Mitochondrial Genetics: A Paradigm for Aging and Degenerative Diseases?

Douglas C. Wallace

Studies of diseases caused by mitochondrial DNA mutations suggest that a variety of degenerative processes may be associated with defects in oxidative phosphorylation (OXPHOS). Application of this hypothesis has provided new insights into such diverse clinical problems as ischemic heart disease, late-onset diabetes, Parkinson's disease, Alzheimer's disease, and aging.

There are four basic concepts in the OXPHOS paradigm for degenerative diseases: OXPHOS generates mitochondrial energy in the form of adenosine triphosphate (ATP); OXPHOS is the main source of ATP for a variety of organs and tissues, including brain, striated muscle, kidney, and pancreatic islets; OXPHOS genetics is complex, involving both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) genes; and OXPHOS declines with age.

Oxidative phosphorylation generates mitochondrial ATP by means of five multiple subunit enzyme complexes (I through V) plus the adenine nucleotide translocator (ANT), all localized within the mitochondrial inner membrane. Complexes I to IV constitute the electron transport chain. Reduced nicotinamide adenine dinucleotide (NADH) is oxidized by complex I (NADH dehydrogenase), and succinate is oxidized by complex II (succinate dehydrogenase); the electrons are transferred to ubiquinone (CoQ) to yield ubiquinol (reduced CoQ). The electrons from ubiquinol are transferred to complex III (ubiquinol:cytochrome c oxidoreductase), then to cytochrome c (*cyt c*), then to complex IV (cytochrome c oxidase), and finally to oxygen. The energy released is used to pump protons out of the mitochondrial inner membrane through complexes I, III, and IV, and the resulting electrochemical gradient is exploited by complex V (ATP synthase) to condense adenosine diphosphate (ADP) and inorganic phosphate (P_i) to form ATP. Both ATP and ADP are exchanged across the mitochondrial inner membrane by ANT (*1, 2*).

Oxidative phosphorylation is the primary source of energy for several organ and tissue systems, including the brain, muscle, heart, kidney, liver, and pancreatic islets. Chronic poisoning of OXPHOS with respiratory inhibitors and uncouplers such as cyanide, sodium azide, and dinitrophenol results in optic atrophy, deafness, ataxia, seizures, myoclonus, basal ganglia degeneration, and movement disorders. Hypoxia caused by asphyxiation or ischemia preferentially affects the brain, kidney, heart, and liver. Ischemia also produces a characteristic muscle pathology, mitochondrial myopathy, in which the oxidative type I muscle fibers degenerate and accumulate aggregates of abnormal mitochondria. Because these mitochondrial aggregates stain red by Gomori modified trichrome, the affected muscle fibers are called ragged-red fibers (RRF) (*1*). Streptozotocin, which selectively damages pancreatic islet mtDNAs, induces diabetes in rats, and respiratory inhibitors block insulin production in isolated islets. Hence, the islets of Langerhans are also highly oxidative (*3*). Taken together, these data indicate that as inhibition of OXPHOS increases, mitochondrial ATP production declines until it falls below the minimum energy levels (thresholds) necessary for more oxidative tissues and organs to function. In this manner, systemic OXPHOS defects can result in tissue-specific diseases (*1*).

OXPHOS genetics is complex because the genes for component peptides are distributed throughout both the nDNA and mtDNA. Human mtDNA is a 16,569–base pair (bp) closed circular molecule located within the mitochondrial matrix, which encodes 13 OXPHOS subunits plus the mitochondrial ribosomal RNAs (rRNA) and transfer RNAs (tRNA) required for their expression (Fig. 1). Respiratory complex I consists of over 30 polypeptides, seven encoded by the mtDNA (these are the NADH dehydrogenase genes *ND1, ND2, ND3, ND4L, ND4, ND5*, and *ND6*); complex II consists of four nDNA-encoded polypeptides; complex III encompasses ten polypeptides, one encoded by the mtDNA [cytochrome b (*cyt b*)]; complex IV incorporates 13 polypeptides, three from the mtDNA (cytochrome c oxidase genes *COI, COII*, and *COIII*); and complex V includes 12 polypeptides, two from the mtDNA (ATP synthase genes *ATPase* 6 and 8) (Fig. 1). The expression of the mtDNA OXPHOS genes requires functional mitochondrial replication, transcription, and translation systems, and all of the polypeptides for these processes are encoded by the nDNA. Thus, the biogenesis of OXPHOS requires hundreds of nuclear and cytoplasmic genes (*1, 4*).

The genetics of OXPHOS is additionally complicated by the unique characteristics of mtDNA inheritance (*1, 5*). The mtDNA is predominantly maternally inherited; less than 0.1% of the mtDNAs are contributed by the sperm. Each cell contains hundreds of mitochondria and thousands of copies of the mtDNA genome. Therefore, cells can harbor mixtures of mutant and normal mtDNAs (heteroplasmy), and each time a heteroplasmic somatic or germ-line cell divides, the mutant and normal mtDNAs are randomly segregated into the daughter cells. Thus, the mtDNA genotype fluctuates from one cell division to the next, and over multiple cell divisions the proportion of mutant mtDNAs drifts toward either predominantly mutant or normal mtDNAs (homoplasmy). The severity of the OXPHOS defect resulting from an mtDNA mutation is a product of the nature of the mtDNA mutation and the proportion of mutant mtDNAs within the cell. Because the phenotype of a patient is related to the severity of the OXPHOS defect and the energetic thresholds of the various organs and tissues, the clinical symptoms of the maternal relatives in heteroplasmic pedigrees vary according to the proportion of mutant mtDNAs that each individual inherits. Because the mutation rate of the mtDNA genes is substantially higher than that of most nDNA polypeptide genes, diseases resulting from heteroplasmic and homoplasmic mtDNA mutations are relatively common (*1, 5*).

The final basic concept in the OXPHOS paradigm is that the OXPHOS system declines with age. The number of skeletal and heart muscle fibers deficient in cytochrome c oxidase increases progressively with age (*6*), and the respiration rates and specific activities of respiratory complexes I and IV decline with age in both skeletal muscle and liver tissue (*7*). One possible cause of this age-related decline in OXPHOS as well as the high mtDNA mutation rate is damage of mtDNA by oxygen free radicals (superoxide anion and hydrogen peroxide). Oxygen radicals are a natural by-product of OXPHOS because electrons can be transferred directly from reduced flavin dehydrogenases, CoQ, and *cyt b* to oxygen. As a

The author is the Robert W. Woodruff Professor of Molecular Genetics and the Chairman of the Department of Genetics and Molecular Medicine, Emory University School of Medicine, Atlanta, GA 30322.

result, about 1 to 4% of oxygen uptake is converted to oxygen radicals. The mtDNA appears to be a common target for oxygen radical damage, accumulating 16 times more oxidative damage than nDNA. Because aging is associated with the accumulation of mtDNA rearrangements in rats, and life span in animals is inversely proportional to basal metabolic rate (oxygen consumption) and directly related to the extent of oxygen radical scavenging systems, the decline in OXPHOS with age may be a product of the accumulation of oxidative damage to the mitochondria and mtDNA (8).

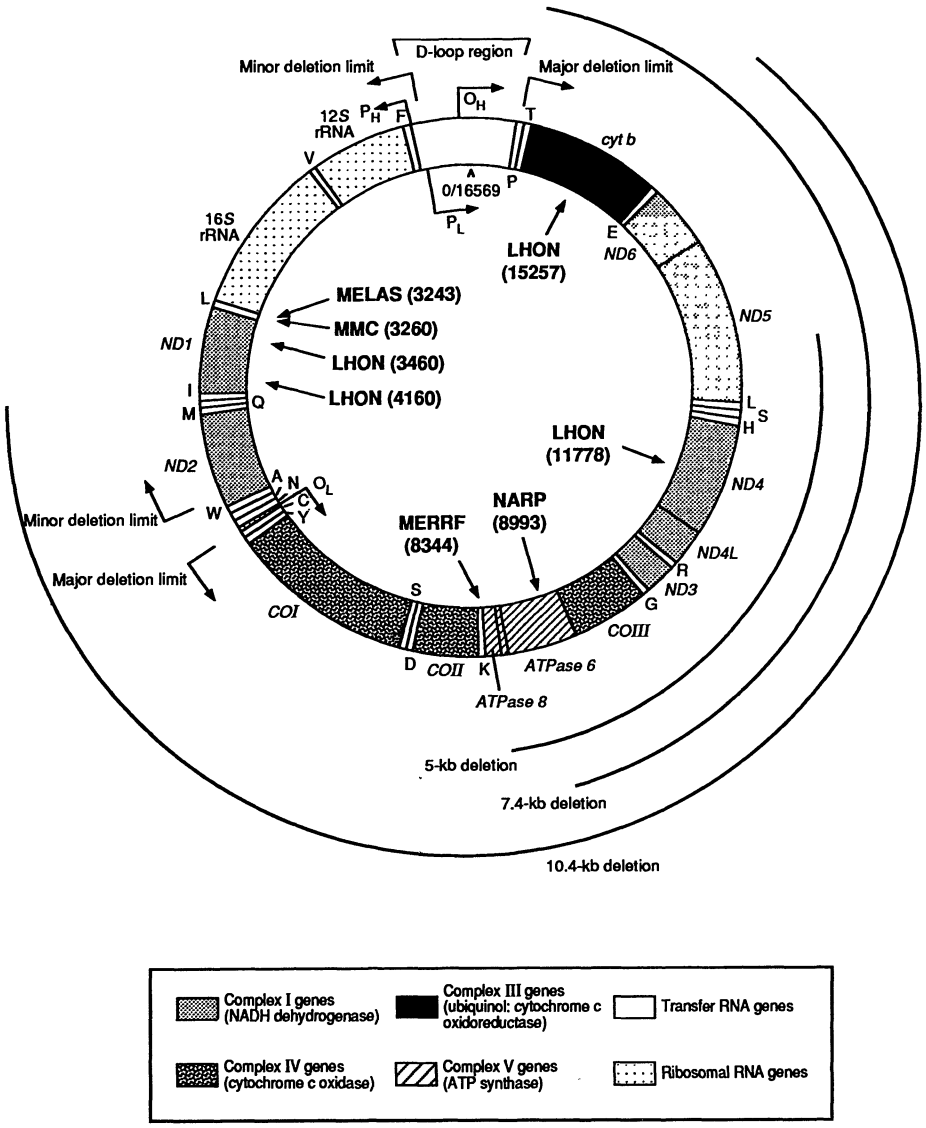

Fig. 1. Human mtDNA map that shows the location of major disease mutations. Nucleotides 0 to 16569 of the mtDNA sequence are numbered counterclockwise from the center of the D-loop. Shaded regions indicate the location of the rRNA and protein genes described in the text. These genes are punctuated by tRNA genes (clear sectors) whose cognate amino acids are indicated by the adjacent amino acid abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. H-strand replication starts at O_H and proceeds clockwise two-thirds of the way around the circle, displacing the parental H-strand. When O_L is exposed, L-strand replication initiates and proceeds counterclockwise back around the free parental H-strand. Transcription initiates at the H-strand and L-strand promoters (P_H and P_L, respectively) within the D-loop. The P_H promoter transcribes all genes but *ND6* and eight tRNAs. The P_L promoter directs transcription of these genes and synthesizes the primer for H-strand DNA replication (*1, 4*). Disease mutation base substitutions are shown inside the circle, and deletion mutations are shown outside. The nucleotide positions of the base substitution mutations are given in parentheses, and only the four primary LHON mutations are shown. The regions removed by the 5-kb (4977-bp), 7.4-kb (7436-bp), and 10.4-kb (10423-bp) deletions are indicated by the external arcs. The outer limits for CEOP, KSS, and Pearson's syndrome deletions are encompassed by two arcs bracketed by O_H and O_L. Most deletions occur within the major deletion limit, which extends clockwise in the direction of H-strand replication from O_H to O_L (positions 16085 through 5786). The remainder of the deletions occur within the minor deletion limit, which extends from O_L to O_H (positions 5448 through 470) and which can remove P_H but not P_L.

The Nature of OXPHOS Diseases

The relevance of the OXPHOS paradigm to degenerative diseases has been shown through studies of individuals with mtDNA mutations. A number of deleterious mtDNA) mutations have been identified (*1, 4*), including base substitution mutations in both the tRNA and protein coding genes, mtDNA insertion and deletion mutations, nuclear mutations that predispose cells to mtDNA deletions, and alterations of mtDNA copy number.

Diseases resulting from mutations in mitochondrial tRNA genes most clearly demonstrate the principles of the OXPHOS paradigm. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is the result of a heteroplasmic mtDNA mutation at nucleotide 8344 in the $tRNA^{Lys}$ gene (Fig. 1). The MERRF mutation is maternally inherited, and severely affected individuals have myoclonic epilepsy (uncontrolled jerking), mitochondrial myopathy, and deficiencies in respiratory complexes I and IV associated with a defect in mitochondrial protein synthesis (9).

The clinical manifestations of the MERRF mutation are variable among maternal relatives and directly proportional to the associated complex I and IV defects. Within a family, as OXPHOS declines, maternal relatives sequentially acquire electrophysiological aberrations of the central nervous system, mitochondrial myopathy, neurosensory deafness, myoclonic epilepsy, progressive dementia, hypoventilation, cardiac insufficiency, and renal dysfunction. The severity of the OXPHOS defect and phenotype is determined by two variables: percentage of mutant mtDNAs and age. In a comparison of family members with similar proportions of mutant mtDNAs, the specific activity of their respiratory complexes decreases, and the severity of their phenotype increases, with age. An individual with 15% normal mtDNAs can have a mild phenotype at age 20 but a severe phenotype by age 60. Consequently, in MERRF pedigrees it is common for individuals having high percentages of mutant mtDNAs to have normal childhoods but to become severely affected as adults (9).

Variable phenotypes associated with heteroplasmy have also been reported in mitochondrial encephalomyopathies resulting from mutations in the mtDNA $tRNA^{Leu(UUR)}$ gene. These include the mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms (MELAS) mutation at nucleotide 3243,

which also inactivates the overlapping transcriptional terminator sequence, and the maternally inherited myopathy and cardiomyopathy (MMC) mutation at nucleotide 3260 (*10*) (Fig. 1).

Diseases caused by mtDNA missense mutations also demonstrate the age-related expression of OXPHOS diseases and the association between the degree of OXPHOS defect and the severity of clinical phenotype. Leber's hereditary optic neuropathy (LHON) is a maternally inherited form of sudden-onset vision loss caused by missense mutations in mtDNA electron transport genes. Individuals with the most common LHON mutation are generally normal throughout childhood but have an increasing probability of going blind as they age. Their average age of vision loss is 27.6 ± 14.2 years, but the range is from 8 to 60 years (*11*). Four mtDNA mutations have been identified that are sufficient in themselves to cause vision loss (Fig. 1). The most common of these mutations is a G to A transition at nucleotide 11778 (LHON11778) that is present in over 50% of cases. This mutation converts the conserved Arg^{340} in *ND4* to a His^{340}. In large pedigrees with many affected individuals, this mutation is essentially homoplasmic and transmitted to all maternal relatives. However, over 50% of LHON11778 cases have no family history, and 14% of the families are heteroplasmic, with the phenotype appearing in the pedigree when the percentage of mutant mtDNAs approaches 100%. This result suggests that a significant number of LHON11778 patients may have mutations that have only recently appeared (*12*). The three remaining mutations that can cause LHON are at position 3460 in *ND1* (*13*), 4160 in *ND1* (*14*), and 15257 in *cyt b* (*15*). All of these alter conserved amino acids.

Five additional mutations appear to contribute to LHON, though only in combination with other LHON mutations. These mutations alter less conserved amino acids and include mutations at nucleotide 4216 in *ND1* (*16*), 4917 in *ND2* (*16*), 5244 in *ND2* (*15*), 13708 in *ND5* (*15, 16*), and 15812 in *cyt b* (*15*). These mutations appear to be additive and in the right combination can reduce OXPHOS sufficiently to cause vision loss. This synergy is best demonstrated in one mtDNA lineage in which four mtDNA mutations have accumulated sequentially with the probability of blindness increasing with each mutation. The successive genotypes were LHON13708, which is present in 8% of non-LHON11778 patients but also in 5% of normal controls; LHON13708 + LHON15257, present in 8% of patients and 0.3% of controls; LHON13708 + LHON15257 + LHON15812, present in 4% of patients and 0.1% of controls; and LHON13708 + LHON15257 + LHON15812 + heteroplasmic LHON5244, present in 4% of patients but in less than 0.05% of controls (*15*). Other possible examples of synergy include a decrease in the severity of the phenotype of the LHON4160 mutation with a second *ND1* mutation at nucleotide 4136 (*14*) and male bias and variability in the expression of LHON as a result of interaction between the mtDNA mutations and a deleterious X-linked allele (*17*). Thus, studies of LHON have shown that OXPHOS disease symptoms may not appear until late in life and that the more severe the OXPHOS defect, the greater the probability that symptoms will develop.

The quantitative nature of OXPHOS diseases is corroborated by maternally inherited neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP), which is associated with seizures, dementia, and developmental delay. This disease is caused by a missense mutation at nucleotide 8993 in the mtDNA *ATPase* 6 gene (Fig. 1). This mutation is heteroplasmic in families, and the proportion of mutant mtDNAs correlates with the severity of the symptoms (*18*).

Diseases caused by mtDNA insertion-deletion mutations also show the relation between quantitative reduction in OXPHOS and the severity of the disease phenotype, and their study has revealed a second mechanism for OXPHOS disease progression. These mtDNA insertion-deletion mutations result in the Kearns-Sayre syndrome (KSS), chronic external ophthalmoplegia plus (CEOP), and Pearson's marrow/pancrease syndrome (*19*). These diseases are generally spontaneous, which suggests that most deletion mutations are somatic in origin (*1*). KSS and CEOP patients are clinically variable, though virtually all have ophthalmoplegia (paralyses of the eye muscles), ptosis (droopy eyelids), and mitochondrial myopathy. Mild cases have only these symptoms and are designated CEOP, whereas severe cases can also have retinitis pigmentosa, hearing loss, heart conduction defects, ataxia, and dementia, and are designated KSS. Pearson's syndrome is a generally fatal childhood disorder involving pancytopenia (loss of all blood cells), pancreatic fibrosis, and splenic atrophy (*1*). Individuals who survive Pearson's syndrome progress to KSS (*20*).

Deletions in the mtDNA are highly heterogeneous in both size and position. However, in CEOP, KSS, and Pearson's syndrome, the deletions always spare the two origins of replication, the heavy (H)-strand origin and light (L)-strand origin (O_H and O_L, respectively), which are located on opposite sides of the genome (*1, 4*). This confines the deletions to two arcs, the "major" arc encompassing two-thirds of the genome and sustaining 95% of the deletions and the "minor" arc encompassing one-third of the genome and the remaining deletions (Fig. 1). Presumably, deletions that eliminate origins retard replication and do not accumulate in somatic tissues.

Deletions of mtDNA are often associated with direct repeats. The most common deletion is 4977 bp (5 kb) and accounts for 30 to 50% of cases. It occurs between the 13-bp direct repeats (5′-ACCTCCCTCACCA) at positions 8470 and 13447 (*4, 21*) (Fig. 1). A second, less common 7436-bp (7.4-kb) deletion occurs between the 12-bp direct repeat (5′-CATCAACAACCG) at positions 8637 and 16073 (*4, 22*) (Fig. 1).

Patients with spontaneous cases of CEOP, KSS, and Pearson's syndrome have only one type of deleted mtDNA, which implies that their diseases are the result of a single deletion event occurring early in development (*1, 4, 19, 21*). In these patients, the proportion of mutant and normal mtDNAs varies markedly between tissues (*23*). Presumably, the distribution of deleted mtDNAs is determined by the time during development that the deletion occurred and the subsequent vagaries of replicative segregation. Thus, variation in the severity of the disease probably reflects variation in the proportion of deleted mtDNAs among the tissues.

In fact, many KSS and CEOP patients are phenotypically normal as children, but symptoms appear later in life and progress. Analysis of muscle samples from individual patients over time has shown that the proportion of deleted mtDNAs progressively increases with age (*24*). Moreover, deleted molecules accumulate in localized regions of muscle fibers (*25*). Because all mtDNA replication enzymes are encoded by the nucleus, the rate of replication of mtDNA is directly proportional to the length of the molecule, so that deleted molecules may have a replicative advantage and consequently increase in number over time (*1*). Some mtDNA duplications have also been reported; these appear to duplicate both replication origins (*26*) and may become enriched because they replicate twice as often (*1*).

The genetic complexity of OXPHOS diseases is further increased by defective nuclear-cytoplasmic interactions. In some KSS pedigrees, the disease is inherited as an autosomal-dominant mutation, and affected family members have a variety of mtDNA deletions instead of the single mtDNA deletion found in spontaneous cases of the disease. Presumably, these KSS cases are the product of a nuclear mutation in a mtDNA replication enzyme (*27*). In

some children with lethal infantile respiratory failure, the amount of the mtDNA in specific tissues is reduced as much as 98%. These mtDNA depletion syndromes can be familial and transmitted through males, thus indicating that they are the result of nuclear mutations (*28*).

The OXPHOS Paradigm as a Hypothesis for Degenerative Diseases

The study of mtDNA diseases has suggested a hypothesis for aging and age-related degenerative diseases. Each individual is born with an initial OXPHOS capacity that subsequently declines with age. When this OXPHOS capacity falls below the energetic threshold of an organ, disease symptoms appear. Although genotypically normal people start with a high OXPHOS capacity and generally die before most tissue thresholds are traversed, individuals born with deleterious OXPHOS mutations start at lower OXPHOS levels and cross expression thresholds within their lifetimes.

Given this paradigm, elucidation of the molecular basis of the age-related decline in OXPHOS is of great importance. The most likely mechanism is the accumulation of damage to mtDNA by oxidation and the progressive enrichment of deleted mtDNAs through their tendency to replicate more rapidly than full-size mtDNA. Evidence that mtDNA damage accumulates with age comes from analysis of heart and brain mtDNAs for the presence of the 7.4- and 5-kb deletions (Fig. 1). The percentage of hearts that have the 7.4-kb deletion increases with age (*22*), and the amount of the 5-kb deletion increases in histologically normal hearts after age 40, reaching 0.0035% by age 80 (*29, 30*). The 5-kb deletion also accumulates in brain tissue; it is absent in children but present in adults (*29*). If all forms of mtDNA damage could be assessed, these data suggest that a significant proportion of the mtDNAs may be defective in elderly people.

Because symptoms appear earlier in patients with OXPHOS defects, affected organs might accumulate higher proportions of mutant mtDNAs. In ischemic heart disease, for instance, OXPHOS is chronically inhibited because atherosclerotic plaques occlude the coronary arteries, which deprives the heart mitochondria of substrates and oxygen. Chronic cardiac ischemia has been associated with an 8- to 2200-fold increase in the 5-kb deletion and an induction of OXPHOS transcript levels for both nDNA (ANT and the ATP synthase β subunit) and mtDNA (rRNAs and *cyt b*) genes in affected hearts (*30*). Similarly, the 7.4-kb deletion was found to be increased in the heart of a 16-year-old girl who had the MELAS mutation and died of hypertrophic cardiomyopathy (*30*). The hearts of cardiomyopathy patients are generally more likely to have the 7.4-kb deletion than those of controls (*22*), hearts from 30% of nonischemic heart failure patients have increased levels of the 5-kb deletion (*30*), and most defective hearts have increased levels of OXPHOS gene transcripts (*30*). Thus, at least some forms of cardiomyopathy can involve OXPHOS deficiency, and chronic inhibition of OXPHOS can be associated with increased secondary damage to mtDNA and a compensatory induction of OXPHOS gene expression.

Some cases of diabetes mellitus may also be a result of OXPHOS defects. Late-onset diabetes mellitus is a heterogeneous group of disorders in which the probability that the affected parent will be the proband's mother increases with the proband's age at onset of the disease (*31*). Recent evidence suggests that this phenomenon might in part be attributable to mtDNA mutations (*32*). Analysis of a large pedigree with maternally inherited diabetes and deafness but with no other manifestation of mitochondrial disease revealed a generalized muscle mitochondrial OXPHOS deficiency associated with a severe mitochondrial protein synthesis defect. Molecular analysis showed that all affected individuals had a systemic, heteroplasmic, 10.4-kb mtDNA deletion flanked by a 10-bp direct repeat (5′-CACCCCATCC) at nucleotide positions 14812 and 4389. This extraordinary deletion removed O_L and all of the mtDNA genes except the rRNAs, *ND1*, part of *cyt b*, and the adjacent tRNAs (*32*) (Fig. 1). The stable maintenance and maternal inheritance of this deletion is unique and suggests that the loss of O_L may offset the replicative advantage of the shorter molecule. The resulting chronic deficiency in OXPHOS appears to inhibit insulin production by the pancreatic islets in a manner analogous to the induction of diabetes by streptozotocin in rats.

Parkinson's, Alzheimer's, and Huntington's diseases may also be associated with OXPHOS defects. Parkinson's disease is a common, late-onset disease associated with movement disorders and loss of dopaminergic neurons in the substantia nigra. The disease can be induced by the complex I inhibitor MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydrophyrine) (*33*), and complex I deficiency has been reported in Parkinson's disease brain (*34*), platelet (*35*), and muscle (*36, 37*) mitochondria. Muscle mitochondrial studies have also shown that patient OXPHOS defects are heterogeneous. Out of six patients in one study, two had complex I deficits, one had a complex I and III defect, one had a complex IV defect, and two had normal activity (*36*). Brains of patients with Parkinson's disease also contain mtDNA deletions (*38*) and Parkinson's disease is genetically heterogeneous. A small number of families are compatible with autosomal-dominant inheritance, but most cases have only occasional affected relatives or are spontaneous (*39*). Thus, Parkinson's disease is both biochemically and genetically heterogeneous, as might be expected if the symptoms were the product of the genetic or environmental inhibition of OXPHOS.

Alzheimer's disease is a common late-onset disease associated with progressive dementia and cortical atrophy. Like Parkinson's disease, Alzheimer's is biochemically and genetically heterogeneous. Five out of six patients with Alzheimer's have been reported to have OXPHOS complex IV defects in platelet mitochondria (*40*). Most Alzheimer's cases are spontaneous, though some are autosomal dominant and a few of these are associated with a β-amyloid polymorphism (*41*). Hence, some Alzheimer's cases could also be the product of systemic OXPHOS defects.

Huntington's disease is a rare movement disorder associated with degeneration of the basal ganglia. OXPHOS enzyme assays have revealed complex IV defects in the basal ganglia of patients with this disease (*42*) and complex I defects in platelet mitochondria (*43*). Huntington's disease is autosomal dominant, but the age of onset and severity of the symptoms can vary, depending on the sex of the affected parent (*1, 44*). Because another disease involving movement disorders and basal ganglia degeneration (LHON and dystonia) is maternally inherited (*45*), the variable age of onset of Huntington's disease might be the product of an mtDNA modifying gene.

These and other age-related degenerative diseases could all fit the OXPHOS paradigm. However, to prove this association, one would have to identify mutant OXPHOS genes that predispose individuals to these conditions. If such mutations can be found, they will permit disease-specific presymptomatic tests. Because metabolic therapies are already being developed for mtDNA OXPHOS diseases (*1*), preventative therapy may ultimately allow individuals at risk for a variety of degenerative diseases to avoid developing symptoms.

REFERENCES AND NOTES

1. D. C. Wallace, *Hosp. Pract.* **21**, 77 (1986); in *Medical and Experimental Mammalian Genetics: A Perspective*, V A McKusick, T. H. Roderick, J. Mori, N. W. Paul, Eds. (Liss, New York, 1987), vol. 23, pp. 137–190; *Trends Genet.* **5**, 9 (1989); J. M. Shoffner and D. C. Wallace, *Adv. Hum. Genet.* **19**, 267 (1990).
2. Y. Hatefi, *Annu. Rev. Biochem.* **54**, 1015 (1985); A. E. Senior, *Physiol. Rev.* **68**, 177 (1988).
3. N. Welsh, S Pacebo, M. Welsh, *Diabetologica* **34**, 626 (1991); S. Y. K. Yousufzai, M. W. Bradford,

E. Shrago, R. B. L. Ewart, *FEBS Lett.* **137**, 205 (1982).
4. D. C. Wallace, M. T. Lott, A. Torroni, J. M. Shoffner, *Cytogenet. Cell Genet.* **58**, 1103 (1991); S. Anderson *et al.*, *Nature* **290**, 457 (1981).
5. R. E. Giles, H. Blanc, H. M. Cann, D. C. Wallace, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6715 (1980); D. C. Wallace, *Somatic Cell Mol. Genet.* **12**, 41 (1986); W. M. Brown, M. George, Jr., A. C. Wilson, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1967 (1979); D. C. Wallace *et al.*, *Curr. Genet.* **12**, 81 (1987); U. Gyllensten, D. Wharton, A. Josefsson, A. C. Wilson, *Nature* **352**, 255 (1991).
6. J. Muller-Hocker, *Am. J. Pathol.* **134**, 1167 (1988); *J. Neurol. Sci.* **100**, 14 (1990).
7. I. Trounce, E. Byrne, S. Marzuki, *Lancet* i, 637 (1989); T.-C. Yen, Y.-S. Chen, K.-L. King, S.-H. Yeh, Y.-H. Wei, *Biochem. Biophys. Res. Commun.* **165**, 994 (1989).
8. A. W. Linnane, S. Marzuki, T. Ozawa, M. Tanaka, *Lancet* i, 642 (1989); B. Bandy and A. J. Davison, *Free Radical Biol. Med.* **8**, 523 (1990); B. N. Ames, *Mutat. Res.* **214**, 41 (1989); L. Piko, A. J. Hougham, K. J. Bulpitt, *Mech. Ageing Dev.* **43**, 279 (1988).
9. D. C. Wallace *et al.*, *Cell* **55**, 601 (1988); J. M. Shoffner *et al.*, *ibid.* **61**, 931 (1990); D. C. Wallace *et al.*, *Am. J. Hum. Genet.* **38**, 461 (1986); A. Chomyn *et al.*, *Mol. Cell. Biol.* **11**, 2236 (1991); J. M. Shoffner, M. T. Lott, D. C. Wallace, *Rev. Neurol.* **147**, 431 (1991).
10. Y. Goto, I. Nonaka, S. Horai, *Nature* **348**, 651 (1990); Y. Kobayashi *et al.*, *Biochem. Biophys. Res. Commun.* **173**, 816 (1990); J. F. Hess, M. A. Parisi, J. L. Bennett, D. A. Clayton, *Nature* **351**, 236 (1991); M. Zeviani *et al.*, *Lancet* **338**, 143 (1991).
11. N. J. Newman and D. C. Wallace, *Amer. J. Ophthalmol.* **109**, 726 (1990); N. J. Newman, M. T. Lott, D. C. Wallace, *ibid.* **111**, 750 (1991).
12. D. C. Wallace *et al.*, *Science* **242**, 1427 (1988); G. Singh, M. T. Lott, D. C. Wallace, *N. Engl. J. Med.* **320**, 1300 (1989); M. T. Lott, A. S. Voljavec, D. C. Wallace, *Am. J. Ophthalmol.* **109**, 625 (1990).
13. K. Huoponen, J. Vilkki, P. Aula, E. K. Nikoskelainen, M. L. Savontaus, *Am. J. Hum. Genet.* **48**, 1147 (1991); N. Howell *et al.*, *ibid.* **49**, 939 (1991).
14. N. Howell, I. Kubacka, M. Xu, D. A. McCullough, *ibid.* **48**, 935 (1991).
15. M. D. Brown, M. T. Lott, A. S. Voljavec, A. Torroni, D. C. Wallace, *ibid.* **49** (suppl.), 183 (1991); M. D. Brown *et al.*, *Genetics* **130**, 163 (1992).
16. D. R. Johns and J. Berman, *Biochem. Biophys. Res. Commun.* **174**, 1324 (1991).
17. J. Vilkki, J. Ott, M. L. Savontaus, P. Aula, E. K. Nikoskelainen, *Am. J. Hum. Genet.* **48**, 486 (1991); X. Bu and J. I. Rotter, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8198 (1991).
18. I. J. Holt, A. E. Harding, R. K. Petty, J. A. Morgan-Hughes, *Am. J. Hum. Genet.* **46**, 428 (1990).
19. I. J. Holt, A. E. Harding, J. A. Morgan-Hughes, *Nature* **331**, 717 (1988); P. Lestienne and G. Ponsot, *Lancet* i, 885 (1988); A. Rotig *et al.*, *ibid.* ii, 567 (1988); M. Zeviani *et al.*, *Neurology* **38**, 1339 (1988).
20. M. A. McShane *et al.*, *Am. J. Hum. Genet.* **48**, 39 (1991).
21. E. A. Schon, *Science* **244**, 346 (1989); J. M. Shoffner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7952 (1989).
22. T. Ozawa *et al.*, *Biochem. Biophys. Res. Commun.* **170**, 830 (1990); K. Hattori *et al.*, *Am. Heart J.* **121**, 1735 (1991).
23. S. Shanske *et al.*, *Neurology* **40**, 24 (1990); V. Cormier *et al.*, *J. Pediatr.* **117**, 599 (1990).
24. N. G. Larsson, E. Holme, B. Kristiansson, A. Oldfors, M. Tulinius, *Pediatr. Res.* **28**, 131 (1990).
25. S. Mita, B. Schmidt, E. A. Schon, S. DiMauro, E. Bonilla, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9509 (1989); E. A. Shoubridge, G. Karpati, K. E. Hastings, *Cell* **62**, 43 (1990).
26. J. Poulton, M. E. Deadman, R. M. Gardiner, *Lancet* i, 236 (1989).
27. M. Zeviani *et al.*, *Nature* **339**, 309 (1989); V. Cormier *et al.*, *Am. J. Hum. Genet.* **48**, 643 (1991).
28. C. T. Moraes *et al.*, *Am. J. Hum. Genet.* **48**, 492 (1991).
29. G. A. Cortopassi and N. Arnheim, *Nucleic Acids Res.* **18**, 6927 (1990).
30. M. Corral-Debrinski *et al.*, *J. Am. Med. Assoc.* **266**, 1812 (1991).
31. G. Dorner, A. Mohnike, E. Steindel, *Endokrinologie* **66**, 225 (1975); ———, A. Plagemann, H. Reinagel, *Exp. Clin. Endocrinol.* **89**, 84 (1987); N. Freinkel *et al.*, *Horm. Metab. Res.* **18**, 427 (1986).
32. S. W. Ballinger *et al.*, *Nature Genetics* **1**, 11 (1992).
33. T. P. Singer *et al.*, *J. Neurochem.* **49**, 1 (1987).
34. A. H. V. Schapira *et al.*, *ibid.* **54**, 823 (1990).
35. W. D. Parker, Jr., S. J. Boyson, J. K. Parks, *Ann. Neurol.* **26**, 719 (1989).
36. J. M. Shoffner, R. L. Watts, J. L. Juncos, A. Torroni, D. C. Wallace, *ibid.* **30**, 332 (1991); D. C. Wallace, J. M. Shoffner, R. L. Watts, J. L. Juncos, A. Torroni, *ibid.*, in press.
37. L. A. Bindoff *et al.*, *J. Neurol. Sci.* **104**, 203 (1991).
38. S.-I. Ikebe *et al.*, *Biochem. Biophys. Res. Commun.* **170**, 1044 (1990).
39. W. G. Johnson, *Neurology* **41**, 82 (1991).
40. W. D. Parker, Jr., C. M. Filley, J. K. Parks, *ibid.* **40**, 1302 (1990).
41. J. L. Haines, *Am. J. Hum. Genet.* **48**, 1021 (1991); A. Goate *et al.*, *Nature* **349**, 704 (1991).
42. W. A. Brennan, Jr., E. D. Bird, J. R. Aprille, *J. Neurochem.* **44**, 1948 (1985).
43. W. D. Parker, Jr., S. J. Boyson, A. S. Luder, J. K. Parks, *Neurology* **40**, 1231 (1990).
44. R. H. Myers *et al.*, *Lancet* i, 208 (1983); R. H. Myers *et al.*, *Am. J. Hum. Genet.* **37**, 511 (1985).
45. E. J. Novotny, *Neurology* **36**, 1053 (1986).
46. I thank M. T. Lott, J. M. Shoffner, and A. Torroni for their many intellectual contributions to this work. These studies were supported by NIH grants NS21328, HL45572, GM46915, and AG10139 and a Muscular Dystrophy Foundation Clinical Research grant.

RESEARCH ARTICLES

Solution Structure of a Calmodulin-Target Peptide Complex by Multidimensional NMR

Mitsuhiko Ikura,* G. Marius Clore,† Angela M. Gronenborn,† Guang Zhu, Claude B. Klee, Ad Bax†

The three-dimensional solution structure of the complex between calcium-bound calmodulin (Ca^{2+}-CaM) and a 26-residue synthetic peptide comprising the CaM binding domain (residues 577 to 602) of skeletal muscle myosin light chain kinase, has been determined using multidimensional heteronuclear filtered and separated nuclear magnetic resonance spectroscopy. The two domains of CaM (residues 6 to 73 and 83 to 146) remain essentially unchanged upon complexation. The long central helix (residues 65 to 93), however, which connects the two domains in the crystal structure of Ca^{2+}-CaM, is disrupted into two helices connected by a long flexible loop (residues 74 to 82), thereby enabling the two domains to clamp residues 3 to 21 of the bound peptide, which adopt a helical conformation. The overall structure of the complex is globular, approximating an ellipsoid of dimensions 47 by 32 by 30 angstroms. The helical peptide is located in a hydrophobic channel that passes through the center of the ellipsoid at an angle of ~45° with its long axis. The complex is mainly stabilized by hydrophobic interactions which, from the CaM side, involve an unusually large number of methionines. Key residues of the peptide are Trp^4 and Phe^{17}, which serve to anchor the amino- and carboxyl-terminal halves of the peptide to the carboxyl- and amino-terminal domains of CaM, respectively. Sequence comparisons indicate that a number of peptides that bind CaM with high affinity share this common feature containing either aromatic residues or long-chain hydrophobic ones separated by a stretch of 12 residues, suggesting that they interact with CaM in a similar manner.

Calmodulin (CaM) is a ubiquitous Ca^{2+} binding protein of 148 residues that is involved in a wide range of cellular Ca^{2+}-dependent signaling pathways, thereby regulating the activity of a large number of proteins including protein kinases, a protein phosphatase, nitric oxide synthase, inositol triphosphate kinase, nicotinamide adenine dinucleotide kinase, cyclic nucleotide phosphodiesterase, Ca^{2+} pumps, and proteins involved in motility (*1*). Binding domains for CaM have been isolated from a number of CaM-dependent enzymes and have been shown to comprise peptide sequences with a high propensity for helix formation (*2*). In addition, both circular dichroism (*3*) and nuclear magnetic resonance (NMR) (*4*, *5*) studies have shown that many naturally occurring CaM binding peptides, such as mellitin and mastoporan, as well as synthetic peptides corresponding to CaM binding domains adopt a helical