DISEASES ONLY YOUR MOTHER CAN GIVE YOU

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INTRODUCTION

Genetic diseases are transmitted in a variety of patterns, commonly including autosomal dominant, autosomal recessive and X-linked transmission. In all of these instances, paternal contributions to the genetic disorder occur. In contrast, another mode of inheritance - strict maternal transmission - has been suggested for a number of years, but convincing evidence for such a pattern has only recently been demonstrated, in the form of mitochondrial-transmitted disorders. As the genetic basis for these disorders is cytoplasmic, rather than nuclear, maternally-transmitted mitochondrial diseases do not obey Mendelian patterns, and specifically, paternal transmission does not occur. A typical pedigree is shown in Figure 1.

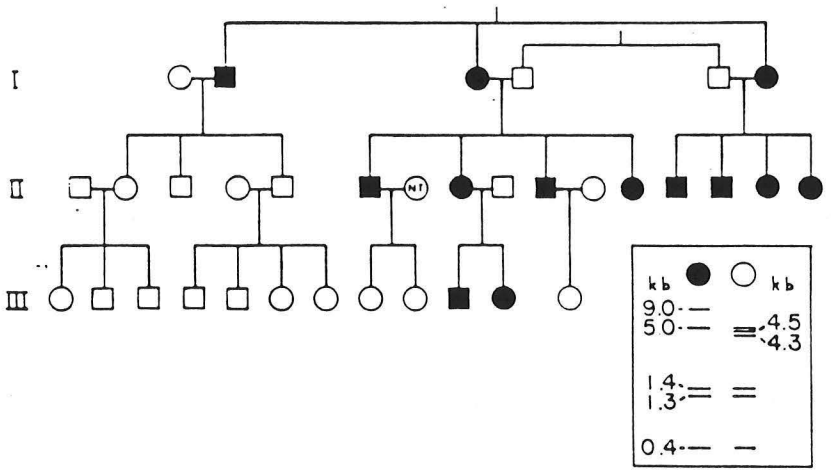

FIGURE 1: MATERNAL INHERITANCE OF HUMAN mtDNA
Mitochondrial DNA from kindred members was digested with the restriction enzyme HaeII, yielding two distinct patterns of DNA fragment lengths (lower right insert). The pattern designated by a filled symbol is only maternally-transmitted (Giles).

The concept of mitochondrial diseases, i.e. disorders which owe to intrinsic mitochondrial dysfunction, was first advanced by Ernster and Luft. In 1962, these investigators identified a woman whose clinical condition was characterized by easily provoked fatigue and euthyroid hypermetabolism (Luft). The mitochondrion was implicated in the pathogenesis of this syndrome, but these observations were not fully explicable, as the proband case predated the exact definition of the mechanism of mitochondrial energy production. Although, the disease is extremely rare, with only two reported cases (Luft; DiMauro, 1976), Luft's Syndrome called attention to the mitochondrion as a locale of disease.

During the 1960's, several advances converged to further our understanding of the molecular basis of mitochondrial diseases. Identification of the mitochondrion as being a source of ATP production, and elucidation of the biochemical machinery was critical. In parallel, mounting numbers of isolated case reports of degenerative muscular and neurological disorders were analyzed in the context of the biochemistry of the mitochondria, leading to the notion of mitochondrial myopathies and encephalomyopathies, characterized by the familiar feature of ragged-red fibers. Independently, it was discovered that mitochondria have a genome which is physically (and functionally) distinct from that of the nuclear genome - an observation which lent potential primacy to the role of mitochondria in disease pathogenesis. That is, rather than being a target of a (genetic) pathophysiologic process, the mitochondrion itself could be the essential perpetrator of pleomorphic, systemic diseases.

Not to discount arduous biochemical studies, diseases of the mitochondria have, in the past four years, been given final definition, with elucidation of the genetic basis of several disorders. Whereas, to date, only a handful of syndromes have been so defined - and often, these have been extremely rare disorders known only to subspecialists - it is apparent that a totally new disease mechanism has emerged. Ongoing studies implicate the mitochondria not only in predictable neuromuscular disorders, but also (with varying degrees of plausibility) in Parkinson's Disease, primary cardiomyopathies, and certain tumors. Indeed, ongoing research suggests that cellular senescence, and systemic aging itself may be related to 'spontaneous' mitochondrial degeneration.

This review focuses upon principles of the maternally-inherited disorders of mitochondrial function. Selected examples of genetically-defined diseases are discussed in the context of their origin and pathogenesis, and special emphasis is placed on the principles of these disorders to explain the basis of mitochondrial-transmitted diseases.

THE MITOCHONDRIAL GENOME

Extra-nuclear DNA within the mitochondrial compartment has been found to be a consistent feature of eukaryotic cells (DeGiorgi; Wallace, 1987). Important features of the mitochondrial genome are listed in Table 1.

TABLE 1: UNIQUE FEATURES OF MITOCHONDRIAL DNA

1. Mitochondrial DNA is maternally inherited
2. The mitochondrial genome differs from that of the nucleus in being circular, and utilizing unique codons
3. Mitochondrial DNA is the only eukaryotic genome which is fully mapped
4. Mitochondrial DNA has a mutation rate ten times higher than nuclear DNA
5. Individual mitochondria contain multiple copies of their genome
6. All structural genes of the mitochondrial DNA encode components of oxidative phosphorylation

Unlike nuclear DNA, each mitochondrion contains multiple copies of a full genetic complement, ranging from 2-10 copies per organelle (Michaels). Given the abundance of mitochondria (Johnson), this means that a given cell carries thousands of copies of mitochondrial DNA (Shmookler). As a perspective, however, a single copy of human mitochondrial DNA is composed of only 16,569 bp (Anderson), and thus only about 1% of the total cellular DNA is borne within this extra nuclear compartment.

From a structural standpoint, the 16.5 kb of the mitochondrial DNA (mtDNA) exists as a covalently-linked, circularized, supercoiled DNA. The mtDNA is double stranded, and classically the two strands are designated heavy (H) and light (L), owing to the relative abundance of guanine (MW = 151) in the former, and cytosine (MW = 111) in the latter (Berk). Shown in Table 2 is a list of gene products encoded by the mtDNA, and in Figure 2, a map of the mitochondrial genome. It is notable that <u>every</u> gene product has been accounted for, and thus, unlike nuclear DNA, the mitochondrial genome is completely mapped. Unlike nuclear DNA, this system is virtually devoid of introns or even minimal intervening sequences and almost every nucleotide participates in a coding function. Thus mutation of almost any nucleotide bears the potential of affecting oxidative phosphorylation, either directly (through a mutation in one of the structural proteins) or indirectly (through misfunction of the protein synthesizing apparatus, i.e. ribosomes and tRNAs).

TABLE 2: GENES OF MITOCHONDRIAL DNA

GENES	FUNCTION
16srRNA gene } 12srRNA gene	Constituents of mitochondrial ribosomes
22 transfer RNA genes	Decoding system for mitochondrial messages
13 polypeptides genes	Seven subunits of the NADH dehydrogenase (complex I) One subunit of the cytochrome bc_1-complex (complex III) Three subunits of cytochrome *c* oxidase (complex IV) Two subunits of the F_1F_0 ATP-synthase complex (complex V)

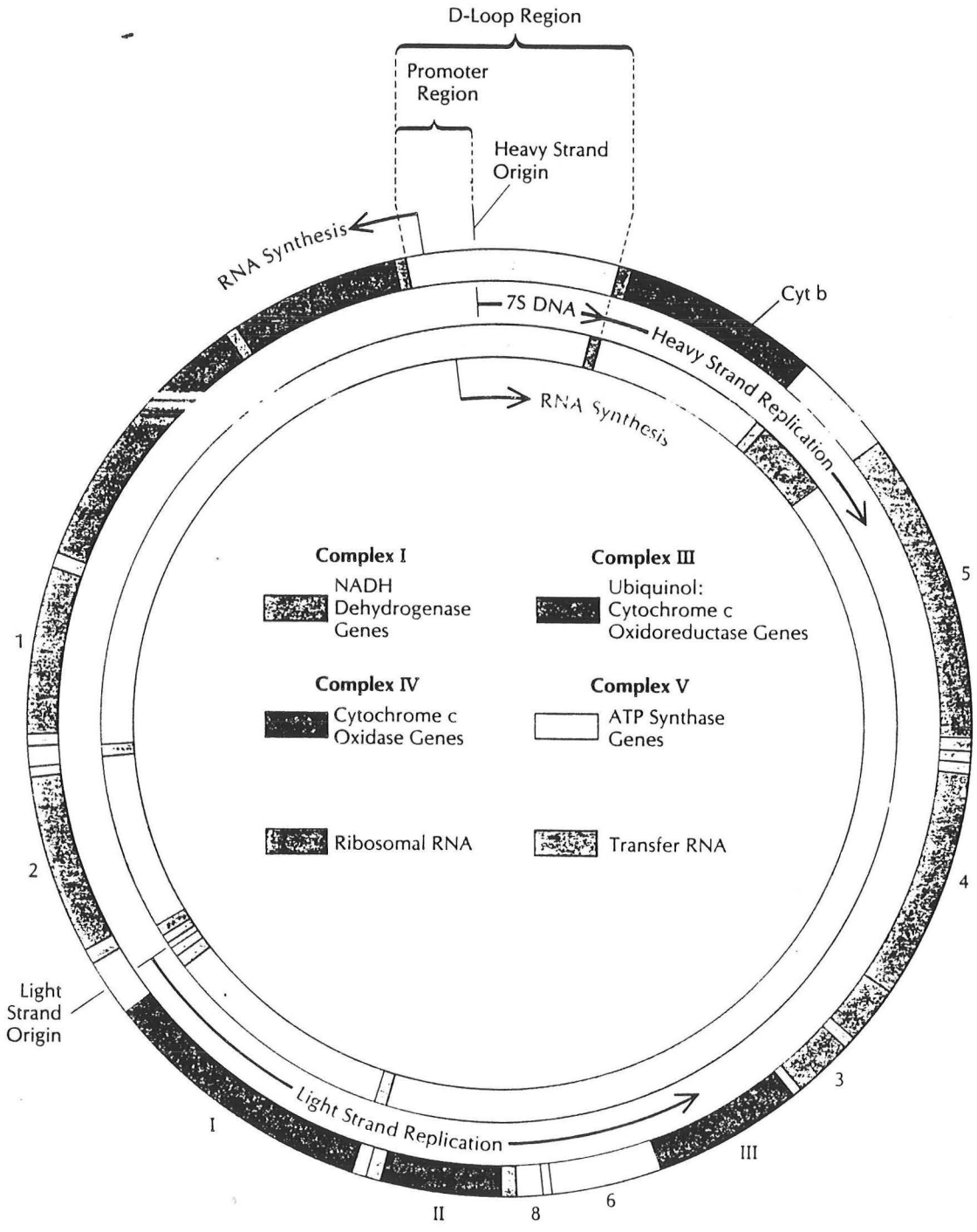

FIGURE 2: MAP OF HUMAN MITOCHONDRIAL DNA
The outer circle represents the heavy (H) strand, the inner circle, the light (L) strand.

Replication of the mtDNA proceeds by means of an unusual process. In mammalian cells, a unique control region, the displacement loop (D-loop) is the locale of initiation of H strand replication, and contains the promotor sites for both H and L strands (Brown, 1986). Structurally, the D loop contains a stable, triplex DNA. Replication of the H stand beings at the D loop, and after approximately two thirds of the H strand has been synthesized, L strand synthesis begins at a site designated "origin of L strand replication", in a direction opposite to H strand synthesis. Separation of the strands within the supercoiled structure is facilitated by topoisomerases (Castora; Fairfield). Once synthesis has been completed, a DNA ligase links the 3' and 5' end of each replicated strand (Lestienne).

Transcription is also an asymmetrical process in that the L strand transcription rate is 2-3 fold higher than that of the H strand. Transcription results in the production of polycistronic mRNAs, which are subsequently processed to result in mature mRNA, tRNA, and rRNA, with the (minimal) noncoding RNA being degraded (Clayton,1982; Clayton,1984). Examination of Figure 2 reveals that each of the 'structural' genes is interspaced between tRNA genes, and it has been suggested that the secondary structure imposed by the tRNAs may serve as a signal for the cleavage of the polycistronic message into its ultimate component parts.

Perhaps the mitochondrial genome is best appreciated by a comparison with the more familiar nuclear DNA. As noted previously, the mtDNA is circular, the nuclear, linear. The mtDNA violates the 'universal' genetic code, in that the nuclear UGA stop codon, encodes for tryptophan in mtDNA, and AUA is used for methionine instead of isoleucine. Further, AGA and AGG encode stop codons instead of arginine, as is the case with nuclear DNA. Indeed, the entire human mtDNA is translated by only 22 tRNAs, instead of the 32 tRNAs required for nuclear DNA initiated processes (Anderson). It has been noted that this diversification bears certain implications, most notably the maintenance of compartmental integrity and efficiency. By evolving a unique system for start and stop codons, translation of the mitochondrial RNA within the cytosol, and vice versa, is precluded. Moreover, the extreme efficiency of the mtDNA (which lacks significant untranslated regions) imparts a selective advantage to the replication of an organelle, which is dependent upon foreign (nuclear) aid for its existence (Wallace, 1982).

The mtDNA is semi-autonomous: whereas the mitochondrial genome directs the synthesis of tRNAs, rRNAs and structural protein products, completion of its mission, (mitochondrial replication) requires a tight, symbiotic relationship with nuclear DNA. Thus, nuclear endonucleases are required for processing of the polycistronic message, and additional, nuclear-encoded subunits for completing the synthesis of the machinery of oxidation phosphorylation. As listed in Table 2, thirteen subunits of complexes I, III, IV and V are mitochondrial derived. Actually, Complexes I-V are comprised of at least 67 polypeptides; the remaining 54 components are nuclear encoded, synthesized within the cytoplasm, and imported into the mitochondria. Recently, considerable insight has emerged into the mechanism by which nuclear encoded proteins are selectively targeted and imported into

the mitochondrion (Schatz). Although this discussion is primarily focused upon disorders of the mitochondrial genome, per se, it is apparent that nuclear DNA mutations can affect the mitochondrion, either through structural mutations within proteins comprising the rest of the respiratory chain, or indirectly, by mutations disrupting the import machinery itself.

THE MITOCHONDRION DURING CELL DIVISION

As noted previously, disorders owing to mutations within the mitochondrial genome are inherited exclusively by maternal transmission. The explanation for this phenomenon owes, in part, to differences in the process of gametogenesis of the sperm and oocyte. Essentially, spermatogenesis entails a process of progressive cytoplasmic contraction, and that of oogenesis, cytoplasmic expansion. As a consequence, it has been estimated that the mature human sperm contains between 10-20 mitochondria, which are confined to the midpiece. In contrast, the human oocyte contains hundreds of mitochondria, and about 2.6×10^5 copies of mtDNA (Michaels).

Thus a simple, dilutional effect might be expected to account for the maternal dominance of transmission of mitochondrial genomes. In fact, the process is probably more complicated, and it has been suggested that there is a selective destruction of the few paternally-derived mitochondria within the cytoplasm of the fertilized egg. Several possible mechanisms have been suggested to explain this event: sperm-derived mitochondria might be targeted to regions of the egg destined to become extra embryonic tissue; alternatively, the paternally-derived mitochondria might be debilitated through either replication block (Vaughn) or enzymatic destruction (Sager).

Irrespective of the mechanism, several investigators have presented evidence that there is absolutely no transmission of paternal mitochondrial DNA, in that paternal mitochondrial DNA could not be detected in the mature organism (Case; Francisco; Giles; Hutchinson; Kroon). Most recently, this view has been challenged, with the demonstration of paternal inheritance of mitochondrial DNA in mice. Through use of the polymerase chain reaction (PCR), paternally-inherited mitochondrial DNA was detected at a frequency of 10^{-4}, relative to the maternal contribution (Gyllensten). This observation has sparked considerable speculation in terms of "matriarchal liberation" (Avise). Potentially, such transmission could allow for paternal transmission of mitochondrial diseases, as well as subtle events such as physical recombination between mitochondrial DNAs from separate parents. However, it is most important to note that evidence for paternal transmission of mitochondrial DNA has only been demonstrated in the artificial setting of inter-strain crosses (conducted with mice, Drosophila, and mussels). At present, paternal leakage in humans awaits demonstration.

Cellular divisions require replication of mitochondria so that daughter cells carry approximately the same number as the mother cell. As alluded to previously, mitochondrial DNA replication is supported by elements encoded by both the mitochondrial (eg, ribosomes, tRNAs) and nuclear (reverse transcriptase) genomes. The

actual replication of mitochondria during cell division is poorly described in humans. It appears, however, that the doubling of mitochondrial number is somehow synchronized with nuclear DNA replication, and that the ultimate number of mitochondria in the daughter cells is influenced by the surface to volume ratio of the cells, inherent, tissue-specific requirements for oxidative phosphorylation, and likely hormonal regulation.

A most essential feature of the mitochondrial DNA composition of a cell, namely, heteroplasmy. This term describes the co-existence within a single cell of more than one population of mitochondria with differences in their respective genomes, and is illustrated in Figure 3.

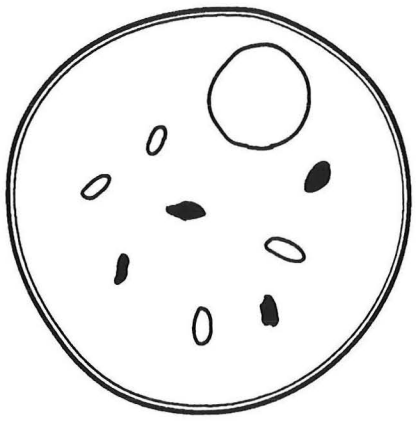

FIGURE 3: HETEROPLASMY
A single cell can carry mitochondria with differences in their respective genomes, as depicted by the open and filled mitochondria.

During oogenesis, it is apparent that mitochondria individually, and perhaps as an entire population, undergo several transformations. First, available evidence suggests that the copy number of the mitochondrial genome is reduced from 2-10 copies per mitochondrion to one. Second, there is an expansion of the number of mtDNA molecules, such that oocytes contain roughly 100 times the number of mtDNA molecules found in somatic cells (Michaels). Third, during blastogenesis and embryogenesis, these mitochondria are sorted. This occurs, in part, because the mtDNA of the oocyte does not replicate until the blastocyst stage (Pollak), and only 3 of the 64 blastocyst cells ultimately give rise to the embryo (Markert). Thus, only a small proportion of the mtDNA of the oocyte is passed on to the next generation. As a result, this process entails a segregation of mitochondria during oogenesis, such that the mature oocytes can have a highly skewed proportion of mitochondria with one particular genotype, as shown in Figure 4.

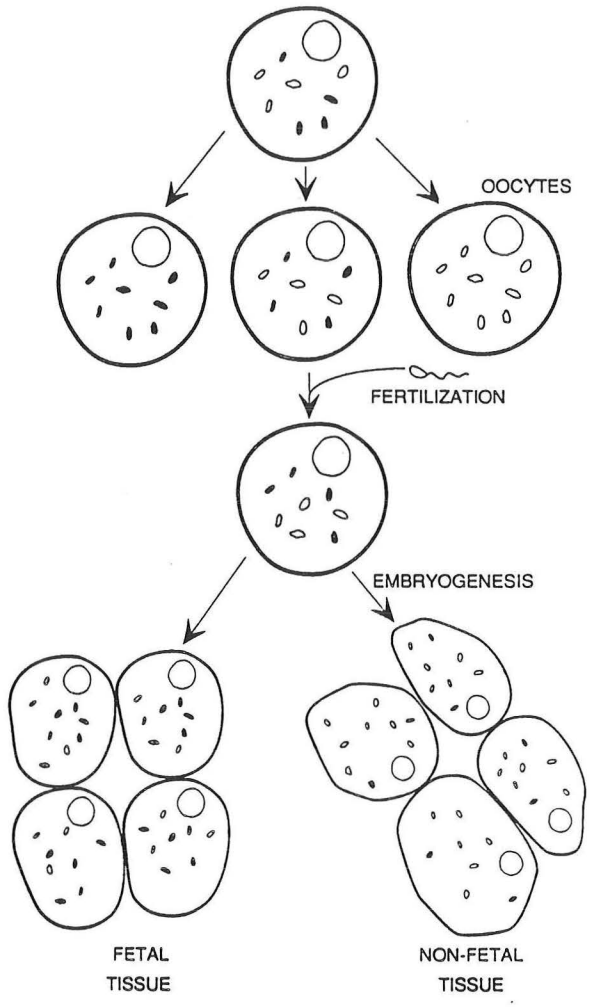

FIGURE 4: MITOCHONDRIAL SEGREGATION DURING OOGENESIS AND EMBRYOGENESIS
Assortment of mitochondria during pre- or post-fertilization cell division can result in daughter cells with a degree of heteroplasmy different from the parental cell.

As a consequence, the segregation pattern of these mitochondria during cell division can result in daughter cells with marked differences in the percentages of each type of mitochondria, ie, a change in the percentage of heteroplasmy (Howell; White). Indeed, it has been shown that the entire mitochondrial genome can switch in a single generation by this mechanism (Hauswirth). This difference resulting from mitochondrial segregation pattern has several important consequences, and can account for the variability in phenotype amongst related offspring that has been observed with maternally inherited disorders, as well as for tissue differences in the distribution of the genetic defect (and hence, the ability to carry out oxidative phosphorylation). At present, the mechanism by which this segregation occurs is not understood and arguments for both a random process (Wallace, 1987), as well as one which is under selective pressure, have been advanced (Harding, 1991; Upholt).

The incidence of heteroplasmy itself is not well defined. Mitochondrial DNA has a

mutation rate which is ten fold higher than that of nuclear DNA (Brown, 1979; Wilson, 1977), therefore, it would be expected that heteroplasmy of the mitochondrial genome would be widespread. Few attempts have been made to define the degree of polymorphism in the mitochondrial genome within individuals. Limited RFLP analysis indicates that within an individual, mtDNA is homogeneous, and that heteroplasmy is seldom encountered in normal individuals. However, such an analysis actually studies only about 5% of the total DNA, because phenotypically-silent polymorphisms are much less likely to be encountered in the mitochondrial DNA, owing to the absence of introns (Vilkki, 1990).

OXIDATIVE PHOSPHORYLATION

As all structural genes in the mitochondrial genome encode for components of the respiratory chain, it is necessary to review essential elements (Racker; Tzagoloff) of this process.

Since the discovery that the mitochondrion is the site of respiration and ATP production, considerable knowledge of the coupling of these two events has occurred. From a narrow point of view, the most essential reaction conducted by mitochondria is ATP synthesis. This process requires specialized transport proteins, as well as a membrane which is relatively impermeable to ions and electrical gradients. As shown in Figure 5, the essential components of this system are five hetero-oligomeric protein complexes (Complexes I-V), which are embedded in the inner mitochondrial membrane. The first four of these complexes constitute the respiratory (or electron transport) chain; Complex V, the terminal step in oxidative phosphorylation, is the actual site of ATP synthesis.

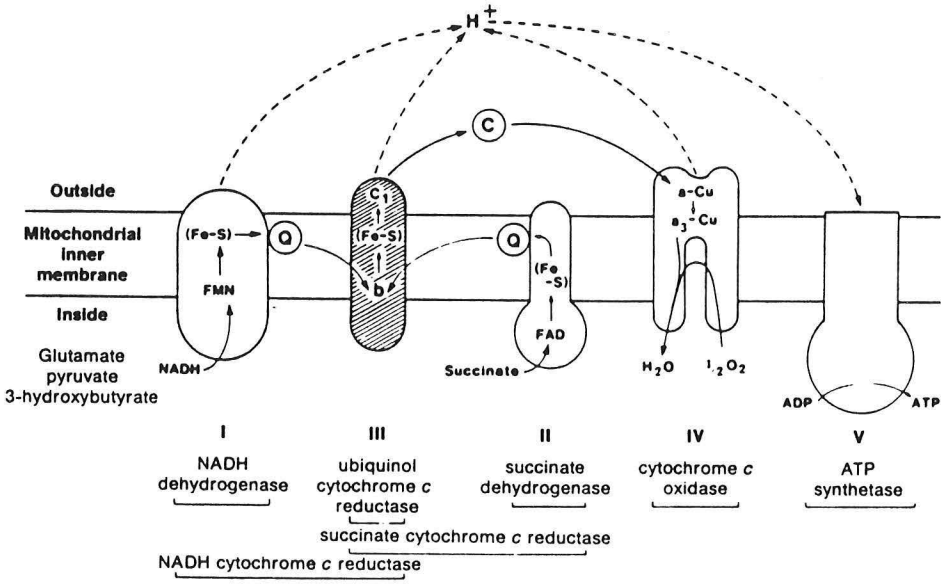

FIGURE 5: MECHANISM OF OXIDATIVE PHOSPHORYLATION

High energy compounds (eg, NADH) donate a portion of their energy in the form of electrons to Complex I and these electrons are sequentially passed along the chain to Complex IV, where the electrons combine with oxygen to yield water (and hence, oxygen is consumed). Importantly, the energy of the electrons within the complexes is in part transduced, and is used to drive protons, or hydrogen ions, from the interior of the mitochondrion to the exterior of the inner membrane. As a result, a proton and electrical gradient is established across the inner membrane. Complex V, the ATP synthetase, uses the energy of this chemical and electrical gradient to fuel ATP synthesis. Specifically, protons enter Complex V through a special pore (F_0) and the energy accompanying the downhill movement of protons is transformed into chemical energy by a second portion of Complex (F_1). Essentially, the energy in the H^+ and electrical gradients supplies the energy needed to unite inorganic phosphate (Pi) with ADP, to yield ATP. Two other transporters (which are nuclear encoded) are important to complete the cycle: an ATP/ADP exchanger which serves to supply ADP (substrate) to Complex V, and to extrude ATP (product) for cytosolic utilization; and a phosphate (Pi) transporter, which facilitates the entry of Pi into the mitochondria so that it can be united with ADP to form ATP.

Many disorders of the mitochondrial function have now been explained in light of this reaction (Capaldi; DiMauro, 1985 and 1987). The specific details of the biochemistry of these syndromes and isolated case reports are much less important than the realization that interference with any step of this chain reaction results in decreased ATP production, which is the final effector in the pathogenesis of these disorders.

The mitochondrion is not the sole site of cellular ATP production; glycolysis, a cytosolic process, also results in the production of ATP, albeit with less efficiency than the former process. In fact, most cells derive energy from both glycolysis and oxidative phosphorylation, but there exist rather radical differences in the extent to which each process dominates. Neural tissue (CNS in particular), skeletal muscle, cardiac muscle, the kidney and liver are highly dependent upon mitochondrial function. This explains, in part, the peculiar organ distribution present in these syndromes; that is, a tissue-specific threshold of mitochondrial dysfunction must be exceeded before pathology occurs (Wallace, 1987). The CNS, which is highly O_2 dependent, with little glycolytic reserve, can tolerate very little in the way of mitochondrial dysfunction, whereas erythrocytes (which lack like mitochondria and have no requirement for oxidative phosphorylation) are unaffected.

The interplay and relative rates of oxidative phosphorylation and glycolysis is of further consequence. In tissues such as muscle, which are capable of substantial rates of both processes, impaired mitochondrial ATP production results in an increased reliance upon glycolysis. As a side product of the glycolytic pathway, lactate is produced, accounting for the elevated levels of lactate found in many of these disorders (DiMauro, 1985).

MITOCHONDRIAL DISEASES

Mitochondrial disorders are characterized by extreme heterogeneity with respect to the mode of inheritance, the extent of disease progression, the tissues affected, and, as discussed below, the molecular mechanism accounting for the disorders. Until recently, classification systems of these disorders were also quite heterogenous, and loose taxonomies have been presented based upon apparent mode of inheritance, pathologic findings, and clinical manifestations. A typical classification is shown in Table 3. Whereas many of these diseases have undergone a (painstaking) partial biochemical definition, this has not necessarily served as an intelligible matrix.

TABLE 3: BIOCHEMICAL CLASSIFICATION OF MITOCHONDRIAL DISEASES

1. DEFECTS OF TRANSPORT
   - (a) CPT deficiency
   - (b) Carnitine deficiency
   - (c) Defect of FAD uptake (?)

2. DEFECTS OF SUBSTRATE UTILIZATION
   - (a) Pyruvate carboxylase deficiency
   - (b) Pyruvate dehydrogenase complex deficiency
   - (c) Defects of β-oxidation

3. DEFECTS OF THE KREBS CYCLE
   - (a) Fumarase deficiency
   - (b) α-Ketoglutarate dehydrogenase (dihydrolipoyl dehydrogenase) deficiency

4. DEFECTS OF OXIDATION-PHOSPHORYLATION COUPLING
   - (a) Luft's syndrome (loose coupling of muscle mitochondria)

5. DEFECTS OF THE RESPIRATORY CHAIN
   - (a) Complex I deficiency
   - (b) Complex II deficiency
   - (c) Complex III deficiency
   - (d) Complex IV deficiency
   - (e) Complex V deficiency
   - (f) Combined defects of respiratory chain components

Most recently, several mitochondrial disorders have undergone molecular definition (Grossman; Harding, 1989 and 1991; Wallace, 1991) and these are shown in Table 4. Although this listing is not comprehensive, as compared with Table 3, it has the dual advantages of (1) being a precise categorization and (2) allowing for a discussion of basic

principles of these disorders based upon their respective defects (Harding, 1991). Rather than reviewing an extremely diverse group of rare disorders in an encyclopedic fashion, the following discussion is based upon the cases of Table 4, with emphasis upon certain heuristic features of each.

TABLE 4: GENETIC DEFECTS IN MITOCHONDRIAL DISEASES

	DISEASE
mtDNA Defects	
Point mutations of mtDNA	LHON RP, Ataxia, dementia, neurogenic weakness Mitochondrial myopathies (MERRF, MELAS)
Large deletions of mtDNA	Mitochondrial myopathies (KSS) Pearson's syndrome
Nuclear Defects	Probably mitochondrial myopathies Mitochondrial myopathies with multiple mtDNA deletions

DISORDERS ARISING FROM POINT MUTATIONS WITHIN THE MITOCHONDRIAL GENOME

LEBER'S HEREDITARY OPTIC NEUROPATHOLOGY

This disorder is characterized clinically by acute (or subacute) bilateral loss of vision due to severe bilateral optic atrophy. Development of loss of vision, which has an onset ranging from adolescent to adult (median age of onset is 20-24 years), is preceded by the development of tortuosity of the retinal vessels, due to retinal swelling (Nikoskelainen, 1984 and 1987; Went). In addition, these patients frequently develop cardiac arrhythmias, related to a high incidence of Wolff-Parkinson-White (WPW) Syndrome and Lown-Ganong-Levine (LGL) Syndrome (Nikoskelainen, 1987; Rose). Several kindred studies indicated that the mode of inheritance of this disorder was strictly maternal, suggesting the

possibility that this disease was due to a mutation in the mitochondrial genome (Erickson; Nikoskelainen, 1987; Wallace, 1987). A typical pedigree is shown in Figure 6.

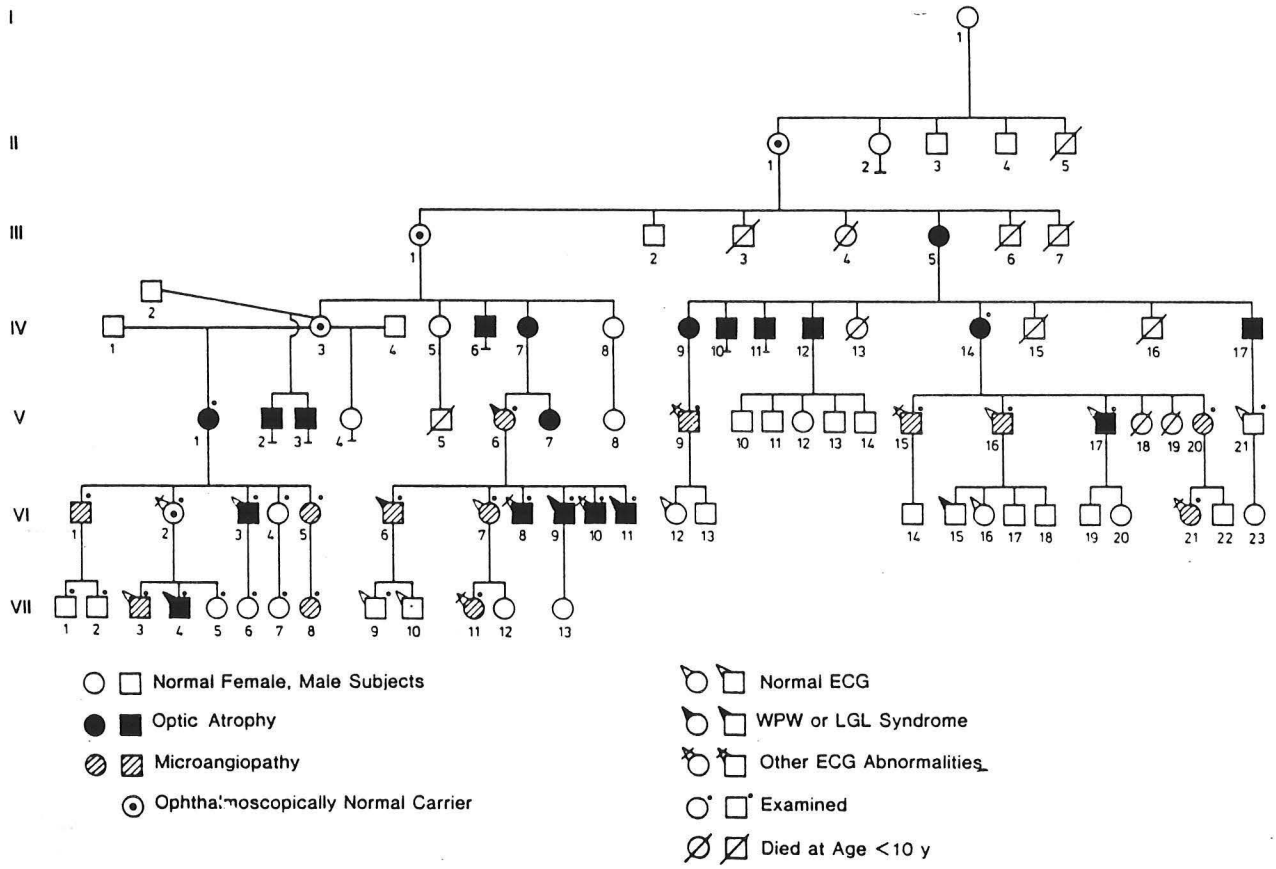

FIGURE 6: PEDIGREE OF A FAMILY WITH LHON

At first glance, this is a surprising conclusion, given the facts that mitochondria are present in all tissues, yet the pathology of Leber's Syndrome is confined largely to an optic neuropathy. In support to this hypothesis, however, several studies suggested that this disease might owe to a defect in oxidative phosphorylation. First, studies indicate that smoking-related cyanide exposure can lead to an acceleration in the blindness of LHON, as well as neuro-ophthalmic disorders (tobacco amblyopia) in general (Vogel; Brierley). Cyanide is a well described, potent inhibitor of Complex I of the respiratory chain and it is reasonable to speculate that an exogenous inhibitor, such as cyanide, would be likely to aggravate selectively a preexisting genetic defect at this site. Second, toxicology studies using known inhibitors of oxidative phosphorylation indicate that the central nervous system is the most susceptible to poisoning. Most particularly, chronic treatment of monkeys with azide (an inhibitor of Complex V, the mitochondrial ATP synthetase) results in a relatively selective demyelination of the optic nerve (Hurst). Thus, the peculiar pathology of the syndrome can be explained, in part, by an interplay of a

specific genetic defect in oxidative phosphorylation with a tissue (optic nerve) which has a high dependence upon oxygen-supported energy production.

In 1988 and 1989, studies from the laboratory of Wallace defined the genetic basis of LHON as being due to a point mutation at base 11778 of the mitochondrial genome (Wallace, 1988b; Singh). This mutation results in the substitution of histidine for an arginine at the 340th amino acid of subunit 4 of NADH dehydrogenase - a mitochondrial DNA encoded subunit of Complex 1 in the respiratory chain. In support of the conclusion that this amino acid substitution was causally related to the clinical symptoms, a biochemical analysis performed on mitochondria harvested from platelets of patients with LHON clearly demonstrated a reduction in total NADH-Q, oxidoreductase activity- a specific assay of Complex I activity. Importantly, the activities of the other four complexes were normal, indicating that the defect of Complex I did not owe to nonspecific mitochondrial dysfunction (Parker).

The particular mutation identified by Wallace (Δ Arg$^{340}\rightarrow$His) has the fortuitous effect of eliminating a recognition site for the restriction endonuclease Sfa N1 (Wallace, 1988b). As shown in Figure 7, this restriction fragment length polymorphism (RFLP) was found in maternally-related individuals within the kindred, whether or not they were affected. This latter point is interesting, in that studies of families with LHON have revealed that whereas 85% of male carriers are symptomatic, only about 18% of female carriers are affected. The basis for the sex-related differences in the expression of the disease has not been demonstrated. Speculations as to the reason include a "second hit" requirement for disease manifestation which is possibly carried on the X-chromosome (Harding, 1991). Likely important are the nuclear-mitochondrial symbiotic interactions noted previously.

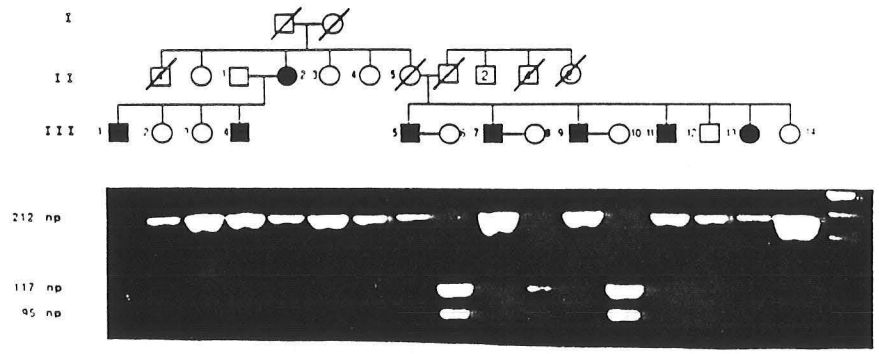

FIGURE 7: LHON PEDIGREE WITH RFLP CHARACTERIZATION OF KINDRED MEMBERS
Digests with the restriction endonuclease SfaN1 reveals that all third generation members of the kindred carry the mutation resulting in the diagnostic DNA fragment of 212 bp, despite the fact that mainly male members are affected.

Since the identification by Wallace of the $\Delta Arg^{340} \rightarrow His$ mutation, two other groups have identified kindreds with LHON in which this mutation cannot be detected. In the United Kingdom (Holt, 1989) and Finland (Vilkki, 1989), only about 50% of families with LHON have the mutation identified by Wallace. Although the causal mutation in these families has not been detected, this heterogeneity is of clinical importance, in that patients with alternative mutations have experienced improvement in their vision, whereas those with the $\Delta Arg^{340} \rightarrow His$ mutation have not.

RETINITIS PIGMENTOSA, DEMENTIA, SEIZURES, NEUROGENIC WEAKNESS

The second example of a maternally-transmitted mitochondrial disorder caused by a point mutation in the mtDNA was identified in 1990 (Holt, 1990). This disorder, which lacks an eponym, consists of a variable constellation of signs, including developmental delay, retinitis pigmentosa, dementia, seizures, ataxia, proximal muscle weakness and sensory neuropathy. These patients do not have lactic acidosis, and pathologic examination of muscle fibers revealed only subtle changes in the mitochondria and no ragged-red fibers.

Genetic definition was obtained by first identification of a unique RFLP pattern in the mtDNA of peripheral leukocytes. Subsequently, a PCR based strategy revealed that the mutation occurred at position 8993 of the mitochondrial genome, resulting in a change from a highly conserved leucine to arginine in subunit 6 of the mitochondrial ATP synthase. This particular subunit constitutes a portion of the hydrophilic proton channel of mitochondrial F_o. All maternally-related individuals within the kindred exhibited heteroplasmy in the mtDNA of muscle and leukocytes.

MELAS: MITOCHONDRIAL ENCEPHALOMYOPATHY, LACTIC ACIDOSIS AND STROKE-LIKE EPISODE

Whereas LHON Syndrome was characterized by a very circumscribed pattern of tissue involvement, without muscle myopathy, one perhaps associates disorders of mitochondrial function with more global pathology, and in particular, with a classic pattern of ragged red fibers on muscle biopsy. Ragged red fibers refers to a characteristic pattern observed when skeletal muscle specimens from patients with myopathies are dyed with modified Gomori trichrome stain. The term mitochondrial myopathies actually encompasses a wide array of disorders, but many individuals with such syndromes fall into one of the three following categories: MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, MERRF (myoclonus epilepsy with ragged red fibers), and KSS (Kearns-Sayre Syndrome). All three of these disorders have now been defined at a molecular level, with the former two owing to point mutations in the mitochondrial genome and the latter to large scale deletions of the same. Their clinical features are contrasted in Table 5 (DiMauro, 1985).

TABLE 5: FEATURES OF THREE MAJOR ENCEPHALOMYOPATHIES

Features	KSS	MERRF	MELAS
Ophthalmoplegia	+	-	-
Retinal degeneration	+	-	-
Heart block	+	-	-
CSF protein >100 mg/dl	+	-	-
Myoclonus	-	+	-
Ataxia	+	+	-
Weakness	+	+	+
Seizures	-	+	+
Dementia	+	+	+
Short stature	+	+	+
Episodic vomiting	-	-	+
Cortical blindness	-	-	+
Hemiparesis, hemianopia	-	-	+
Sensorineural hearing loss	+	+	+
Lactic acidosis	+	+	+
Positive family history	-	+	+
Ragged-red fibers	+	+	+
Spongy degeneration	+	+	+

MELAS Syndrome was categorized as a distinct disorder in 1984 (Pavlakis). Patients so afflicted are usually normal at birth, but subsequently exhibit stunted growth, episodic vomiting, seizures, and recurrent cerebral events resembling strokes, which result in hemiparesis, hemianopia and/or cortical blindness. Lactic acidosis is a prominent feature. This syndrome is distinguished from the other two major types of mitochondrial myopathies, MERRF and KSS, by the presence of the stroke-like episodes and vomiting.

Kindred analysis of MELAS has been very limited, however, available pedigrees exhibit patterns consistent with maternal inheritance. In investigation of the possibility that MELAS Syndrome represented a genetic defect of mitochondrial DNA, two groups independently sequenced the mitochondrial genome of patients afflicted with this disease. Both identified a single print mutation in the dihydrouridine loop of mitochondrial $tRNA^{Leu(UUR)}$ (Kobayashi, 1990; Goto), as shown in Figure 8. In both studies the mutation was relatively specific to patients with MELAS Syndrome, yet causality could not be demonstrated because of the limited size of the kindreds. Subsequently, muscle cells from affected patients were isolated and subcloned to yield two groups: those which were normal, and those which had impaired respiration. Sequencing of the mtDNA from both groups revealed that the point mutation in $tRNA^{LEU}$ was exclusively confined to the

cloned muscle cells which were deficient in respiration. In addition, it was demonstrated that levels of cytochrome oxidase were negligible in the mutant cells (Kobayashi, 1991).

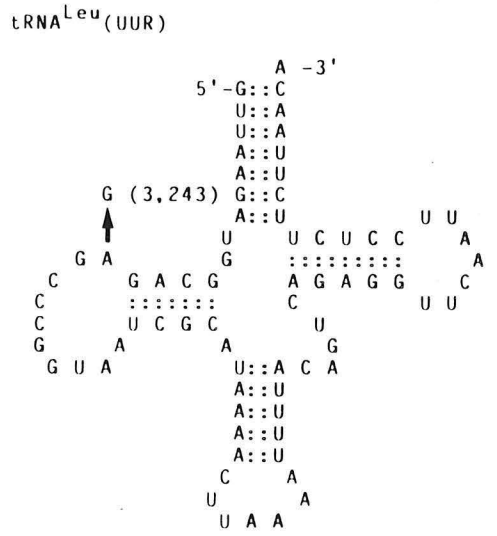

FIGURE 8: SECONDARY STRUCTURE OF tRNA-Leu AND MUTATION SITE IN MELAS

MERRF: MYOCLONUS, EPILEPSY, AND RAGGED-RED FIBERS

In 1980, the constellation of myoclonus, epilepsy, mental deterioration and ragged-red fibers in muscle biopsy specimens was designated as a distinct clinical syndrome (Fukuhara, 1980). With respect to its relationship with the other two main classes of mitochondrial myopathy (MELAS and KSS), the syndrome can be distinguished by the presence of myoclonus, ataxia, weakness and seizures in the absence of ophthalmoplegia, retinal degeneration, heart block, elevated CSF protein, episodic vomiting and cortical blindness, as shown in Table 5. In most instances, the onset of clinical symptoms is before the age of 20, and there is often a positive family history of a similar condition. Although maternal transmission was suspected with this syndrome (Fukuhara, 1983; Rosing), conclusive evidence was lacking due to relative small kindred sizes.

One such kindred allowed for the elucidation of the genetic defect in this disorder by Wallace and co-workers. In a series of reports, both the biochemical and genetic aspects of the disease were characterized (Wallace, 1988; Schoffner, 1989 and 1990). The disorder was found to owe to a point mutation in nucleotide 8344; which is part of the coding region for the mitochondrial $tRNA^{LYS}$. This mutation was found in three independent MERRF pedigrees, and was absent in 75 controls. All MERRF patients, and their less affected maternal relatives, bore between 98 and 73% MERRF type

mitochondrial DNAs. Patients with higher percentages of the mutant genome generally had more severe disease and an earlier onset of symptoms.

Biochemical analysis of the mitochondria harvested from affected individuals revealed dysfunction at two sites: Complexes I and V. This is not surprising, given that a mutation within a tRNA would be expected to have more pleotrophic effects than a point mutation in a structural gene.

Subsequently the observation of Wallace was confirmed in an independent study of several kindreds. The latter study, however, revealed that not all cases of MERRF owe to this particular mutation. At present, the second putative site has not been identified (Zeviani, 1991).

DISORDERS ARISING FROM DELETIONS WITHIN THE MITOCHONDRIAL GENOME

KSS: KEARNS-SAYRE SYNDROME

Unlike the previously described disorders, which owes to point mutations within the mitochondrial genome, Kearns-Sayre Syndrome results from large deletions within the 16.5 kb of mitochondrial DNA. This was the first demonstration of a genetic disorder owing to disruption of the mitochondrial genome and it is somewhat paradoxical that this was accomplished in a disorder which is not maternally inherited (Holt, 1988). This syndrome is characterized by the progressive external ophthalmoplegia, pigmentary degeneration of the retina and onset before the age of 15 years. In addition, patients may have a number of other disorders, including heart block, ataxia, and/or high cerebrospinal fluid protein (DiMauro, 1985; Kenny; Petty). Lactic acidosis and ragged-red fibers place the syndrome within the grouping of mitochondrial myopathies, and features of the disease are contrasted with those of MERRF and MELAS in Table 5.

Importantly, all cases of this disorder have been sporadic, except for possibly one family clustering (Harding, 1991). Recently, the molecular basis for Kearns-Sayre Syndrome has been elucidated, accounting for this inheritance pattern. In 1988, Holt, et al determined that large scale deletions of mitochondrial DNA (up to 7kb) could be detected in the muscles of affected patients, with the proportion of abnormal muscle mitochondrial DNA ranging from 20-70%. Subsequently, several reports have appeared documenting the presence of deletions in this disorder (Larsson; Mita; Moraes; Nakase; Ponzetto; Shanske; Zeviani, 1990a). It is notable that there is extreme heterogeneity in the size of the deletions (Figure 9), despite the fact that the symptoms and signs of Kearns-Sayre Syndrome are reasonably consistently. This is explained by the fact that these large deletions result not only in abnormal structural proteins of oxidative phosphorylation, but, more importantly, loss of tRNAs essential for mitochondrial protein synthesis. Thus, biochemical analysis of mitochondria harvested from affected individuals reveals global

dysfunction of Complexes I-V. As expected from the loss of tRNAs, transcription proceeds normally in the non-deleted mitochondrial genes, but translation is grossly impaired.

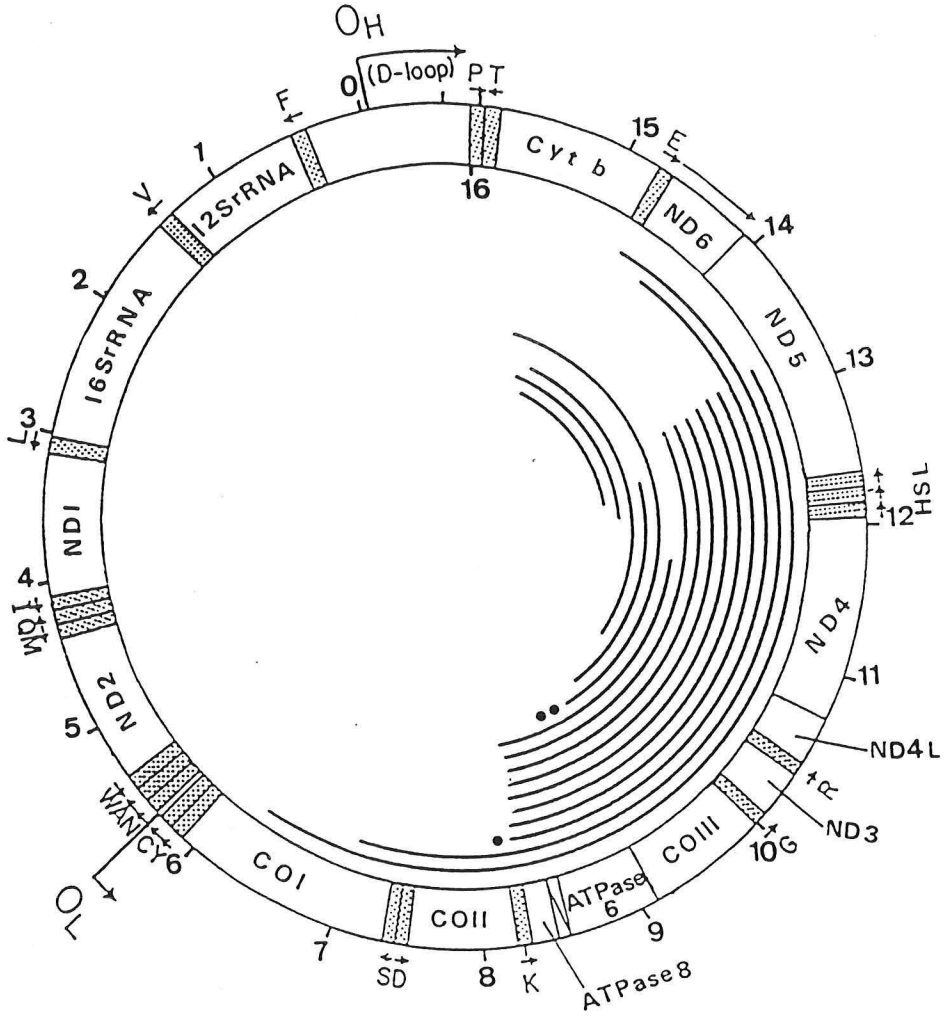

FIGURE 9: HETEROGENEITY OF DELETIONS IN KSS
The inner arcs depict the length and locations of mapped deletions.

The pathogenesis of this disorder is best explained by either germ line (oocyte) or early somatic mutations during embryogenesis. This is depicted in Figure 10. Because of the segregation of mitochondria during cell division, peculiar tissue distributions of mitochondrial deletions have been reported. In some individuals, most tissue examined carried the deletions of the mitochondrial DNA, which in other instances, deletions were identified in muscle, but not leukocytes. The latter situation likely derived from a somatic cell mutation during embryogenesis at a point past the differentiation of myoblast and leukocyte precursors. Alternatively, it has been suggested that this disparate distribution owes to the facts that deleted mitochondrial DNA molecules can survive in muscle fibers,

because the number of fibers does not increase significantly after early fetal life. In contradistinction, frequent cell division in leukocyte precursors would be expected to select against cells containing defective mitochondria (Harding, 1991).

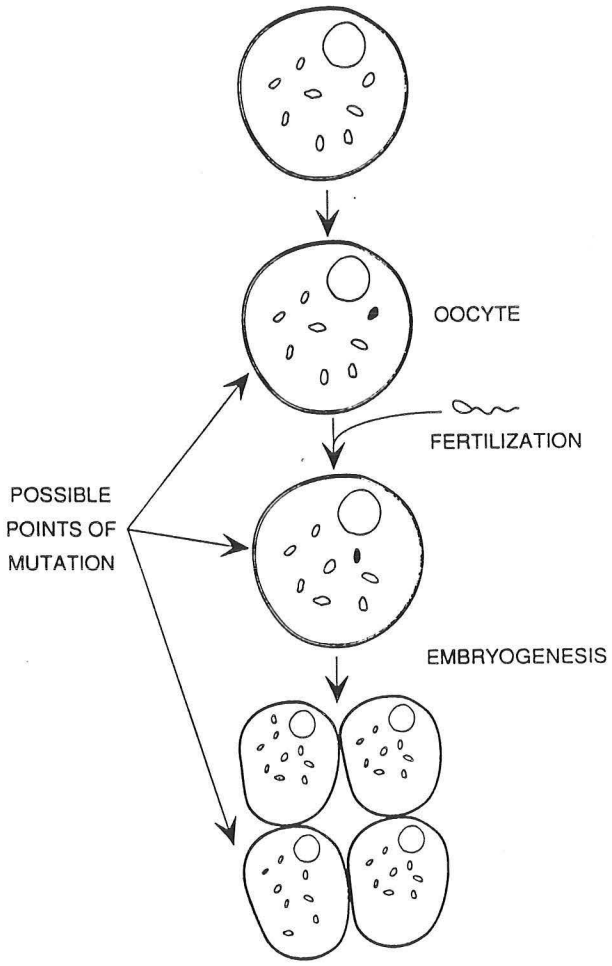

FIGURE 10: POTENTIAL ORIGINS OF SOMATIC MITOCHONDRIAL MUTATIONS

PEARSON'S SYNDROME

Pearson's Syndrome is a rare disorder characterized by the childhood onset of refractory sideroblastic anemia, thrombocytopenia, neutropenia, pancreatic insufficiency and hepatic dysfunction. In addition, patients may also have a systemic lactic acidosis. The disorder has an early onset, and death before the age of three is not unusual (Pearson, Stoddard). Initially, this disorder was thought to be inherited in an autosomal recessive pattern, but this view was discounted with the elucidation, in 1988, of the mitochondrial basis of the disease. Holt determined that the primary defect owed to large scale deletions of one

population of mitochondrial DNA, with variable degrees of heteroplasmy (Holt, 1988), and this has since been confirmed (Corimer; Moreas, 1991).

It is interesting to note that the same basic pathogenesis (large scale mitochondrial deletions) underlies both Kearns-Sayre Syndrome and Pearson's Syndrome. As discussed previously, the deletions of mitochondrial DNA in patients with the former result in impaired tRNA synthesis, translational arrest and global dysfunction of Complexes I-V. Thus, the distinctive features of the two syndromes is somewhat surprising. Recently, a patient was described with the classic findings of Pearson's Syndrome, but at age five developed full blown manifestations of Kearns-Sayre Syndrome. This indicates that the two disorders likely have a common molecular basis, and that the different phenotypes are probably a function of the initial proportions of heteroplasmy and differences in the tissue distribution of the genetic defect (McShane). Given the fact that patients with Pearson's Syndrome frequently die before the age of three years, it would appear that the distribution of defective mitochondrial DNA is more global in this disorder than in Kearns-Sayre Syndrome, which has a mean age of onset some ten years later in life.

NUCLEAR DNA ENCODED MITOCHONDRIAL DISORDERS

As noted previously, only 13 of the 67 components of Complexes I-V are encoded by the mitochondrial genome. The remainder are nuclear gene products which are imported into the mitochondrion. It has now become clear that mutations within these nuclear-derived subunits can result in mitochondrial dysfunction (Cormier,1991; Zeviani, 1989). As would be expected, these nuclear-encoded defects are not maternally-transmitted, but rather follow classic Mendelian transmission patterns.

One such syndrome, described by Zeviani, is characterized by progressive external ophthalmoplegia (PEO) and lactic acidosis, and has been demonstrated in multiple kindreds to be transmitted in an autosomal dominant pattern, including paternal transmission (Zeviani, 1989 and 1990). Molecular characterization has revealed that this disorder owes to multiple deletions of the mitochondrial DNA, beginning at the D-loop initiation site. The pathology of this disorder likely owes to a mechanism similar to that of Kearns-Sayre and Pearson's Syndromes - namely defective translation of the mitochondrial encoded structural proteins of oxidative phosphorylation due to loss of tRNA function. At present, the nuclear defect responsible for this phenomenon has not been elucidated, but this example seems to underscore the "cross-talk" of nuclear and mitochondrial DNA in the biogenesis of mitochondria.

SPECULATIONS: MITOCHONDRIA AND CANCER, DEATH, AND THE ORIGIN OF THE HUMAN SPECIES

Definition of the predictable role of the mitochondrial genome in neuromuscular disorders has fostered a search for other, less obvious diseases which may owe, in part, to intrinsic

mitochondrial dysfunction. These include tumors, and the aging process itself. At the present time, there has been no causal link established between mtDNA mutations and diseases, other than those discussed previously. However, exploration of this area is in its infancy, and it is most likely that the mitochondrial genome will be found to be a participant in the pathogenesis of unexpected disorders.

As an example, ongoing investigations have led to the definition of a molecular alteration of mitochondrial DNA which is associated with human renal oncocytomas (Welter). In one study, a restriction fragment length polymorphism was found in 6/6 of these tumors, but not in adjacent, normal renal parenchyma or in renal cell carcinomas harvested from other patients. It is not known whether this represents a primary, causal link, or an irrelevant epiphenomenon reflective of the tumor process in oncocytomas.

Speculations as to how the mitochondrial DNA may become mutated in 'acquired' disorders generally center upon oxidative stress, in the form of a superoxide radical attack on basic DNA structure (Bandy; Linnane; Miguel; Richter; Zglinicki). In its most wide-reaching formulation, this hypothesis has been extended to the processes of cellular senescence and systemic aging. Arguments marshalled in support of this proposal include the high mutation rate of mtDNA (which might lead to decreased efficiency of oxidative phosphorylation) and scattered reports of changes in mitochondrial activity and structure as a function of age. In its simplest form, these views tend to tie the fatigue of the elderly to a decreased ability to produce ATP. Clearly, direct molecular studies are required to test these hypotheses.

In a different vein, mapping of mtDNA polymorphisms has recently been applied to physical anthropology, with an end of determining the geographic and temporal origins of the human species (Vigliant). The central assumption of this approach is that the degree of (mitochondrial) genetic divergence between two groups is a direct function of the time period the groups have been separated. The mtDNA is uniquely suited to this use because of its high mutation rate (which generates the necessary polymorphism) and because it is exclusively maternally transmitted (and thus not subject to recombinational events which could greatly accelerate divergence). The resultant phylogenetic tree, indicates that the human species had its origin in Africa between 166,000 and 249,000 years ago. Although not fully accepted with regard to its final conclusions (Kruger), this analysis demonstrates the broad utility of the mitochondrial genome in investigation of phylogenetic relationships.

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