

## REVIEW ARTICLE

## MECHANISMS OF DISEASE

## Mitochondrial Respiratory-Chain Diseases

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**M**ORE THAN A BILLION YEARS AGO, AEROBIC BACTERIA COLONIZED primordial eukaryotic cells that lacked the ability to use oxygen metabolically. A symbiotic relationship developed and became permanent. The bacteria evolved into mitochondria, thus endowing the host cells with aerobic metabolism, a much more efficient way to produce energy than anaerobic glycolysis. Structurally, mitochondria have four compartments: the outer membrane, the inner membrane, the intermembrane space, and the matrix (the region inside the inner membrane). They perform numerous tasks, such as pyruvate oxidation, the Krebs cycle, and metabolism of amino acids, fatty acids, and steroids, but the most crucial is probably the generation of energy as adenosine triphosphate (ATP), by means of the electron-transport chain and the oxidative-phosphorylation system (the “respiratory chain”) (Fig. 1).

The respiratory chain, located in the inner mitochondrial membrane, consists of five multimeric protein complexes (Fig. 2B): reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase–ubiquinone oxidoreductase (complex I, approximately 46 subunits), succinate dehydrogenase–ubiquinone oxidoreductase (complex II, 4 subunits), ubiquinone–cytochrome *c* oxidoreductase (complex III, 11 subunits), cytochrome *c* oxidase (complex IV, 13 subunits), and ATP synthase (complex V, approximately 16 subunits). The respiratory chain also requires two small electron carriers, ubiquinone (coenzyme Q10) and cytochrome *c*.

ATP synthesis entails two coordinated processes (Fig. 2B). First, electrons (actually hydrogen ions derived from NADH and reduced flavin adenine dinucleotide in intermediary metabolism) are transported along the complexes to molecular oxygen, thereby producing water. At the same time, protons are pumped across the mitochondrial inner membrane (i.e., from the matrix to the intermembrane space) by complexes I, III, and IV. ATP is generated by the influx of these protons back into the mitochondrial matrix through complex V (ATP synthase), the world’s tiniest rotary motor.<sup>1,2</sup>

Mitochondria are the only organelles of the cell besides the nucleus that contain their own DNA (called mtDNA) and their own machinery for synthesizing RNA and proteins. There are hundreds or thousands of mitochondria per cell, and each contains approximately five mitochondrial genomes. Because mtDNA has only 37 genes, most of the approximately 900 gene products in the organelle are encoded by nuclear DNA (nDNA) and are imported from the cytoplasm.

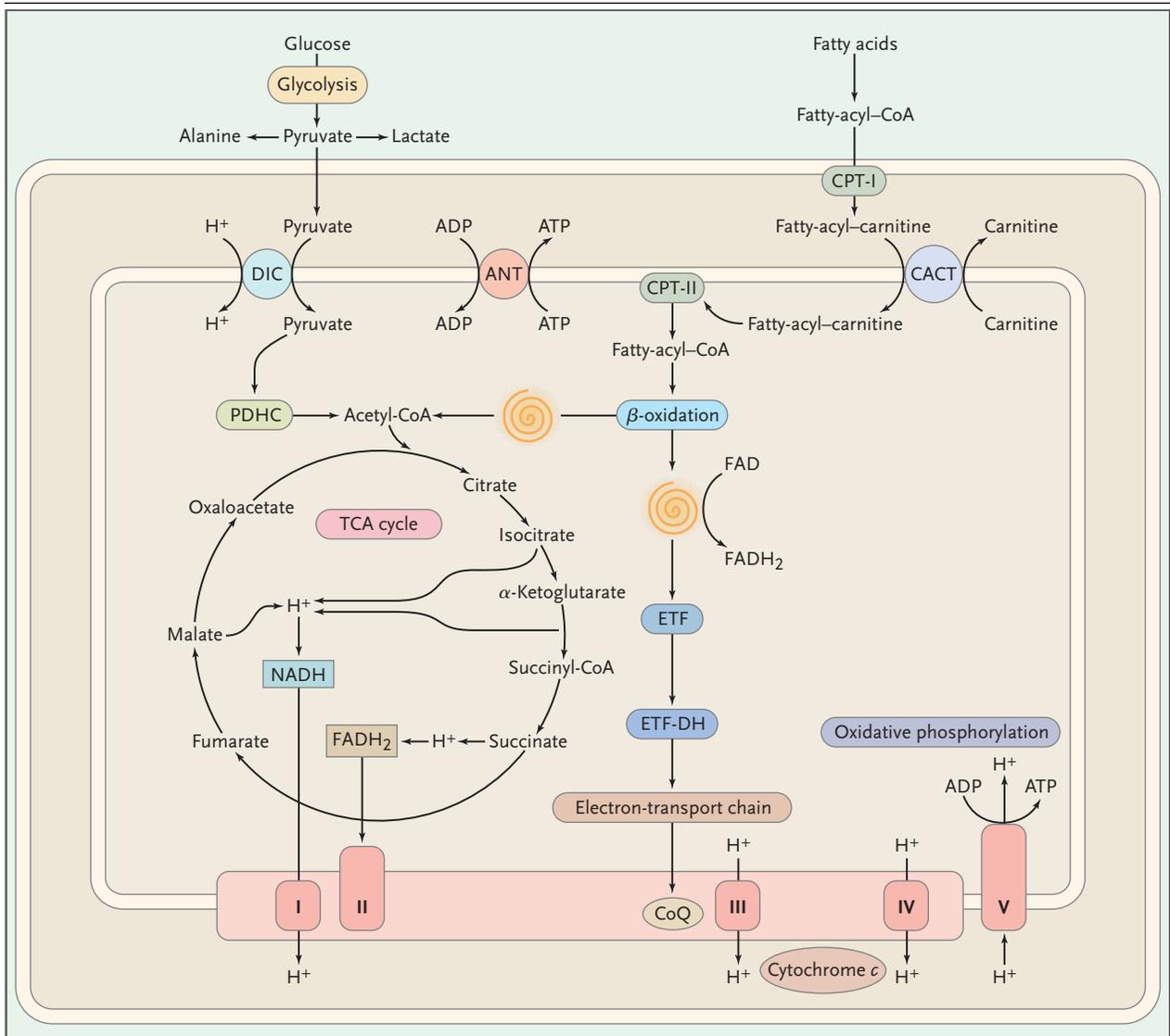
Defects in any of the numerous mitochondrial pathways can cause mitochondrial diseases, but we will confine our discussion to disorders of the respiratory chain. Because many of them involve brain and skeletal muscle, these disorders are also known as mitochondrial encephalomyopathies. The fact that the respiratory chain is under dual genetic control makes these disorders particularly fascinating, because they involve both mendelian and mitochondrial genetics. Moreover, these diseases are not as rare as commonly believed; their estimated prevalence of 10 to 15 cases per 100,000 persons is similar to that of better known neurologic diseases, such as amyotrophic lateral sclerosis and the muscular dystrophies.<sup>3</sup>

The genetic classification of the primary mitochondrial diseases distinguishes dis-

orders due to defects in mtDNA, which are inherited according to the rules of mitochondrial genetics, from those due to defects in nDNA, which are transmitted by mendelian inheritance. Since the discovery of the first pathogenic mutations in human mtDNA a mere 15 years ago,<sup>4,5</sup> the increase in our knowledge of the role of mitochondria in disease has exceeded all expectations.

### MITOCHONDRIAL GENETICS

The human mtDNA is a 16,569-bp, double-stranded, circular molecule containing 37 genes (Fig. 2A). Of these, 24 are needed for mtDNA translation (2 ribosomal RNAs [rRNAs] and 22 transfer RNAs [tRNAs]), and 13 encode subunits of the respiratory chain: seven subunits of complex I (ND1, 2, 3, 4,



**Figure 1. Selected Metabolic Pathways in Mitochondria.**

The spirals represent the spiral of reactions of the  $\beta$ -oxidation pathway, resulting in the liberation of acetyl-coenzyme A (CoA) and the reduction of flavoprotein. ADP denotes adenosine diphosphate, ATP adenosine triphosphate, ANT adenosine nucleotide translocator, CACT carnitine-acyl-carnitine translocase, CoQ coenzyme Q, CPT carnitine palmitoyltransferase, DIC dicarboxylate carrier, ETF electron-transfer flavoprotein, ETF-DH electron-transfer dehydrogenase, FAD flavin adenine dinucleotide, FADH<sub>2</sub> reduced FAD, NADH reduced nicotinamide adenine dinucleotide, PDHC pyruvate dehydrogenase complex, TCA tricarboxylic acid, I complex I, II complex II, III complex III, IV complex IV, and V complex V.

4L, 5, and 6 [ND stands for NADH dehydrogenase]), one subunit of complex III (cytochrome *b*), three subunits of cytochrome *c* oxidase (COX I, II, and III), and two subunits of ATP synthase (A6 and A8). Mitochondrial genetics differs from mendelian genetics in three major aspects: maternal inheritance, heteroplasmy, and mitotic segregation.

#### MATERNAL INHERITANCE

As a general rule, all mitochondria (and all mtDNAs) in the zygote derive from the ovum. Therefore, a mother carrying an mtDNA mutation passes it on to all her children, but only her daughters will transmit it to their progeny. Recent evidence of paternal transmission of mtDNA in skeletal muscle (but not in other tissues) in a patient with a mitochondrial myopathy<sup>6</sup> serves as an important warning that maternal inheritance of mtDNA is not an absolute rule, but it does not negate the primacy of maternal inheritance in mtDNA-related diseases.

#### HETEROPLASMY AND THE THRESHOLD EFFECT

There are thousands of mtDNA molecules in each cell, and in general, pathogenic mutations of mtDNA are present in some but not all of these genomes. As a result, cells and tissues harbor both normal (wild-type) and mutant mtDNA, a situation known as heteroplasmy. Heteroplasmy can also exist at the organellar level: a single mitochondrion can harbor both normal and mutant mtDNAs. In normal subjects, all mtDNAs are identical (homoplasmy). Not surprisingly, a minimal number of mutant mtDNAs must be present before oxidative dysfunction occurs and clinical signs become apparent: this is the threshold effect. The threshold for disease is lower in tissues that are highly dependent on oxidative metabolism, such as brain, heart, skeletal muscle, retina, renal tubules, and endocrine glands. These tissues will therefore be especially vulnerable to the effects of pathogenic mutations in mtDNA.

#### MITOTIC SEGREGATION

The random redistribution of organelles at the time of cell division can change the proportion of mutant mtDNAs received by daughter cells; if and when the pathogenic threshold in a previously unaffected tissue is surpassed, the phenotype can also change. This explains the age-related, and even tissue-related, variability of clinical features frequently observed in mtDNA-related disorders.

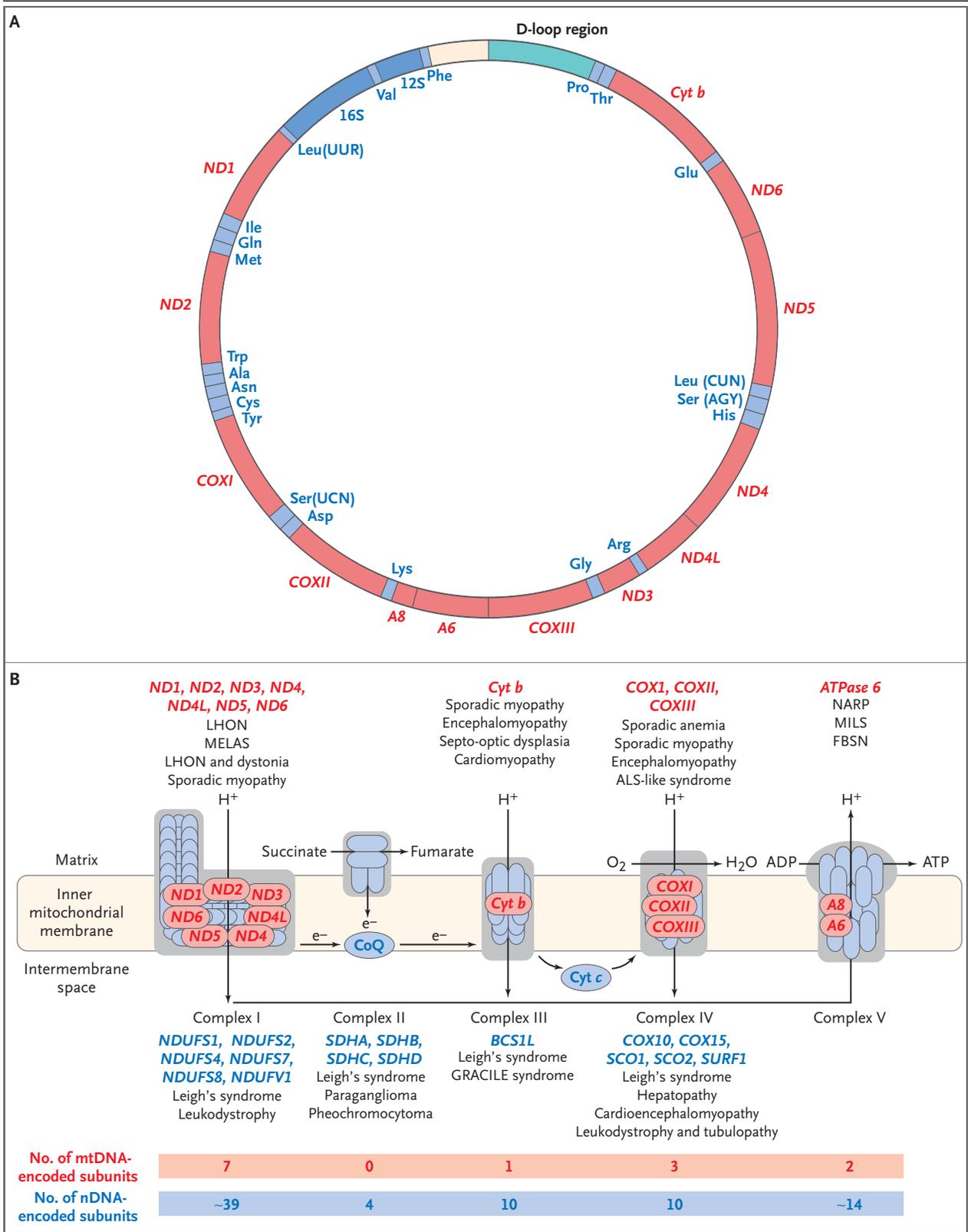
### RESPIRATORY-CHAIN DISORDERS DUE TO DEFECTS IN MTDNA

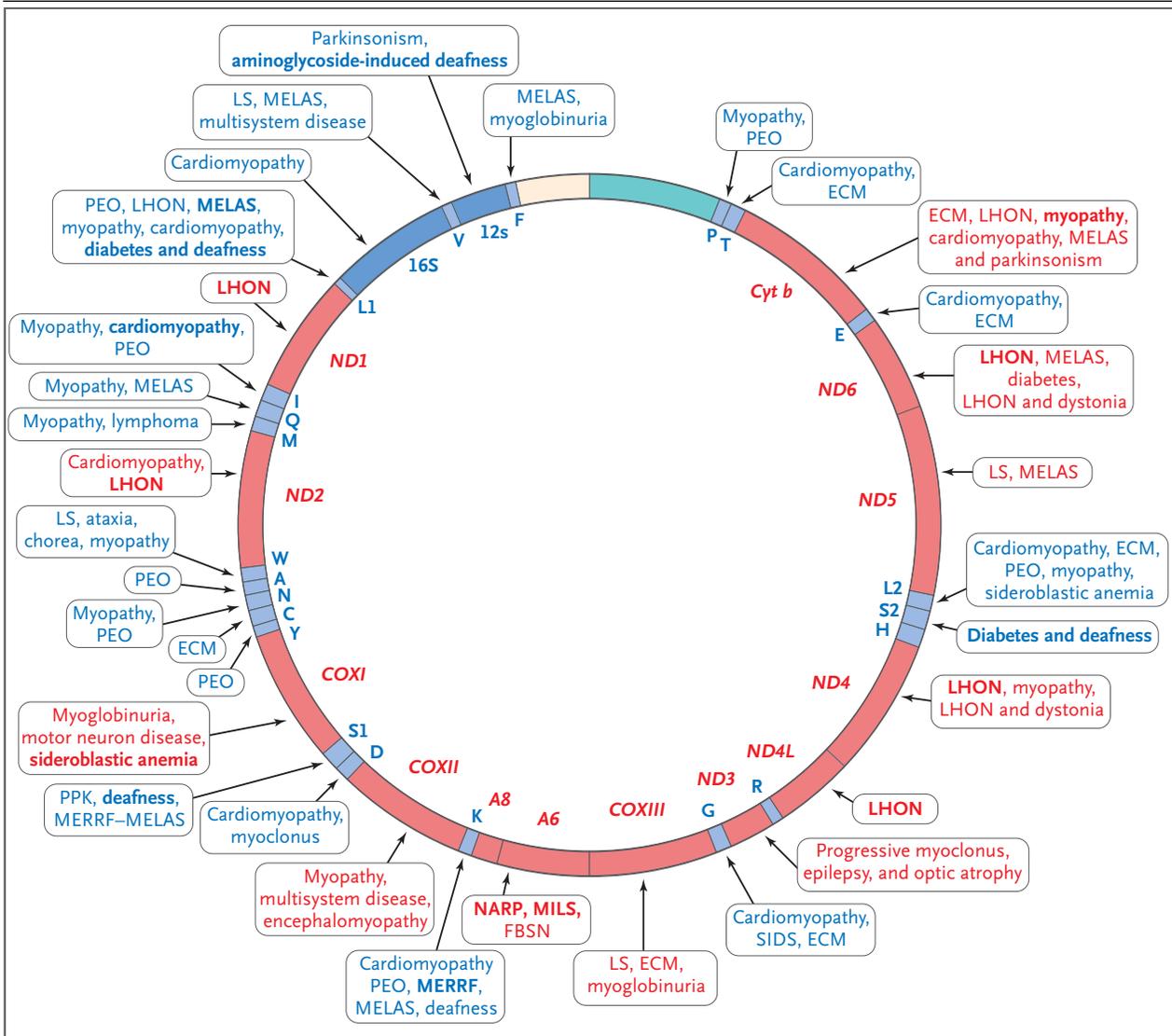
The small mitochondrial genome contains many mutations that cause a wide variety of clinical syndromes, as shown in Figure 3.<sup>7</sup> The genome is peppered with mutations, although a few “hot spots” stand out. Relatively few mutations have been found in rRNA genes, and all these are confined to the 12S rRNA. Not shown on the map are the hundreds of different pathogenic giant deletions of 2 to 10 kb, which invariably delete tRNA genes.

Although clinically distinct, most mtDNA-related diseases share the features of lactic acidosis and massive mitochondrial proliferation in muscle (resulting in ragged-red fibers). In muscle-biopsy specimens, the mutant mtDNAs accumulate preferentially in ragged-red fibers, and ragged-red fibers are typically negative for cytochrome *c* oxidase activity<sup>8</sup> (Fig. 4).

#### Figure 2 (facing page). Mitochondrial DNA (mtDNA) and the Mitochondrial Respiratory Chain.

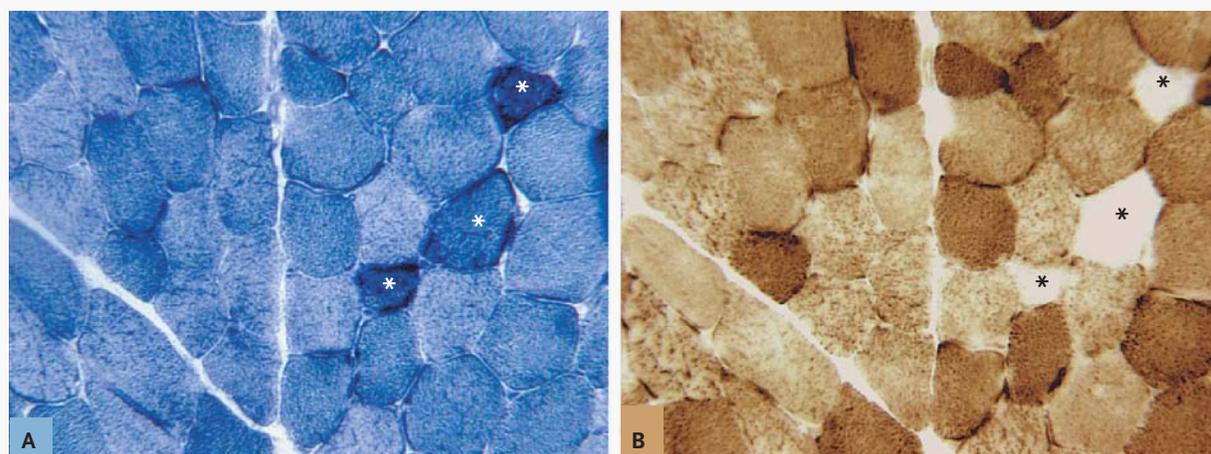
Panel A shows the map of the human mitochondrial genome. The protein-coding genes — seven subunits of complex I (ND), three subunits of cytochrome *c* oxidase (COX), the cytochrome *b* subunit of complex III (Cyt *b*), and two subunits of adenosine triphosphate (ATP) synthase (A6 and A8) — are shown in red. The protein-synthesis genes — the 12S and 16S ribosomal RNAs and the 22 transfer RNAs (three-letter amino acid symbols) — are shown in blue. The D-loop region controls the initiation of replication and transcription of mtDNA. Panel B shows the subunits of the respiratory chain encoded by nuclear DNA (nDNA) in blue and the subunits encoded by mtDNA in red. As electrons (e<sup>-</sup>) flow along the electron-transport chain, protons (H<sup>+</sup>) are pumped from the matrix to the intermembrane space through complexes I, III, and IV and then back into the matrix through complex V, to produce ATP. Coenzyme Q (CoQ) and cytochrome *c* (Cyt *c*) are electron-transfer carriers. Genes responsible for the indicated respiratory-chain disorders are also shown. *ATPase 6* denotes ATP synthase 6; *BCS1L* cytochrome *b-c* complex assembly protein (complex III); *NDUF* NADH dehydrogenase-ubiquinone oxidoreductase; *SCO* synthesis of cytochrome oxidase; *SDHA*, *SDHB*, *SDHC*, and *SDHD* succinate dehydrogenase subunits; *SURF1* surfeit gene 1; *FBSN* familial bilateral striatal necrosis; *LHON* Leber's hereditary optic neuropathy; *MELAS* mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; *MILS* maternally inherited Leigh's syndrome; *NARP* neuropathy, ataxia, and retinitis pigmentosa; *GRACILE* growth retardation, aminoaciduria, lactic acidosis, and early death; and *ALS* amyotrophic lateral sclerosis.





**Figure 3. Mutations in the Human Mitochondrial Genome That Are Known to Cause Disease.**  
 Disorders that are frequently or prominently associated with mutations in a particular gene are shown in boldface. Diseases due to mutations that impair mitochondrial protein synthesis are shown in blue. Diseases due to mutations in protein-coding genes are shown in red. ECM denotes encephalomyopathy; FBSN familial bilateral striatal necrosis; LHON Leber's hereditary optic neuropathy; LS Leigh's syndrome; MELAS mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; MERRF myoclonic epilepsy with ragged-red fibers; MILS maternally inherited Leigh's syndrome; NARP neuropathy, ataxia, and retinitis pigmentosa; PEO progressive external ophthalmoplegia; PPK palmo-plantar keratoderma; and SIDS sudden infant death syndrome.

Mutations in mtDNA can affect specific proteins of the respiratory chain or the synthesis of mitochondrial proteins as a whole (mutations in tRNA or rRNA genes, or giant deletions). There is no straightforward relation between the site of the mutation and the clinical phenotype, even with a mutation in a single gene. For example, mutations in the tRNA<sup>Leu(UUR)</sup> gene are usually associated with the mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS) syndrome, but they cause other syndromes as well. Conversely, mutations in different genes can cause the same syndrome; MELAS, again, is a prime example (Fig. 3). There are exceptions: virtually all patients who have the myoclonus epilepsy with ragged-red fibers (MERRF) syndrome have mutations in the tRNA<sup>Lys</sup>



**Figure 4.** Serial Cross-Sections of Muscle from a Patient with the Kearns–Sayre Syndrome, Showing Increased Mitochondrial Activity in Ragged-Red Fibers on Staining with Succinate Dehydrogenase (Asterisks in Panel A;  $\times 120$ ) and the Absence of Activity on Staining with Cytochrome *c* Oxidase (Asterisks in Panel B;  $\times 120$ ).

gene,<sup>9</sup> all patients with Leber's hereditary optic neuropathy have mutations in ND genes,<sup>10</sup> and most mutations in the *cytochrome b* gene cause exercise intolerance.<sup>11</sup>

Because mitochondria are ubiquitous, every tissue in the body can be affected by mtDNA mutations, which is why mitochondrial diseases are often multisystemic. Table 1 lists the most common mtDNA-related syndromes. Certain constellations of symptoms and signs are characteristic of these syndromes, and the diagnosis is relatively easy to establish in patients with these typical features. However, as a result of heteroplasmy and the threshold effect, different tissues harboring the same mtDNA mutation may be affected to different degrees, thus explaining the frequent occurrence of oligosymptomatic or asymptomatic carriers of the mutation within a family. Selective organ involvement can also occur, presumably as a result of skewed heteroplasmy (i.e., disproportionately high levels of the mutation in a given tissue), as in mitochondrial diabetes,<sup>12</sup> mitochondrial cardiomyopathies,<sup>13</sup> mitochondrial myopathies,<sup>8</sup> and mitochondrial deafness.<sup>14</sup>

Although most mtDNA-related diseases are maternally inherited, there are exceptions. The occurrence of giant deletions is almost always sporadic and probably takes place in oogenesis or early embryogenesis. Oocytes from normal women contain about 150,000 mtDNA molecules, some of which may harbor deletions.<sup>15</sup> A "bottleneck" between

ovum and embryo allows only a minority of maternal mtDNAs to populate the fetus. On rare occasions a partially deleted mtDNA (or its progeny, depending on when in oogenesis the deletion occurred) may slip through. A few mtDNAs with giant deletions in the blastocyst can then enter all three germ layers and result in the Kearns–Sayre syndrome (a multisystem disorder), segregate to the hematopoietic lineage and cause Pearson's syndrome, or segregate to muscle and cause progressive external ophthalmoplegia (Table 1). In these three cases, all mutated mtDNAs in the patient are identical, because they are a clonal expansion of the original molecule. Mutations in protein-coding genes of myogenic stem cells, presumably occurring after germ-layer differentiation, result in isolated myopathies<sup>11</sup>; 15 of the 17 known mutations in the *cytochrome b* gene fall into this category (however, paternal inheritance of mtDNA in these cases must be ruled out<sup>6</sup>).

The pathogenesis of these disorders is unclear, although impaired production of ATP most likely has a central role. This concept has been borne out by studies using cytoplasmic hybrid ("cybrid") cell cultures, which are established human cell lines that are depleted of their own mtDNA and then repopulated with the patient's mitochondria containing mutated genomes.<sup>16</sup>

The extraordinary variability of clinical presentations can largely be attributed to the peculiar rules of mitochondrial genetics, especially heteroplasmy

**Table 1. Clinical and Genetic Heterogeneity of Disorders Related to Mutations in Mitochondrial DNA (mtDNA).\***

Symptoms, Signs, and Findings	Giant Deletions in mtDNA			Mutation in Transfer RNA		Mutation in Ribosomal RNA	Mutation in Messenger RNA		
	KSS	PEO	PS	MERRF	MELAS	AID	NARP	MILS	LHON
<b>Central nervous system</b>									
Seizures	-	-	-	+	+	-	-	+	-
Ataxia	+	-	-	+	+	-	+	±	-
Myoclonus	-	-	-	+	±	-	-	-	-
Psychomotor retardation	-	-	-	-	-	-	-	+	-
Psychomotor regression	+	-	-	±	+	-	-	-	-
Hemiparesis and hemianopia	-	-	-	-	+	-	-	-	-
Cortical blindness	-	-	-	-	+	-	-	-	-
Migraine-like headaches	-	-	-	-	+	-	-	-	-
Dystonia	-	-	-	-	+	-	-	+	±
<b>Peripheral nervous system</b>									
Peripheral neuropathy	±	-	-	±	±	-	+	-	-
<b>Muscle</b>									
Weakness and exercise intolerance	+	+	-	+	+	-	+	+	-
Ophthalmoplegia	+	+	±	-	-	-	-	-	-
Ptosis	+	+	-	-	-	-	-	-	-
<b>Eye</b>									
Pigmentary retinopathy	+	-	-	-	-	-	+	±	-
Optic atrophy	-	-	-	-	-	-	±	±	+
<b>Blood</b>									
Sideroblastic anemia	±	-	+	-	-	-	-	-	-
<b>Endocrine system</b>									
Diabetes mellitus	±	-	-	-	±	-	-	-	-
Short stature	+	-	-	+	+	-	-	-	-
Hypoparathyroidism	±	-	-	-	-	-	-	-	-
<b>Heart</b>									
Conduction disorder	+	-	-	-	±	-	-	-	±
Cardiomyopathy	±	-	-	-	±	+	-	±	-
<b>Gastrointestinal system</b>									
Exocrine pancreatic dysfunction	±	-	+	-	-	-	-	-	-
Intestinal pseudo-obstruction	-	-	-	-	+	-	-	-	-
<b>Ear, nose, and throat</b>									
Sensorineural hearing loss	±	-	-	+	+	+	±	-	-
<b>Kidney</b>									
Fanconi's syndrome	±	-	±	-	±	-	-	-	-
<b>Laboratory findings</b>									
Lactic acidosis	+	±	+	+	+	-	-	±	-
Ragged-red fibers on muscle biopsy	+	+	±	+	+	-	-	-	-
<b>Mode of inheritance</b>									
Maternal	-	-	-	+	+	-	+	+	+
Sporadic	+	+	+	-	-	-	-	-	-

\* Characteristic constellations of symptoms and signs are boxed. Plus signs indicate the presence of a symptom, sign, or finding; minus signs the absence of a symptom, sign, or finding; and plus-minus signs the possible presence of a symptom, sign, or finding. KSS denotes the Kearns–Sayre syndrome; PEO progressive external ophthalmoplegia; PS Pearson's syndrome; MERRF myoclonic epilepsy with ragged-red fibers; MELAS mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; AID aminoglycoside-induced deafness; NARP neuropathy, ataxia, and retinitis pigmentosa; MILS maternally inherited Leigh's syndrome; and LHON Leber's hereditary optic neuropathy.

and the threshold effect. For example, different mutational loads readily explain the different degrees of severity between the neuropathy, ataxia, and retinitis pigmentosa syndrome and maternally inherited Leigh's syndrome, two encephalomyopathies caused by the same genetic defect in the *ATPase 6* gene.<sup>17</sup> What is difficult to explain is the distinct "tissue proclivity" of seemingly similar mutations, especially in the brain — for example, the strokelike episodes in MELAS, the myoclonus in MERRF, and the pigmentary retinopathy in the Kearns–Sayre syndrome. High concentrations of each mutation in cerebral small vessels, in the dentate nucleus of the cerebellum, and in the retinal pigment epithelium, respectively, do not explain why the mutation is in that particular area of the brain. Conversely, many patients with mitochondrial diabetes have symptoms even though they have a relatively small amount of mutation, suggesting that the pathogenetic mechanism goes beyond a mere energy deficit. For example, diabetes may be the result of subtle interactions among oxidative energy levels and the glucose sensor,<sup>18</sup> the NADH shuttle,<sup>19</sup> and even uncoupling proteins.<sup>20</sup> Even more puzzling, mutations that are ubiquitous often have tissue-specific effects (e.g., deafness due to mutations in 12S rRNA, cardiopathy due to mutations in tRNA<sup>Leu</sup>, and optic atrophy due to mutations in ND genes). Studies of animal models with mtDNA mutations ("mito-mice")<sup>21,22</sup> may provide some answers to these riddles.

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#### RESPIRATORY-CHAIN DISORDERS DUE TO DEFECTS IN nDNA

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In recent years, interest has shifted toward mendelian genetics in mitochondrial disease. This shift is understandable, not only because most of the 75-plus respiratory-chain proteins are encoded by nDNA (Fig. 2B), but also because proper assembly and function of respiratory chain complexes require approximately 60 additional (ancillary) nucleus-encoded proteins. Mutations in these genes can also cause mitochondrial disease.<sup>23</sup>

#### MUTATIONS IN STRUCTURAL COMPONENTS OF THE RESPIRATORY CHAIN

Mutations in structural components of the respiratory chain have thus far been found only in complexes I<sup>24</sup> and II<sup>25–27</sup> and have generally been associated with severe neurologic disorders of childhood, such as Leigh's syndrome and leukodystrophy.<sup>28</sup>

However, mutations in complex II have also been associated with paragangliomas<sup>29,30</sup> and pheochromocytomas.<sup>31</sup> Although its genetic basis remains unknown and it is likely to be heterogeneous, coenzyme Q10 deficiency is emerging as an important cause of autosomal recessive encephalomyopathies, ranging from predominantly myopathic forms (with recurrent myoglobinuria) to predominantly encephalopathic forms (with ataxia and cerebellar atrophy).<sup>32</sup>

#### MUTATIONS IN ANCILLARY PROTEINS OF THE RESPIRATORY CHAIN

No pathogenic mutations have been identified in any nDNA-encoded subunits of complex III, IV, or V, but defects of complexes III<sup>33,34</sup> and IV<sup>35,36</sup> have been related to mutations in ancillary proteins required for the assembly or insertion of cofactors. Cytochrome *c* oxidase deficiency is generalized in disorders that are due to mutations in genes required for the assembly of complex IV, but the enzyme defect and the symptoms are more severe in certain tissues; for example, mutations in the surfeit gene (*SURF1*) predominantly affect the brain and cause Leigh's syndrome, mutations in a gene required for the synthesis of cytochrome oxidase (*SCO2*) or in cytochrome *c* oxidase 15 (*COX15*)<sup>37</sup> cause infantile cardiomyopathy in addition to brain disease, and mutations in cytochrome *c* oxidase 10 (*COX10*) and another gene for the synthesis of cytochrome oxidase (*SCO1*) affect kidney and liver tissues, respectively. Although pathogenic mutations may occur in structural subunits of complexes III, IV, and V, they may be lethal in utero because there is no metabolic compensation, and complex V is the sole site of oxidative phosphorylation<sup>38</sup> (Fig. 2B). This concept would apply only to the "all-or-none" type of mendelian inheritance, since mutations in mtDNA-encoded components of complexes III, IV, and V do indeed occur but are expressed incompletely because of heteroplasmy.

#### DEFECTS IN INTERGENOMIC SIGNALING AFFECTING RESPIRATORY FUNCTION

In the course of evolution, mitochondria lost their independence, and mtDNA is now the slave of nDNA, depending on numerous nucleus-encoded factors for its integrity and replication.<sup>39</sup> Mutations in these factors affect mtDNA directly, either quantitatively or qualitatively, and cause diseases that are inherited as mendelian traits.

A quantitative alteration is exemplified by abnor-

mal reductions in the number of mtDNA molecules (both per cell and per organelle) — mtDNA-depletion syndromes. A qualitative alteration is exemplified by multiple deletions (in contrast to the single mtDNA deletions in sporadic Kearns–Sayre syndrome, progressive external ophthalmoplegia, and Pearson’s syndrome).

Ophthalmoplegia is the clinical hallmark of multiple mtDNA deletions,<sup>40</sup> but patients with autosomal dominant progressive external ophthalmoplegia often have proximal limb weakness, peripheral neuropathy, sensorineural hearing loss, cataracts, endocrine dysfunction, and severe depression.<sup>41,42</sup> Autosomal recessive progressive external ophthalmoplegia has two main clinical presentations: one consists of cardiomyopathy and ophthalmoplegia,<sup>43</sup> and the other of peripheral neuropathy, gastrointestinal dysmotility, and leukoencephalopathy (mitochondrial neurogastrointestinal encephalomyopathy).<sup>44</sup>

Both quantitative and qualitative defects may result from impairment of the integrity of the mitochondrial genome. Such impairment can be direct (e.g., affecting proteins required for the replication and maintenance of mtDNA) or indirect (e.g., affecting proteins required to maintain nucleotide pools in mitochondria). For example, some families with autosomal dominant progressive external ophthalmoplegia have mutations in Twinkle, a mitochondrial protein similar to bacteriophage T7 primase/helicase, whereas other families have mutations in the mitochondrial adenine nucleotide translocator 1 (ANT1).<sup>45,46</sup> Mutations in mitochondrial-specific DNA polymerase  $\gamma$  have been associated with both dominant and recessive multiple-deletion disorders.<sup>47</sup>

Mitochondrial neurogastrointestinal encephalomyopathy is clearly due to the loss of function of thymidine phosphorylase, resulting in markedly increased concentrations of thymidine in the blood.<sup>48</sup> Thymidine phosphorylase is not a mitochondrial protein, yet it appears to have a selective effect on mitochondrial nucleotide pools required for maintaining the integrity and abundance of mtDNA. The role of nucleotides is bolstered by the pathogenicity of the ANT1 mutations and by recent findings that mutations in mitochondrial thymidine kinase<sup>49,50</sup> and deoxyguanosine kinase<sup>51,52</sup> are associated with the myopathic and hepatocerebral forms of mtDNA depletion. Knowledge of these mutations makes prenatal diagnosis feasible for some families with the infantile mtDNA-depletion syndromes and

may offer new approaches to therapeutic intervention (e.g., lowering blood thymidine concentrations in patients with mitochondrial neurogastrointestinal encephalomyopathy).

#### DEFECTS OF THE MEMBRANE LIPID MILIEU

Except for cytochrome *c*, which is located in the intermembrane space, all components of the respiratory chain are embedded in the lipid milieu of the inner mitochondrial membrane, which is composed predominantly of cardiolipin. Cardiolipin is not merely a scaffold but is an integral and indispensable part of some respiratory-chain components.<sup>53</sup> It therefore stands to reason that defects in cardiolipin would cause respiratory-chain dysfunction and mitochondrial disease. This concept is exemplified by an X-linked disorder, Barth syndrome (mitochondrial myopathy, cardiomyopathy, growth retardation, and leukopenia).<sup>54</sup> The mutated gene in Barth syndrome, *G4.5*, encodes a family of acyl-coenzyme A synthetases (tafazzins) that must have an important role in cardiolipin synthesis, because cardiolipin concentrations are markedly decreased in skeletal and cardiac muscle and in platelets from affected patients.<sup>55</sup>

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#### DISORDERS WITH INDIRECT INVOLVEMENT OF THE RESPIRATORY CHAIN

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Indirect involvement of mitochondria has been documented or suggested in many conditions, including normal aging, late-onset neurodegenerative diseases, and cancer. However, the precise role of mitochondrial dysfunction in these conditions remains controversial.

#### DEFECTS OF MITOCHONDRIAL PROTEIN IMPORTATION

Cytosolic proteins destined for mitochondria have mitochondrial targeting signals that enable them to be routed to the appropriate compartment within the organelle, where they are then refolded into an active configuration.<sup>56</sup> Although a number of mutations in mitochondrial targeting signals have been found, few errors in the importation machinery itself are known, perhaps because they may be lethal.<sup>57</sup> However, at least one such defect has been identified, the deafness–dystonia syndrome (Mohr–Tranebjaerg syndrome), an X-linked recessive disorder characterized by progressive neurosensory deafness, dystonia, cortical blindness, and psychiat-

ric symptoms,<sup>58</sup> features that are strikingly similar to those of the primary mitochondrial diseases. This disorder is due to mutations in *TIMM8A*, encoding the deafness–dystonia protein (DDP1), a component of the mitochondrial-protein–import machinery in the intermembrane space. According to a recent report, an autosomal dominant form of hereditary spastic paraplegia is associated with mutations in the mitochondrial import chaperonin HSP60.<sup>59</sup>

#### DEFECTS IN MITOCHONDRIAL MOTILITY

Mitochondria are not static organelles; they are propelled within the cell by energy-requiring dynamins along cytoskeletal microtubule “rails.”<sup>60</sup> Mutations in a gene encoding a mitochondrial dynamin-related guanosine triphosphatase (*OPA1*) are associated with an autosomal dominant form of optic atrophy, which together with Leber’s hereditary optic neuropathy, is a major cause of blindness in young adults.<sup>61,62</sup>

#### NEURODEGENERATIVE DISEASES

A few neurodegenerative disorders are due to mutations in proteins that target the mitochondria. These include Friedreich’s ataxia, at least one form of hereditary spastic paraplegia, and Wilson’s disease. Friedreich’s ataxia is due to expansion of trinucleotide (GAA) repeats in the *FRDA* gene, which encodes frataxin, a mitochondrion-targeted protein involved in iron homeostasis.<sup>63</sup> Excessive free iron resulting from decreased concentrations of frataxin may damage proteins containing iron-sulfur groups, including complexes I, II, and III, and aconitase, a Krebs-cycle enzyme. An autosomal recessive form of hereditary spastic paraplegia is due to mutations in the *SPG7* gene, which encodes paraplegin, a mitochondrial protein similar to yeast metalloproteases.<sup>64</sup> Impairment of the respiratory chain is suggested by the presence of ragged-red fibers and fibers deficient in cytochrome *c* oxidase in muscle from affected patients. In Wilson’s disease, an autosomal recessive disease characterized by movement disorder and liver failure, there are mutations of the *ATP7B* gene, which encodes a copper-transporting ATPase, one isoform of which is localized in mitochondria.<sup>65</sup> The pathogenesis of Wilson’s disease may involve either direct damage to copper-containing enzymes, such as cytochrome *c* oxidase, or more generic oxidative damage to the cell owing to the accumulation of copper.

Mitochondrial dysfunction may be a final com-

mon pathogenic mechanism in aging and in Alzheimer’s disease, amyotrophic lateral sclerosis, Huntington’s disease, progressive supranuclear palsy, and Parkinson’s disease.<sup>66</sup> Rearrangements of and point mutations in mtDNA may accumulate over time, eventually surpassing the pathogenic threshold in multiple tissues (aging) or in specific areas of the central nervous system (neurodegenerative disorders). Although mtDNA mutations do increase in postmitotic tissues of healthy elderly persons, there is no convincing evidence that they reach deleterious levels.<sup>67</sup> The role of mtDNA mutations in neurodegenerative disorders, whether as the cumulative effect of generic mutations or as the specific effect of putative pathogenic mutations, is even more controversial.<sup>68</sup>

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#### THERAPEUTIC APPROACHES

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Therapy for mitochondrial diseases is woefully inadequate. In the absence of a clear understanding of basic pathogenetic mechanisms, treatments have been palliative or have involved the indiscriminate administration of vitamins, cofactors, and oxygen-radical scavengers, with the aim of mitigating, postponing, or circumventing the postulated damage to the respiratory chain.<sup>69</sup>

Rational therapies, on the whole, remain elusive, but the broad outlines of such approaches are beginning to emerge. For the mtDNA-related disorders, the most promising approach is to reduce the ratio of mutated to wild-type genomes (“gene shifting”).<sup>70</sup> Such shifting might be accomplished by pharmacologic,<sup>71</sup> physiological,<sup>70</sup> or even surgical<sup>72</sup> approaches. Genetic approaches to treatment are particularly daunting, since they will require targeting not only affected cells, but also the organelles within them. Nevertheless, some headway is being made here as well, although it is limited to *in vitro* systems. These include the selective destruction of mutant mtDNAs through importation of a restriction enzyme into mitochondria,<sup>73</sup> the replacement of a mutant mtDNA-encoded protein with a genetically engineered normal equivalent expressed from the nucleus (allotopic expression),<sup>74–76</sup> the replacement of a defective respiratory-chain complex with a cognate complex from another organism,<sup>77</sup> and the importation of a normal tRNA to compensate for a mutation in the corresponding organellar tRNA.<sup>78</sup>

As for nuclear mutations, the problems of gene therapy are the same as those encountered in oth-

er mendelian disorders. However, pharmacologic treatments might be of use in special cases, such as lowering thymidine concentrations in patients with mitochondrial neurogastrointestinal encephalomyopathy.<sup>79</sup> Reports that cytochrome *c* oxidase deficiency can be reversed by the supplementation of copper in cultured cells with a mutation in *SCO2*, a copper chaperone, suggest that copper supplementation may be useful in some patients.<sup>80,81</sup>

The concept of mitochondrial disease was introduced 41 years ago, when Luft and coworkers described a young woman with severe, nonthyroidal hypermetabolism due to loose coupling of oxidation and phosphorylation in muscle mitochondria.<sup>82</sup> Only one other patient with Luft's syndrome has been identified.<sup>83</sup> Yet nowadays, the possibility of mitochondrial dysfunction needs to be taken into account by every medical subspecialty. Although caution is required in discriminating primary from secondary mitochondrial involvement, progress in this field has been striking enough to amply justify the term "mitochondrial medicine,"<sup>84</sup> used by Luft in the title of a review written 32 years after he first described "his" syndrome.

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## REFERENCES

1. Elston T, Wang H, Oster G. Energy transduction in ATP synthase. *Nature* 1998;391:510-3.
2. Noji H, Yoshida M. The rotary machine of the cell, ATP synthase. *J Biol Chem* 2001;276:1665-8.
3. Chinnery PF, Turnbull DM. Epidemiology and treatment of mitochondrial disorders. *Am J Med Genet* 2001;106:94-101.
4. Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 1988;331:717-9.
5. Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 1988;242:1427-30.
6. Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA. *N Engl J Med* 2002;347:576-80.
7. Servidei S. Mitochondrial encephalomyopathies: gene mutation. *Neuromuscul Disord* 2002;12:101-10.
8. DiMauro S, Bonilla E. Mitochondrial encephalomyopathies. In: Rosenberg RN, Prusiner S, DiMauro S, Barchi RL, eds. *The molecular and genetic basis of neurological disease*. 2nd ed. Boston: Butterworth-Heinemann, 1996:201-35.
9. DiMauro S, Hirano M, Kaufmann P, et al. Clinical features and genetics of myoclonic epilepsy with ragged red fibers. In: Fahn S, Frucht SJ, eds. *Myoclonus and paroxysmal dyskinesia*. Philadelphia: Lippincott Williams & Wilkins, 2002:217-29.
10. Carelli V. Leber's hereditary optic neuropathy. In: Schapira AHV, DiMauro S, eds. *Mitochondrial disorders in neurology*. 2nd ed. Boston: Butterworth-Heinemann, 2002:115-42.
11. Andreu AL, Hanna MG, Reichmann H, et al. Exercise intolerance due to mutations in the cytochrome *b* gene of mitochondrial DNA. *N Engl J Med* 1999;341:1037-44.
12. Kadowaki T, Kadowaki H, Mori Y, et al. A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. *N Engl J Med* 1994;330:962-8.
13. Hirano M, Davidson MM, DiMauro S. Mitochondria and the heart. *Curr Opin Cardiol* 2001;16:201-10.
14. Fischel-Ghodsian N. Mitochondrial mutations and hearing loss: paradigm for mitochondrial genetics. *Am J Hum Genet* 1998;62:15-9.
15. Chen X, Prosser R, Simonetti S, Sadlock J, Jagiello G, Schon EA. Rearranged mitochondrial genomes are present in human oocytes. *Am J Hum Genet* 1995;57:239-47.
16. King MP, Attardi G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. *Science* 1989;246:500-3.
17. Tatum Y, Christodoulou J, Feigenbaum A, et al. Heteroplasmic mtDNA mutation (T→G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. *Am J Hum Genet* 1992;50:852-8.
18. Matschinsky FM, Glaser B, Magnuson MA. Pancreatic beta-cell glucokinase: closing the gap between theoretical concepts and experimental realities. *Diabetes* 1998;47:307-15.
19. Eto K, Tsubamoto Y, Terauchi Y, et al. Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. *Science* 1999;283:981-5.
20. Langin D. Diabetes, insulin secretion, and the pancreatic beta-cell mitochondrion. *N Engl J Med* 2001;345:1772-4. [Erratum, *N Engl J Med* 2002;346:634.]
21. Inoue K, Nakada K, Ogura A, et al. Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion in zygotes. *Nat Genet* 2000;26:176-81.
22. Sligh JE, Levy SE, Waymire KG, et al. Maternal germ-line transmission of mutant mtDNAs from embryonic stem cell-derived chimeric mice. *Proc Natl Acad Sci U S A* 2000;97:14461-6.
23. Shoubridge EA. Nuclear gene defects in respiratory chain disorders. *Semin Neurol* 2001;21:261-7.
24. Triepels RH, Van Den Heuvel LP, Trijbels JM, Smeitink JA. Respiratory chain complex I deficiency. *Am J Med Genet* 2001;106:37-45.
25. Bourgeron T, Rustin P, Chretien D, et al. Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. *Nat Genet* 1995;11:144-9.
26. Parfait B, Chretien D, Rotig A, Marsac C, Munnich A, Rustin P. Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. *Hum Genet* 2000;106:236-43.
27. Birch-Machin MA, Taylor RW, Cochran B, Ackrell BA, Turnbull DM. Late-onset optic atrophy, ataxia, and myopathy associated with a mutation of a complex II gene. *Ann Neurol* 2000;48:330-5.
28. Shoubridge EA. Nuclear genetic defects of oxidative phosphorylation. *Hum Mol Genet* 2001;10:2277-84.
29. Baysal BE, Ferrell RE, Willett-Brozick JE, et al. Mutations in *SDHD*, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000;287:848-51.
30. Niemann S, Muller U. Mutations in *SDHC* cause autosomal dominant paraganglioma, type 3. *Nat Genet* 2000;26:268-70.
31. Gimm O, Armanios M, Dziema H, Neumann HP, Eng C. Somatic and occult germline mutations in *SDHD*, a mitochondrial complex II gene, in nonfamilial pheochromocytoma. *Cancer Res* 2000;60:6822-5.
32. Musumeci O, Naini A, Slonim AE, et al. Familial cerebellar ataxia with muscle coenzyme Q10 deficiency. *Neurology* 2001;56:849-55.
33. de Lonlay P, Valnot I, Barrientos A, et al. A mutant mitochondrial respiratory chain assembly protein causes complex III deficiency in patients with tubulopathy, encephalopathy and liver failure. *Nat Genet* 2001;29:57-60.
34. Visapaa I, Fellman V, Vesa J, et al. GRACILE syndrome, a lethal metabolic disorder with iron overload, is caused by a point mutation in *BCS1L*. *Am J Hum Genet* 2002;71:863-76.

35. Shoubridge EA. Cytochrome c oxidase deficiency. *Am J Med Genet* 2001;106:46-52.
36. Zeviani M. The expanding spectrum of nuclear gene mutations in mitochondrial disorders. *Semin Cell Dev Biol* 2001;12:407-16.
37. Antonicka H, Mattman A, Carlson CG, et al. Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy. *Am J Hum Genet* 2003;72:101-14.
38. Sue CM, Schon EA. Mitochondrial respiratory chain diseases and mutations in nuclear DNA: a promising start? *Brain Pathol* 2000;10:442-50.
39. Hirano M, Marti R, Ferreira-Barros C, et al. Defects of intergenomic communication: autosomal disorders that cause multiple deletions and depletion of mitochondrial DNA. *Semin Cell Dev Biol* 2001;12:417-27.
40. Hirano M, DiMauro S. ANTI1, Twinkle, POLG, and TP: new genes open our eyes to ophthalmoplegia. *Neurology* 2001;57:2163-5.
41. Servidei S, Zeviani M, Manfredi G, et al. Dominantly inherited mitochondrial myopathy with multiple deletions of mitochondrial DNA: clinical, morphologic, and biochemical studies. *Neurology* 1991;41:1053-9.
42. Suomalainen A, Majander A, Wallin M, et al. Autosomal dominant progressive external ophthalmoplegia with multiple deletions of mtDNA: clinical, biochemical, and molecular genetic features of the 10q-linked disease. *Neurology* 1997;48:1244-53.
43. Bohlega S, Tanji K, Santorelli FM, Hirano M, al-Jishi A, DiMauro S. Multiple mitochondrial DNA deletions associated with autosomal recessive ophthalmoplegia and severe cardiomyopathy. *Neurology* 1996;46:1329-34.
44. Nishino I, Spinazzola A, Papadimitriou A, et al. Mitochondrial neurogastrointestinal encephalomyopathy: an autosomal recessive disorder due to thymidine phosphorylase mutations. *Ann Neurol* 2000;47:792-800.
45. Kaukonen J, Juselius JK, Tiranti V, et al. Role of adenine nucleotide translocator 1 in mtDNA maintenance. *Science* 2000;289:782-5.
46. Spelbrink JN, Li FY, Tiranti V, et al. Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat Genet* 2001;28:223-31. [Erratum, *Nat Genet* 2001;29:100.]
47. Van Goethem G, Dermaut B, Lofgren A, Martin JJ, Van Broeckhoven C. Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. *Nat Genet* 2001;28:211-2.
48. Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science* 1999;283:689-92.
49. Saada A, Shaag A, Mandel H, Nevo Y, Eriksson S, Elpeleg O. Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy. *Nat Genet* 2001;29:342-4.
50. Mancuso M, Salviati L, Sacconi S, et al. Mitochondrial DNA depletion: mutations in thymidine kinase gene with myopathy and SMA. *Neuropathology* 2002;59:1197-202.
51. Mandel H, Szargel R, Labay V, et al. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat Genet* 2001;29:337-41. [Erratum, *Nat Genet* 2001;29:491.]
52. Salviati L, Sacconi S, Mancuso M, et al. Mitochondrial DNA depletion and dGK gene mutations. *Ann Neurol* 2002;52:311-7.
53. Schlame M, Rua D, Greenberg ML. The biosynthesis and functional role of cardiolipin. *Prog Lipid Res* 2000;39:257-88.
54. Barth PG, Wanders RJ, Vreken P, Janssen EA, Lam J, Baas F. X-linked cardioskeletal myopathy and neutropenia (Barth syndrome) (MIM 302060). *J Inher Metab Dis* 1999;22:555-67.
55. Schlame M, Towbin JA, Heerdt PM, Jehle R, DiMauro S, Blanck TJ. Deficiency of tetralinoleoyl-cardiolipin in Barth syndrome. *Ann Neurol* 2002;51:634-7.
56. Okamoto K, Brinker A, Paschen SA, et al. The protein import motor of mitochondria: a targeted molecular ratchet driving unfolding and translocation. *EMBO J* 2002;21:3659-71.
57. Fenton WA. Mitochondrial protein transport—a system in search of mutations. *Am J Hum Genet* 1995;57:235-8.
58. Roesch K, Curran SP, Tranebjaerg L, Koehler CM. Human deafness dystonia syndrome is caused by a defect in assembly of the DDP1/TIMM8a-TIMM13 complex. *Hum Mol Genet* 2002;11:477-86.
59. Hansen JJ, Durr A, Courru-Rebeix I, et al. Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60. *Am J Hum Genet* 2002;70:1328-32.
60. Boldogh IR, Yang HC, Nowakowski WD, et al. Arp2/3 complex and actin dynamics are required for actin-based mitochondrial motility in yeast. *Proc Natl Acad Sci U S A* 2001;98:3162-7.
61. Alexander C, Votruba M, Pesch UE, et al. OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet* 2000;26:211-5.
62. Delettre C, Lenaers G, Griffioen JM, et al. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 2000;26:207-10.
63. Cavadini P, O'Neill HA, Benada O, Isaya G. Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. *Hum Mol Genet* 2002;11:217-27.
64. Casari G, De Fusco M, Ciarmatori S, et al. Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell* 1998;93:973-83.
65. Lutsenko S, Cooper MJ. Localization of the Wilson's disease protein product to mitochondria. *Proc Natl Acad Sci U S A* 1998;95:6004-9.
66. Beal MF. Mitochondria in neurodegeneration. In: Desnuelle C, DiMauro S, eds. *Mitochondrial disorders: from pathophysiology to acquired defects*. New York: Springer, 2002:17-35.
67. DiMauro S, Tanji K, Bonilla E, Pallotti F, Schon EA. Mitochondrial abnormalities in muscle and other aging cells: classification, causes, and effects. *Muscle Nerve* 2002;26:597-607.
68. Schon EA, Manfredi G. Neuronal degeneration and mitochondrial dysfunction. *J Clin Invest* 2003;111:303-12.
69. DiMauro S, Hirano M, Schon EA. Mitochondrial encephalomyopathies: therapeutic approaches. *Neurol Sci* 2000;21:S901-S908.
70. Taivassalo T, Fu K, Johns T, Arnold D, Karpati G, Shoubridge EA. Gene shifting: a novel therapy for mitochondrial myopathy. *Hum Mol Genet* 1999;8:1047-52.
71. Manfredi G, Gupta N, Vazquez-Memije ME, et al. Oligomycin induces a decrease in the cellular content of a pathogenic mutation in the human mitochondrial ATPase 6 gene. *J Biol Chem* 1999;274:9386-91.
72. Clark KM, Bindoff LA, Lightowers RN, et al. Reversal of a mitochondrial DNA defect in human skeletal muscle. *Nat Genet* 1997;16:222-4.
73. Tanaka M, Borgeld HJ, Zhang J, et al. Gene therapy for mitochondrial disease by delivering restriction endonuclease SmaI into mitochondria. *J Biomed Sci* 2002;9:534-41.
74. Manfredi G, Fu J, Ojaimi J, et al. Rescue of a deficiency in ATP synthesis by transfer of MTATP6, a mitochondrial DNA-encoded gene, to the nucleus. *Nat Genet* 2002;30:394-9.
75. Guy J, Qi X, Pallotti F, et al. Rescue of a mitochondrial deficiency causing Leber hereditary optic neuropathy. *Ann Neurol* 2002;52:534-42.
76. Ojaimi J, Pan J, Santra S, Snell WJ, Schon EA. An algal nucleus-encoded subunit of mitochondrial ATP synthase rescues a defect in the analogous human mitochondrial-encoded subunit. *Mol Biol Cell* 2002;13:3836-44.
77. Bai Y, Hajek P, Chomyn A, et al. Lack of complex I activity in human cells carrying a mutation in MtDNA-encoded ND4 subunit is corrected by the *Saccharomyces cerevisiae* NADH-quinone oxidoreductase (NDI1) gene. *J Biol Chem* 2001;276:38808-13.
78. Kolesnikova OA, Entelis NS, Mireau H, Fox TD, Martin RP, Tarassov IA. Suppression of mutations in mitochondrial DNA by tRNAs imported from the cytoplasm. *Science* 2000;289:1931-3.

79. Spinazzola A, Marti R, Nishino I, et al. Altered thymidine metabolism due to defects of thymidine phosphorylase. *J Biol Chem* 2002;277:4128-33.
80. Jaksch M, Paret C, Stucka R, et al. Cytochrome c oxidase deficiency due to mutations in SCO2, encoding a mitochondrial copper-binding protein, is rescued by copper in human myoblasts. *Hum Mol Genet* 2001;10:3025-35.
81. Salviati L, Hernandez-Rosa E, Walker WF, et al. Copper supplementation restores cytochrome c oxidase activity in cultured cells from patients with SCO2 mutations. *Biochem J* 2002;363:321-7.
82. Luft R, Ikkos D, Palmieri G, Ernster L, Afzelius B. A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical, and morphological study. *J Clin Invest* 1962;41:1776-804.
83. DiMauro S, Bonilla E, Lee CP, et al. Luft's disease: further biochemical and ultrastructural studies of skeletal muscle in the second case. *J Neurol Sci* 1976;27:212-32.
84. Luft R. The development of mitochondrial medicine. *Proc Natl Acad Sci U S A* 1994;91:8731-8.

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