

Why Do We Still Have a Maternally Inherited Mitochondrial DNA? Insights from Evolutionary Medicine

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Abstract

The human cell is a symbiosis of two life forms, the nucleus-cytosol and the mitochondrion. The nucleus-cytosol emphasizes structure and its genes are Mendelian, whereas the mitochondrion specializes in energy and its mitochondrial DNA (mtDNA) genes are maternal. Mitochondria oxidize calories via oxidative phosphorylation (OXPHOS) to generate a mitochondrial inner membrane proton gradient (ΔP). ΔP then acts as a source of potential energy to produce ATP, generate heat, regulate reactive oxygen species (ROS), and control apoptosis, etc. Interspecific comparisons of mtDNAs have revealed that the mtDNA retains a core set of electron and proton carrier genes for the proton-translocating OXPHOS complexes I, III, IV, and V. Human mtDNA analysis has revealed these genes frequently contain region-specific adaptive polymorphisms. Therefore, the mtDNA with its energy controlling genes may have been retained to permit rapid adaptation to new environments.

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Mitochondrion: a eukaryotic α -proteobacterial symbiotic organism that mediates energy transformations via maintenance of an inner membrane potential ($\Delta P = \Delta \Psi + \Delta \mu^{H+}$)

INTRODUCTION

Life is the interplay between structure, energy, and information. For man and other multicellular plants and animals, energy flow is mediated by intracellular symbiotic bacteria. In plants symbiotic cyanobacteria make up the chloroplasts, which collect the sun's photons and use the energy to split water into hydrogen and oxygen (1) (**Figure 1**). The hydrogen is bound to carbon to generate glu-

cose, which can be stored as starch. The oxygen (O_2) is released into the atmosphere.

In animals and plants symbiotic α -proteobacteria constitute the mitochondria. When animals eat plants, glucose is absorbed through the gut and delivered to the mitochondria via the bloodstream. Atmospheric oxygen is absorbed through the lungs and carried to the mitochondria via hemoglobin. In the mitochondrion, hydrogen atoms are stripped from the plant hydrocarbons and reacted with the oxygen to regenerate water, thus releasing the sun's energy. The recovered energy is stored as potential energy in the mitochondrial electrochemical gradient which can be converted to the high-energy phosphate bounds in ATP for use in powering work or is released as heat to maintain the endothermic body temperature (**Figure 1**). Since energy can be used only once, the mitochondria mediate animal energy allocation (2, 3).

Prior to the symbiosis, the protomitochondrion and the protohost cell both had genomes sufficient for their independent lifestyles. However, once the nuclear cytosol and mitochondrial symbiosis became established, the cytosol became the mitochondrial universe (2–5).

As the mitochondrial symbiont became more established, selective pressure developed to drive the mitochondrial DNA (mtDNA) to be uniparentally and, in most cases, maternally inherited. This is because biparental inheritance of cytoplasmic genomes permits deleterious mutations with a replicative advantage to spread throughout the population and erode species fitness. Nuclear mutations that restrict the transmission of organelle genomes to a single parent limit this possibility (6–8).

The number of mtDNA lineages transmitted from maternal parent to offspring also needs to be restricted. This is because in a mixed population of mtDNA molecules (heteroplasmy), an mtDNA mutation that has a replicative advantage but that is also deleterious to the host, can come to predominate. Each subsequent deleterious mutation with a

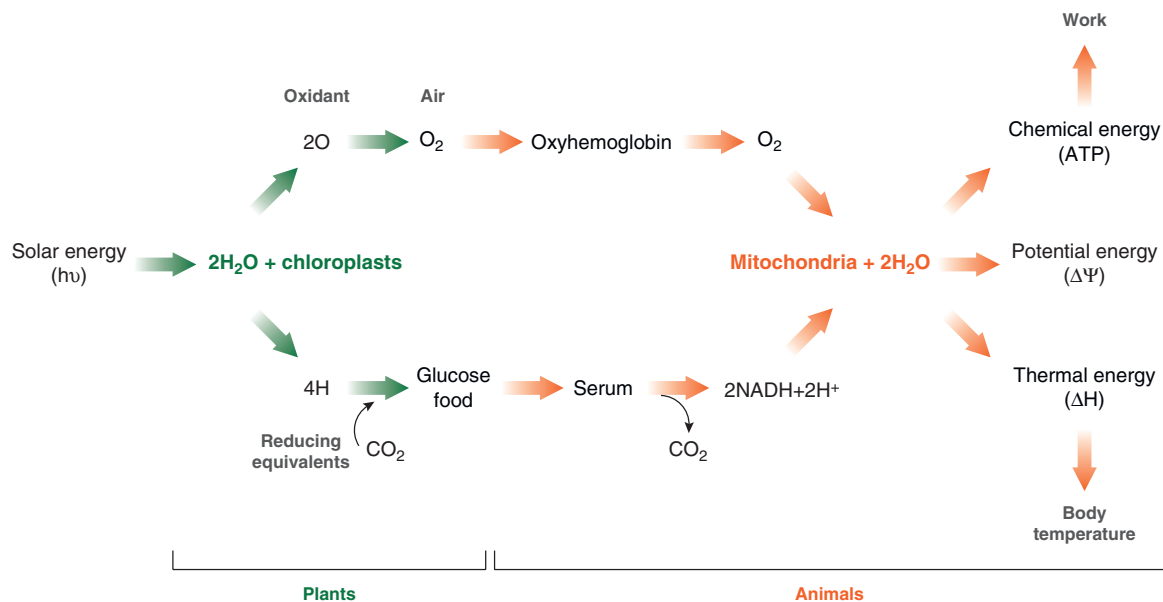


Figure 1

Transformation of the sun's energy by the plant cell symbiotic chloroplast and the animal cell symbiotic mitochondria. The chloroplast, derived from cyanobacteria, uses the sun's energy to split water, releasing the oxygen in the atmosphere and fixing the hydrogen to carbon to generate glucose. Animals eat the plants to acquire the glucose and collect the oxygen from the atmosphere by breathing. In the animal mitochondria, the hydrogen is extracted from the hydrocarbons and reacted with the oxygen to regenerate water. The energy released is used to generate a mitochondrial inner membrane electrochemical potential, $\Delta\Psi$, the potential energy of which can be used for multiple purposes, including ATP generation and heat production (2).

replicative advantage will supplant the previous one until the lineage dies, a process known as "Muller's ratchet" (9). The introduction of a germ line bottleneck restricting the number of mtDNAs transmitted between generations ameliorates this problem by increasing the rate that mutant mtDNAs become purified in the maternal germ line by genetic drift. Thus, new mtDNA mutations are rapidly segregated to homoplasmy so that they can be tested by natural selection, and the deleterious mutations are eliminated from the population before they can become widely disseminated (10, 11).

Ultimately, these evolutionary genetic factors led to the exclusive maternal inheritance of the mtDNAs. In humans and animals, this is achieved, in part, by the dilution of the sperm mtDNAs by ovarian mtDNAs at fer-

tilization, the oocyte having over 200,000 mtDNAs whereas the sperm has hundreds (12–15). Furthermore, residual paternal mtDNAs are selectively removed in animals. During spermiogenesis in the Japanese Medaka fish (*Oryzias latipes*), the sperm mtDNAs are reduced about fivefold to about 10 nucleoids per sperm. Following fertilization, the remaining sperm mtDNA nucleoids disappear within an hour (16). In mammals, the sperm mitochondrial outer membrane protein, prohibitin, is ubiquitinated, presumably permitting the sperm mitochondria to be recognized as foreign by the oocyte and degraded within 24 h of fertilization (17, 18). This recognition process is also active when sperm mitochondria and mtDNAs are injected into mammalian mtDNA-deficient (ρ^0) somatic cells (19). In mouse intraspecific crosses, the sperm

Mitochondrial DNA (mtDNA):

DNA located in oxidative mitochondria that encodes key membrane polypeptides for the proton-translocating OXPHOS complexes

Oxidative phosphorylation (OXPHOS): a mitochondrial process that oxidizes calories to generate a membrane potential for use in generating ATP and heat and to energize other processes

mitochondria and mtDNAs are selectively destroyed by the zygote (20), but in interspecific crosses, the sperm mtDNAs can persist (21). Thus, maternal inheritance must be the result of a genetically encoded, species-specific, concerted process.

Animal mtDNAs have been found to have a high mutation rate, evolving about 20 times faster than comparable function nDNA gene sequences (22–24). The human mtDNA mutation rate has been estimated at about 2.5 substitutions per site per million years (25, 26), but that of the nematode *Caenorhabditis elegans* is about 8.9 substitutions per site per million years (27).

Mutations in the mtDNA can be deleterious, neutral, or occasionally advantageous. Because the mtDNA genes are all essential for oxidative phosphorylation (OXPHOS) and thus critical for life and health, the high mtDNA mutation rate means that diseases of the mtDNA are common (28). The importance of uniparental inheritance in limiting the adverse consequences of deleterious mtDNA mutations has been seen in the association between abnormal human embryos and the presence of paternal mtDNAs (29) as well as in one reported case of paternal transmission of an mtDNA. In the later case, the child had retained the father's mtDNA in his muscle, but the paternal mtDNA had acquired a microdeletion causing mitochondrial myopathy (30). A few of the maternal and paternal mtDNAs had also recombined in the child's muscle (31).

A large mtDNA encoding many genes would place many important functions at risk for degeneration via Muller's ratchet. Therefore, there was strong selective pressure to transfer critical gene functions to the nDNA where they could be protected from mutational decay by sexual reproduction and recombination (32, 33). Once the mtDNA replication apparatus was transferred to the nDNA, and thus was common to all cellular mtDNAs, the mtDNA replication time became proportional to the length of the mtDNA molecule. Consequently, the shorter

mtDNAs replicated faster and outcompeted longer mtDNAs, further selecting for small mtDNAs (3, 34).

For these reasons, once the α -protobacterial symbiont became established, the mtDNA genes required for a free-living lifestyle were rapidly deleted, as appears to be occurring in the intracellular parasitic α -protobacteria *Rickettsia prowazekii* (35, 36). Subsequently, systems evolved by which functionally important mtDNA genes could be transferred to the nDNA, where they could be replicated and transcribed, and then the proteins translated on cytosolic ribosomes, and the resulting polypeptides selectively transported back into the mitochondrion. The resulting virtually unidirectional transfer of genes from organelle DNAs to the nDNA has been thoroughly documented for both mtDNAs and chloroplast DNAs (3, 5, 37).

The steps involved in the transfer of a functional mtDNA gene to the nDNA include the transfer of the nucleic acid information from the mtDNA to the nDNA by either direct DNA transfer or by the release of mtDNA mRNAs into the cytosol and the reverse transcription of the mRNA into DNA with its subsequent integration into the nDNA (38, 39). The newly integrated gene must acquire the signals for nDNA transcription and cytosolic translation as well as the signals that permit the cytosolically synthesized protein to be reinserted back into the mitochondrion. One mechanism by which this could be achieved is for the mtDNA protein-coding sequence to insert in the first intron of a preexisting nDNA-encoded mitochondrial gene that is targeted to the mitochondrion by an N-terminal targeting peptide. An in-frame fusion of the mtDNA coding region to the first exon would result in the acquisition of the appropriate transcription, translation, and mitochondrial targeting information. The sequence of the new nDNA-encoded mitochondrial gene sequence could then adapt to the nucleo-cytosol coding rules (5, 37).

Although the molecular machinery to integrate the mtDNA genes into the nDNA must

have existed in the protohost cell at the time of the initial symbiosis, the machinery for importing cytosolically synthesized proteins into the mitochondrion probably did not. In modern eukaryotes, proteins synthesized on cytosolic 80S ribosomes and destined for the mitochondrion are tagged by either an N-terminal, positively charged, amphiphilic targeting peptide, which is cleaved off during import, or alternatively by internal mitochondrial targeting sequences that are retained in the mature polypeptide. The three main mitochondrial protein import complexes used in modern cells are the translocase of the outer membrane (TOM) and two translocases of the inner membrane (TIM22 and TIM23). The TOM complex imports all cytosolically synthesized proteins through the outer mitochondrial membrane. The diameter of the TOM channel is estimated to be 20–25 Å and encompasses the core translocase proteins Tom40, Tom22, and Tom7. This channel can import either linear polypeptides or hairpin loop polypeptides as occurs during the import of certain inner membrane carrier proteins, such as the adenine nucleotide translocator (ANT). The TIM22 complex, with Tim22 being the core translocase, is responsible for the insertion of polypeptides with internal targeting sequences into the mitochondrial inner membrane from the intermembrane space side. The TIM23 complex, in which Tim23 and Tim17 form the core translocase, is responsible for importing proteins with N-terminal targeting peptides into the mitochondrial inner membrane from the matrix side or in association with the PAM complex to import and release soluble proteins into the matrix. The diameter of the Tim23 channel is estimated to be 13–24 Å. Finally, a SAM complex is located in the mitochondrial outer membrane and is responsible for assembling the TOM complex and for inserting other proteins into the outer mitochondrial membrane (40, 41).

Current data indicate that the SAM and PAM complexes were carried forward from

the α -proteobacterial symbiont. However, the core components of the TOM, TIM22, and TIM23 translocases do not have bacterial counterparts. Hence, these components must have evolved de novo during the early stages of solidification of the α -proteobacterial symbiosis (41). Therefore, the nature of the TOM, TIM22, and TIM23 complexes must have been shaped by the characteristics of the proteins that were encoded by the nDNA and needed to be imported into the mitochondrion.

The co-occurrence of the transfer of genes from the mtDNA to the nDNA and the evolution of the mitochondrial protein import system ultimately resulted in the integration of virtually all of the α -proteobacterial genes into the animal cell nDNA. The nDNA-encoded mitochondrial genes now comprise all of the mitochondrial biogenesis genes, including the mtDNA and RNA polymerases, ribosomal proteins, elongation factors, tRNA synthetases, all mitochondrial intermediary metabolism genes, and virtually all of the genes for the OXPHOS complexes, i.e., the four subunits of respiratory complex II (*sdhA-D*), a total of ≈ 1500 genes (2, 28). The only genes that now remain in the mammalian mtDNA are *nad1-4*, *nad4L*, *nad5* and *-6*, which encode seven (ND1–5, ND4L, ND5, and ND6) of the 45 polypeptides of complex I; *cob*, which encodes cytochrome *b* of the 11 subunits of complex III; *cox1-3*, which encode three (COI, II, and III) of the 13 polypeptides of complex IV; and *atp6* and *-8*, which encode 2 of the ~ 16 subunits (ATP6 and -8) of complex V (Figure 2). The mammalian mtDNAs also encode a 12S and 16S rRNA and 22 tRNAs, the latter apparently sufficient to translate the mtDNA proteins (Figure 2). The mammalian mtDNA also includes a 1121-nucleotide control region encompassing the promoters for transcription of the C-rich light-strand (P_L), the G-rich heavy-strand (P_H), and the origin for H-strand replication (O_H). The origin of L-strand replication (O_L) is separate, located within the coding region (39, 42).

Complex I (CplxI) (NADH):

NADH dehydrogenase or NADH-ubiquinone oxidoreductase

Complex II (CplxII) (SDH):

succinate dehydrogenase or succinate-ubiquinone oxidoreductase

Complex III (CplxIII):

bc₁ complex or ubiquinol-cytochrome *c* oxidoreductase

Complex IV

(CplxIV) (COX): cytochrome *c* oxidase or ferrocyclochrome *c*-oxygen oxidoreductase

Complex V

(CplxV): ATP synthase or H^+ -translocating ATP synthase

Complexes I, III, IV, and V:

multiple subunit mitochondrial OXPHOS inner membrane complexes that couple electron transport to proton gradient generation and synthesis of ATP

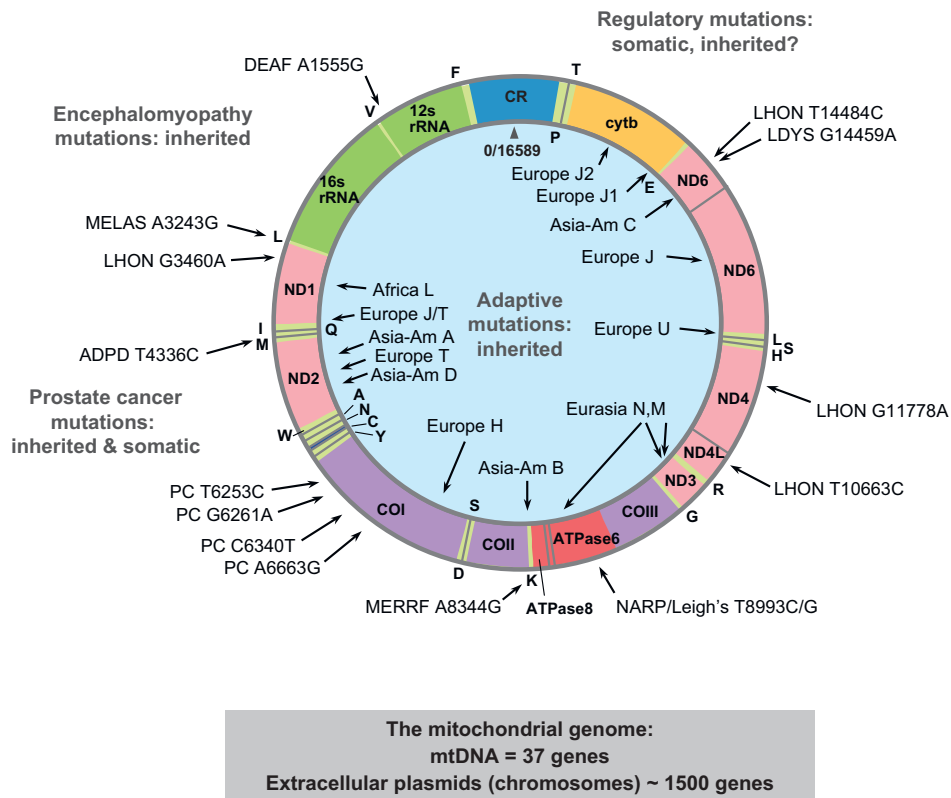


Figure 2

The human mtDNA map. The human mtDNA encompasses three classes of clinically relevant mutations: recent maternally inherited disease-causing mutations, examples of which are shown on the outside of the circular map; ancient geographically correlated and frequently adaptive polymorphic variants, examples presented inside the circle; and somatic mutations that accumulate with age in postmitotic tissues and provide the aging clock. Letters around the outside perimeter indicate cognate amino acids of the tRNA genes. Letters within the ring represent the proteins encoded by the gene sector, all of which are integral membrane components of the proton-translocating complexes of OXPHOS. The polypeptides, corresponding gene, and complexes are ND1-4, -4L, -5, and -6 (*nad1-4*, *-4L*, *-5*, and *-6* gene) of complex I; cytb or cytochrome *b* (*cob* gene) of complex III; COI-III (*cox1-3* genes) of complex IV; and ATP6 and ATP8 (*atp6* and *atp8* genes) of complex V. The CR is the control region, which encompasses the H-stand and L-strand promoters (P_H and P_L) and the H-strand origin of replication (O_H). Arrows associated with abbreviations followed by numbers around the outside of the circle indicate representative pathogenic mutations, and the number is the nucleotide position of the mutation. The arrows followed by continent names and associated letters on the inside of the circle indicate the position of defining polymorphisms of selected region-specific mtDNA lineages. Haplogroup markers shown include: macrohaplogroup L, 3594T (L0, L1, L2) versus 3594C (L3, M, N); macrohaplogroup N, 8701A (versus M and L0-3, 8701G) plus 10398A (European lineages T/W/X/K2 are 10398A, and L/J/I/K1 are 10398G); macrohaplogroup M, 10400T; and haplogroup A, 4824G; haplogroup B, 8271:8281 (9 bp deletion); haplogroup C, 14318C; haplogroup D, 5178A; haplogroup H, 7028C; haplogroup J/T, 4216C; haplogroup J, 13708A; haplogroup J1, 14798C; haplogroup J2, 15257A; haplogroup T, 4917G; and haplogroup U, 12308G. Abbreviations: Am, America; DEAF, deafness; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; LHON, Leber's hereditary optic neuropathy; ADPD, Alzheimer's and Parkinson's diseases; MERRF, myoclonic epilepsy and ragged red fiber disease; NARP, neurogenic muscle weakness, ataxia, retinitis pigmentosa; LDYS, LHON plus dystonia; PC, prostate cancer. (Modified from References 2 and 43.)

Given the overwhelming number of genes that now reside in the nDNA, there must have been strong selective pressure to retain the remaining 13 polypeptides on the mtDNA. The question is why? In this review, I address this question in light of two observations: (a) Similar arrays of membrane subunits for the proton-translocating OXPHOS complexes I, III, IV, and V are retained by the mtDNAs of virtually all organisms that continue to use the complex. (b) Intraspecific variation in human mtDNA polypeptides is both prevalent and adaptive. On the basis of these facts, I argue that mtDNA genes have been retained so that those proteins that contribute to the mitochondrial inner membrane proton gradient will coevolve as the organism adapts to changing energetic environments.

MITOCHONDRIAL OXPHOS AND THE OXPHOS GENES

Mitochondrial OXPHOS consists of two subsystems, the electron transport chain (ETC), which encompasses complexes I–IV, and the ATP synthase, complex V (**Figure 3**). The ETC reacts hydrogen extracted from dietary hydrocarbons with oxygen to generate water. Hydrogens (reducing equivalents) collected from carbohydrates and organic acids by the tricarboxylic acid (TCA) cycle or from fats via β -oxidation are transferred to the carrier molecule NAD^+ to generate $\text{NADH} + \text{H}^+$. The electrons from NADH are then donated to complex I (NADH dehydrogenase). Electrons from the TCA cycle intermediate succinate are transferred to complex II (succinate dehydrogenase, SDH). The electrons from complexes I and complex II are then transferred to the lipid-soluble carrier, ubiquinone or coenzyme Q_{10} . A first electron reduces ubiquinone to ubisemiquinone and a second electron reduces ubisemiquinone to ubiquinol. Ubiquinol then transports its two electrons within the mitochondrial inner membrane to complex III (bc_1 complex). These electrons transit complex III and then reduce cytochrome c . Reduced cytochrome

c then carries the electrons to complex IV (cytochrome c oxidase, COX), where four electrons reduce a molecule of oxygen (O_2) to give two H_2O molecules. The energy that is released as the electrons flow down the ETC is used to pump protons out of the mitochondrial inner membrane through complexes I, III, and IV, but not through complex II. This creates a proton electrochemical gradient ($\Delta P = \Delta \Psi + \Delta \mu^{\text{H}^+}$) that is acidic and positive on the outside (in the intermembrane space) and negative and alkaline on the inside (in the matrix). The potential energy stored in ΔP is then used for multiple purposes, one of which is to drive complex V (ATP synthase) to condense $\text{ADP} + \text{P}_i$ to make ATP. Matrix ATP is then exchanged for cytosolic ADP by the inner membrane ANT_s (**Figure 3**) (2).

Recent structural and biochemical analyses of complexes I, III, IV, and V are beginning to clarify the roles of the mtDNA-encoded polypeptides. The crystal structure has been solved for complex III (44). This complex pumps protons out of the mitochondrial inner membrane via the Q cycle in which cytochrome b is the central component. In the Q cycle, the fully reduced ubiquinol binds to cytochrome b at the coenzyme Q-binding site on the outside of the inner membrane adjacent to the intermembrane space (Q_o). One electron is transferred to the Rieske iron-sulfur protein and then on to cytochrome c_1 and cytochrome c . However, the second electron is transferred to the low-potential cytochrome b and then to the high-potential cytochrome b , where it reduces a ubiquinone bound to the cytochrome b coenzyme Q_{10} -binding site on the inside of the inner membrane adjacent to the matrix (Q_i). This reduces the ubiquinone to ubisemiquinone. As a consequence, the coenzyme Q bound to the Q_o site has lost two negative charges, causing it to release its two protons into the intermembrane space. A second ubiquinol then replaces the ubiquinone at the Q_o site, and one electron is passed to the Rieske iron-sulfur protein, and the other is added to the ubisemiquinone in the Q_i site, reducing it to ubiquinol. This coenzyme

Life = structure + energy

Energy: fats + sugars + oxygen = energy (heat + work) + CO_2 + H_2O

Reactive oxygen species: mitochondrial combustion \rightarrow oxygen radicals

Apoptosis: energy \downarrow + ROS $\downarrow \rightarrow$ mtPTP activated \rightarrow cell death (apoptosis)

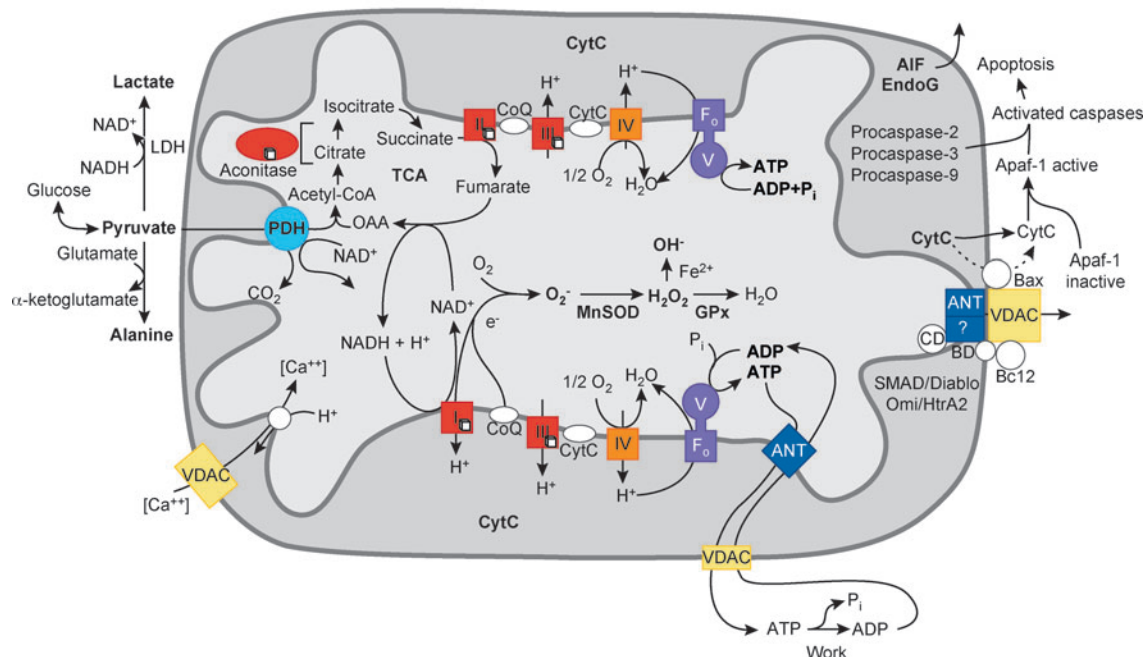


Figure 3

Mitochondrial energy production and its relation to the pathophysiology of disease. Three features of mitochondrial metabolism are central to the pathophysiology of the common age-related diseases: (a) energy production by oxidative phosphorylation (OXPHOS), (b) reactive oxygen species (ROS) generation as a by-product of OXPHOS, and (c) regulation of apoptosis through activation of the mitochondrial permeability transition pore (mtPTP). OXPHOS complex I is composed of 45 polypeptides, 7 (ND1, -2, -3, -4L, -4, -5, and -6) encoded by the mammalian mtDNA; complex II consists of 4 nDNA-encoded polypeptides; complex III consists of 11 polypeptides, 1 (cytochrome *b*) encoded by the mtDNA; complex IV is composed of 13 polypeptides, 3 (COI, -II, -III) encoded by the mtDNA; and complex V is composed of 16 polypeptides, 2 (ATP6 and -8) encoded by the mtDNA. Abbreviations: Acetyl-CoA, acetyl-coenzyme A; ADP or ATP, adenosine di- or triphosphate; ANT, adenine nucleotide translocator; Apaf-1, apoptotic protease activating factor-1; cytc, cytochrome *c*; GPx, glutathione peroxidase-1; LDH, lactate dehydrogenase; MnSOD, manganese superoxide dismutase; NADH, reduced nicotinamide adenine dinucleotide; SMAD/Diablo, antagonizes inhibitors of apoptosis (IAPs); Omi/HtrA2, serine protease 24; TCA, tricarboxylic acid cycle; VDAC, voltage-dependent anion channel; I, II, III, IV, and V, OXPHOS complexes I to V. Reprinted from References 2 and 43 with permission.

Q now has two negative charges, which are then neutralized by absorbing two protons from the matrix. The net result of this Q cycle is to transport two protons out across the mitochondrial inner membrane for every two electrons that traverse complex III (28, 44–46).

The crystal structure of complex IV is also known (47) and the COI–III polypeptides are also central to coupling electron transport to proton pumping. The COI protein harbors the two cytochromes, *a* and *a*₃, and the copper B center (CuB) forming the trinuclear reaction center. O_2 binds at the cytochrome

a_3 -CuB binuclear center where it is reduced by four electrons to generate two H_2O s. COI obtains its electrons from COII, which harbors two copper atoms that form the CuA binuclear center. COII, in turn, forms the binding pocket for reduced cytochrome c from which it acquires two electrons. The precise function of COIII is not known, but it appears to form an aqueous channel, which may allow protons to move through the membrane (28, 45–47).

The crystal structure of complex I has not been solved. Therefore, the specific functions of the mtDNA-encoded inner membrane ND1-4, -4L, -5, and -6 subunits remain uncertain. However, the mtDNA subunits are definitely important because disease-associated mutations have been identified in every one of the mtDNA subunits (28). Electrons are donated to complex I from NADH and are transferred through flavin mononucleotide and a series of iron-sulfur centers to ultimately reduce coenzyme Q. One possibility is that the proton-pumping capacity of complex I might involve a Q cycle to which the mtDNA-encoded subunits contribute (28, 46, 48, 49). Such a model is supported by functional studies of pathogenic mutations that affect several of the mtDNA complex subunits. For example, the nucleotide 14459G>A (A72V) mutation in subunit ND6, which causes Leber's hereditary optic neuropathy (LHON) and generalized dystonia, is associated with an altered complex I affinity for coenzyme Q analogues (28, 46, 50, 51).

The proton gradient established by ETC complexes I, III, and IV is utilized as a source of potential energy by complex V to drive the condensation of ADP + Pi to form ATP. Structurally, the ATP synthase (complex V) is composed of the soluble globular F1 ATPase, which projects into the matrix, a stalk, and a membrane base, the F0 component. The mtDNA ATP6 protein forms the proton channel of complex V, coupling the proton gradient to ATP synthesis. Functionally, the ATP synthase can be divided into two parts, "rotor" and "stator." The rotor consists of a

wheel in which 10 to 12 c subunit spokes (the bacterial equivalent of the ATP9) are bound to an axle composed of ϵ and γ subunits. The wheel lies in the plane of the membrane, and the axle projects into the interior of the barrel-shaped F1, which is composed of three pairs of alternating α and β subunits. On the outside edge of each of the ATP9 subunit spokes is a negatively charged carboxy amino acid (ATP9 amino acid Glu⁵⁸). The stator consists of the ATP6 subunit (subunit a in bacterial nomenclature), which spans the inner membrane and is juxtaposed to the Glu⁵⁸ ends of the ATP9 spokes of the rotator. Bound to ATP6 is the long thin b subunit, which links ATP6 to the F1 via the δ subunit. The ATP6 subunit encompasses two half proton channels, offset from each other, one open to the intermembrane space having a high proton concentration and the other open to the matrix with a low proton concentration. The negatively charged carboxyl groups of the ATP9 subunits rotate past the two half channels, traveling through an electronically neutral pocket formed in mammalian ATP6 by Leu¹⁵⁶. Thus, as a ATP9 subunit passes the half channel open to the intermembrane space, the negatively charged Glu⁵⁸ picks up a proton, neutralizing the spoke and permitting it to rotate into the lipid bilayer. The protonated spoke rotates all of the way around the wheel until it returns to ATP6 and encounters the half channel that is open to the alkaline matrix. This pulls the proton off the Glu⁵⁸. A displaced positively charged arginine, Arg¹⁵⁹ in mammals, then attracts the deprotonated and negatively charged Glu⁵⁸ spoke toward the intermembrane space-associated half channel. Therefore, the wheel's spin direction is determined by attraction of the deprotonated subunit ATP9 Glu⁵⁸ to the displaced Arg¹⁵⁹, and the speed of the wheel is determined by the electrostatic potential of the proton gradient ΔP . As the wheel rotates, the ϵ - γ axle spins inside the F1 barrel striking the β subunits and driving them to condense ADP + Pi to ATP. The function of ATP8 is currently unclear (28, 46, 52–54).

MnSOD (*Sod2*):
mitochondrial
manganese
superoxide dismutase

mtPTP:
mitochondrial
permeability
transition pore

Pathogenic mutations have been identified in all of the human mtDNA-encoded complex I, III, IV, and V subunits (28, 43). For example the nucleotide 11778 (G>A) mutation in the mtDNA *nad4* gene (R340H) causes a mid-life, sudden onset blindness syndrome, LHON (28, 55), while an nt 8993 T>G mutation in the *atp6* gene (L156R) causes the neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP) and Leigh syndromes (55, 56). It has been suggested that this mutation, which converts Leu¹⁵⁶ to Arg¹⁵⁶ in ATP6, places a positive charge on ATP6 adjacent to the Glu⁵⁸ of ATP9. This would then inhibit the spinning of the wheel and reduce ATP production (57–59).

OXPHOS complexes I and III have also been found to generate reactive oxygen species (ROS). The first of the mitochondrially generated ROS is the superoxide anion ($O_2^{\cdot-}$), which results from the transfer of one electron from these two complexes directly to O_2 . The $O_2^{\cdot-}$ is detoxified by the nDNA-encoded mitochondrial Mn superoxide dismutase (MnSOD), which dismutates two $O_2^{\cdot-}$ s into a hydrogen peroxide (H_2O_2). H_2O_2 , which is relatively stable, can then diffuse out of the mitochondrion where it can be degraded by catalase located in the peroxisomes. However, H_2O_2 can also acquire an additional electron from a reduced transition metal to generate the highly reactive hydroxyl radical ($\cdot OH$) (Figure 3). Mitochondrial ROS damages mitochondrial lipids and proteins and mutagenizes the mtDNA. However, mitochondrial ROS is also an important signal transduction molecule, which allows the status of mitochondrial metabolism to be monitored by the nucleus (2, 60–62). When mitochondrial damage becomes sufficiently severe to limit normal cellular functions, the mitochondrial permeability transition pore (mtPTP) is activated, initiating programmed cell death (apoptosis). This destroys the cell with its malfunctioning mitochondria. The mtPTP has been proposed to be composed of inner membrane proteins, including the ANT; the outer membrane channel pro-

tein; the voltage-dependent anion channel (VDAC); proapoptotic members of the Bax gene family; antiapoptotic members of the Bcl-2 family; the benzodiazepine receptor; and the calcium sensing protein cyclophilin D. The mtPTP can be activated by a decline in ΔP , reduced ADP and ATP, increased mitochondrial uptake of Ca^{2+} , or increased mitochondrial oxidative stress. Once activated, the mtPTP opens a channel between the cytosol and the mitochondrial matrix collapsing ΔP . The outer mitochondrial membrane then becomes permeable to the release from the intermembrane space into the cytosol of sequestered proapoptotic proteins, including cytochrome *c*, procaspase-9, apoptosis initiating factor (AIF), and endonuclease G. This initiates the caspase cascade and chromatin degradation (Figure 3) (2, 63).

COMPARATIVE mtDNA GENOMICS AND MEMBRANE SUBUNITS OF COMPLEXES I, III, IV, AND V

The transfer of genes from the α -prokaryotic genome to the host genome has occurred to different extents in different unicellular eukaryotic species, the protists (Table 1). Mitochondria come in two common physiological varieties, those that are found in oxidative organisms and use O_2 as their terminal electron acceptor and those that occur in anaerobic organisms and use organic acids and protons as their terminal electron acceptors and are known as hydrogenosomes (5, 64–66).

Among the mitochondria from oxidative eukaryotes, the protist whose mtDNA retains the most ancestral bacterial genes is *Reclinomonas americana* (Ram in Table 1). The *R. americana* mtDNA encodes 67 protein genes; 26 tRNAs; a large, small, and 5S rRNA; and a RNase P RNA. Included in the *R. americana* mtDNA protein coding genes are the genes (*rpo A-D*) for the four subunits of the bacterial-type RNA polymerase ($\alpha_2\beta\beta'\sigma$). This is in marked contrast to all other

Table 1 Genes encoded by the mtDNAs of oxidative protists, plants, and animals reveal a core set of OXPHOS genes involved in generating, maintaining, and utilizing the mitochondrial inner membrane proton gradient (ΔP)

Animal - fungal				Protists										Multicellular plants															
- Plastids				+ Plastids																									
Multicellular animals				Fungi				Yeasts				Chlorophyta			Streptophyta				Embryophytes										
												Rhodophytes	Haptophytes	Prasinophytes	Trebouxioophytes	Chlorophytes	Charophytes												
Gene	Vrtb	Dya	Nem	Hdi	Ncr	Scy	Mbr	Apa	Hmf	Ppo	Ram	Ppy	Ppu	Ehu	Mvi	Nol	Pmi	Pwi	Cre	Ceu	Cvu	Mpo	Osa	Tae	Zma	Ath	Bvu	Bna	Nla
Complex I																													
	nad1	+	+	+	+	+	+/−	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	nad2	+	+	+	+	+	+/−	+		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	nad3	+	+	+	+	+	+/−	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	nad4	+	+	+	+	+	+/−	+		+	+		+	+	+	+	+	+											
	nad4L	+	+	+	+	+	+/−	+		+	+		+	+	+	+	+	+											
	nad5	+	+	+	+	+	+/−	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	nad6	+	+	+	+	+	+/−	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	nad7								+	+	+				+														
	nad8								+		+																		
	nad9							+							+														
	nad10										+					+													
	nad11										+																		
Complex II																													
	sdh2										+		+																
	sdh3										+		+		+						+							ψ	+
	sdh4										+		+		+						+							ψ	+
Complex III																													
	cob	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Complex IV																													
	cox1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	cox2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	cox3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	cox11										+																		
Complex V																													
	atp1										+				+						+					+	+	+	+
	atp3										+																		
	atp4													+															
	atp6	+	+	+	+	+	+	+					+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	atp8	+	+	+	+	+	+	+			+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	atp9						+	+			+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CytcBg																													
	ccmB										+										+					+	+	+	+
	ccmC										+										+					+	ψ	+	+
	ccmFN																				+					+	+	+	+
	ccmFC										+															+	+	+	+

Table 1 (Continued)

Animal - fungal				Protists										Multicellular plants									
				- Plastids				+ Plastids															
								Chlorophyta										Streptophyta					

^aMitochondria can be divided into those residing in aerobic and anaerobic organisms. This table reports the mtDNA polypeptide gene sets of the aerobic organisms. The only mtDNA sequence that has been determined from an anaerobic organism is from *Nyctotherus ovalis*. Although the sequence is incomplete, this mtDNA is known to encode complex I subunits but probably not complex III or IV subunits (65). In the table, the top of the columns gives the overall phylogenetic relationship of the various organisms. Plastids are chloroplasts or their evolved derivatives. The Streptophyta represent the land plants (Embryophyta) and their algal progenitors (Charophytes). The Chlorophyta are the other green algae. The Rhodophytes are red algae. *Plasmodium* (Pyo), the malarial parasite, which is from the phylum Apicomplexa, all of whose members contain an additional DNA-containing organelle, the apicoplast. The multicellular animal mtDNAs were derived from yeast-fungal progenitors giving the animal-fungal mtDNA lineage. The organism with the most mtDNA protein-coding genes is *Reclinomonas americana* (Ram), which is placed between the nonplastid and the plastid-containing lineages, with the plant and animal mtDNAs positioned on opposite sides of the table. The three-letter codes along the top of the table are abbreviations for the individual species or groups of organisms whose mtDNA gene constituents are listed.

^bThe entries in the columns of the table indicate the presence of genes (+) in the mtDNAs of the organism or group of organisms indicated at the top of the columns. The entries along the left side of the table indicate the mitochondrial protein complexes of interest and the individual genes within that complex that have been found in the mtDNAs of various organisms.

^cAbbreviations: Cnplex, OXPHOS complex; Cytb, cytochrome *c* biosynthetic pathway; SmRib, the small subunit of the mitochondrial ribosome; LrgRib, the large subunit of the mitochondrial ribosome; other, additional genes found in the mtDNAs of an organism that are rarely found in other mtDNAs; *Nad*, NADH dehydrogenase genes; *adh*, succinate dehydrogenase genes; *cob*, cytochrome *b* gene; *coxI*-3, COI-III genes; *atp6* and -8, ATP6 and ATP8 genes; *camB*, *D*, *FN*, *FC*, cytochrome *c* biogenesis genes; *S5*, ribosomal protein gene; *rps*, ribosomal protein small subunit genes; *rpl*, ribosomal protein large subunit genes; *mat- τ* , functional gene; *mtb*, (*TarC*, *OrfX*) inner membrane protein export channel gene; *dnm*, a DNA methyltransferase gene; *ymf39*, functional gene. The abbreviations of the organism mtDNAs reported are Vrtb, vertebrate animals (69-71); Dya, *Drosophila yakuba* (72); Nem, class Secernentea, nematodes, including *C. elegans* and *Steinernema carpocapsae* (73); Hdi, *Hymenolepis diminuta*, Tapeworm (74); Ncr, *Neurospora crassa*, *atp9* homologue in both nDNA and mtDNA (75-78); ScY, *Schizosaccharomyces octosporus*, *Schizosaccharomyces japonicus*, *Saccharomyces cerevisiae*, which lack a proton-translocating complex I (79) versus *Yarrowia lipolytica*, which retains a proton-translocating complex I (80); Mbr, *Monoiga brevicollis*, choanoflagellate (81); Apa, *Amoebidium parasiticum*, ichthyosporan (81); Hmf, Hemoflagellates: *Leishmania tarentolae*, *Critidia fasciculata*, *Trypanosoma brucei*, *Trypanosoma borreli* (82); Ppo, *Physarum polycephalum* (83); Ram, *Reclinomonas americana* (67, 68); Pyo, *Plasmodium yoelii* (84); Ppu, *Porphyra purpurea*, Rhodophyte alga (85); Ehu, *Emiliania huxleyi*, Haptophyte alga (86); Mvi, *Mesostigma viride*, Prasinophyte alga (87); Noli, *Nephroselmis olivacea*, Prasinophyte alga (88); Pmi, *Palinomonas minor*, Prasinophyte alga (88); Pwi, *Prototheca wickerhamii*, Chlorophyte alga (89); Cre and Ceu, *Chlamydomonas reinhardtii* and *Chlamydomonas eugametos*, Chlorophyceae, Chlamydomonadales algae (88, 90); Cvu, *Chara vulgaris*, Charophyte alga (91); Mipo, *Marchantia polymorpha*, liverwort, Bryophyte (92); Osa, *Oryza sativa* L., Rice (93); Tae, *Triticum aestivum*, Wheat (94); Zma, *Zea mays*, Corn (95); Ath, *Arabidopsis thaliana* (96); Bvu, *Beta vulgaris* L., Beet (97); Bna, *Brassica napus* L., Rapeseed (98); Nia, *Nicotiana tabacum*, Tobacco (99).

^dThe "core" *cob* and *coxI* genes have been found in every aerobic eukaryotic mtDNA analyzed to date and are highlighted in orange; the *cox2* and -3 genes have been found in most mtDNAs and are highlighted in green; the complex 1 genes found in most mtDNAs (*atp1*-4, -4L, -5, -6) are highlighted in blue; and the complex V genes commonly found in most mtDNAs (*atp6*, -8, and -9) are highlighted in purple.

mitochondria studied to date, which employ an nDNA-encoded T bacteriophage-like RNA polymerase. *R. americanus* mtDNA also encodes a translation factor, a secretory pathway protein, and 27 ribosomal proteins. In addition, the *R. americanus* mtDNA encodes 12 subunits of complex I (*nad1-4*, *4L*, *5-11*), 3 of the 4 subunits of complex II (*sdb2-4*), 1 subunit of complex III (*cob*), 3 structural subunits of complex IV (*cox1-3*), plus the complex IV assembly gene *cox11*, 5 subunits of complex V (*atp1*, *atp3*, *atp6*, *atp8*, *atp9*), and four genes involved in cytochrome *c* biogenesis (*ccmB*, *C*, *FN*, *FC*) (67, 68).

The mtDNAs of other protists retain various subsets of the *R. americanus* mtDNA gene complement. All organelle genomes sequenced to date have been found to harbor a large and small rRNA, and many encode one or more tRNAs, although some or all of the mitochondrial tRNAs may be nDNA-encoded (100).

The organisms with the least number of mtDNA polypeptide genes are the Chlorophyte Chlamydomonads and the partially related human apicomplexan parasites *Plasmodium* and *Toxoplasma* (101).

Chlamydomonas reinhardtii and *Chlamydomonas eugametos* mtDNAs encode *cob* and *cox1* genes, as well as *nad1*, *-2*, *-4*, *-5* and *-6* genes. They do not retain the *nad 3* or *-4L* genes, which are the smallest of the complex I polypeptides. The *cox2* gene is nuclear, having been split into two separate genes, *cox2a* and *cox2b* (COXIIA and COXIIB polypeptides) (102), and the *cox3* gene (103) and the *atp6* genes are also nDNA-encoded (104).

Like the Chlamydomonads, the apicomplexan parasites have a split *cox2* gene (*cox2a* and *cox2b*). Because apicomplexan *cox2a* and *cox2b* genes (105) are homologous to those of *C. reinhardtii*, it is possible that they were acquired from the Chlorophyte alga by lateral transfer (101). The apicomplexan parasites derive their name from the presence of an additional DNA-containing organelle, the apicoplast. The apicoplast DNA encodes

30 proteins, rRNAs, and tRNAs and contributes to type II fatty acid biosynthesis, isoprenoid biosynthesis, and heme biosynthesis (105–108).

The best characterized of the apicomplexan parasite mtDNAs is that of *Plasmodium yoelii*, an intracellular parasite of blood erythrocytes, which also parasitizes its insect vector and thus lives in two different but otherwise relatively stable environments. The *P. yoelii* mtDNA is highly reduced, having been reported to generate six major mtDNA transcripts, five ascribed to *cox1*, *cox3*, *cob*, and small and large rRNA genes (84). The genomic sequence of the nDNA has revealed all of the genes for the F1 catalytic component of complex V as well as the F0 “c” (*atp9*) polypeptide. Although the a (*atp6*) and b subunits of the complex V F0 have not yet been found, the presence of the *atp6* in the nDNA of the Chlamydomonads (104) suggests that these genes might be present in a modified form in the *Plasmodium* nDNA. The *Plasmodium* nDNA and the mtDNA also lack the genes for a proton-pumping complex I. Instead, the nDNA encodes a single polypeptide NADH dehydrogenase that can reduce coenzyme Q from NADH but does not couple the energy released to proton transport (105). A similar single polypeptide NADH dehydrogenase has also been found in the fission yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (109), though not in the yeast *Yarrowia lipolytica* whose mtDNA retains the *nad 1-4*, *nad4L*, and *nad5* and *-6* genes of the proton pumping complex I (80).

The hemoflagellate (*Trypanosoma*, *Leishmania*, *Crithidia*) mtDNAs also lack complex V subunits and are obligate parasites, with mammalian and insect hosts (Table 1). However, the hemoflagellate mtDNAs retain the *cob*, *cox1-3*, and *nad1*, *-3*, *-4*, and *-5* genes (Table 1).

All other mitochondria from oxidative organisms have been analyzed to date, including protists and higher animals and plants that have the same “core” array of protein genes.

These are *nad1-4*, *-4L*, *-5*, and *-6* of complex I; *cob* of complex III; *cox1-3* of complex IV; and *atp6*, *-8*, and *-9* of complex V. In addition, many protists and all higher plant mtDNAs encode multiple proteins of the small subunit (*rps*) and large subunit (*rpl*) ribosomal proteins. While multiple ribosomal protein genes are found in the multicellular plant (Embryophyta) mtDNA, they are strikingly absent in the multicellular animal and fungal mtDNAs. The Charophyte algae, *Chara vulgaris*, which is encompassed within the Streptophyta and thought to be the closest algal ancestor to the Embryophyta, has an mtDNA with many features similar to those of the higher plants (91).

The striking variability in protista mtDNA gene content, but the relative uniformity of the plant mtDNAs with their high polypeptide gene numbers (92–99), and the animal mtDNAs with their low polypeptide gene numbers (69, 72–74), supports the hypothesis that interorganellar gene exchange continued as an evolutionary strategy in the radiation of the single-cellular protists, but slowed in multicellular eukaryotes, which shifted their adaptive strategy from intracellular gene reorganization to the elaboration of increasingly complex multicellular structures (3). By this logic, multicellularity arose within the Charophyte algae at an earlier stage in the evolutionary reduction of the mtDNA than occurred when multicellularity arose in the fungal-animal lineage. Thus, the plant mtDNAs became established with more mtDNA genes remaining.

Still, mtDNA gene loss continues in both the animal and the plant mtDNA lineages. *Nicotiana tabacum* (99) still retains two of the complex II genes (*sdb3* and *sdb4*) as does *C. vulgaris*, whereas all of the other multicellular plants [*Marchantia polymorpha* (92), *Zea mays* (95), *Oryza sativa* (93), *Triticum aestivum* (94), *Beta vulgaris* (97), *Brassica napus* (98), *Arabidopsis thaliana* (96)] have lost the functional copies of these genes. Similarly, *C. vulgaris* has almost all of the ribosomal protein genes found in multicellular plant mtDNAs, but various

plant species have lost one or more of these proteins.

That gene transfer actually can occur in multicellular plants has been documented by the discovery that a functional *cox2* gene has been transferred to the nDNA in certain legumes. An nDNA *cox2* gene has been cloned and characterized from soybean (110) and in cowpea (38). In both cases, the nDNA *cox2* copy is the RNA-edited copy from the mitochondrion, indicating that the gene transfer occurred via RNA intermediated retrotransposition. In the nDNA *cox2* gene, the “mitochondrial” *cox2* amino acid sequence is extended with an N-terminal peptide with features of a mitochondrial targeting peptide. In the nDNA, this targeting peptide is separated from the “mitochondrial” amino acid sequence by an intron, suggesting that the “mitochondrial” cDNA became a functional nDNA-encoded mitochondrial gene by integration into an exon downstream from a preexisting nDNA “mitochondrial targeting exon.” The mtDNA copy of *cox2* in soybean is still present and has the appearance of a functional gene, although its expression has not yet been shown (110). By contrast, an mtDNA copy of *cox2* in cowpea can no longer be detected (38). Hence, the *cox2* genes of the two legumes appear to be at different stages in the process of transition from mtDNA gene, to mtDNA + nDNA gene, to exclusively nDNA gene (5).

Although the fungal-animal lineage has many fewer genes, the yeast and fungi mtDNAs can encode one ribosomal protein that is lost in multicellular animals. Likewise, the fission yeast have both *atp8* and *atp9* genes, but in all animal cells, the *atp9* gene is nDNA encoded. Interestingly, *N. crassa* has two *atp9* genes, one in the mtDNA and the other in the nDNA copy. Both appear functional; the nDNA copy is expressed in vegetative cells, whereas the mtDNA copy is expressed during conidial germination (75). Perhaps the soybean nDNA and mtDNA *cox2* genes are also expressed at different developmental times or stages.

Finally, within the multicellular animal lineage the *atp8* gene appears in the mtDNAs of every animal group except the class Secernentea (nematodes) (73). Hence, this gene must have been transferred to the nucleus during the evolution of this class.

All of the above organisms are aerobic and presumably use OXPHOS to generate ATP at some time in their life cycle. Because O₂ is their terminal electron acceptor, they must retain mitochondrial complexes III and IV and, consequently, the *cob* and *cox1* genes. However, other eukaryotic cells have become specialized for life in anaerobic environments, necessitating a major change in the mitochondrial physiology, and giving rise to the hydrogenosomes.

Most hydrogenosomes lack an organellar DNA (64). However, the double membrane hydrogenosomes of *N. ovalis*, an anaerobic ciliate that inhabits the cockroach hindgut, have cristae, cardiolipin, and an organellar DNA. A portion of the *N. ovalis* hydrogenosome DNA has been sequenced and found to encode a 12S rRNA, three large ribosomal subunit proteins, and the complex I subunit genes *nad2*, *nad4L*, *nad5*, and *nad7*. The host cell nucleus has also been found to encode additional complex I subunits, the complex II subunits *sdhA* and *sdhB*, and multiple other typical mitochondrial metabolic and protein import genes. Therefore the *N. ovalis* hydrogenosome is clearly of mitochondrial origin.

N. ovalis performs fermentations by donating a portion of its electrons to organic compounds such as pyruvate and fumarate to generate lactate and succinate. Other electrons are transferred to protons to produce molecular hydrogen. *N. ovalis* hydrogenosomes have an active rotenone-sensitive complex I, which oxidizes NADH and transfers the electrons to rhodoquinones 8 and 9. The energy that is released is used to pump protons out of the hydrogenosome inner membrane, generating an inner membrane ΔP . This is accomplished without complex III or IV, both of which *N. ovalis* lacks (64–66).

Four general principles can be gleaned from this genomic analysis. First, there is strong selective pressure for genes to be transferred from the mtDNA to the nDNA. This is apparent from the large drop in the number of genes from the genome of *R. prowazekii* to the mtDNA of *R. americanus* and from the mtDNA of *R. americanus* to the mtDNAs of other protists, plants, and animals. It is also demonstrated by the transfer of *atp6* and *cox3* to the nDNA of *C. reinhardtii* and the transfer of *cox 2* to the nDNA in *C. reinhardtii*, the apicomplexa, and the legumes.

Second, the rate of gene transfer from the mtDNA to the nDNA virtually stops when it reaches a relatively uniform set of integral inner membrane polypeptide genes. These commonly retained genes are all inner membrane subunits of the four proton-translocating OXPHOS complexes: *nad1–4*, *-4L*, *-5*, and *-6* for complex I, *cob* for complex III, *cox1–3* for complex IV, and *atp6*, *-8* and *-9* for complex V.

Third, depending on the species' niche, certain proton-translocating complexes can become unnecessary and lost, resulting in the deletion of both the nDNA and mtDNA subunits. The proton-translocating complex I has been deleted from some yeasts, although not others, and the proton-translocating complexes III and IV have been deleted from the mtDNA and, presumably, the nDNA of the *N. ovalis* hydrogenosome.

Finally, in organisms that occupy relatively stable environments, all of the genes for certain complexes can be moved to the nucleus. This is the case for the complex V genes of the apicomplexan and hemoflagellate parasites. It is probably also true for the complex I gene subunits in those anaerobic organisms in which the hydrogenosome has lost its mtDNA.

These four principles suggest that two major factors may oppose the selective drive to transfer the mtDNA OXPHOS genes to the nDNA. First, the genes retained by the mtDNA probably play an integral role in

inner membrane proton translocation. Second, organisms that must cope with variable environments are more likely to retain genes of the proton-translocating complexes in their mtDNAs than organisms that live in more uniform environments.

PREVIOUS HYPOTHESES ON WHY THERE ARE mtDNA GENES

Four main hypotheses have been put forward to explain why certain membrane subunits of the OXPHOS complexes have been retained in the mtDNA (5). Three of these hypotheses have been strongly influenced by the retention of the *cob* and *cox1* genes in all oxidative organism mtDNAs.

The first hypothesis is that the current mtDNA polypeptide genes became “frozen” in the mtDNAs because the mtDNA genetic codes diverged from that of the nDNA, thus rendering the mtDNA polypeptide genes uninterpretable by the nuclear-cytosolic system (3, 111). The second hypothesis is that certain core mtDNA proteins have been retained to ensure that the OXPHOS complexes are only assembled in the mitochondrial inner membrane, either because the mtDNA proteins provide an essential scaffold for assembly of the complexes or because relocation of the OXPHOS complexes at inappropriate sites could generate ROS that would be destructive (34, 112). The third hypothesis is that the retained mtDNA proteins are simply too hydrophobic to be translated in the cytosol and then to be appropriately incorporated in the mitochondrial inner membrane. Rather, they might be misdirected to another compartment such as the endoplasmic reticulum secretory apparatus (113). The final hypothesis argues that the remaining mtDNA-encoded polypeptides contain regions (mesohydrophobic regions) that are too hydrophobic to be imported into the mitochondrion through the mitochondrial protein import machinery (114, 115).

Are the mtDNA Genes Retained Because of Altered Genetic Codes?

The initial discovery that both yeast (116) and human (69) mtDNAs had altered genetic codes suggested that all mtDNAs might have altered genetic codes, rendering the remaining mtDNA genes unable to function in the nucleus (3). The key genetic code alteration that made this argument compelling was the suppression of the *opal* stop codon, 5'-UGA, by modification of the tryptophan tRNA. In the fungal-animal mtDNAs, the anticodon of the tryptophan tRNA is changed from 3'-ACC to 3'-ACU, permitting it to read both the traditional tryptophan codon, 5'-UGG, as well as the adjacent *opal* codon, 5'-UGA.

The suppression of the UGA *opal* stop codon is also seen in the mtDNAs of the protists thought to be the progenitors of the fungal-animal lineage (*Amoebidium parasiticum* and *Monosiga brevicollis*), as well as in other protist groups (81), including the hemoflagellate mtDNAs. The hemoflagellates import their tRNAs from the nDNA, and the anticodon of the nDNA-encoded tRNA^{Trp} is edited in the mitochondrion to suppress the *opal* stop codon (117).

However, it was subsequently found that the majority of protist mtDNAs and all of the plant mtDNAs use the same genetic code as the nucleus-cytosol (100). Yet the core mtDNA genes are still retained by these mtDNAs. Therefore, the retention of the core mtDNA polypeptides cannot have been caused by alterations in the mtDNA genetic code.

If the suppression of the *opal* (UGA) stop codon is not universal, why has it arisen repeatedly in different mtDNA lineages? One possibility is that the preferential use of the *opal* stop codon for tryptophan by the mtDNA would block the spurious translation of mtDNA-encoded mRNAs that escape into the cytosol (3). This concept is supported because there is a 15-fold predilection of the mammalian mtDNA to use the UGA codon

for tryptophan and because mammalian mtDNAs have deleted the tRNA^{Arg}(AGG/AGA) gene, thus creating two new stop codons. Since the cytosol commonly uses the AGG and AGA codons, the loss of the tRNA^{Arg}(AGG/AGA) gene would effectively block the translation of cytosolic mRNAs in the mitochondrial compartment (3).

Are the Core Proteins Retained to Assure Regional Complex Assembly?

It is clearly essential that the mitochondrial OXPHOS complexes assemble in the correct compartment (34, 112, 113). However, the efficiency and fidelity of the modern mitochondrial protein import systems appear more than adequate to accomplish this task (40, 41). Hence, this probably is not the reason why organisms have retained an mtDNA.

Are the Core mtDNA Proteins Retained Because of Mesohydrophobicity?

Considerable experimental evidence has been accumulated over the past 10 years that regional hydrophobicity in the mtDNA-encoded polypeptides can block their import into the mitochondrion. This is particularly true for cytochrome *b*, which has eight transmembrane domains, and presumably is also true for COI, which has twelve. Two parameters are used to determine the likelihood that a protein will be imported by the current mitochondrial import apparatus, the maximum hydrophobicity of any 17-amino acid stretch $\langle H \rangle_{17}$ and the mesohydrophobicity, the maximum average hydrophobicity over a range of 60–80 amino acids $\langle H \rangle_{60-80}$. These two parameters have been calculated for a range of mitochondrial proteins, both mtDNA or nDNA encoded, and the values plotted with $\langle H \rangle_{17}$ as the abscissa and $\langle H \rangle_{60-80}$ as the ordinate axis. This generates an arc across the plot such that proteins that fall above the arc are less likely to be imported into the mitochondrion, whereas those that fall below the arc

are more likely to be imported (114, 115). For example, the yeast cytochrome *b* polypeptide falls above the arc, and the nDNA-encoded *Chlamydomonas* COXIII (103, 118) and ATP6 (104) polypeptides fall in the intermediate zone.

Direct experimental analysis has confirmed that the mesohydrophobic status of a protein can predict its capacity to be imported into existing mitochondria. The yeast mtDNA-encoded bI4 maturase protein has been recoded to conform to the nuclear-cytosol genetic code and inserted into a plasmid for introduction into the nucleus. To the bI4 maturase, a mitochondrial-targeting peptide can be added to the N-terminal end, and different segments of the recoded cytochrome *b* can be added to the C-terminal end. Such constructs were transformed into yeast that were respiratory deficient because of mutations in the mtDNA-encoded BI4 maturase gene, thus causing a defect in splicing of the appropriate mtDNA mRNA. If the addition of the cytochrome *b* protein sequences cause the inhibition of mitochondrial import of the bI4 maturase, then splicing cannot occur, and the yeast will remain respiratory deficient and not able to grow on oxidizable substrates. Using this system, it was shown that addition of all eight of the cytochrome *b* transmembrane domains blocked mitochondrial import as did addition of cytochrome *b* transmembrane domains 1 to 5 or 6 to 8. Duplication of the N-terminal targeting sequences increased import but was still insufficient to permit import of the entire cytochrome *b* protein. Therefore, the mesohydrophobicity of the cytochrome *b* transmembrane domains 5 to 8 proved to be too high to permit import by this yeast mitochondrial polypeptide through the protein import system (115).

In mammalian systems, difficulty has also been reported in introducing the recoded human mtDNA *cob* and *nad4* genes into the nucleus and having the cytochrome *b* and ND4 proteins imported into mitochondria. This was true even when the mtDNA genes were fused to the 43-amino acid N-terminal

targeting peptide from the highly hydrophobic nicotinamide nucleotide transhydrogenase gene. However, the human *atp8* gene was successfully introduced into the nucleus, and its protein was transported back into the mitochondrion (119). Furthermore, the above negative results for the ND4 protein have been countered by an independent study that reported the successful recoding of the human mtDNA *nad4* gene, its transduction into the nDNA, and the import of the ND4 back into the mitochondrion (120). Moreover, another study reported the successful recoding of the human mtDNA *atp6* gene, its introduction into the nDNA, and its successful transport back into the mitochondrion (121).

From a clinical perspective, these studies are of great importance, for there are a wide range of pathogenic mtDNA mutations (28), which could potentially be treated by simply introducing a normal copy of the gene into the nucleus and having the functional protein imported back into the mitochondrion to complement the biochemical defect (122). Common mtDNA diseases that could be treated in this manner are LHON, resulting from the mtDNA *nad4* nt 11778 G>A mutation (55, 120), and the NARP and Leigh syndromes, resulting from the *atp6* nt 8993T>G mutation (56, 121). Unfortunately, many pathogenic mutations have also been found in the mtDNA *cob* and *cox1* genes (28), including four germ line *cox1* mutations associated with prostate cancer (123). The prostate cancer *cox1* variants are of particular relevance because by causing a male-specific disease, there is no strong selective pressure for the elimination of the pathogenic alleles from the maternal lineage (124). Therefore, developing a system to transfect prostate tissue with an nDNA-encoded functional *cox1* might have provided a valuable cancer prevention therapy.

Although mesohydrophobicity is of considerable relevance to the development of mitochondrial gene therapy, it may not be centrally important to the question of why OXPHOS genes have been retained in the

mtDNA. Just because the current mitochondrial protein import systems cannot import the mtDNA-encoded cytochrome *b* polypeptide does not mean that it is the physical nature of the cytochrome *b* protein that blocked its transfer to the nDNA. It is just as likely that it was evolutionarily beneficial to retain the *cob* and *cox1* genes in the mtDNA, and thus it was unnecessary for evolution to devise a mitochondrial protein import system to import the cytochrome *b* and COI polypeptides into the mitochondrion. After all, the key import translocases (TOM, TIM22, and TIM23) did not preexist in the α -proteobacterium but evolved as the symbiosis became established (41). Therefore, if it had been advantageous for the *cob* and *cox1* genes to be in the nDNA, the TOM and TIM complexes could have evolved to make this practicable.

This contention is supported by direct experimental evidence. The mammalian *atp8* (119), *atp6* (121), and *nad4* (120) genes have already been recoded and introduced into the nDNA, and the proteins successfully inserted back into the mitochondrial inner membrane. Similarly, the yeast (*S. cerevisiae*) mtDNA *atp8* and *atp9* genes and the *Aspergillus nidulens* mtDNA *atp8* gene have been successfully recoded, fused to one or two N-terminal targeting peptides, inserted into the yeast nDNA, and successfully imported back into the mitochondrion (125–127).

More significantly, yeast cells respiratory deficient owing to bI4 maturase mutations and harboring an nDNA fusion gene containing the bI4 maturase fused to the cytochrome *b* hydrophobic domains 5 + 6 (pS56HA1) were selected for growth in non-fermentable substrates, and mutants were isolated. These respiratory competent mutants were found to have acquired the ability to import the maturase-cytochrome *b* fusion protein into the mitochondrion. One mutant overexpressed a truncated form of the karyopherin β Pse1p/Kap121p protein (*PSE1*), retaining the N-terminal 600 amino acids of the 1089-amino acid protein (*PSE1-t*). In cells that did not overexpress *PSE1* or *PSE1-t*

alleles, 90% of the maturase-cytochrome *b* chimera remained stuck on the outside of the mitochondria. However, in cells that overexpressed *PSE1* or *PSE1-t*, there was a five- to sevenfold increase in mitochondrial import of the bI4 maturase-cytochrome *b* domains' 5 + 6 chimeric protein, and 50% of the chimeric precursor protein was processed to the mature form and became resistant to exposure of the intact mitochondria to protease (128). Thus, with relatively minor modifications, major changes can be made in the ability of the mitochondrial protein import machinery to take up mesohydrophobic proteins.

Given over a billion years of mutational trials, occurring in countless eukaryotic cells, it seems likely that a mitochondrial protein import apparatus would have evolved that was capable of importing the cytochrome *b* and COI proteins if it had been evolutionarily beneficial for the organism to have the *cob* and *cox1* genes in the nucleus. The fact that the *nad1-4*, *-4L*, *-5*, and *-6* genes of complex I, the *cob* gene of complex III, the *cox1-3* genes of complex IV, and the *atp6*, *-8*, and *-9* genes of complex V have been consistently retained in the mtDNA indicates that there has been some selective advantage for keeping these genes in the mtDNA.

What advantage might have been derived from retaining selected membrane components of complexes I, III, IV, and V in the mtDNA? The answer may lie with the reaction product that they all share in common, ΔP . Clearly, the proton conductance of all four complexes must be balanced if ΔP is to be a stable source of potential energy for cellular energetics. If the proton channel of one of the ETC complexes mutated to become more leaky to the back flow of protons, this would short-circuit ΔP for the other complexes.

For the proton channels of these four complexes to remain in electrical balance, they must coevolve. For them to coevolve, they must be linked on a nonrecombining genomic element, the mtDNA. Because the mtDNA is maternally inherited, it can only change by sequential mutation. Therefore, each new mu-

tation that changes the coupling efficiency of one complex will influence the common reaction product, ΔP , of all of the complexes. Consequently, each new mutation will be tested by natural selection in the context of the pre-existing polymorphisms of the other proteins linked on the same mtDNA.

If there was one universal optimal set of proton conductances for complexes I, III, IV, and V, then the best strategy would have been to transfer all of the OXPHOS genes to the nDNA. There the optimal polypeptide sequences could be stabilized via the independent assortment of new mutation alleles and their elimination by natural selection. Indeed, this might explain why in certain species all of the complex V genes appear to be nDNA encoded. In these species, it may be beneficial to keep the ATP synthase genes invariant at the highest possible coupling efficiency and thus for the *atp6*, *-8*, and *-9* genes to be nuclear. Variation in the remaining mtDNA-encoded ETC proton channel proteins could still provide significant variation in mitochondrial coupling efficiency to permit adaptation to many environmental changes. Similarly, for eukaryotes living in anaerobic environments, variability in OXPHOS genes may no longer be adaptive. Therefore, the mtDNAs of the hydrogenosomes of most anaerobic organisms could transfer all of their genes to the nDNA and disappear.

By contrast, the vital role of mitochondrial energy production to free-living oxidative life forms would make it essential that mitochondrial energy metabolism be able to vary in concert with changes in the energetic demands of the local environment. Because mitochondrial ΔP impacts many cellular processes, including generation of ATP, production of heat, energization of cation (Ca^{++}) and protein import, provision of alternative redox and pH microenvironments, ROS generation, mtPTP stabilization, etc., the relative importance of these various uses of ΔP must differ in different environments. Therefore, to permit adaptation to varying energy demands, the retention of the genes for key proteins

in OPXPHOS coupling on the mtDNA and the maintenance of a relatively high mtDNA mutation rate to generate necessary variation make sense. Such intraspecific variation would not be apparent in comparisons of individual mtDNA sequences from very different species. Rather, mutation and selection act at the individual level. Therefore, adaptive variation would only be apparent in studies of intraspecific mtDNA variation. Fortunately, such data have been accumulated for *Homo sapiens*.

IMPORTANCE OF HUMAN mtDNA VARIATION IN ADAPTATION

Because of the strict maternal inheritance (129) and high mutation rate (23) of the human mtDNA, human mtDNAs have sequentially accumulated mutations on radiating maternal lineages since the time of their last common mtDNA ancestor, who existed between 150,000 and 200,000 years before present (YBP) (130–135). As a result, the mutational history of human mtDNA sequence variation can be reconstructed as a single sequential mutational tree radiating from a common origin (136).

As the mtDNA mutation tree was elucidated, it was discovered that the branches of the mtDNA tree correlated dramatically with the geographic origin of indigenous human populations. Although originally attributed to genetic drift, considerable evidence has since accrued that supports the conclusion that the nonrandom distribution of mtDNA variation is the result of natural selection, not statistical fluctuation (135, 137–141).

Human mtDNA Phylogeny of Polymorphic Variants

The unique correlation between mtDNA variation and human geographic origin was first observed when samples from Africans, Asians, and Europeans were analyzed for *HpaI* mtDNA restriction site polymorphisms,

and the mtDNAs of each population were found to have region-specific polymorphisms (137). Further characterization of restriction site polymorphisms revealed that all mtDNA types fit into a single tree (130), with the greatest mtDNA variation and root of the tree being in Africa (130–132) and with specific branches radiating into the different continents (138). The sequence variation found in a particular mtDNA is designated the mtDNA haplotype, and a group of related haplotypes, which form a discrete branch of the tree, is called a haplogroup. Haplogroups are defined by ancient sequence polymorphisms that occurred at the base of that particular branch of the mtDNA tree (133–135, 138) (**Figure 4**).

The most diverse and thus most ancient mtDNAs are found in Africa, with the sequence divergence giving an estimate of African occupation of between 150,000 to 200,000 YBP. African mtDNAs fall into four major haplogroups: L0 (oldest), L1, L2, and L3 (youngest). L0, L1, and L2 represent about 76% of all sub-Saharan African mtDNAs and are defined by a *HpaI* restriction site at nt 3592 (nt 3594T) (**Figure 4**). In northeastern Africa, two mtDNA lineages, M and N, arose from L3 about 65,000 YBP. These were the only mtDNA lineages that succeeded in leaving sub-Saharan Africa for Eurasia to give all of the Eurasian mtDNAs. In Europe, haplogroup N gave rise to haplogroups H (about 45% of European mtDNAs), T, U, V, W, and X (about 2%), as well as I, J (about 9%), and K (Uk). The European lineages arose about 40,000–50,000 YBP. In Asia, lineages M and N radiated to give rise to a plethora of mtDNA lineages. These include from N, haplogroups A, B, F, and others, and from M, haplogroups C, D, G, and others (135, 138).

As Asians migrated northeast into Siberia, haplogroups A, C, and D became progressively enriched until they predominated in the indigenous peoples of extreme northeastern Siberia, Chukotka. When the Bering land bridge appeared about 20,000 to 30,000 YBP, people harboring these mtDNA haplogroups were in a position to migrate into the

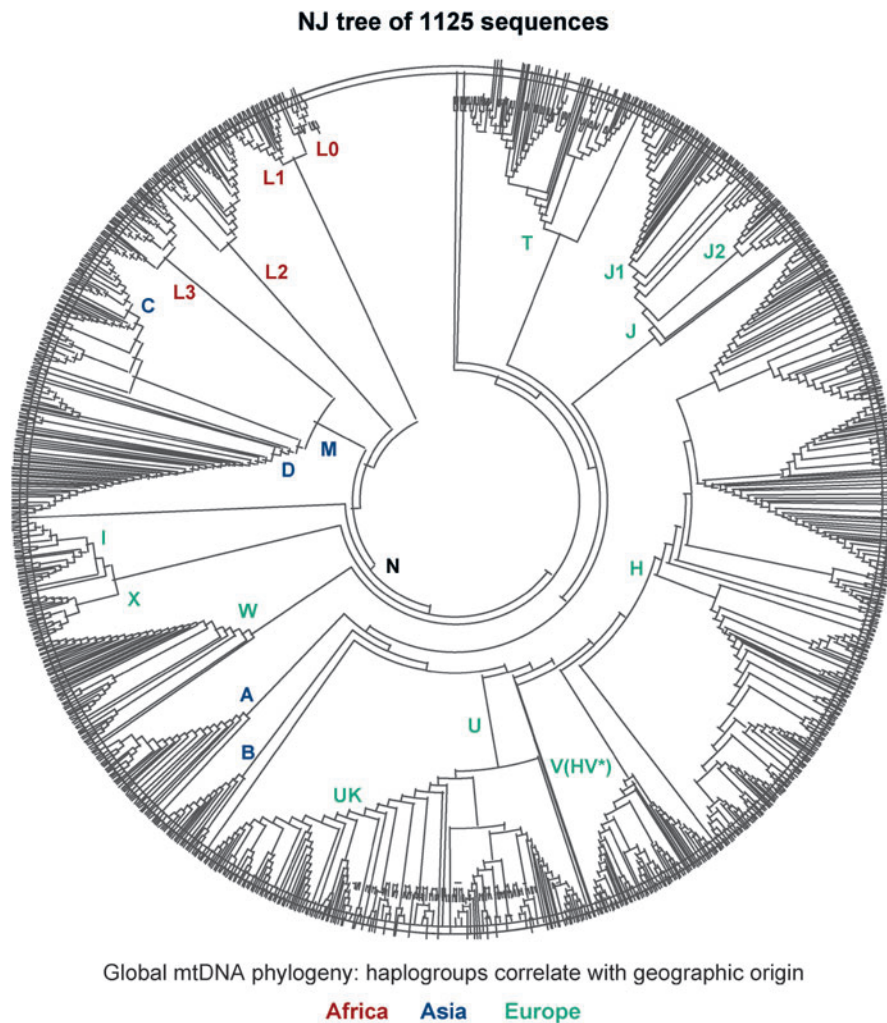


Figure 4

Phylogenetic tree of human mtDNA coding sequence variants, demonstrating regional association of haplogroups. Haplogroups, groups of related haplotypes, are derived from a founding haplotype, harboring characteristic mtDNA sequence polymorphisms. Each haplogroup is designated by a letter with or without a subdividing letter. In this tree, the ticks around the perimeter of the circle represent the individual mtDNA sequences. The internal radial lines that connect the ticks represent the relative number of mtDNA nucleotide changes that separate one mtDNA sequence from another. The total number of mutational differences between two mtDNAs is related to the sum of the lengths of the radial lines necessary to trace a path from one mtDNA to the other. The mtDNA haplogroups have proven to be highly geographically associated. The African haplogroups are L0–L3. These cluster together on the deepest branches of the tree, demonstrating the African origin of the mtDNAs (131, 132). Only two mtDNAs, M and N, left Africa and colonized all of Eurasia. M gave rise to the Asian mtDNA lineages C and D as well as multiple others. N gave rise to the Asian mtDNA lineages A and B plus others; all of the European lineages I, U, Uk, V(HV*), H, J, J1, J2, and T; as well as the Eurasian mtDNA haplogroup X. Of all of the Eurasian mtDNAs, only representatives of haplogroups A, C, D, and X survived the Arctic to found the Native American populations. Haplogroup B joined these haplogroups in the Americas by a sub-Arctic route (135, 139, 140). Abbreviation: NJ, neighbor joining. Reprinted from the supplemental material of Reference 139.

New World, where they founded the Paleo-Indians. After the land bridge submerged, haplogroup G arose in central Asia and moved into northeastern Siberia to populate the area around the Sea of Okhotsk. About 12,000 to 15,000 YBP, a migration carrying haplogroup B started from eastern Central Asia and moved along the coast to the New World, bypassing Arctic Siberia. Haplogroup B then mixed with A, C, and D in temperate and tropical North, Central, and South America. About 15,000 YBP, haplogroup X crossed the Arctic to the New World in a migration that settled in the Great Lakes region, such that today 25% of the Ojibwa mtDNAs are haplogroup X (138).

Later migrations from northeastern Siberia, carrying a modified lineage of haplogroup A, founded the Na-Déné populations about 9,500 YBP. More recently, immigrants from Siberia, bearing derived lineages of haplogroups A and D, moved along the Arctic Circle to found the Eskimos and Aleuts (138).

Adaptive mtDNA Variants in Human Evolution

The phylogeographic distribution of mtDNA haplogroups reveals two striking discontinuities in human mtDNA diversity. The first occurs between sub-Saharan Africa and Eurasia. Virtually all of the sub-Saharan African mtDNA diversity remained in Africa, whereas only derivatives of lineages M and N colonized temperate Eurasia. The second occurs between temperate Central Asia and Arctic Siberia and America, where the plethora of Asian mtDNA types is markedly reduced to only three ancient mtDNA lineages (A, C, and D). Thus, the major transitions in mtDNA types correlate most strongly with latitude. This suggests that climatic selection may have enriched for mtDNA variants that changed the coupling efficiency from predominantly ATP production to increased heat production, thus permitting humans to survive in the colder northern climates (135, 139–141).

Evidence that climatic adaptation has influenced the geographic distribution of

mtDNA diversity was first obtained by analyzing the amino acid replacement (nonsynonymous, NS) (K_a) to silent (synonymous, S) (K_s) mutation ratios (K_a/K_s) of the 13 mtDNA polypeptide genes in mtDNAs that were associated with tropical and subtropical Africa, temperate Europe, and Arctic Siberia. This revealed that the predicted amino acid sequence of the *atp6* gene was highly variable in the Arctic but was strongly conserved in the tropics and temperate zone; the predicted polypeptide sequence of the *cob* gene was hypervariable in temperate Europe but conserved in the tropics and Arctic; and the predicted amino acid sequence of the *cox1* gene was variable in tropical and subtropical Africa but invariant in the temperate and Arctic regions. Regional variation was also observed in the predicted amino acid sequences of various *nad* subunit genes (135). Such regional gene-specific variation would not be expected if all mtDNA mutations were neutral and had accumulated purely by genetic drift.

The geographic constraints on mtDNA protein variation were further validated by arranging all of the mtDNA variants from over two thousand complete mtDNA coding sequences in a sequentially mutational tree (136, 139). On the basis of the position of the sequence variant in the tree and the effect that the variant had on the protein function, the mtDNA variants could be divided in to three classes: (a) recent deleterious mutations, many of which altered functionally important amino acids and thus are potentially deleterious and pathogenic; (b) ancient neutral mutations, which were either synonymous or altered weakly conserved amino acids and thus were likely to be neutral or near neutral variants; and (c) ancient adaptive mutations, which changed highly evolutionarily conserved amino acids yet persisted in the population and thus were adaptive. The conclusion that this third class of variants represented adaptive mutations follows from findings that these amino acid substitutions changed amino acids that were at least as highly evolutionarily conserved as those

known to be pathogenic mutations, yet they persisted and expanded in the human population in the face of purifying selection. Specifically, the average interspecific amino acid conservation index (CI) of 22 known human pathogenic mtDNA replacement mutations is 93%. Of the ancient missense mutations, about 74% are weakly conserved with an average CI of 23% and probably represent neutral or near neutral variants. However, about 26% of the ancient amino acid substitutions alter highly conserved amino acids with an average CI of 85%. Thus, the distribution of ancient potentially adaptive polymorphisms substantially overlaps that of the known pathogenic mutations (139).

Confirmation that these polymorphic mtDNA variants must have been adaptive has come from multiple observations. First, these ancient missense mutations frequently initiate geographically constrained branches of the mtDNA tree, meaning that individuals carrying these mutations were able to survive and multiply in new geographic regions. For example, Macro-haplogroup N, which left tropical Africa to colonize temperate Eurasia, is founded by two missense mutations, the ND3 G10398A (which causes an A114T amino acid substitution) plus the ATP6 G8701A (which causes an A59T substitution), and these variants correlate with increased matrix Ca^{++} and reduced pH (184). Second, the relative frequency of ancient NS to S mutations increases from south to north, indicating that the change in environment has resulted in an increased rate of fixation of missense mutations. This differential is particularly striking for haplogroups that reside in the Arctic and sub-Arctic. The mean internal branch NS/S ratio for northeastern Siberian-North American haplogroups A, C, D, and X was 0.61, much higher than the mean ratio of 0.39 for the non-Arctic haplogroups, the mean of 0.31 for the African L haplogroups, or the mean of 0.38 for the Native American haplogroup B, which bypassed the cold selection of Siberia. Furthermore, the CI of the ancient missense mutations for mtDNAs

from colder climates is higher than that of mtDNAs from warmer climates with the CIs of 51% for the ancient variants of the Arctic haplogroups A, C, D, and X; 39% for all other global haplogroups; 36% for macrohaplogroup L; and 31% for haplogroup B (139, 140).

A similar trend is seen for the European branches of the mtDNA tree. The frequency of ancient replacement mutations (NS/S) for haplogroup H was 0.48, J was 0.66, and IWX was 0.63, whereas that for Africa was 0.31. The NS/S ratio for haplogroup T is only 0.31, but that is because T was founded by a single highly conserved founding replacement mutation in the *nad2* gene (np 4917) with a very high CI of 20.3. Thus, adaptive changes fall into two categories: either (a) the lineage accumulates multiple missense mutations, each changing a reasonably conserved amino acid, or (b) the lineage is founded by only a few amino acid substitution mutations, but each changing a highly conserved amino acid (139, 140).

Examples of the adaptive mtDNA mutations that occurred in the Arctic mtDNAs include two replacement mutations [*nad2* np 4824G (T119A) and *atp6* np 8794T (H90Y)] for haplogroup A; two replacement variants [*nad4* np 11969A (A404T) and *cob* np 15204C (I153T)] for haplogroup C; a *nad2* np 5178A (L237M) variant for haplogroup D; and a *nad5* np 13708A (A458T) variant for a sublineage of haplogroup X, which is also seen in haplogroup J (139, 140).

The European sister haplogroups J and T provide the clearest example of the two classes of adaptive mutation strategies: several less conserved mutations versus a few highly conserved mutations (**Figure 4**). Haplogroups J and T share a common root involving two amino acid substitutions: *nad1* np 4216C (Y304H) and *cob* np 15452A (L236I). The two haplogroups then diverge. Haplogroup T is founded by the single nodal adaptive mutation, *nad2* np 4917G (N150D), the most conserved ND2 polymorphism found (139).

Haplogroup J has two replacement mutations at its root: *nad3* np 10398G (T114A) and *nad5* np 13708A (A458T); the second is the same variant found in haplogroup X. Haplogroup J then splits into two subhaplogroups J1 and J2, each defined by a major *cob* mutation. The J2 *cob* variant is at np 15257A (D171N), and the J1 *cob* variant is at np 14798C (F18L). The np 14789C mutation is also found at the root of subhaplogroup Uk. The np 15257 and np 14789 variants alter well-conserved amino acids with CIs of 95% and 79%, respectively. The 15257 variant alters the Q_o site of complex III, which contacts the Rieske iron-sulfur protein, whereas the np 14798 site alters the Q_i of complex III (139). Because the Q_o and Q_i binding sites are essential for complex III proton pumping via the Q cycle, the np 14798 and 15257 variants may disconnect the electron flow through complex III with proton pumping. This would reduce the coupling efficiency of mitochondrial OXPHOS by one third, decreasing ATP production while proportionately increasing heat generation (139, 140).

That the internal branch mtDNA missense mutations are functionally relevant has been demonstrated by comparing the sperm mobility of males harboring different mtDNA haplogroups. Sperm flagellar motion is driven primarily by ATP, generated from the mitochondria in the midpiece. Therefore, sperm with partially uncoupled mitochondria should swim slower than those with coupled mitochondria. As expected, sperm from haplogroup H subjects swam significantly faster than those from T subjects (142), and those of haplogroup U that harbor *cob* missense mutations swam significantly more slowly than those without (143). Moreover, individuals that harbor uncoupling *cob* missense mutations occur in higher frequencies in northern Europe than in southern Europe. This is consistent with people, who generate on average more heat, wishing to live in colder climates (143). Thus, the functional mtDNA variants that founded specific mtDNA lin-

eages appear to affect mitochondrial physiological functions.

Further evidence for the physiological importance of the ancient mtDNA polymorphic variants comes from clinical observations. Multiple studies have demonstrated that the European haplogroup J is enriched in the extremely old and thus seems to slow the aging process (144–147). A similar observation has been made for haplogroup D in the Japanese (148, 149).

Haplogroups J and Uk have been found protective for Parkinson's disease (150, 151), and haplogroup T is underrepresented in Alzheimer's disease patients (152–154). The frequent association of haplogroups J1 and Uk with longevity and neuroprotection correlates with the fact that both subhaplogroups independently acquired the same *cob* polymorphism at nt 14798. Conversely, haplogroup T is associated with increased risk for bipolar affective disorder (155), and haplogroup J increases the penetrance of the milder mtDNA mutations associated with LHON (156–159).

That the same mtDNA polymorphisms can be associated with increased life span and protection against neurodegenerative diseases on the one hand, yet increase the predilection for developing other degenerative diseases on the other hand, might be explained by the interaction between mitochondrial energy production, ROS production, and the mtPTP activation of apoptosis. Assuming that the haplogroup J and Uk polymorphisms partially uncouple OXPHOS, they would cause the mitochondria to burn excess calories to generate heat, thus leaving fewer excess electrons to generate ROS. This would be protective of neurodegenerative diseases and aging, which are thought to be caused by the accumulation of somatic mtDNA mutations resulting from elevated mitochondrial ROS damage (2, 160–162). By contrast, these same partial uncoupling mutations would reduce the efficiency of ATP production. This would render the individuals more prone to diseases caused by ATP deficiency (2).

Because the human mtDNA also encodes the tRNA and rRNA genes necessary for mitochondrial protein synthesis, it would be logical that certain sequence variants in tRNA and rRNA genes would also be adaptive. Applying similar principles to the human mtDNA tRNA and rRNA gene polymorphisms as were used for the polypeptide polymorphisms, it was found that about 19% of the tRNA stem variants and 13% of the tRNA loop variants were potentially adaptive (163). Several tRNA and rRNA polymorphisms are particularly noteworthy. The tRNA^{Leu(CUN)} variant nt 12308A>G is a founding sequence variant of the large European haplogroup U. The tRNA^{Gln} nt 4336 A>G variant defines a subhaplogroup of H. Similarly, the 16S rRNA nt 1811A>G defines a major subhaplogroup of U, which encompasses the clinically important lineage Uk. Furthermore, the 16S rRNA nt 3010G>A variant is a defining polymorphism for mtDNA haplogroups and subhaplogroups C, L2a, U3, H1, J1, and D4. In fact, this same variant has recurred 15 times in 10 different haplogroups, 6 of which are in internal branches of the mtDNA tree.

That these protein synthesis variants are functionally significant again comes from clinical studies. The tRNA^{Leu(CUN)} nt 12308A>G has been reported to affect the pathogenicity of mtDNA rearrangement mutations (164), the tRNA^{Gln} nt 4336A>G variant was correlated with increased risk for Alzheimer's and Parkinson's diseases (2, 165), and the 16S rRNA 1811A>G variant was associated with alterations in sperm motility (143, 163).

Given that mtDNA variation can be adaptive in humans, what are possible local environmental factors for which mtDNA variation could prove beneficial? On a global scale, mtDNA variants that caused changes in coupling efficiency would shift the calorie allocation between ATP production and heat generation. Generally, then, individuals who live in uniformly warm environments would benefit from having tightly coupled OXPHOS, which maximizes ATP production and min-

imizes heat generation. By contrast, individuals exposed to extreme cold, such as in the Arctic, could benefit from shifting calorie utilization away from maximum ATP production and toward greater heat production to survive periods of extreme cold stress.

The regulation of calorie allocation between ATP and heat production may be only one of a large number of potentially adaptive effects of changes in OXPHOS coupling efficiency. For example, it has been observed that individuals harboring haplogroup H are more likely to survive sepsis (166). This could be because the more tightly coupled haplogroup H mitochondria would generate more ROS, which could inhibit systemic infection.

The adaptive consequences of mtDNA variation appear to be acting at the cellular as well as the organismal level. A meta-analysis of the somatic mtDNA mutations, found in cancer cells, has revealed that 52% of the polypeptide mutations, 83% of tRNA mutations, 38% of rRNA mutations, as well as 85% of the control region mutations proved to be the same sequence variants as those found to be polymorphic in population studies. This suggests that a significant proportion of cancer mtDNA mutations may be "adaptive." This follows logically from the fact that cancer cells must pass through two energetic stages in their evolution. The initial mtDNA mutations may inhibit OXPHOS, thus increasing ROS production and driving tumor cell proliferation (62). However, once the tumor starts to grow, then it will deplete surrounding caloric substrates and O₂. Consequently, new adaptive mtDNA mutations would be selected to permit the cancer cell to continue to survive, grow, and metastasize in ever changing environments (167, 168).

The high mutation rate of the animal mtDNA means that functionally significant mutations arise frequently in the population. For populations living in an environment for which they are optimally adapted, most of these mutations will be less fit than the norm and result in disease, which is probably why mtDNA disease is so common in humans

(28). However, this same high mutation rate would provide considerable population variation, which could permit some individuals to survive a sudden change in the environment. Hence, the high mammalian mtDNA germ line mutation rate may represent a compromise between generation of sufficient adaptive variation to increase the likelihood that the species will survive catastrophic environmental change, versus the suppression of the mtDNA mutation rate sufficiently to ensure that the accumulation of deleterious mutations along maternal lineages does not lead to extinction as a result of Muller's ratchet.

The high mammalian mtDNA germ line mutation and segregation rates plus the pivotal role of OXPHOS in regulating energy metabolism make the mtDNA the ideal system for fostering adaptation to rapidly changing environments. Therefore, the selective pressure for the retention in the mtDNA of the membrane subunits of the proton-translocating OXPHOS complexes (*nad1-4*, *-4L*, *-5*, and *-6* subunits of complex I, *cob* of complex III, *cox1-3* of complex IV, and *atp6*, *-8* and *-9* of complex V) may be to provide a pliable system that permits rapid adaptation to environmental change by eukaryotic organisms.

Although the transfer of genes to the nDNA greatly decreases their rate of sequence variation, it does not mean that nDNA-encoded mitochondrial genes do not play a role in adaptation. It just means that the time frame is much greater. In fact, an analysis of the predicted polypeptide sequence variation of the 39 nDNA-encoded and 7 mtDNA-encoded complex I genes for the great ape lineages (human, chimpanzee, gorilla, and orangutan) revealed significant adaptive changes in the nDNA genes for the NDUFA1 and NDUFC2 proteins between the root of the great ape lineage and other mammals and for the nDNA genes for the NDUFA1 and NDUFA4 proteins when the human-chimp-gorilla lineage diverged from the orangutan. Possible evidence was also found for the co-evolution of nDNA and mtDNA subunits.

The nDNA-encoded NDUFC2 polypeptide cys39 was found to change in parallel with of the mtDNA *nad5* gene cys330 in interspecific comparisons (169). Comparable data have also been found for adaptive evolution of primate OXPHOS genes for complexes III, IV, and V, but not for complex II (170-174). Therefore, adaptive radiation of mtDNA-encoded OXPHOS genes may be especially important for intraspecific adaptation, whereas radiation of nDNA-encoded OXPHOS genes may be particularly important for interspecific radiation.

Since both mtDNA and nDNA OXPHOS genes can be polymorphic and adaptive, their interaction might be important in speciation. It is possible that rapid adaptation of mtDNA genes to regional environmental change may make certain mtDNA genotypes incompatible with common nDNA OXPHOS alleles in the parental population. This would inhibit subsequential introgression of these mtDNA lineages back into the parent population and lead to speciation (175).

These considerations might also explain why higher plant mtDNAs are large (96), have high recombination rates (94, 99), and have generally low sequence evolution rates (5). Given that plants are sedentary, function within a relatively defined ecological niche, divide energy metabolism between the mitochondria and chloroplast, and, in regions with adverse seasonal environments, become dormant might mean that they are not as reliant on variation in mitochondrial energy metabolism as an adaptive strategy as are animals. In situations where the species environment is more uniform, then it would be advantageous for the mtDNA gene sequences to remain constant. This could be accomplished by transferring some mtDNA genes to the nDNA as has been seen for *cox2*. In addition, the species could strongly reduce the mtDNA mutation rate and increase the mtDNA recombination rate to rapidly segregate and eliminate functional variants.

However, different plant species should be influenced by environmental fluctuation

PGC-1:

peroxisome-proliferating-activated receptor γ (PPAR γ) coactivator-1

to differing degrees depending on life cycle and niche. Therefore, some plant species may continue to need mtDNA variation to rapidly adapt to environmental energetic changes. This might explain why the plants of the family *Geraniaceae* were found to have higher mtDNA sequence evolution rates than other plant families. Moreover, different genera of this family have different mtDNA sequence evolution rates (176). One genus, *Plantago*, which includes a large group of cosmopolitan weeds, exceeds the mtDNA sequence evolution of the slower plant mtDNAs by $\approx 4,000$ -fold and is even higher than the sequence evolution rate of animal mtDNAs (177).

MITOCHONDRIAL ROLE IN SHORT-TERM ENERGETIC ADAPTATION

That rapid adaptation to changing energetic environments is critical to animals is demonstrated by the elaborate regulatory circuitry that animals have evolved to adapt to sudden changes in their thermal and caloric environments. Rodents, exposed to an acute temperature drop, activate β -adrenergic neurons, which innervate brown adipose tissue (BAT). BAT cells contain lipid droplets plus large numbers of mitochondria. Activation of the β -adrenergic neurons activates adenylyl cyclase in the BAT cells. The increased cAMP activates protein kinase A, which phosphorylates the cAMP response element binding protein (CREB), causing it to migrate to the nucleus. In the nucleus, the phosphorylated CREB binds to the cAMP response element (CRE) in the promoter of the peroxisome proliferation-activated receptor γ (PPAR γ) coactivator-1 α (PGC-1 α) gene (178). Induction of PGC-1 α then activates PPAR γ , which strongly induces the uncoupler protein 1 (UCP1) gene *ucp1* (179). UCP1 is imported into the mitochondrial inner membrane where it introduces a proton channel, uncoupling OXPHOS. Freed of the constraints of complex V by the drop of ΔP , the ETC starts burning the calories in the stored lipids and generating heat. The heat is

then distributed throughout the body by the circulatory system, and the animal becomes thermal neutral.

Thermogenesis is just one of a large number of interrelated pathways that have been found to regulate mitochondrial energy metabolism in response to environmental cues (180–183). To make sense of this complexity, it is necessary to recognize that there is an “energy anatomy” for human and animal organs. The “energy-utilizing tissues” are those most reliant on mitochondrial ATP production for function. These include the central nervous system, heart, muscle, kidney, and endocrine systems. The “energy storage tissues” include white adipose tissue (WAT) to store energy to fuel mitochondrial ATP production and BAT to store energy for mitochondrial thermal regulation. The “energy homeostasis tissue,” the liver, regulates serum glucose levels. The “energy sensing tissue,” the pancreatic α and β cells, monitors serum glucose levels and signals the availability of calories to the rest of the body’s organs by secretion of insulin from the β cells and glucagon from the α cells (2). Serum glucose is the prime indicator of energy availability because that is what plants produce during the growing season. When animals eat the glucose-rich plant foods, their blood sugar rises. The high serum glucose stimulates the pancreatic β cells to secrete insulin into the circulation. This signals the energy-utilizing cells to down-regulate OXPHOS because there is ample glucose to generate energy by glycolysis. The excess energy is then stored as fat (**Figure 5**). In the nongrowing season, the pancreatic β cells decrease insulin secretion, and the low serum glucose levels stimulate the pancreatic α cells to secrete glucagon. Glucagon signals the WAT to mobilize fats and secrete lipids into the bloodstream. It also signals the energy-utilizing cells to up-regulate mitochondrial OXPHOS to start burning fat to generate ATP by OXPHOS to survive the nongrowing season (2) (**Figure 5**).

At the energy-utilizing target cells, insulin binds to the insulin receptor on the plasma

membrane (**Figure 6**). This activates the insulin receptor tyrosine kinase, which phosphorylates insulin target proteins. These proteins activate the PI3 kinase pathway, which activates protein kinase B (PKB). PKB then phosphorylates the FOXO (mammalian forkhead transcription factor) proteins, excluding them from the nucleus, thus inactivating them as transcription factors.

When not phosphorylated and thus active, the FOXOs bind to insulin response elements (IREs) in the promoters of nuclear genes, including PGC-1 α , whose promoter contains three IREs. PGC-1 α , in turn, interacts with a variety of nuclear transcription factors and up-regulates mtDNA gene transcription for mitochondrial biogenesis genes such as mtTFA. The mtTFA migrates to the mitochondrion and up-regulates mtDNA transcription and replication (**Figure 6**) (right side). IREs are also likely found in the promoters of antioxidant genes, such as MnSOD and catalase, and active FOXOs up-regulate their transcription. Because of this regulatory pathway, during the growing season when animal serum glucose is high, insulin secretion is high, and the FOXOs are phosphorylated and inactivated. FOXO inactivation means that they cannot bind to the IREs. As a result, PGC-1 α and MnSOD transcription is reduced. Hence, the mitochondrial OXPHOS and antioxidant defenses are down-regulated, shifting the animal's energy generation from OXPHOS to glycolysis.

By contrast, during the nongrowing season, the low animal serum glucose reduces serum insulin levels, inactivating PKB and leaving the FOXOs unphosphorylated and active. The active FOXOs migrate to the nucleus and bind to the IREs, thus up-regulating PGC-1 α , mitochondrial OXPHOS, and the mitochondrial antioxidant defenses. Down-regulation of insulin also mobilizes the secretion of WAT fat stores into the bloodstream to serve as fuel for the mitochondria (**Figure 6**).

At the same time, the reduced serum glucose stimulates the pancreatic α cells to secrete the glucagons. Glucagon binds to the

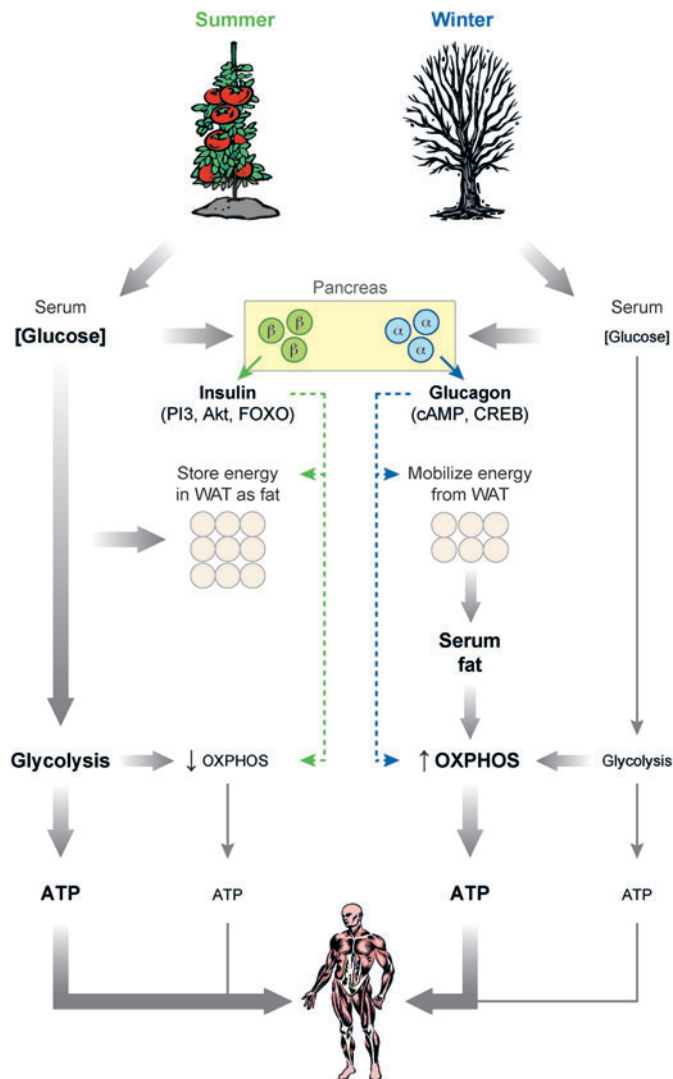


Figure 5

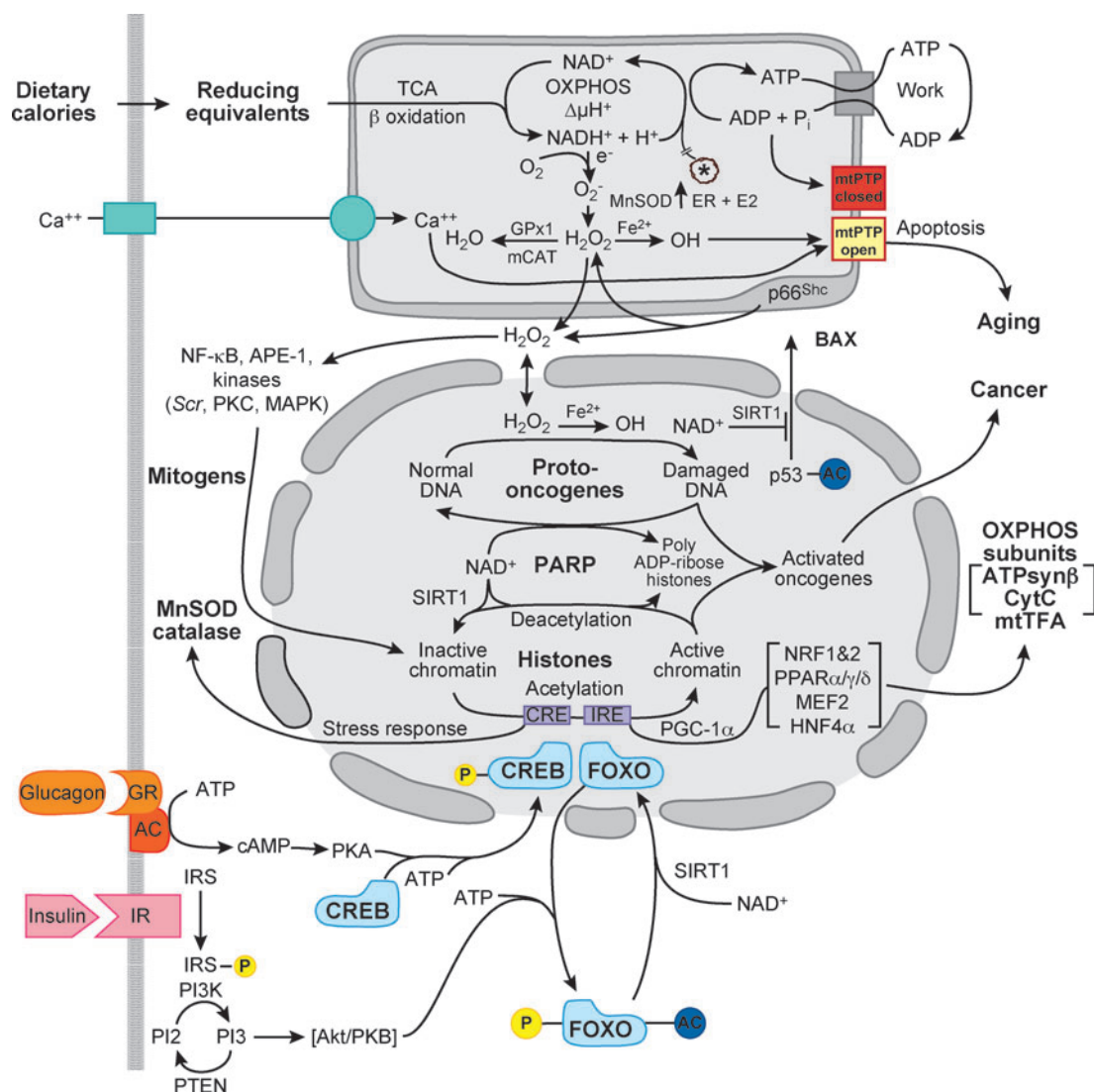
Proposed interaction between seasonal plant glucose production and animal metabolic modulation, mediated by the pancreatic hormones insulin and glucagon. Consumption of the high plant glucose generated during the plant growing season results in high serum glucose in animals. The high glucose level signals the pancreatic β cells to secrete insulin, which instructs the energy-utilizing tissues (central nervous system, heart, muscle, and kidney) to reduce mitochondrial OXPHOS and generate the low levels of energy necessary by emphasizing glycolysis. Insulin also instructs the energy storage tissue, white adipose tissue (WAT), to store the excess energy as fat. During the nongrowing season when plants do not make glucose, animal serum glucose levels decline, insulin production declines, and the pancreatic α cells secrete glucagon. Glucagon interacts with the WAT and instructs this tissue to mobilize fat and secrete it into the bloodstream. Glucagon instructs the energy-utilizing tissues to up-regulate mitochondrial OXPHOS to burn fat, generating energy to survive the winter (2).

FOXO: mammalian forkhead transcription factor

plasma membrane glucagon receptor activating adenylyl cyclase. The resulting rise in cAMP activates protein kinase A, which phosphorylates CREP. Phosphorylated CREP migrates into the nucleus and binds the CRE in the PGC-1 α promoter, up-regulating mitochondrial OXPHOS.

When calories are in excess relative to the energetic requirements for ATP production and/or thermal regulation, the ETC remains in a chronically reduced state. The resulting excess electrons in the presence of saturating

O₂ favors increased ROS production. Chronically increased ROS increases the probability of mutagenesis of the mtDNA and activation of the mtPTP, resulting in premature cell death and aging. Furthermore, mitochondrial H₂O₂, which is relatively stable, diffuses out of the mitochondrion and into the cytosol and the nucleus. In the nucleus, H₂O₂ can be converted to a hydroxyl radical. Hydroxyl radicals can then increase the mutation rate of the proto-oncogenes, converting them to oncogenes and initiating tumor formation



(Figure 6). Moreover, mitochondrial H_2O_2 can activate cytosolic and nuclear mitogenic pathways including NF- κ B, AP-1, MAP kinases, etc., driving the cell into mitosis, thus promoting tumor growth. As a consequence, animals that ingest excess calories over those needed for ATP and heat production are more prone to metabolic syndrome, premature aging, and cancer (2).

Thus, animal mitochondrial energy metabolism is coupled to plant chloroplast photosynthesis through glucose availability. The rapid seasonal adjustment of animal energy metabolism to environmental fluctuations in available substrates augments the more fundamental changes in the efficiency of OXPHOS energy metabolism resulting from

region-specific mtDNA adaptive sequence variants. These factors in turn interact with the environmental availability of calories plus thermal and other environmental stresses to modulate aging rate, predisposition to degenerative and metabolic disease, and cancer (2).

MITOCHONDRIAL EXPANSION OF THE BIOMEDICAL PARADIGM

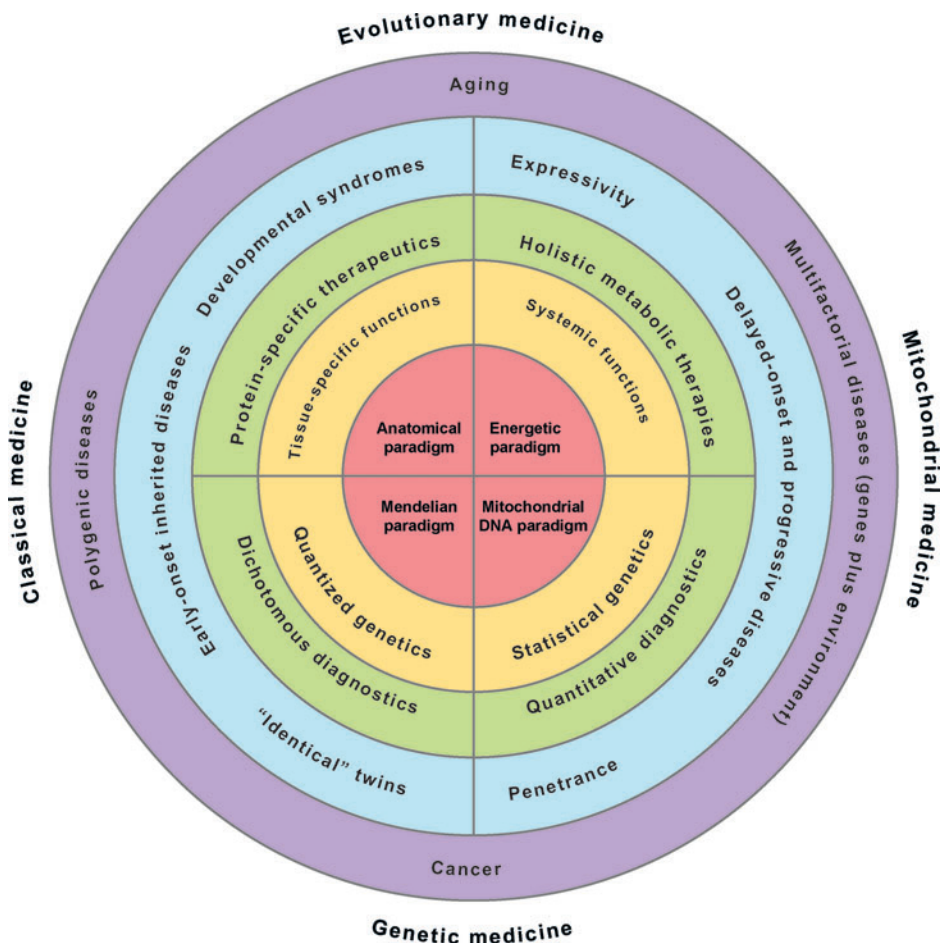
The addition of the functions and genetics of the human mitochondrial organism to our understanding of those already identified for the human nuclear-cytosol organism can now complete our understanding of human cell

Figure 6

Model for the environmental modulation of energy metabolism and the mitochondrial physiological basis of age-related diseases and aging. The diagram shows the primary interactions between mitochondrial energy production, ROS generation, and initiation of apoptosis through activation of the mtPTP. These components of energy metabolism are modulated by environmental constraints such as calorie availability and thermal stress through the regulation of the FOXO (forkhead) and CREB (cAMP response element-binding) protein transcription factors. The FOXO transcription factors are inactivated through phosphorylation by protein kinase B (PKB), which is up-regulated by insulin binding to the insulin receptor through activation of phosphatidyl inositol signaling. FOXOs can also be inactivated by their acetylation via NAD-dependent SIRT1. SIRT1 activity is modulated by the NADH/NAD ratio and thus by the metabolic status of the cell. The CREB transcription factor is activated by phosphorylation by protein kinase A (PKA), which, in turn, is activated by cAMP, generated in response to the binding of glucagons by the glucagon receptor. The peroxisome proliferation-activated receptor γ (PPAR γ) coactivator-1 (PGC-1) α factor is a nuclear transcriptional coactivator that interacts and enhances the action of multiple transcription factors, causing the coordinated up-regulation of genes for mitochondrial biogenesis, including the mtDNA transcription factor mtTFA. The promoter of the PGC-1 α factor has three insulin response elements (IREs) that bind the dephosphorylated and deacetylated FOXOs and a cAMP response element (CRE) that binds phosphorylated CREB. When serum glucose is high, insulin is high; the FOXOs are phosphorylated and inactive; and CREB is not phosphorylated and is inactive. Consequently, PGC-1 α is down-regulated causing mitochondrial OXPHOS to be down-regulated in association with the down-regulation of the antioxidant defenses, MnSOD and catalase. When serum glucose is low, insulin signaling declines; the FOXOs are dephosphorylated and activated and then migrate to the nucleus where they bind to IREs and activate transcription of PGC-1 α and the antioxidant enzymes. Glucagon binds the glucagon receptor, which activates adenyl cyclase and generates cAMP. The cAMP-activated PKA then phosphorylates and activates CREB, which binds to CREs and up-regulates PGC-1 α and the antioxidant enzymes. The Sir2 system further modulates the apoptosis effectors as well as transcription and replication mediators in relation to the metabolic status of the cell. Abbreviations: PARP, poly ADP-ribose polymerase; SIRT1, the mammalian homologue to Sir2; FOXO3, the most ubiquitous mammalian forkhead transcription factor; P, a phosphorylated protein; Ac, an acetylated protein; ILL, insulin-like ligand; ILR, insulin-like growth factor receptor; PI3K, the PI3 kinase; PI2, the membrane-bound phosphatidyl-inositol diphosphate; PI3, membrane-bound phosphatidyl-inositol triphosphate; AKT, the AKT kinases and also known as protein kinase B (PKB); GR, glucagon receptor; AC, adenyl cyclase. Reprinted with modifications from Reference 2.

Figure 7

New biomedical paradigm that combines the traditional structural biology and Mendelian genetics of the nuclear-cytosolic organism with the energy biology and mtDNA genetics of the mitochondrial organism. The four sectors indicate the different aspects of human biology and genetics determined by the functional and genetic components of the two different human symbiotic organisms.



biology, biochemistry, and molecular genetics as they relate to health and disease. Until now, the prevailing paradigms in classical Western medicine have focused primarily on the structural biology of cells, tissues, and organs and on Mendelizing genetic traits, both characteristic of the nuclear-cytosol organism. The classical anatomical paradigm asserts that organ-specific symptoms are due to defects in tissue-specific functions and thus tissue-specific genes (**Figure 7**, upper left quadrant). The Mendelian paradigm posits that genes behave exclusively according to the laws of inheritance, elaborated by Gregor Mendel, which are mirrored in the behavior of the chromosomes (**Figure 7**, lower left quadrant).

In Mendelian genetics, autosomal genes are present in two copies, which permit only three states (two healthy genes, $+/+$; one healthy and one damaged gene, $+/-$; or two damaged genes, $-/-$). Mendelian genetics is thus quantized. Furthermore, in identical twins, all Mendelian genes are identical, whereas in fraternal twins, only half of the genes are identical. Therefore, it is assumed that if a human clinical trait is more concordant among identical twins than fraternal twins, it has a genetic component. By contrast, phenotypic traits that are equally concordant in identical and fraternal twins are assumed to be the result of environmental influences (**Figure 7**, lower left quadrant).

However, many clinical phenotypes are clearly familial but do not follow the rigid rules of Mendelian inheritance. Examples include late-onset Alzheimer's disease, Parkinson's disease, migraines, cardiovascular disease, diabetes, obesity, cataracts, macular degeneration, cancer, inflammatory disease, etc. Such complex familial traits have been interpreted as resulting from allelic variants in multiple genes, referred to as "polygenic" diseases (**Figure 7**, left). Although single-gene defects have been discovered in rare familial cases of Alzheimer's and Parkinson's diseases, cardiovascular disease, and various familial cancers, many cases have not been found to be associated with mutations in these same genes. Moreover, the major risk factor for development of symptoms for the metabolic syndrome (which encompasses diabetes, hypertension, obesity, and cardiovascular disease), all of the neurodegenerative diseases, all of the solid tumors, and aging itself is age. Yet nothing in the quantized Mendelian genetics can explain why a person should be normal when young and then become increasingly ill as he or she ages.

The addition of the mitochondrial organism to human biology now augments the nuclear-cytosol structural and Mendelian paradigms with the additional mitochondrial energetic and mtDNA genetic paradigms (**Figure 7**, right). Mitochondrial function is important to all tissues. Hence, mitochondrial defects are systemic but result in organ-specific deficiencies due to the differential energy requirements of the human tissues. Therefore, the systemic mitochondrial energy biology of mitochondrial medicine can complement the organ-specific defects of classical medicine permitting a more rational explanation for multisystem disease.

Similarly, mtDNA genetics involves uniparental inheritance of thousands of copies of each gene. Hence, mtDNA genetics is a quantitative genetics in which the phenotype reflects the average genotype summed over

thousands of copies of each allele. Hence, from the mtDNA perspective, identical twins generated from the fertilization of a heteroplasmic oocyte can have different mtDNA genotypes and thus different clinical phenotypes. By contrast, identical twins and fraternal twins, which inherit the same homoplasmic mtDNA variant, will give the same phenotype, a concordance, which would be assumed to be nongenetic if it were interpreted using only the Mendelian paradigm. Finally, because there are thousands of copies of the mtDNA per cell, and they continually replicate and accumulate mutations in the post-mitotic tissues throughout an individual's lifetime, the mtDNA can act as a mutational clock in which the progressive accumulation of somatic mutations erodes cellular energetics until the cell crosses the bioenergetic threshold and dies by apoptosis. The progressive loss of cells due to this stochastic process can then account for the delayed onset and progressive course of many age-related diseases and aging. Therefore, the quantitative and stochastic genetics of mtDNA genes complements the qualitative and deterministic genetics of the nDNA genes. Consequently, organ-specific diseases can result from systemic mitochondrial defects, but to identify the molecular basis of these diseases requires a totally different set of approaches, leading to very different strategies for diagnosis and treatment.

Therefore, the addition of the mitochondrial organism to the nuclear-cytosol organism now makes human biology and genetics complete. The combination of the quantized Mendelian genetics and the quantitative mtDNA genetics now provides a comprehensive genetic medicine. Similarly, the interaction of structural biology and energetic biology can now account for the different ways in which humans can interact with their environment, providing explanations of the characteristics of human genetic variation and creating core principles for evolutionary medicine.

Mitochondrial medicine: a new medical discipline that pertains to all clinical problems that involve the mitochondria

Evolutionary medicine: a branch of medicine that uses human evolutionary information to understand gene-environment interactions in common disease processes

SUMMARY POINTS

1. Life is the interplay between structure, energy, and information. Because the mitochondria provide most of the cellular energy, to fully understand human health and disease, we must understand mitochondrial biology and genetics.
2. The mitochondria are ancient endosymbiotic bacteria with their own mitochondrial DNAs (mtDNAs). Although the original mitochondrial genome encoded all of the genes necessary for a free-living bacterium, most of the original mitochondrial genes have been transferred to the nucleus. Today, the mammalian mtDNA encodes only 13 polypeptides, all inner membrane components of the mitochondrial proton-translocating oxidative phosphorylation (OXPHOS) complexes. These are ND1-4, -4L, -5, and -6 (*nad1-4*, *-4L*, *-5*, and *-6* genes) of OXPHOS complex I; *cytb* (*cob* gene) of complex III; COI-III (*cox1-3* genes) of complex IV; and ATP6 and ATP8 (*atp6* and *atp8* genes) of complex V.
3. Mitochondrial OXPHOS consists of the ETC complexes I-IV, which oxidize dietary calories (hydrogen) with atmospheric oxygen to generate water and utilize the energy released to pump protons out across the mitochondrial inner membrane through complexes I, III, and IV, generating a transmembrane electrochemical gradient ($\Delta P = \Delta \Psi + \Delta \mu^{H+}$). ΔP is then used as a source of potential energy for multiple purposes, including ATP synthesis by proton flux through the mitochondrial ATP synthase (complex V), heat generation, ion or protein transport, subcellular compartmentalization, ROS production, and regulation of cell growth and death. The coupling efficiency is the ATP yield per calorie oxidized, and the difference is dissipated primarily as heat.
4. Comparative genomics of the protist, plant, and animal mtDNAs indicates that there has been a strong evolutionary tendency for genes to be transferred from the mtDNA to the nDNA. Various protist mtDNAs have lost mtDNA genes to different extents, but the mtDNA gene complement of plant and animal mtDNAs appears to have become stabilized with the advent of multicellularity, with plant mtDNAs having retained more genes than animal mtDNAs.
5. All mtDNAs analyzed to date contain *cob* and *cox1* genes, which are central to coupling electron transport to proton pumping through complexes III and IV. Most mtDNAs also retain the *cox2* and *cox3* genes, which deliver electrons and protons to *cox1*. Those mitochondria that have a proton-pumping complex I generally retain the *nad1-4*, *-4L*, *-5*, and *-6* genes, and those that have an ATP synthase usually retain *atp6*, *-8*, and *-9* in their mtDNAs.
6. On the basis of the consistent retention of the genes for integral inner membrane polypeptides of the proton-translocating OXPHOS complexes (I, II, III, IV, and V), I hypothesize that these core genes are maintained in the mtDNA because they share a common function in the generation, maintenance, and utilization of the mitochondrial ΔP . By retaining these genes in the hypervariable mtDNA, potentially adaptive mutations can accumulate in the proton-translocating polypeptides that reallocate the energy stored in ΔP to differing purposes, depending on local environmental conditions. By keeping the genes linked through exclusive maternal inheritance of the mtDNA, all of the genes associated with ΔP will be selected as a functional unit and thus will optimally coevolve.

7. Analysis of intraspecific mtDNA variation in *H. sapiens* has confirmed that the mtDNA polypeptides are highly polymorphic and that a significant proportion of mtDNA variants are adaptive. Moreover, these adaptive variants found geographically correlated branches of the human mtDNA tree, and multiple mtDNA variants have been shown to recur repeatedly in mtDNA lineages around the world, demonstrating convergent evolution and proving the adaptive nature of multiple mtDNA functional variants.
8. Mammalian mitochondrial OXPHOS has also been found to be highly regulated, and this regulation correlates with the availability of plant calories in the form of glucose in the environment as well as with fluctuations in environmental temperature. Therefore, energy adaptation at both the gene regulation and genetic mutation levels appears to be critical for individual health and species survival.
9. These evolutionary considerations demonstrate that energy metabolism and therefore mtDNA variation play a major role in human biology and thus individual health and well-being. Therefore, to understand the pathophysiology of disease and the inheritance of clinical traits, we must consider the biology and genetics of both human organisms: the nucleus-cytosol and the mitochondrion.

FUTURE ISSUES

1. We must determine the precise function of the mtDNA-encoded complex I subunits.
2. We must better define the biochemical characteristics of human adaptive mtDNA polymorphisms.
3. We need to determine the importance of human deleterious, adaptive, and somatic mtDNA mutations in common human age-related diseases.
4. We must clarify the adaptive changes that became established in our ancient ancestors in response to their environments so that we can better tailor our modern environment to suit individual genetic heritage.

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