Mitochondria play a pivotal role in cellular metabolism and in energy production in particular. Predictably, defects of mitochondrial metabolism have a deleterious effect on cell function and survival, especially in highly energy-dependent tissues such as brain and skeletal muscle. Although a multitude of biochemical reactions occur within mitochondria, the oxidative phosphorylation (OXPHOS) system is the most important in terms of adenosine triphosphate generation and in its association with human disease.

The OXPHOS system is located on the inner mitochondrial membrane and comprises the respiratory chain (complexes I-IV) and adenosine triphosphate synthase (complex V). It is responsible for proton pumping, producing the transmembrane electrochemical gradient ($\psi$, $\Delta m$), and generating adenosine triphosphate by aerobic metabolism. The 5 protein complexes of the OXPHOS system comprise approximately 82 subunits, 13 of which are encoded by mitochondrial DNA (mtDNA). Human mtDNA is a 16.5-kilobase circular double-stranded molecule that codes for 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs), subunits ND1 through ND6 and ND4L of complex I, cytochrome b of complex III, COI-III of complex IV (cytochrome oxidase [COX]), and subunits 6 and 8 of complex V. Thus, complexes I, III, IV, and V are unique in that they are the products of 2 different genomes—nuclear and mitochondrial (Figure). Nuclear-encoded subunits are translated on cytoribosomes and transported to mitochondrial compartments via a complex import and intramitochondrial sorting system incorporating targeting sequences and membrane receptors (see Ryan and Jensen1 for review).

Deficiencies of the OXPHOS system can be considered as primary, ie, inborn genetic defects affecting either the nuclear or mitochondrial genomes, or secondary and induced by either endogenous or exogenous toxins. In addition, defects of OXPHOS enzymes and/or mutations of mtDNA have been identified in a number of neurodegenerative diseases. It would be premature at present to classify these as primary or secondary abnormalities.

**PRIMARY DEFECTS: mtDNA**

Each mitochondrion contains 2 to 10 molecules of mtDNA. Mitochondrial DNA is virtually exclusively maternally inherited. This feature, together with its high polymorphism rate, has enabled mtDNA to be used in genetic studies of evolution and population migration, as well as more recent applications in forensic science. The first report of mtDNA mutations in human disease described deletions in patients with chronic progressive external ophthalmoplegia or Kearns-Sayre syndrome.2,3 The deleted mtDNA molecules coexist with normal wild-type molecules, a situation referred to as heteroplasmy. The proportion of mutant molecules is known to vary between patients and between different tissues of the same patient, although deletions are rarely found in blood. It appears from in vitro studies that for deletions, a threshold of 60% mutant-mtDNA must be exceeded for biochemical deficiency to ensue.4 However, there is often little correlation in patients between deletion load and clinical presentation. The reasons for this are ob-
scure. Mitochondrial DNA deletions in muscle are associated with ragged red, succinate dehydrogenase (SDH)-positive and COX-negative fibers. In situ hybridization studies have demonstrated that the deleted molecules are present in highest proportion in these abnormal fibers. Deletions may also be found in association with mtDNA duplications, although recent evidence suggests that the duplicated molecules do not cause respiratory chain deficiency.3

Point mutations in protein coding genes have been found in Leber hereditary optic neuropathy (LHON), neurogenic weakness ataxia and retinitis pigmentosa, and Leigh disease. Leber hereditary optic neuropathy is characterized by bilateral sequential visual loss with onset in young adulthood, optic atrophy, and variable recovery. Three mutations in complex I genes (G11778Abp in ND4, T14484Cbp in ND6, and A3460Tbp in ND1) are considered primary as they are found only in patients with LHON, while a number of secondary (possibly modifying) base changes have been identified in LHON, but also in some control subjects.6 Mutations in LHON are systematically distributed, often in high proportion to wild-type, and readily detected in the blood. The majority of persons with LHON have disease confined to the optic nerve although a multiple sclerosis–like illness has been described in some women with the G11778Abp mutation;7 and patients with LHON-dystonia mutations6,8 have been described. Several features of LHON remain unexplained, eg, restriction of clinical deficit to the optic nerve, the excess of male patients, and the presence of asymptomatic carriers (usually females) often with as high levels of mutation in the blood as affected siblings. These factors have led to the suggestion that there may be modifying factors on the biochemical expression of the mtDNA mutations, eg, X-linked susceptibility genes, environmental factors, or autoimmunity. Neurogenic weakness ataxia and retinitis pigmentosa and Leigh disease may be associated with point mutations in the adenosine triphosphate synthase 6 gene at position 8993. Interestingly, muscle biopsy specimens from patients with LHON, neurogenic weakness ataxia and retinitis pigmentosa, or Leigh disease are not commonly associated with ragged red fibers, leading to the suggestion that these morphological abnormalities are more often the consequence of mutations involving tRNA genes. Leigh disease may be associated with other mtDNA mutations, pyruvate dehydrogenase deficiency, and other biochemical abnormalities.10

Point mutations in tRNA genes are found in a number of clinical syndromes, including myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS), myoclonic epilepsy with ragged red fibers, and encephalomyopathies encompassing limb myopathy, dementia, ataxia, and deafness. In addition, syndromes including cardiomyopathy, diabetes mellitus, and multiple lipomatosis have been described. The relationship between genotype and phenotype is not strict, although MELAS is most often associated with rRNAleu(UUR) mutations (particularly the A3243G mutation) and myoclonic epilepsy with ragged red fibers with the A8344Gbp rRNAlys mutation. Mutations in tRNA are heterogeneous and can usually be detected in blood. In vitro studies suggest that greater than 90% mutant-mtDNA load is required before biochemical defects appear, although this may vary from one tissue to another.11 The mechanisms by which tRNA mutations cause cell dysfunction are unknown but inevitably must involve translational abnormalities.

A polymorphism at 1555 in the 12S rRNA of mtDNA has been associated with increased sensitivity to deafness induced by aminoglycoside antibiotics.12 Aminoglycosides target the 16S rRNA of Escherichia coli to impair “housekeeping” translational activity, the 1555 polymorphism probably results in enhanced binding of aminoglycosides to the human mtDNA 12S analog in the cochlear cells, and consequent enhanced toxicity. Aminoglycoside-induced deafness is relatively common and it has been estimated that 17% of affected patients have this polymorphism. Interestingly, this same polymorphism has been identified in families with maternally inherited non-syndromic deafness.

**PRIMARY DEFECTS: NUCLEAR DNA**

Several families with autosomal dominant mitochondrial myopathy have been described.13 Typically, autosomal dominant mitochondrial myopathy is characterized by chronic progressive external ophthalmoplegia with limb myopathy and ragged red fibers on biopsy. Mitochondrial DNA analysis shows multiple deletions in tissues from patients, which contrasts with the single deletions of patients with sporadic chronic progressive external ophthalmoplegia or Kearns-Sayre syndrome. To date, 2 loci on chromosomes 3 and 10 have been identified by linkage analysis but the respective genes have not been identified, although a number of likely candidates have been excluded.14,15

Mitochondrial DNA depletion syndrome presents in early infancy with hepatic failure, hypotonia, and failure to thrive; death is common before 1 year of age.8 Mitochondrial DNA levels in severely affected individuals may be 10% or less of age-matched control subjects. Family studies are limited but have suggested autosomal inheritance, probably recessive in most cases. Nuclear involvement has been confirmed in recent studies on 2 different patients that demonstrated restoration of mtDNA.

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**Schematic representation of the mitochondrial respiratory chain and oxidative phosphorylation system.** Each hexagon is the polypeptide product of a single gene. Yellow hexagons are encoded by nuclear genes, and green hexagons represent subunits encoded by mitochondrial DNA.
levels following fusion of cells containing remnant patient mtDNA with mtDNA-less (p0) cells containing normal nuclei. The molecular mechanisms involved in mtDNA depletion are not known and may vary from one family to another. However, a genetic defect, perhaps in a developmentally regulated gene controlling mtDNA copy number, seems at least one likely explanation.

The only mutation identified in a nuclear gene encoding a respiratory chain subunit is a missense base change in the flavoprotein of SDH (complex II). The mutation was identified in 2 sisters with Leigh disease and severe SDH deficiency in muscle and cultured tissues. To date, this mutation has not been identified in other patients with SDH defects.

**MITOCO NDRIAL DYSFUNCTION IN NEURODEGENERATION**

**Parkinson Disease**

Numerous toxins inhibit mitochondrial function and many of these are used in agriculture as herbicides and pesticides. Attention has focused on mitochondrial toxins that cause human disease and several of these are now known to produce models of neurodegenerative disease. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is probably the most studied of these; it is known to induce parkinsonism in humans and other primates. MPTP targets the dopaminergic neurones of the substantia nigra through specific uptake and conversion pathways and is then concentrated within mitochondria.

1-Methyl-4-phenylpyridinium (MPP+), the active metabolite of MPTP, is a selective complex I inhibitor and is thought to induce cell death through adenosine triphosphate depletion. However, there is good evidence that free radicals, including nitric oxide, participate with complex I inhibition in MPP+ toxicity. A remarkable parallel between MPTP toxicity and idiopathic Parkinson disease (PD) was drawn when complex I deficiency was identified in the substantia nigra of patients who had died with PD. This defect, together with other biochemical abnormalities, including oxidative stress and damage, is thought to participate in the pathogenesis of nigral cell loss in PD. The complex I defect appears to be isolated in the substantia nigra within PD brain, although several reports have also documented a decrease in complex I activity (with and without defects in complexes II-IV) in platelets from patients with PD. The presence of an OXPHOS defect in PD skeletal muscle is more contentious. One recent report has suggested that the platelet complex I deficiency in some patients with PD may be caused by mtDNA mutations. This finding implies that complex I deficiency may be of primary etiological importance, at least in some patients with PD.

**Huntington Disease**

Accidental ingestion of 3-nitropropionic acid, a contaminant of mildewed sugar cane, was found to cause striatal necrosis and chorea with dystonia in survivors. 3-Nitropropionic acid is a complex II inhibitor and when injected into primates produces striatal pathologic features strikingly similar to those of Huntington disease (HD). Malonate, another complex II inhibitor, induces similar effects to 3-nitropropionic acid. Severe deficiency of complex II and III activity has been identified in HD caudate and thus provides another example of a direct link between a mitochondrial toxin and human neurodegenerative disease. In HD, the mitochondrial defect must be secondary to the abnormal CAG expansion mutation in the huntingtin gene (IT15). The absence of the complex II and III deficiency in tissues that express mutant huntingtin indicates that it must be related to specific biochemical or pharmacological features in the striatum. Nitric oxide synthase–positive neurons and fibers are present in the striatum in addition to glutaminergic fibers and this has led to the suggestion that excitotoxicity may participate in neuronal cell death in HD. Nitric oxide is a potent inhibitor of complex II and III and so the mitochondrial defect may be secondary to an excitotoxic mechanism.

**Friedreich Ataxia**

Clinically, Friedreich ataxia (FA) is characterized by onset in early adolescence of ataxia, areflexia, and pyramidal features in association with kyphoscoliosis and a cardiomyopathy. Patients usually die of cardiac failure within 15 years of diagnosis. Pathologically, the main feature in FA is a “dying back” neuropathy affecting dorsal sensory neurons. Friedreich ataxia is an autosomal recessive disorder caused by an expanded GAA repeat in intron 1 of the X25 gene on chromosome 9. The repeat causes reduced transcription of the X25 gene and therefore decreased expression of its product, frataxin. Frataxin is widely expressed but its function is not known. However, frataxin has significant sequence homology with a yeast protein now termed YFH-1. Deletion of the gene for YFH-1 results in defective energy production and mtDNA depletion. This yeast knockout model also accumulates iron in the mitochondrial matrix. Studies with fluorescent tags have localized frataxin to the mitochondria. Thus, there is overwhelming evidence that frataxin is a mitochondrial protein and that in yeast, at least, its deficiency causes defective OXPHOS, a loss of mtDNA, and accumulation of iron. Some of these features are reminiscent of the situation in the PD substantia nigra (see above).

**Alzheimer Disease**

Several reports have demonstrated COX deficiency both histochemically and biochemically in Alzheimer disease (AD). One group has also demonstrated COX deficiency in AD platelets. p0 cells have been used to show that the platelet COX defect is transmitted with AD mtDNA transfer and that this deficiency is associated with certain base changes in mtDNA COX genes. Although these “mutations” were also found in controls, they were present at higher frequency in patients with AD. If confirmed, these observations could have important implications for etiology and inheritance, in at least a proportion of patients with AD.
Dystonia

Three groups have reported on platelet mitochondrial function in platelets from patients with focal or generalized dystonia. The first study found marked complex I deficiency in both groups of patients; the second showed normal mitochondrial function. The third study looked at sporadic focal dystonia and DYT-1-positive and -negative families with generalized dystonia; a complex I deficiency was seen in the focal group, but no abnormality was detectable in the patients with generalized dystonia linked or not to DYT-1.

NEURODEGENERATION: CONCLUSIONS

Mitochondrial abnormalities in HD and FA are undoubtedly secondary to the respective primary molecular defects in the huntingtin and frataxin genes. The mechanisms by which the mitochondrial defects in HD and the putative abnormalities in FA are induced and the part they play in disease pathogenesis are unknown. In HD, however, the pattern of respiratory chain defect supports the role of excitotoxicity. In PD, AD, and dystonia, the situation is much less clear. Data from mtDNA transfer studies suggest a primary role for mtDNA mutations in PD and AD, but whether this is relevant to the majority or only a small subgroup of patients is not clear.

Since the manuscript was accepted for publication, the mitochondrial DNA mutations reported to be present at increased frequency in AD have subsequently been identified as artifacts of polymerase chain reaction amplification of nuclear DNA, i.e., nuclear-embedded mtDNA pseudogenes. A second nuclear respiratory chain DNA mutation in the gene encoding the 18-kD subunit of complex I has also recently been described.

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