably coordinated by PGC-1α. NEM proteins are imported into the organelles via Translocator Complexes of the Outer and Inner Membrane (TOM and TIM, respectively), and are distributed to the matrix, membranes and the mtDNA expression and maintenance machinery.
and the mature and stable transcripts (mRNA, tRNAs, tRNAs) are excised from the polycistronic transcript, while non-coding RNA is degraded instantaneously [17]. Transcription of mtDNA is under predominant control of nuclear encoded proteins, and a minimal mtDNA transcription machinery was only recently assembled from recombinant purified proteins in vitro [28], consisting of mtRNA polymerase, and two transcription factors termed TFAM and TFBM (transcription factor A and B, mitochondrial, respectively, Fig. 1). The TFBM protein exists in two isoforms with somewhat different affinities for the polymerase, but both isoforms bind tightly to the polymerase and thus they do not seem to be good candidates for transcription regulation. On the other hand, TFAM is a DNA binding protein which directs the polymerase with high specificity to the appropriate initiation sites in vitro and also stimulates the basal transcription rate [29]. Thus, it is generally believed to be the main factor regulating mtDNA transcription, and indeed, import of the recombinant protein into the matrix of liver mitochondria stimulates transcription from both promoters [30,31].

Replication of mtDNA starts, as on nuclear chromosomes, with RNA primers which are elongated by a specific mtDNA polymerase (mtDNA Pol γ). At present, a hot debate on two alternative mechanisms for mtDNA replication is going on. According to a long accepted model established in cultured cells [29], replication takes place by a rather unusual, asymmetric mechanism, while more recent data indicate that in vivo replication follows a conventional strand symmetric pathway [32].

Although it is still unclear how exactly transcription is regulated in vivo by the highly abundant TFAM protein within the mitochondrial nucleoid structure, numerous examples show that increasing mitochondrial mass in a cell is mainly achieved by increasing levels of mitochondrial transcripts (reviewed in Ref. [22]). Since mtDNA is present in multiple copies in a cell, some authors have postulated that regulation of the copy number of mtDNA determines the functional mass of mitochondria, while transcription was considered to be a constitutive process only depending on template availability. This mechanism is unlikely, since (i) comparison of tissues or cell lines with different oxidative capacities has revealed no correlation between RC capacity and copy number of mtDNA [33,34], and (ii) in many experimental models in which mitochondria proliferate, no increase of mtDNA copy number has been found at all [24,25].

5. Regulation of the expression of nuclear encoded, mitochondrial (NEM) genes

5.1. General features

Mitochondrial genes encoded in the nucleus have been called “housekeeping genes”, since they are expressed in every cell due to the vital functions of the organelle. However, since they are regulated under various circumstances and because cell-type specific isoforms of several subunits of the RC are already known, this categorization is somewhat misleading and should be restricted to constitutively expressed genes, if these exist at all. In most cases NEM gene promoters lack a TATA-box, but otherwise they are highly diverse in their structure, size and content of regulatory elements. Many studies were performed to find common regulatory features that coordinate expression of all genes necessary for mitochondrial biogenesis (extensively reviewed in Ref. [35]). Many NEM genes contain one or several GC-motifs binding the zinc-finger protein SP1 known to activate constitutively expressed genes (Fig. 1). SP1 normally is needed for basal promoter activity, but was also shown to mediate physiological adaptation processes [36]. Other transcriptional regulators which were shown to act on some NEM genes are API, NFκB, and YY1, but their importance for coordination of mitochondrial biogenesis remains to be elucidated (for an overview, see Ref. [37]).

The Nuclear Respiratory Factors (NRFs) were the first candidates for being specific transcriptional activators regulating NEM genes (Fig. 1). NRF-1 was identified as the main activator of the cytochrome c promoter, and later on it was shown to transactivate expression of many other mitochondrial genes, including those for subunits of all five respiratory chain complexes [38]. The human nuclear respiratory factor NRF-2 (and its mouse homologue GABP) is structurally not related to NRF-1, but was named so for its comparable function in many NEM genes. The genes for the NEM transcription factors (TFAM and TFBM) necessary for transcription of mtDNA (see above) contain regulatory sites for both NRF-1 and NRF-2, so this provides connecting links between nuclear and mitochondrial gene regulation [38]. The NRFs are especially interesting, as they regulate almost exclusively NEM genes, while other transcriptional activators known to target NEM genes have well-described functions for non-mitochondrial proteins as well.

Another frequent feature of NEM gene promoters are CRE elements (Fig. 1). The cAMP-responsive element binding protein (CREB) and its close relatives ATF-1 and CREM bind to CRE sites upon their phosphorylation, which can be induced by various signals like serum induction [39] or other stimuli leading to cAMP elevation. For several NEM genes, direct transcriptional activation by CREB was shown [40], but as expression of the coactivator PGC-1 (see below) is also regulated by this factor [41], CREB seems to influence NEM genes via multiple pathways.

The activating function of DNA-binding transcriptional regulators is often mediated by coactivators, which interact with DNA-bound regulator proteins and the basal transcription machinery. The best characterized coactivator involved in mitochondrial biogenesis is the PPARγ coactivator-1α (PGC-1α) (Fig. 1). PGC-1α was discovered as the
main regulator of mitochondrial proliferation in adaptive thermogenesis in brown adipocytes, but is expressed in all mitochondria-rich tissues such as slow oxidative skeletal muscle, liver, and the heart [42]. Overexpression of PGC-1α in cultured cells [43] and in skeletal muscle of transgenic mice [44] stimulates mitochondrial biogenesis. PGC-1α is a coactivator interacting with a broad variety of transcriptional regulators including peroxisome proliferator-activated receptor (PPARs), other nuclear receptors like hormone receptors, and NRF-1. Bound to any of these factors, PGC-1α enhances the transcriptional activation by recruiting HAT coactivators (histone acetyltransferases) like CBP/P300, SRC-1, or pCAF [45].

5.2. Regulation of NEM genes during heart development

The mitochondrial mass of cardiomyocytes increases during early fetal development, but especially in the early postnatal phase [46] when a switch of cardiac energy metabolism from glucose and lactate to FAO also takes place [12]. This is probably due to an activation of transcriptional regulators, which induce FAO enzyme expression as well as other NEM genes.

The MEF transcription factor family is crucial for heart-specific gene expression and MEF2 proteins are major regulators of the cardiac differentiation program (Fig. 2). All MEF factors are present in heart with MEF2A being the predominantly expressed form in postnatal cardiac muscle [47]. Direct MEF regulation of NEM genes was shown for the mitochondrial creatine kinase and the cardiac isoform of the CYTOX subunit VII [48,49]. Mice deficient in MEF2A develop a dilated cardiomyopathy after birth with a high probability for sudden death; surviving animals show mitochondrial deficiency and reduced mitochondrial content [50]. MEF2A therefore seems to be involved in upregulation of mitochondrial function in post-natal development and the maintenance of mitochondrial content in the heart.

The peroxisome proliferator-activated receptors (PPARs) are transcription factors involved in fatty acid metabolism: PPARα is known to be the main regulator of genes involved in FAO in muscle tissues and liver, while PPARγ regulates fat accumulation and adipocyte differentiation [51]. In the myocardium, PPARα is the predominant form, underscoring its strong reliance on FAO [52] (Figs. 1 and 2). The hearts of PPARα-deficient mice consume less fatty acids and strongly depend on glucose and lactate [53]. PPARα induces gene expression by dimerization with the retinoid receptor RXR and is activated by various ligands like fatty acids, but also importantly by PGC-1α. PGC-1α thus has an important function not only for mitochondrial biogenesis in brown fat and muscle, but also in heart development. PGC-1α gene expression in the heart is induced by MEF (Fig. 2) and repressed by HDAC (histone deacetylase 5) [54], and its transactival function can be regulated by phosphorylation or interaction with a repressor [55]. Indeed, PGC-1α expression is increased in neonatal mouse heart together with mitochondrial biogenesis [46], and forced overexpression in cardiac myocytes induces strong mitochondrial proliferation [56]. Transgenic approaches have also given evidence for developmental and gender-specific effects of PGC-1α: while overexpression in hearts stimulates mitochondrial biogenesis in neonatal mice, leading to a dramatic increase of mitochondrial mass and size, mitogenesis in adult mice is enhanced much less [57]. Instead, cellular and mitochondrial structure gets disturbed and the animals develop a severe cardiomyopathy. Downregulation of PGC-1α by induction of a constitutive form of its repressor HDAC5 (Tetracycllin system) results in disturbed mitochondrial integrity and sudden cardiac death in male mice, while female mice survive longer but develop a dilated cardiomyopathy [54], which is somehow reversible, if PGC-1α levels are normalized again.

5.3. Physiological adaptations of mitochondrial biogenesis in heart

The signaling pathway of PGC-1α and PPARα seems to be not only involved in developmental upregulation of...
mitochondrial content, but also in later life during physiological adaptations of the heart: fasting leads to PGC-1α upregulation not only in liver, but also in cardiomyocytes [56]. Stress situations like hypoxia and high ROS levels activate p38 Mitogen-Activated Protein Kinase (MAPK) [58]. The resulting phosphorylation of PPARα leads to enhanced interaction with PGC-1α and induced expression of mitochondrial β-oxidation enzymes [59].

5.4. Regulation of NEM genes during heart hypertrophy

While adaptation to regular exercise induces mitochondrial biogenesis in the growing heart cells in athletes’ hearts, which is well coordinated with the rising energy demand of the organ, pathological hypertrophy finally results in maladaptation processes, leading also to a relative decrease in mitochondrial mass.

Members of the transcription factor family of the nuclear factor of activated T-cells (NFAT) play a central role in modulating the gene expression program during hypertrophy [60] (Fig. 2). Acute pressure overload leads to increased activity of calcineurin and CaMKII and accelerated protein biosynthesis [61], including enhanced mitochondrial biogenesis. Activation by dephosphorylation of hyperphosphorylated NFAT3 by the calcium/calmodulin-activated phosphatase calcineurin leads to its translocation to the nucleus, where NFAT activates transcription synergistically with GATA4/6 [62]. Constitutively active NFAT or calcineurin induces strong hypertrophic growth of the heart [63], while mice deficient for the catalytic subunit of calcineurin CnAβ do not develop cardiac hypertrophy even upon pathological stimuli including pressure overload [64]. Other signaling cascades leading to cardiac hypertrophy including angiotensin-triggered PKC activation or ras/MAPK are activated by an increase in intracellular Ca²⁺ levels as well (for an overview on intracellular signalling, see Ref. [60]).

The connection between NFATs and NEM gene expression is not yet clear, but mice deficient in NFAT die in early embryonic stages showing signs of mitochondrial dysfunction [65] and mitochondrial metabolic stress leads to calcineurin/NFAT activation in a postulated retrograde communication pathway [66].

Cardiomyocytes of hearts failing due to pressure-overload seem to reverse the developmental program and re-establish some fetal features: energy metabolism switches from β-oxidation of fatty acids to glucose oxidation and glycolysis, and mitochondrial mass and activity declines. Accordingly, a decrease in transcriptional activators of genes involved in the fatty acid oxidation program is monitored in this state of cardiac hypertrophy. PGC-1α, PPARα, and NRF-1 levels are reduced, while MEF2A and MEF2C levels, predominant MEF factors in adult and fetal heart, respectively, remain unchanged [59,11,12]. In addition, some known repressors of FAO genes such as nuclear receptors and SP factors regain their fetal levels in heart failure [67] (Fig. 2) and accordingly expression of FAO enzymes is strongly reduced under these conditions. This regulatory pathway could also contribute to the down-regulation of all NEM genes, although the mechanisms involved are not yet clear.

In conclusion, the PGC-1α/PPARα proteins are central regulators of mitochondrial biogenesis and induction of FAO gene expression in the heart during development and physiological hypertrophy. Pathological hypertrophy leading to maladaptation of cardiac energy metabolism, on the other hand, probably involves downregulation of these signalling pathways by as yet mostly unknown factors, resulting finally in cardiomyocytes lacking an adequate mitochondrial mass.

6. Hypertrophic cardiomyopathy due to intrinsic defects, especially mutations of mitochondrial genes

In the previous sections we have discussed regulation of mitochondrial proliferation during cardiac development as well as dysregulation during hypertrophy triggered by extrinsic factors. However, hypertrophy can also be caused by intrinsic defects within the myocyte itself, involving dysfunction of a variety of cardiac proteins, which is seen in a number of inherited diseases including mitochondrial disorders.

Non-mitochondrial diseases leading to hypertrophic cardiomyopathy (HCM) include autosomal dominant inherited disorders caused by mutations in genes coding for sarcomeric proteins. The most prevalent of the 11 proteins currently known to belong to this group are β-myosin heavy chain (gene: MYH7), myosin binding protein C (MYBPC3), and cardiac troponin T (TNT2) (for an overview see e.g. Ref. [6]). HCM caused by alterations of other, non-sarcomeric proteins are due to mutations in AMP-activated protein kinase (AMPK) undergoing myotonic dystrophy. They also include storage disorders, e.g. Pompe disease (glycogen storage disease Type II; deficiency of the lysosomal enzyme alpha-1,4 glucosidase (GAA)), and disorders in fatty acid β-oxidation (in particular, carnitine-palmitoyltransferase deficiency CPT2, systemic carnitine deficiency, long-chain 3-hydroxyacyl-CoA-dehydrogenase LCHAD, and very long chain acyl-CoA dehydrogenase VLCAD deficiency).

However, we also know that several primary mitochondrial disorders, i.e. deficiencies of the RC due to mutations in NEM genes or mtDNA, also lead to HCM. In fact, cardiac involvement belongs to the most common manifestations and is evident in 24% of cases in a paediatric patient cohort with RC deficiency [68]. RC dysfunction in the heart was confirmed in endomyocardial biopsies in a
number of such cases and can be considered a primary and not a secondary phenomenon caused by the cardiomyopathy itself [69]. In recent years, several mutations in either of the two genomes have been identified as the underlying molecular basis.

mtDNA alterations leading to HCM comprise mainly point-mutations and in some exceptional cases rearrangements like deletions and/or duplications. Point-mutations have been evidenced in all 3 classes of mtDNA encoded genes, i.e. in tRNA genes (tRNA<sub>Leu(UUR)</sub>, tRNA<sub>Ile</sub>, tRNA<sub>Val</sub>, tRNA<sub>Glu</sub>, tRNA<sub>Gln</sub>), in genes coding for structural RC subunits (Cytc, ATPase6) and in the 12S rRNA (overview, see e.g. Ref. [7]).

Mutations leading to HCM have also been found in nuclear genes coding for structural RC subunits and for proteins involved in RC assembly. The former comprises two genes coding for subunits of Complex I, NDUFS2 and NDUFV2 [7]. Interestingly, HCM was not seen in any of the patients with known mutations in other structural RC subunit genes. Mutations in nuclear genes encoding assembly factors thus far belong to proteins involved exclusively in the biogenesis of CYTOX: SCO2, COX15, COX10 and SURF1. While all patients with SCO2 and COX15 mutations consistently present with HCM as the main clinical phenotype, this has been found only occasionally with COX10 [70] and SURF1 [71]. The latter mutations typically lead to Leigh syndrome, a neurodegenerative disorder showing various lesions in the basal ganglia and other areas of the central nervous system. SCO2 codes for a chaperone protein involved in the delivery of copper to the Cu<sub>A</sub> site of CYTOX subunit COX2p [72], while the protein coded by COX15 is involved in the synthesis of heme A, one prosthetic group of CYTOX [73]. All patients typically present early, very often already in the neonatal period or in the first months of life, and ultimately die from heart failure in the first year or in early infancy.

A clearly distinct presentation with regards to both the clinical and the biochemical phenotype is Friedreich’s ataxia, in which HCM develops in most patients during adolescence and early adulthood. Friedreich’s ataxia is a multisystem neurodegenerative disorder presenting initially with gait and limb ataxia. The disease-causing gene for frataxin, a protein most likely involved in the intramitochondrial synthesis of iron–sulfur (Fe–S) clusters [74]. Loss of frataxin leads to mitochondrial iron accumulation, deficiency in the activities of Fe–S cluster-containing enzymes of the mitochondrial respiratory chain and the tricarboxylic acid cycle, and increased oxidative stress (for an overview, see Ref. [75]). The disclosure of Friedreich’s ataxia as a genuine mitochondrial disorder [76] led to the hypothesis of a pathogenetic concept integrating oxidative stress, Fe–S deficiency, and iron accumulation in a vicious cycle as underlying the progressive course of the disease. Ultimately, this concept allowed development and testing of a pharmacological therapeutic approach to slow down the disease progression by administration of idebenone, a short chain analogue of coenzyme Q<sub>10</sub> with presumably beneficial antioxidant function [77]. This treatment has meanwhile proved efficient in reducing the myocardial hypertrophy in most, but not all, patients treated, but did not lead to major improvements of the neurologic condition. Remarkably, it has been recently shown that the improvement in myocardial function is paralleled by a restoration of the initially deficient activities of Fe–S cluster-containing enzymes in a patient after 5 years of treatment with idebenone [78]. This clearly indicates that Fe–S enzyme deficiency is crucial in the pathogenesis of HCM in Friedreich’s ataxia and that idebenone is able to improve this deficiency.

Another line of evidence that primary mitochondrial dysfunction can lead to cardiomyopathy comes from several animal models created in order to study mitochondrial disorders. Cardiac hypertrophy has been seen in mice after invalidation of the heart/muscle isoform of the adenosine nucleotide translocator (ANT1) [79] and two separate conditional knock-out models (striated muscle and neuron/cardiac muscle-specific) for frataxin [75]. Interestingly, ultrastructural examination of heart tissue showed clear signs for mitochondrial proliferation in the ANT1 knock-out mice but no increase in size or change in the appearance of mutant mitochondria. On the other hand, the frataxin knock-out animals showed clear alteration of structure, size, and integrity of mitochondria in their heart tissue. Notably, the initial cardiac hypertrophy developed into a dilated cardiomyopathy in later life, which can be observed frequently in the course of human disease. Dilated cardiomyopathy was also seen in mice in which Mn-superoxide-dismutase (Sod2), the mitochondrial enzyme responsible for detoxification of the superoxide anion [80], was ablated or in mice in which TFAM, the mitochondrial transcription activator, was ablated specifically in heart [81]. Interestingly, in the TFAM knock-out mice a metabolic switch from fatty acid oxidation to glucose utilisation also occurred [82].
Acknowledgements

Our work on mitochondrial biogenesis and mitochondrial diseases was supported by DFG to R.J.W. (DFG Wi 889/3-3), Stiftung VERUM to R.J.W. and S. G. and Köln Fortune to J.C.v.K.-R, respectively. We apologize to all our colleagues whose work we could not properly quote due to space constraints.

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