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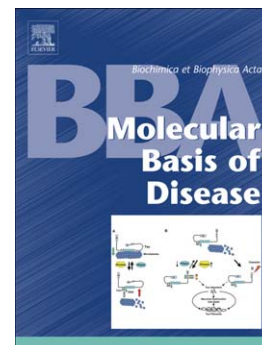
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**A Critical Approach to the Therapy of Mitochondrial Respiratory Chain and Oxidative
Phosphorylation Diseases**

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Summary

Taking advantage of a series of questions raised by an association of patients with mitochondrial disease, this review, after a brief overview of basic concepts of mitochondrial bioenergetics and genetics, discusses the pro and con of a number of practical options in the field of mitochondrial therapy. This makes it clear that, in contrast to the spectacular progress in our understanding of the biochemical and molecular bases of the mitochondrial diseases defined restrictively as disorders due to defects in the mitochondrial respiratory chain, we are still extremely limited in our ability to treat these conditions. We finally discussed the emerging genetic-based strategies that show some promise, even if much work remains to be done.

Considering that mitochondrial dysfunction may originate from mutations in more than 1,000 genes [1], from the deleterious effects of many toxic compounds [2], and even occur spontaneously during ageing [3], it is hardly surprising that human mitochondrial diseases are much more frequent than previously thought [4]. They may be mild or severe, static or progressive, early- or late-onset, tissue-specific or multisystemic [5-7] (Fig. 1). Besides the numerous potential pathogenic mechanisms, additional factors that determine the clinical phenotypes of such these disorders include the type and severity of each defect, and the types of cells or organs involved. As a result, it is very difficult to review the many therapeutic challenges posed by this heterogeneous group of diseases, essentially covering all types of medical specialties. Even if we restrict the definition of mitochondrial diseases to include only those due to defects of the respiratory chain (RC) and oxidative phosphorylation (OXPHOS), we are still faced with a considerable number of diseases.

Arguably the major function of mitochondria is to burn in the flame of oxygen substrates derived from carbohydrates, proteins and fats, thus providing ATP for the cell [8]. The transport of substrates and cofactors into the mitochondrial matrix space is facilitated by various carriers in the mitochondrial membranes (Fig. 2) [9]. Notably, different cell types may have different proportions of matrix enzymes and membrane carriers, which adapt them to the specific metabolic demand of each organ[10]. Similarly, the dehydrogenases either belonging to the RC (complex I, succinate dehydrogenase) or feeding into the RC (ETF, glyceraldehyde 3-phosphate dehydrogenase, etc; Fig. 3A) vary from tissue to tissue, whereas the composition and organization of the terminal cytochrome segment of the RC (from complex III to IV) are much more conserved among tissues [11]. This tissue-specific structural organization of the OXPHOS system can predictably change the clinical consequences of a given defect in

different tissues. The assembly of RC complexes into higher molecular weight entities, the so-called super-complexes [12], further complicates the potential effects of any defect in the OXPHOS pathway. Finally, before considering therapeutic strategies, we should stress that our knowledge of the functions of OXPHOS components is still partial and that we might still discover additional and unpredictable roles for these proteins (as in the case of cytochrome *c*, or the GRIM19 protein), or their substrates (such as tumor-triggering succinate) [13].

To this functional complexity corresponds the genetic complexity of mitochondria (Fig. 3B) [1]. Hundreds of genes are necessary to build the OXPHOS system, of which only a small subset is still entrapped in the mitochondrial matrix. Many more - between 1,500 and 2,000 of the 30,000 genes of a human cell - are necessary to build the whole mitochondrion, and deleterious mutation in these genes may well result in secondary OXPHOS dysfunction. Because of the dual origin (nuclear or mitochondrial) of the RC components, all known types of inheritance have been reported in affected families [14]. Mutations affecting mtDNA are either sporadic or maternally inherited, with a single exceptional (and partial) case of paternal inheritance. However, mendelian inheritance of mtDNA anomalies (deletions or depletion) are due to mutations in nuclear genes encoding proteins involved in mtDNA metabolism. Besides maternal inheritance, the hallmarks of mtDNA genetics include heteroplasmy, threshold effect, and mitotic segregation, because cells are endowed with much more than one mitochondrial genome (in fact, from 1,000 to 1,000,000). When an mtDNA mutation is present, a cell can harbor a mixed population of normal and mutated mtDNA genomes (heteroplasmy) in various proportions. If the mutation is pathogenic, a crucial minimum number of mutated mtDNA is required to cause OXPHOS dysfunction (threshold effect) [15]. As transmission of this mixed population to daughter cell is random, the clinical phenotype

resulting from varying proportion of heteroplasmy may also shift between generations (mitotic segregation).

It follows from the above considerations that mitochondrial diseases can be classified on the basis of clinical presentation, inheritance, or functional impairment. None of these categories are very useful when considering therapy, since a) similar clinical presentations may result from different biochemical and genetic defects (*e.g.* Leigh syndrome); b) conversely, mutation in one and the same gene can result in different diseases (*e.g.* BCS1L mutations); and c) biochemical defects of any RC complex can cause distinct diseases (*e.g.* complex II defect leading to encephalopathy or cancer). Given the puzzling complexity and variability of mitochondrial diseases, treatment strategies also require diversity and specificity, and it is inconceivable that a single “magic bullet” could treat all mitochondrial diseases.

Instead of reviewing all the different therapeutic strategies (palliative, pharmacologic, genetic) available or under investigation, we chose a more practical approach. Taking advantage of a set of questions about real medical situations selected from a French patient’s association (Association contre les Maladies Mitochondriales; AMMi) catalog, and considering the future of therapy for OXPHOS diseases, we will attempt to discuss some major issues related to this topic.

Questions regarding present therapeutic approaches

1. *Is Coenzyme Q₁₀ supplementation recommended in all mitochondrial diseases and under any circumstance?*

What makes CoQ₁₀ so extremely popular in the treatment of mitochondrial diseases is its well-documented safety, even at doses as high as 2,000 mg daily, and its dual role as a component of the RC and as a potent reactive oxygen species (ROS) scavenger [16] (Fig. 4). Also in favor of CoQ₁₀ is experimental evidence from studies of lymphocytes from 12 patients with diverse well-documented RC defects before and after 12 months of supplementation with a “cocktail” that included CoQ₁₀ (350 mg daily), L-carnitine, vitamin B complex, vitamin C, and vitamin K₁ (Phylloquinone). [17] There was a significant increase in ATP synthetic capacity in lymphocytes after treatment, although none of the patients improved clinically. Exposure of control lymphocytes in vitro to the various agents showed that only CoQ₁₀ increased ATP synthesis in a dose-dependent manner.

There are numerous anecdotal reports of the beneficial effect of CoQ₁₀ in mitochondrial diseases, but a rigorous, placebo-controlled, double-blind therapeutic trial is still missing (and sorely needed). Standardized therapeutic trials of CoQ₁₀ have been conducted or are being conducted in neurodegenerative diseases, including Parkinson disease (PD), Huntington disease (HD), and amyotrophic lateral sclerosis (ALS), some of which suggested a trend towards improvement, but none were clearly successful [18]. Not unexpectedly, the results were generally more positive in patients with primary or even secondary CoQ₁₀ deficiencies. For some patients with primary CoQ₁₀ deficiency, supplementation can be life-saving, but it should be tried in all patients with decreased CoQ₁₀ concentration in muscle, in whom substantial improvement is generally observed [19-25].

A synthetic form of CoQ₁₀, called idebenone, penetrates the blood-brain barrier more effectively and has been used in therapeutic trials of Friedreich ataxia (FA) [26] (Fig. 4 E, F). This autosomal recessive disease can be considered a RC defect because the pathogenic trinucleotide repeat in the *frataxin* gene impairs the non-heme iron–sulfur (FeS) proteins that

are part of complexes I, II, and III [27]. While initial studies had suggested a beneficial effect of idebenone only on the cardiopathic component of FA, a recent standardized study showed a dose-related beneficial effect also on the neurological component of FA [28].

To answer the question raised by the title of this subsection, our empirical answer is that high doses of CoQ₁₀ (30 mg/kg in children; at least 600 mg daily in adults) should be tried in all patients with mitochondrial diseases because it is harmless and more often than not at least mildly beneficial.

2. How about other electron acceptors?

In a woman with exercise intolerance, mitochondrial myopathy with ragged-red fibers (RRF) and complex III deficiency (later attributed to a mutation in the *cyt b* gene), two artificial electron acceptors, menadiol diphosphate (40 mg daily) and vitamin C (4 g daily) improved the clinical picture, as documented also by ³¹P-MRS [29]. However, the improvement was not sustained and other patients with complex III deficiency myopathy failed to respond [30]. At this point in time, no other electron acceptor possibly acting as a shunt in the respiratory chain has proven safe and effective in patients with mitochondrial diseases.

3. Is the ketogenic diet indicated for RC defects?

The ketogenic diet (KD) has been in use for many years in children with seizures resistant to conventional antiepileptic drugs (AEDs), but recently it has been advocated also for children with mitochondrial diseases, in whom it has proven safe and largely effective [31]. The mechanism of action of the KD is not clear, but evidence has been provided that it

increases brain energy metabolism by upregulating mitochondrial biogenesis [32]; by increasing ATP and adenosine concentrations and neuron-glia interaction [33]; by inhibiting ROS production [34]; and even by shifting the level of heteroplasmy in cell cultures harboring single mtDNA deletions [35]. Because of this heteroplasmic shifting observed *in vitro*, we are now planning to try the ketogenic diet in patients with large-scale mtDNA deletions (KSS, CPEO, PS).

4. *Is creatine useful?*

While CoQ₁₀ has been used extensively, other energy-boosting compounds, such as creatine, have been less commonly employed. Two randomized studies of creatine monohydrate supplementation have arrived to different conclusions: a smaller cohort of severely affected patients improved [36], whereas a larger cohort of less severely affected patients did not [37].

A recent study has explored the effect of three compounds with different mechanisms of action: increasing ATP production (CoQ₁₀ and creatine), scavenging ROS (CoQ₁₀ and lipoic acid), and providing alternative energy sources (creatine). A randomized, double-blind, placebo-controlled, crossover study in 16 patients with definite or probable mitochondrial diseases showed positive results, including lower blood lactate and urinary 8-isoprostanes and increased muscle strength [38]. Should all patients with weakness be tried on creatine? Given the conflicting results of the formal trials, it does not seem justified to prescribe creatine alone to all weak patients, although it can be usefully associated with CoQ₁₀ in selected patients.

5. *Which drugs in clinical practice should be used with caution in patients with RC defects?*

Another practical question regards common drugs that may interfere with mitochondrial metabolism or biogenesis and either cause or exacerbate mitochondrial dysfunction. The most common drug in this group is valproic acid, a very effective AED often considered in children with Alpers syndrome, in whom, however, it may cause a catastrophic worsening of the liver disease [39]. In patients without POLG deficiency, valproate can be useful in controlling seizure, but liver function should be carefully monitored.

Other drugs to be used with caution are aminoglycoside antibiotics, which can cause hearing loss in individuals harboring the A1555G mutation in the 12S rRNA gene of mtDNA [40, 41]. If aminoglycoside administration is required, it would be prudent to exclude that the patients harbor the A1555G mutation. The problem of antiretroviral drugs impairing mtDNA replication and causing symptomatic mtDNA depletion is well known and has triggered surveillance groups in many countries [42].

An unanswered problem regards anesthetics: on the one hand, patients with mitochondrial diseases often require surgical intervention (cochlear implantation; percutaneous endoscopic gastrostomy; ear tubes placement; tracheotomy); on the other hand, they are notoriously vulnerable to stress. Thus, both preoperative evaluation, with special attention to cardiac function, and the choice of anesthesia (local *vs* general) and anesthetics (is propofol toxic to mitochondria?) have to be carefully considered [43].

6. How do we fight lactic acidosis?

In fighting lactic acidosis (Fig. 5), administration of bicarbonate is the first line of defense. However, on the long run, bicarbonate is not effective and may actually exacerbate cerebral dysfunction. A more specific lactic acid-lowering agent is dichloroacetate (DCA), which acts by inhibiting pyruvate dehydrogenase (PDH) kinase, thus keeping PDG in the

dephosphorylated, active form and favoring pyruvate metabolism and lactate oxidation [44]. Although treatment of MELAS patients harboring the c.3243A>G had to be interrupted because of peripheral nerve toxicity [45] and a demyelinating effect of DCA has been documented in co-cultures of rat Schwann cells and dorsal root ganglia [46], prolonged treatment of 36 young children with lactic acidosis was considered well tolerated [47]. When is it crucial to treat lactic acidosis? Some pediatricians try to quench even mildly increased blood lactic acid. This becomes imperative when lactate levels surpass 10 mmol/l or blood CO₂ falls below 20 mEq/l.

7. Is arginine effective in treating and preventing strokes in MELAS?

The pathogenesis of the “strokelike episodes” that characterize the MELAS syndrome remains unclear, but probably involves both vasogenic and cytotoxic edema. Altered endothelial function of brain arterioles has been suggested long ago [48], by morphology and, more recently, by serial brain imaging [49], and by flow-mediated vasodilation (FMD) [50] and may explain the apparently beneficial effect of L-arginine administration to patients both in the acute phase and as a preventive measure [51, 52]. Although our knowledge of arginine metabolism is limited [53], there is little question on its role in maintaining NO-mediated vasodilator tone. Unfortunately, the beneficial effect of L-arginine has not yet been documented in a standardized therapeutic trial and remains anecdotal.

8. How to handle anecdotal information on isolated cases or from unofficial medical sources.

Anecdotal information has to be handled as interesting data (especially if based on a convincing rationale) waiting for experimental (e.g. double-blinded trial) confirmation. Non-medical data have to be handled with extreme skepticism, both to prevent raising false hopes among anguished parents and to prevent ruthless commercial exploitation of what amounts to quackery. Case in point is the recurrent inquiry on the part of parents about the efficacy of hyperbaric therapy, which has never been proven effective and which – if anything – raises concerns about being deleterious in children with mitochondrial diseases.

9. Dealing with generally multisystemic diseases, does targeting one organ, one system, or one function make any sense?

Although mitochondrial diseases, and especially those due to mtDNA mutations, are often multisystemic, available best standard of care has to be directed to individual affected tissues. Thus, seizures can be controlled with AEDs (with due caution in employing valproic acid, as discussed above); droopy eyelids (ptosis) can be alleviated by frontalis suspension; neurosensory hearing loss can improve dramatically with cochlear implants; endocrine dysfunctions can benefit from appropriate hormone replacement; cardiac block in Kearns-Sayre syndrome (KSS) can be avoided with timely placement of a pacemaker [18]. More controversial is single organ transplantation in generalized diseases. Nonetheless, cardiac transplantation has been employed successfully in some patients with mitochondrial diseases,[54-57], and liver transplantation has been used in a few patients with the hepatocerebral form of mtDNA depletion (usually due to mutations in the *DGUOK* gene), who had severe liver failure but relatively minor brain involvement [58-62]. Longer follow-up of transplanted patients will be necessary to assess the value of this intervention.

10. What is the role of artificial life support in mitochondrial diseases?

Heroic measures to keep mitochondrial patients alive in the face of life-threatening situations (infections; cardiorespiratory failure; liver failure) have to be discussed with adult patients and their families and with parents of pediatric patients well ahead of the crisis, explaining to them that life might be prolonged for days, weeks, or even months, but the disease is relentlessly progressive. This is exemplified by Leigh syndrome, a devastating encephalomyopathy of infancy or childhood with diverse etiologies, including pyruvate dehydrogenase complex (PDHC) deficiency and a variety of RC defects. There is no effective therapy for any form of LS and death rarely occurs later than 5 years of age. It is important to stress that a decision of this magnitude has to be weighed carefully on a case-to-case basis and with the full support of the family.

11. Could some vaccines be dangerous, especially in young children?

The risk of vaccination in children with mitochondrial diseases has taken center stage in the United States after the parents of a child with autism and a purported – but not conclusively documented – mitochondrial disease, were awarded compensation from the federal government because autism was considered the consequence of vaccination. This “cause célèbre” has probably encouraged a dangerous tendency on the part of parents to refuse vaccination for autistic children and children with mitochondrial diseases [63]. While there is evidence that autism may be related to mitochondrial dysfunction in some cases [64], there is absolutely no evidence that vaccination substantially worsens symptoms of mitochondrial diseases or causes autistic traits to manifest. In fact, mitochondrial patients especially need to be protected from infectious diseases that may run a devastating course in energy-challenged individuals. The fear of vaccination may be magnified by a recent report that low-heteroplasmy pathogenic mtDNA mutations are unexpectedly frequent among the normal population [4].

12. Where can general information on safe practices be obtained?

Practical information can be obtained from patients advocate groups, such as the United Mitochondrial Disease Foundation (UMDF) in the United States. The UMDF has just published a primer on mitochondrial diseases called “Mito 101”, which tries to answer most common concerns [65]. Increasingly, centers of excellence in mitochondrial diseases and national or multinational mitochondrial research consortia are appearing throughout the world and are offering patients specialized diagnostic services and state-of-the-art therapeutic support. We would strongly recommend to patients who gather information from easily available websites to check this information with that provided by the above mentioned institutions.

What next?

1. Faithful animal models: do they exist?

One of the most formidable problems of mitochondrial medicine (and especially of “mitochondrial therapy”) has been the lack of faithful animal models, at least for the mtDNA-related disorders. For disorders due to nDNA mutations, there are steadily increasing numbers of transgenic mice (both knockout and knockin) that more or less faithfully recapitulate the human diseases, especially defects of intergenomic signalling. Thus, knockout mice for the *ANT1* gene develop mitochondrial myopathy [66]; two different knockin mice for the *TK2* gene caused mtDNA depletion but different clinical phenotypes [67, 68]; mice carrying heterozygous mutations in the *OPA1* gene show optic nerve pathology [69]; knockin mice for the *PEO1* gene show mitochondrial myopathy and respiratory dysfunction [70]; and mice harbouring an error-prone version of the *POLG* gene (“mutator mice”) accumulate mtDNA mutations and show dramatically premature aging [71]. There is at least one example of

knockout mice for a COX assembly gene, *COX10*, which develop a mitochondrial myopathy [72].

Lately, the progressive ataxic phenotype of the *Harlequin* mouse - a natural mutant due to a retroviral insertion in the first intron of the *Aif* (Apoptosis Inducing Factor) gene [73] – has been ascribed to a defective complex I activity [74]. Both disease progression and phenotypic variability of the *Harlequin* mouse strongly resemble that of human mitochondrial-complex I-deficiency syndromes, including progressive cerebellar ataxia, optic atrophy, growth retardation, and, inconsistently, late-onset cardiac hypertrophy [75]. Thus, the *Harlequin* mouse is a promising model in which to experiment treatments for complex I deficiency syndromes. Targeting the *NDUFS4* subunit of complex I also produced mice that exhibited several features seen in patients with complex I deficiency [76]. However, the severe enzyme defect in these mice causes death at 7 weeks of age and does not allow the animals to express the extraordinary clinical variability of the human disease.

Generating transmitochondrial mice is enormously more difficult because nobody has found a way of putting DNA into mitochondria [77, 78]. The best transmitochondrial mouse was obtained through an ingenious trick by Hayashi and collaborators [79]: these mice harbour and transmit maternally large-scale mtDNA deletions and manifest mitochondrial myopathy, growth retardation, and kidney failure.

2. When will gene therapy become available for mitochondrial diseases?

Although gene therapy for mtDNA-related diseases has been pursued with great fervour in many laboratories and has generated numerous elegant papers, it is fair to say that most of this activity has been confined to the bench and has had few bedside applications [80]. We think that the most viable of the many strategies proposed [18] (Fig. 6) is heteroplasmic shifting towards wild-type mtDNA, in part because clinical experience teaches

us that a relatively small such shift may suffice to lower the mutation load below the pathogenic threshold. Many different approaches have been tried, but their applicability to humans appears remote [81]. These include: (i) inhibiting the replication of mutant mtDNA by selective hybridization with nucleic acid derivatives (such as peptide nucleic acids, PNAs) [82, 83]; (ii) importation of yeast tRNA to replace mutated human tRNA [84]; (iii) importation of wild-type polypeptides (either allotopically or xenotopically expressed) into mitochondria to replace mutated ones [85-88] or to complement faulty function [89, 90]; (iv) importing specific restriction endonucleases that cut mutated but not wild-type mtDNA and act as “silver bullets” [91, 92].

Potentially more applicable are pharmacological or dietary approaches that would specifically inhibit mutated mitochondrial genomes. For example, exposure of cybrid cell lines harbouring single large-scale mtDNA deletions to ketone bodies in the culture medium has resulted in a downward heteroplasmic shift [35]: as the ketogenic diet is already employed in pediatric neurology to control drug-resistant epilepsy, this dietary regimen could be tried in patients with KSS, PEO, or PS.

An even friendlier approach to gene shifting is exercise, but the results are mixed. Aerobic (endurance) exercise has been shown to improve quality of life, physiological features (peak work, V_{O_2max} , peak O_2 extraction) and biochemical features (muscle energetics as reflected by ^{31}P -magnetic resonance spectroscopy, citrate synthase activity, and COX activity) in muscle of patients with mitochondrial diseases [93-95]. However, the effect on heteroplasmy is questionable: one study showed an increase of the mutation load [93], another showed stable heteroplasmy level of single large-scale mtDNA deletions [95]; a third study by the same group showed greater levels of oxidative stress during endurance training [96]. On the other hand, resistance exercise promotes maturation and incorporation of satellite cells, which, harboring lower mutation loads, shift heteroplasmy towards the wild-type [97].

However, the efficacy and safety of resistance exercise in mitochondrial patients remain to be fully assessed.

For mendelian mitochondrial diseases, stem cell therapy offers real promise. This is best illustrated by the success of allogeneic stem cell transplantation (alloSCT) in one woman with MNGIE: her clinical condition improved, TP activity reached heterozygous levels in the buffy coat, blood levels of toxic compounds, thymidine and deoxyuridine, returned to normal, and nerve conduction velocities improved [98]. Almost two years after alloSCT, she continues to improve.

Yet another approach to the therapy of mendelian RC disorders tries to imitate mother nature by promoting mitochondrial biogenesis (and the residual activity of a defective enzyme). The key molecules here are the peroxisome proliferator activated receptors (PPARs), a family of ligand-modulated transcription factors that regulate gene expression programs of metabolic pathways. Mitochondrial biogenesis is regulated by PPAR γ coactivator α (PGC-1 α). The PPAR/PGC-1 α pathway is, in turn, activated by bezafibrate, a drug already used in medicine. Initially, fibrates were used to document that fatty acid oxidation (FAO) could be stimulated in cells with FAO defects, such as carnitine palmitoyltransferase II (CPT II) or very long-chain acyl-CoA (VLCAD) dehydrogenase deficiency [99]. More interesting for us, bezafibrate increased the activities of RC complexes both in normal cultured cells and in cells with RC enzyme defects [100]. Similarly, in a mouse with COX deficiency myopathy due to an engineered mutation in the assembly gene *COX10*, treatment with bezafibrate increased residual COX activity and ATP production in muscle and delayed both the onset of myopathy and the time of death [101]. Because bezafibrate has already been used in humans and is part of our pharmacological armamentarium, it could be tested relatively rapidly in patients with mitochondrial diseases.

An ethically controversial preventive approach to severe mtDNA-related diseases is “ooplasmic transfer”. A woman carrying a mtDNA mutation could have her fertilized oocytes “cleansed” in vitro of the cytoplasm and, with it, of most of the mitochondria. The naked pronucleus can then be transferred to a normal enucleated host oocyte and implanted in the woman’s uterus. If successful, the resulting children would be mitochondrially normal while carrying all the nuclear traits of both parents. Although partial replacement of the cytoplasm of aged oocytes has been used to “rejuvenate” them and improve the success of in vitro fertilization [102], there are strong objections to a more thorough application of the same technique [103], which offers the best hope for carriers of severe mtDNA mutations of having “their own” normal progeny. Fortunately, experimentation of ooplasmic transfer has been approved in the UK and the results are awaited with great expectation.

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Legends to Figures

Figure 1. The many potential features associated with mitochondrial OXPHOS diseases.

The multiple syndromes and organ deficits resulting from mitochondrial dysfunction can be isolated or multi systemic, with early- or late-onset. CIPO, Chronic intestinal pseudoobstruction; GH, Growth hormone; MELAS, Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; MERRF, Myoclonic epilepsy and ragged-red fibers; MNGIE, Mitochondrial neurogastrointestinal encephalomyopathy; PEO, Progressive external ophthalmoplegia.

Figure 2. An oversimplified scheme of the interactions between mitochondrial import and export pathways, matrix metabolism and OXPHOS function.

The products of lipid, carbohydrate, and protein metabolism enter the mitochondria (*top*), are metabolized through various interconnected catabolic pathways, and ultimately provide reduced cofactors utilized by the electron transfer chain coupled to the ATPase to produce ATP (*bottom*). Beside major mitochondria-associated metabolic pathways, *i.e.* the fatty acids β -oxidation spiral (A), the urea cycle (B), the Krebs cycle (C), the malate-aspartate shuttle (D), a number of individual reactions occur in the mitochondria, requiring a battery of enzymes for the import and metabolism of substrates and cofactors. Several membrane-associated proteins (in dark) have been found defective in various human diseases. Finally, mitochondria also release important signal molecules, especially a set of proteins recognized as cell death effectors.

I, II, III, IV, V denote the various complexes of the respiratory chain; ADP, ATP, adenosine di- and tri-phosphate; AIF, Apoptosis Inducing Factor; CoA, coenzyme A; cyt, cytochrome; Fum, fumarate; α -KG, α -ketoglutarate; Mal, malate; OAA, oxaloacetic acid; Succ, succinate;

TIM, TOM, Translocator of the inner and outer membranes; Tpp, thiamine pyrophosphate; UCP, uncoupling protein; VDAC, voltage-dependent anion channel.

Figure 3. The respiratory chain and its dual genetic origin.

A, schematic view of the respiratory chain showing the organization of complexes I, III and IV as a super-complex (respirasome), and the various ubiquinone pools channeling electrons from the different dehydrogenases to complex III. **B**, Dual origin of respiratory chain components. Numerals indicate components encoded by nuclear (left) or mitochondrial (right) genes for each complex; *c*, cytochrome *c*; dark and white arrows indicate electron and proton flows respectively; Ddh, dihydro-orotate dehydrogenase; ETF, Electron Transfer Protein; G3Pdh, Glycerol 3-phosphate dehydrogenase; im, inner membrane; om, outer membrane; UQ, ubiquinone; I-V, the various complexes of the respiratory chain.

Figure 4. What should be known on quinones and mitochondria.

A, Ubiquinone (UQ) is in large excess when compared to any other electron carrier of the respiratory chain. In a functional respiratory chain, only a part of these quinones is usually reduced, depending on which dehydrogenase feeds electrons to the chain. **B**, UQ is found in most cell compartments, but is particularly enriched in golgi, lysosomal, and mitochondrial membranes. UQ content also varies markedly among tissues and is highest in the heart [16]. **C**, Depending on its redox status, UQ can act as a potent reducing agent (reduced form, quinol; right) or a pro-oxidant agent (semi reduced form, semi quinone; centre). The hydrophobic reduced form possibly reacts with oxygen, lipoperoxide (LOO^o) and tocopheryl (2 α -TO^o) radicals to give their respective reduced forms. The more hydrophilic semi reduced form is highly unstable and reacts with oxygen and hydrogen peroxide to give highly reactive radicals. **D**, Comparison the chemical structures of ubiquinone and idebenone. Note the

different lengths of the side chains, the unsaturated double-bonds of the ubiquinone side-chain, and the additional charges of idebenone. E, Predicted locations of UQ (ubiquinone; coenzyme Q10; Q10) and idebenone (ide) in a biological membrane according to their hydrophobicity. Due to their different locations, idebenone will be react more efficiently with hydrophilic superoxides, while UQ will preferably react with lipoperoxide radicals.

Figure 5. Increased formation of lactate in respiratory chain defects.

Respiratory chain defects result in NADH accumulation in the mitochondrial matrix and decreased ability to metabolize pyruvate. The excess pyruvate is reduced to lactate, especially as NADH tends to accumulate in respiratory-deficient cells. This results in increased lactate to pyruvate ratio in the cell cytosol and increased hydroxyl butyrate (OH-but) to aceto-acetate (AcAc) ratio in the mitochondrial matrix. Measuring these ratios in human fluids (blood, cerebrospinal fluid) can pinpoint the defect in the respiratory chain.

Figure 6. Strategies for a future gene therapy of respiratory chain defects.

A, investigating the ability of mitochondria to import exogenous DNA to be used by the mitochondrial transcription/replication machinery [104]; **B**, delivering cytosolic tRNA into mitochondria thanks to the expression of the Leishmania RNA import complex (RIC) [105]; **C**, using the ability of mitochondria to import tRNAs that can complement for defective ones [106]; **D**, attaching mRNAs of interest to the mitochondrial outer membrane through mitochondria-bound polyribosomes to boost the intramitochondrial location of their translation products [88]; **E**, expressing allotopic proteins whose functions complement respiratory chain defects [81, 107]; **F**, boosting the expression of mitochondrial genes thanks to PPAR ligands [101]; **G**, providing antigenomic drugs (PNA, peptide nucleic acid; CMCO:PNA, cell membrane crossing oligomers PNA hybrids), such as peptides that could

enter mitochondria and selectively impair the replication of mutant mtDNA [80]; **H**, targeting to mitochondria endonucleases that would specifically recognize mutant mtDNA.

ACCEPTED MANUSCRIPT

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