Mitochondrial Biology and Genetics

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Mitochondrial origins

Mitochondria are bacterium-sized organelles found in almost all mammalian cells **(Fig. 1)**. They have different shapes and sizes, including spheres (~1 micron in diameter) and tubes, and can be either discrete individual organelles or can be part of an interconnected reticulated network.

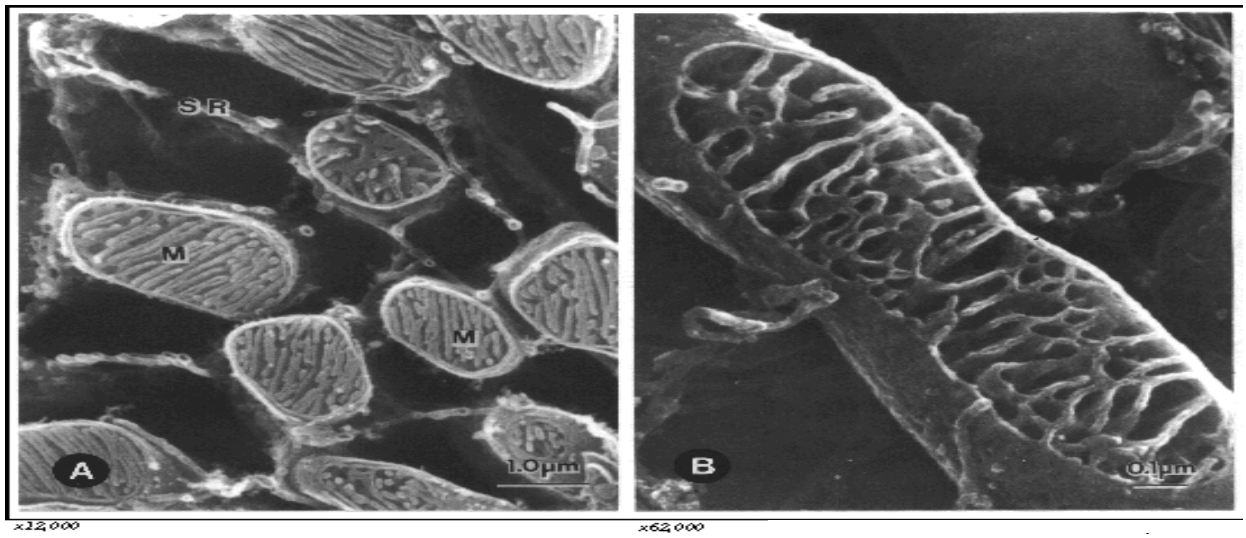

Figure 1. Dog heart mitochondria *(Yoshikane et al. (1986) J. Submicrosc. Cytol. 18:625)*

Mitochondria are the descendents of bacteria that were "captured" by proto-eukaryotic cells early in evolution **(Fig. 2)**. This "endosymbiont hypothesis" postulates that more than a billion years ago, the earth's atmosphere shifted from a reducing one (rich in hydrogen, carbon monoxide, ammonia, and methane) to an oxidizing one (rich in oxygen, nitrogen, and carbon dioxide). The oxygen (also produced as a byproduct of photosynthesis) became a serious problem, because it was toxic. Prokaryotic bacteria were among the first organisms to solve the oxygen problem. Some evolved enzymatic systems to deal with oxygen, converting it into non-toxic forms such as carbon dioxide and nitrate. Importantly, this evolutionary leap had a beneficial side effect - the bacteria increased the efficiency of their energy production by oxidizing a wider array of reduced substrates

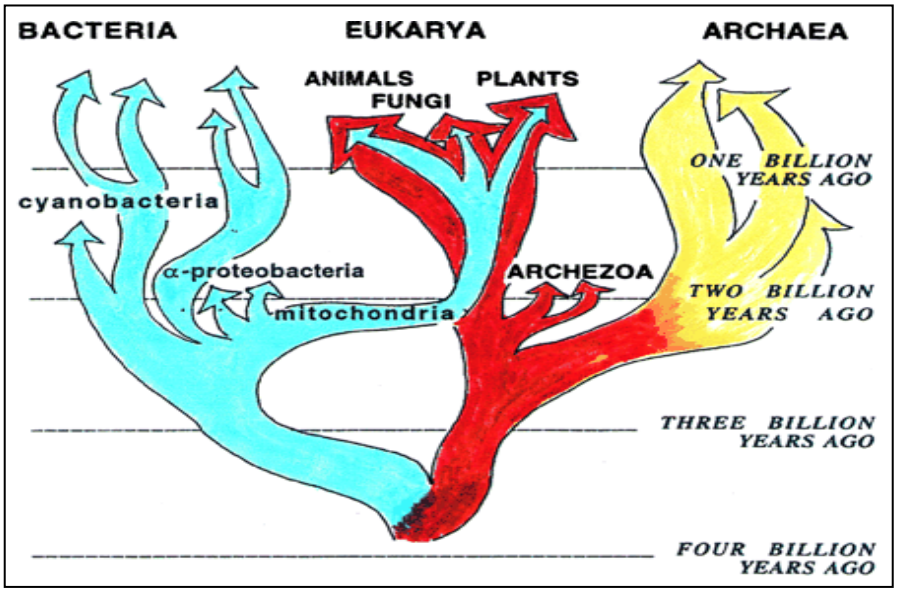

Figure 2. The endosymbiont hypothesis *(Doolittle (1997) PNAS 94:12751)*.

At some point in evolution, these energy-efficient aerobic bacteria were ingested by early single-celled eukaryotes, thereby conferring on the host cells two distinct advantages: increased protection from toxic oxygen and increased efficiency in converting food (e.g. glucose) into energy (e.g. adenosine triphosphate, or ATP). Eventually, the relationship between the host cell and the bacterium became a symbiotic one, in which the eukaryote provided food and shelter, while the bacterium detoxified the oxygen and provided extra oxidative energy. The endosymbiont eventually became a true cellular organelle - today's mitochondrion - due to two key events: the bacterium lost almost all of its genes (many of which became incorporated into the host's nuclear DNA), and the bacterium's residual DNA (now called mitochondrial DNA, or mtDNA) evolved so that its few remaining gene transcripts could omly be translated using a modified genetic code.

Mitochondrial structure

Mitochondria are unique among cellular organelles in that they have a two-membrane structure **(Figs. 1 and 3)**: the *outer mitochondrial membrane* surrounds the *inner mitochondrial membrane*, which invaginates into the *matrix* to form *cristae*; the *intermembrane space* is between the inner and outer membranes.

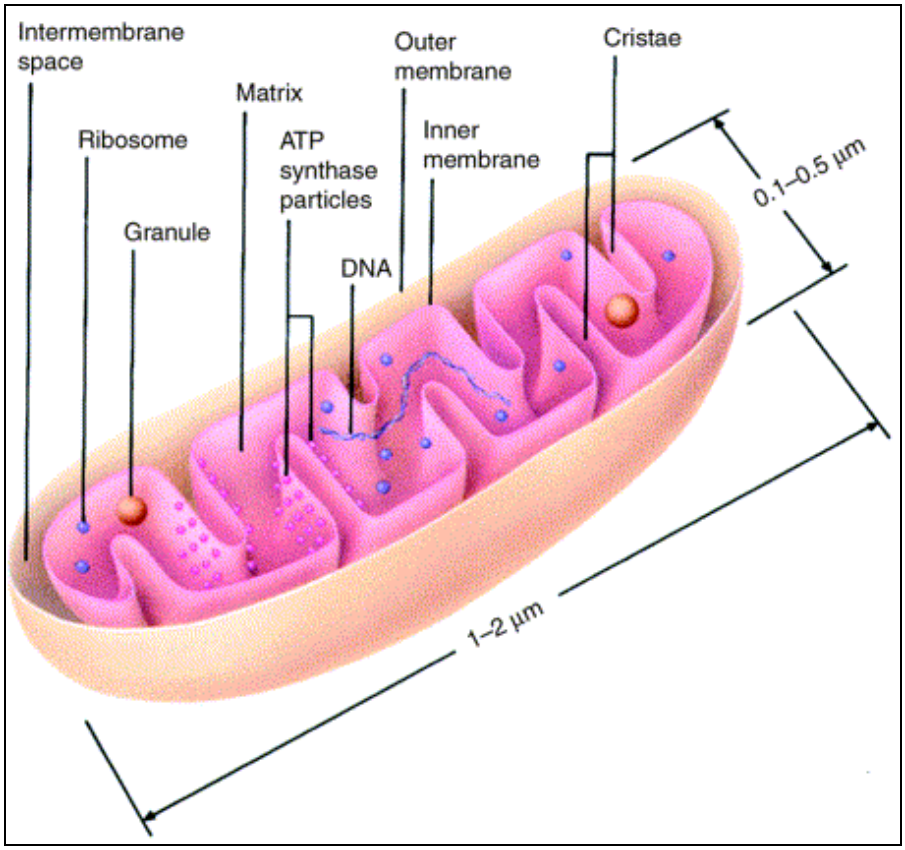

Figure 3. The Palade "baffle " model of mitochondrial structure (*Frey and Mannella (2000) TIBS 25:319)*

Mitochondrial importation

Only a handful of genes remain today in human mtDNA, all associated with oxidative energy production (see below). The rest were either lost or were incorporated into the nuclear DNA. Approximately 1,800 nuclear genes encode proteins that are now synthesized in the cytoplasm but are then imported into mitochondria.

Mitochondrial importation is a complex process, with different pathways for the targeting and sorting of polypeptides to the four main organellar compartments (the outer and inner membranes, the intermembrane space, and the matrix). Some components of the import machinery are members of the so-called "heat shock" protein family, which are "molecular chaperones" that help unfold, and then refold, the mitochondrially-targeted polypeptides as they are inserted through the import receptors and are sorted to the appropriate compartments (**Fig. 4)**.

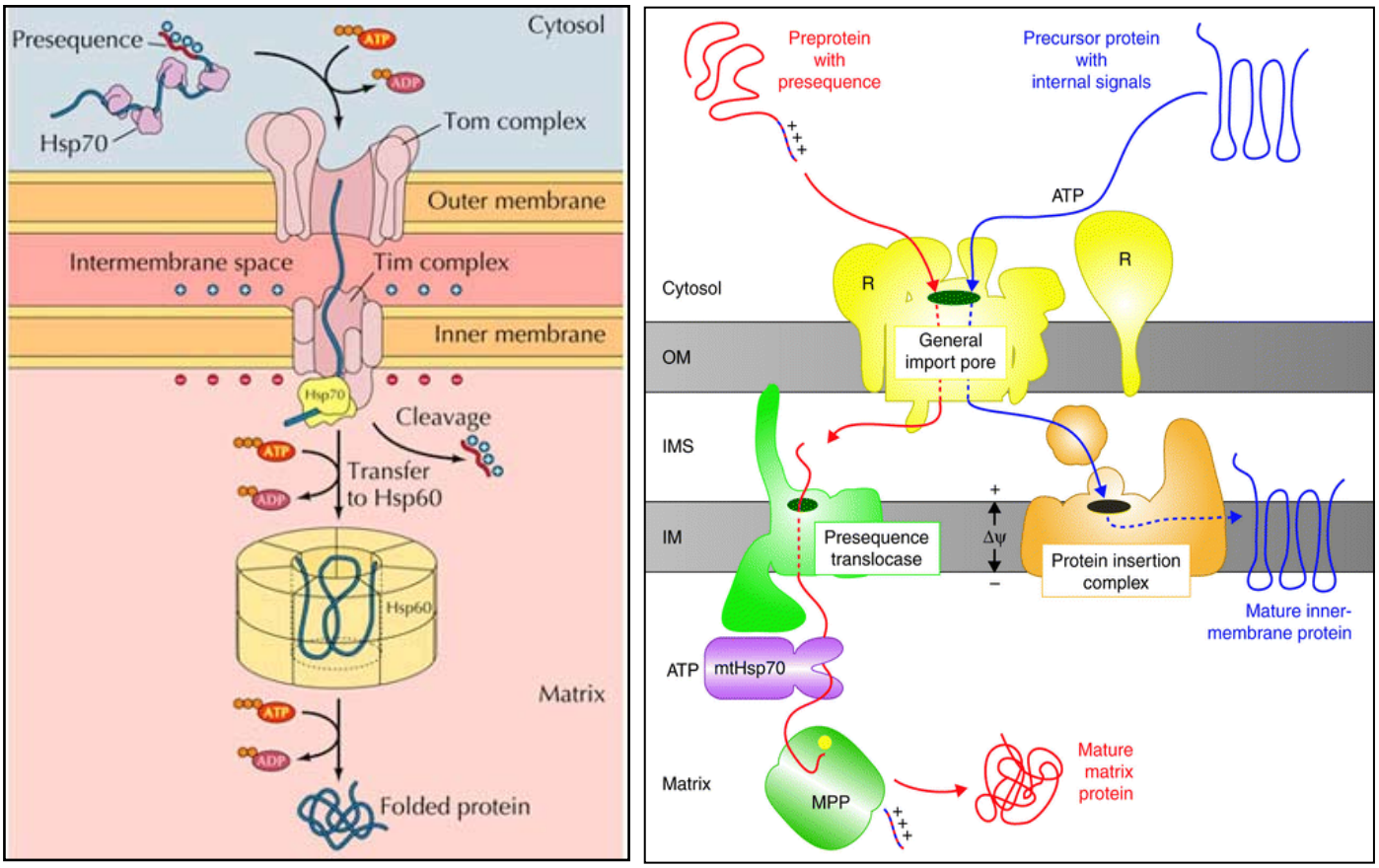

Figure 4. Importation of mitochondrially-targeted proteins. **Left.** Courtesy of Liza Pon, Columbia University. **Right.** From *Pfanner and Wiedemann (2002) Curr. Opin. Cell Biol. 14:400*

These 1,800 proteins are "addressed" to mitochondria via a mitochondrial "targeting signal" (MTS) often located at the N-terminus of the polypeptide. Once inside the organelle, the N-terminal MTS (also called a "leader peptides") is cleaved to release the mature polypeptide. The leader sequence is usually highly basic (i.e. it contains many arginine and lysine and arginine, and few or no aspartate or glutamate residues), and it often contains peptide "motifs" that determine the precise point of cleavage of the presequence inside the organelle.. There are also C-terminal and even "internal" MTS's, but these have been less well characterized.

Mitochondrial functions

Many of these imported proteins are required for maintenance of the mitochondrion's integrity. These would include proteins required for mtDNA synthesis, replication, and transcription, protein translation, maintenance and integrity of organellar membranes, and transport of various molecules (**Table 1)**.

Table 1. Gene products present in human mitochondria.

"Maintenance" functions (881)		"Specialized" functions (1040)	
Protein translation & stability	261	Respiratory chain & OxPhos	235
Organellar morphology & inheritance	190	Signal transduction	168
Carriers & Transporters	160	Lipid metabolism	163
Nucleic acid metabolism	146	Intermediate metabolism	137
Stress response	65	Apoptosis & cell death	130
Protein import & sorting	59	Amino acid & nitrogen metabolism	74
		Miscellanous/Unknown	133

Note: Some gene products have more than one function

Most of the remaining imported proteins are involved in specialized functions that are required for the maintenance of the cell's well-being ("housekeeping" functions). These include enzymes for the oxidation of carbohydrates (e.g. the pyruvate dehydrogenase complex, or PDHC), enzymes for the transport and beta-oxidation of lipids (e.g. carnitine palmitoyltransferases and acyl-CoA synthetases, dehydrogenases, and acetyltransferases), enzymes of the tricarboxylic or citric acid cycle (e.g. citrate synthase, aconitase, fumarase, and succinate dehydrogenase), enzymes for the transport and degradation of amino acids (e.g. transaminases), and enzymes for the metabolism of nitrogen-containing compounds (e.g. monoamine oxidase and ornithine aminotransferase) **(Fig. 5)**.

Other specialized functions of mitochondria, however, are not really "housekeeping" functions at all. These include proteins involved in the cell's response to stress (especially oxidative stress), a surprisingly large number of signal transduction molecules, and mitochondrial proteins involved in the initiation of programmed cell death (apoptosis).

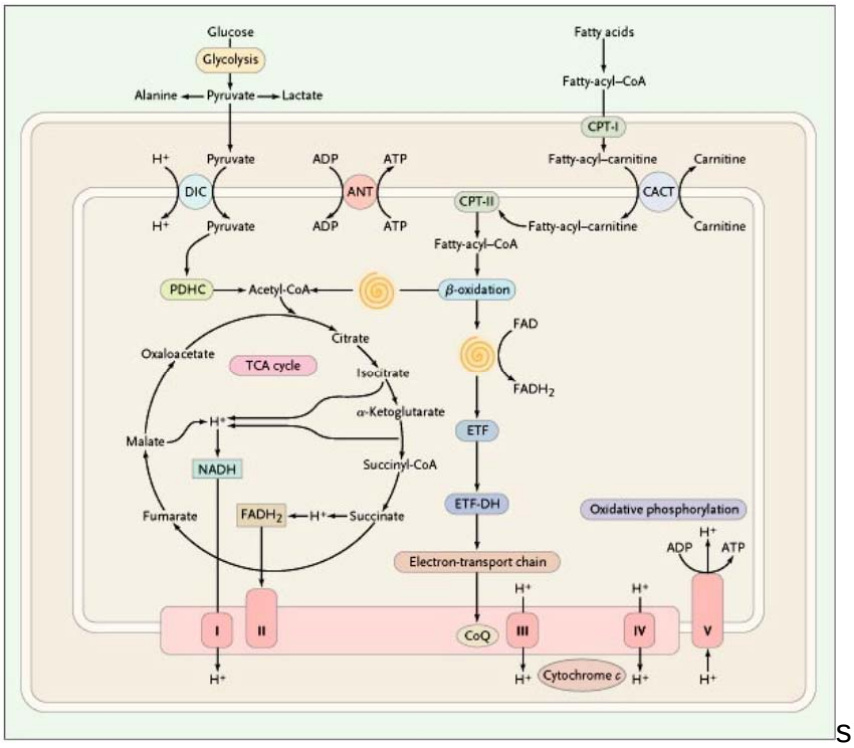

Fig. 5. Mitochondrial biochemistry *(DiMauro and Schon (2003) NEJM 348:2656)*

The one housekeeping function that is of overriding importance to our discussion here is the production of energy in the form of adenosine triphosphate (ATP) via the respiratory chain/oxidative phosphorylation system. This aspect of mitochondrial function is unique, because the production of

oxidative energy is a collaboration between the mitochondrion and the nucleus, as genes from both organelles are required.

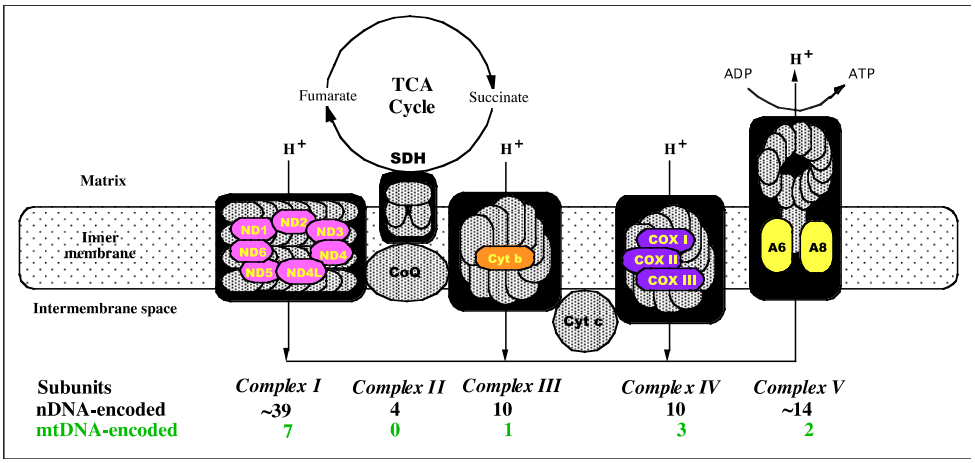

Figure 6. The mitochondrial respiratory chain.

The respiratory chain **(Fig. 6)**, located in the inner mitochondrial membrane, consists of five complexe: NADH dehydrogenase-ubiquinone oxidoreductase (complex I); succinate dehydrogenase-ubiquinone oxidoreductase (complex II); ubiquinone-cytochrome *c* oxidoreductase (complex III); cytochrome *c* oxidase (complex IV); and ATP synthetase (complex V). Complexes -I, III, IV, and V contain polypeptides encoded by both nuclear and mitochondrial DNA; complex II contains only nDNA-encoded subunits. As such, complex II can be used as an "internal control" for the presence, viability, and quantity of mitochondria in those circumstances where mutations in mtDNA render some of the other four complexes inactive.

A subset of nuclear genes are required for the synthesis of many respiratory chain subunits. There are also nuclear genes that encode regulatory elements (these are *not* targeted to mitochondria), such as called nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2). These regulatory elements may be one of the ways in which the nuclear and mitochondrial genomes "communicate" with each other, perhaps by coordinately regulating the transcription of these genes to respond to changing oxidative demands of the cell.

Mitochondrial genetics

Genome organization

The human mitochondrial genome **(Fig. 7)** is a double-stranded circle 16,569 base pairs (bp) in length. The two strands are asymmetric in the composition of their bases, with one strand rich in the nucleotides G and T (called the "heavy" or H strand) and the other correspondingly rich in the nucleotides C and A (called the "light" or L strand). The nomenclature of "heavy" and "light" refers to the differential mobility of the separated strands in alkaline cesium chloride gradients.

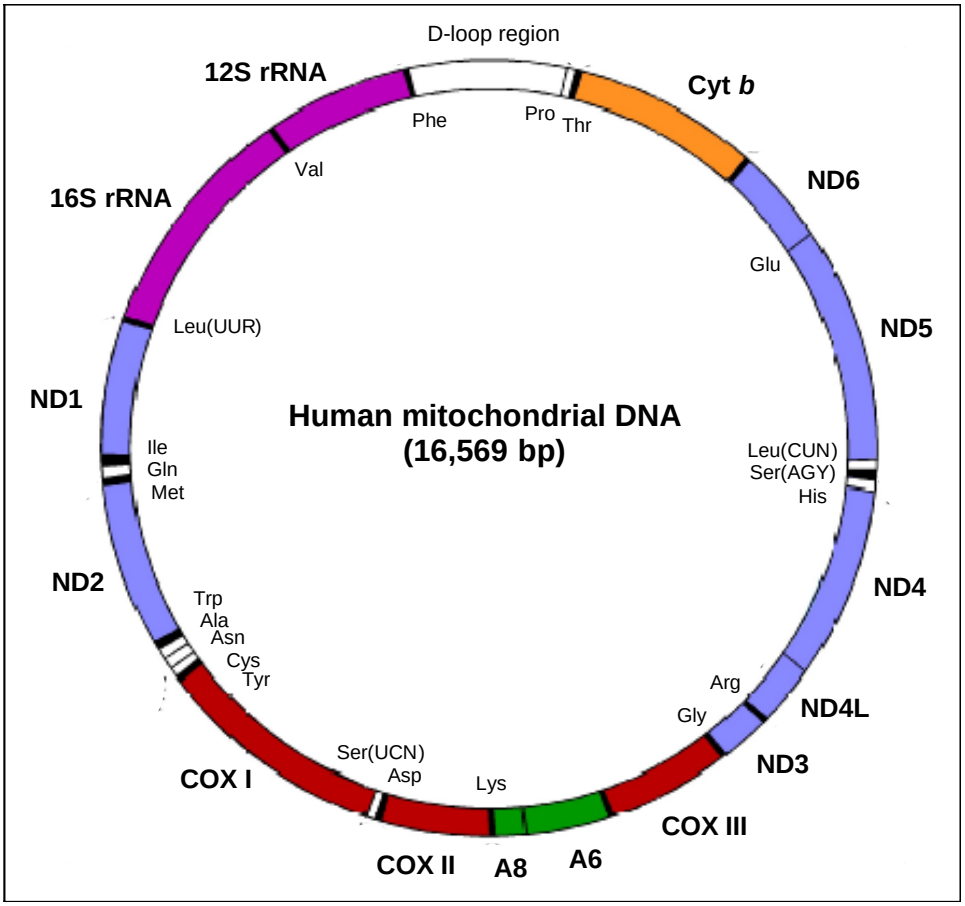

Figure 7. The human mitochondrial genome.

Human mtDNA contains only 37 genes, of which only 13 encode polypeptides; all 13 are subunits of the mitochondrial respiratory chain (Fig. 6), including 7 of the 46 subunits of complex I, 1 of the 11 subunits of complex III, 3 of the 13 subunits of complex IV, and 2 of the 12 subunits of complex V. The other 24 genes are required for translation on mitochondrial ribosomes of the mRNAs specifying these 13 polypeptides: 2 genes encode ribosomal RNAs (called 12S and 16S rRNA), and 22 genes encode transfer RNAs required for incorporation of the 20 amino acids into the growing polypeptide chain (there are 22, rather than 20, tRNAs because there are 2 tRNAs each for both leucine and serine). The tRNA genes are "dispersed" throughout the mtDNA circle, at the borders between the rRNA and most of the polypeptide-coding genes (see also Fig. 9). This "punctuation" is believed to be crucial to the precise transcription of the tRNAs, rRNA, and mRNAs.

Replication of mtDNA

The replication of mammalian mtDNA has a number of features that are reminiscent of bacterial DNA replication. In bacteria with circular genomes, there is a single "origin" of replication, with the synthesis of daughter-strand DNA (from the "top" and "bottom" strands of the parental DNA) beginning bi-directionally from the origin. Replication is controlled by the combined action of RNA polymerase (which synthesizes short RNAs used to "prime" DNA replication) and DNA polymerases (which extend the newly-forming daughter DNA from those primers, using the parental strands as the template), and proceeds simultaneously in opposite directions: clockwise from the "top strand" and counterclockwise from the "bottom strand." Replication ends with the creation of a pair of catenated

circles (each circle is a double helix containing one "old" parental strand and one "new" daughter strand), which are then separated into two circles by the action of an enzyme called topoisomerase II.

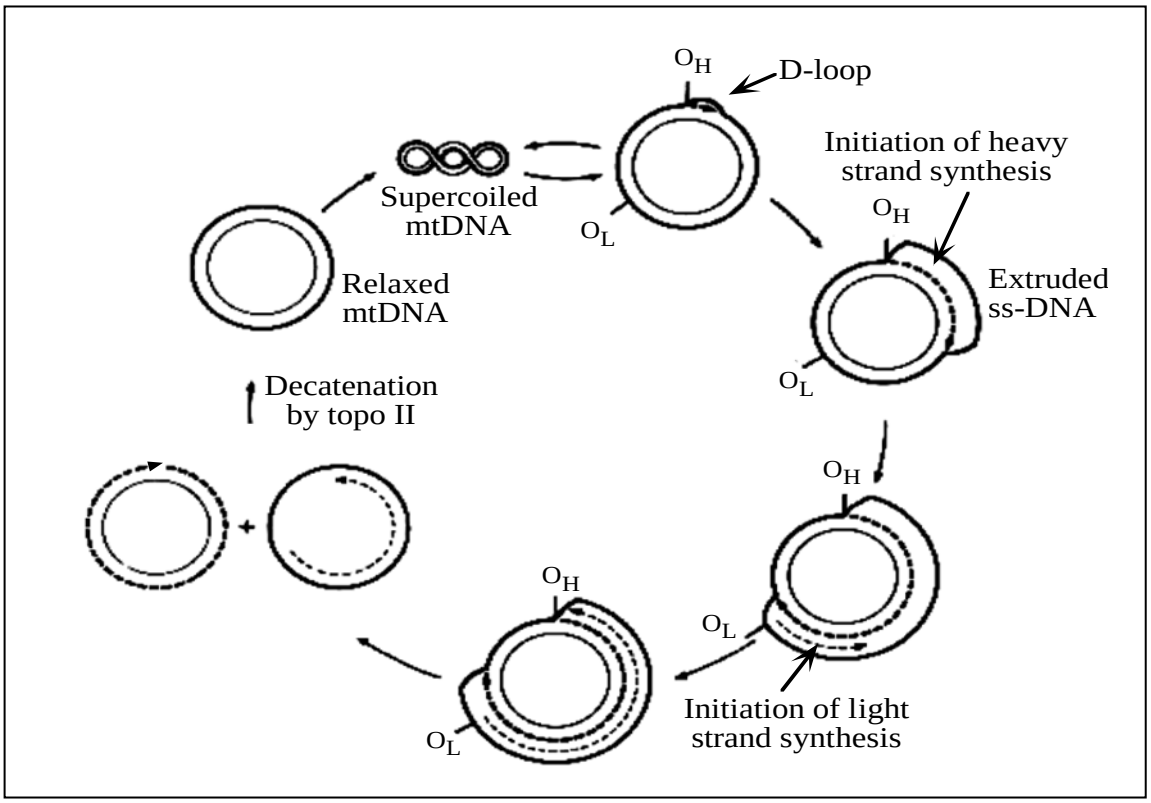

Figure 8. The "Clayton model" of mtDNA replication *(Clayton (1982) Cell 28:693)*.

Current views of the replication of human mtDNA have recently come under challenge. In the "standard" view **(Fig. 8)**, based on the pioneering work of David Clayton of Stanford, the origin of replication is physically separated into two "halves," each controlling synthesis of one of the two daughter DNA strands. Synthesis of one strand, by mitochondrial DNA polymerase γ, begins at the "origin of heavy-strand replication" (called O_H), which is located at "12-o'clock" on the circle, and proceeds in a clockwise direction. There is an initial requirement for the synthesis of a piece of RNA to prime DNA synthesis from O_H. This RNA, plus a short stetch of extended DNA, hybridizes to the L-strand and displaces a portion of the H-strand in the region containing O_H, thereby exposing the so-called "D-loop" (D is for "displacement"). As the DNA polymerase (and the displaced DNA) passes "8-o'clock" on thecircle, synthesis of the other strand begins, at the "origin of light-strand replication"(called O_L). The two oppositely-growing strands continue until they both have completed their respective circles, forming a catenated pair of rings., whichare then decanated by topo II. The entire process takes a surprisingly long time - about two hours.

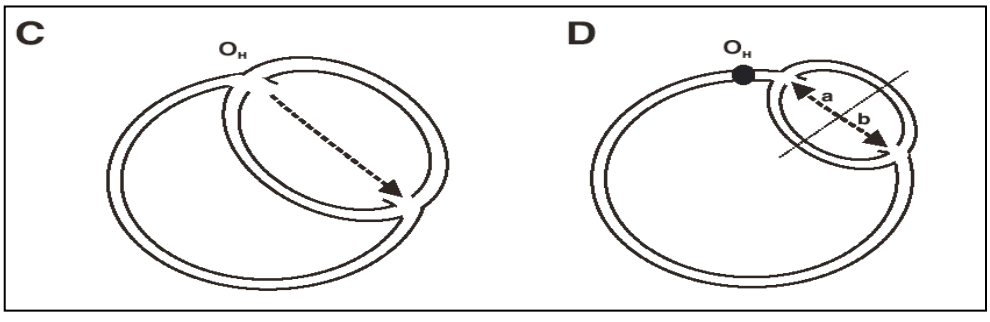

Figure 9. The "Holt model" of mtDNA replication *(Bowmaker et al. (2003) J. Biol. Chem. 278:50961)*.

The competing model, put forth by Ian Holt of Cambridge, is basically a modification of classic bacterial "theta" replication (so called because of the theta structure generated by the bidirectional replication from a single origin) **(Fig. 9)**.

Transcription of mtDNA

Transcription of human mtDNA also "looks" prokaryotic-like. Instead of transcribing each of the 37 genes separately (as would be the case for nuclear DNA), all of the genes are initially transcribed as two giant 16-kb polycistronic precursor transcripts, one encoded by the H-strand and the other by the L-strand **(Fig. 10)**. There are promoters for RNA transcription, both located within the D-loop, controlling transcription off the respective light- and heavy-strands (LSP and HSP). Both rRNAs, 14 tRNAs, and 12 of the 13 polypeptide-coding genes are encoded by the H-strand [meaning that they have the same sequence as the L-strand]); only 1 mRNA and 8 tRNAs are encoded by the L-strand. It is thought that the tRNAs, which "punctuate" the genes around the circle, are excised precisely from the precursor RNAs, thereby releasing not only the tRNAs, but the flanking rRNAs and mRNAs as well. Following cleavage, the 3' termini of the mRNAs are polyadenylated and the tRNAs acquire certain additions and base modifications.

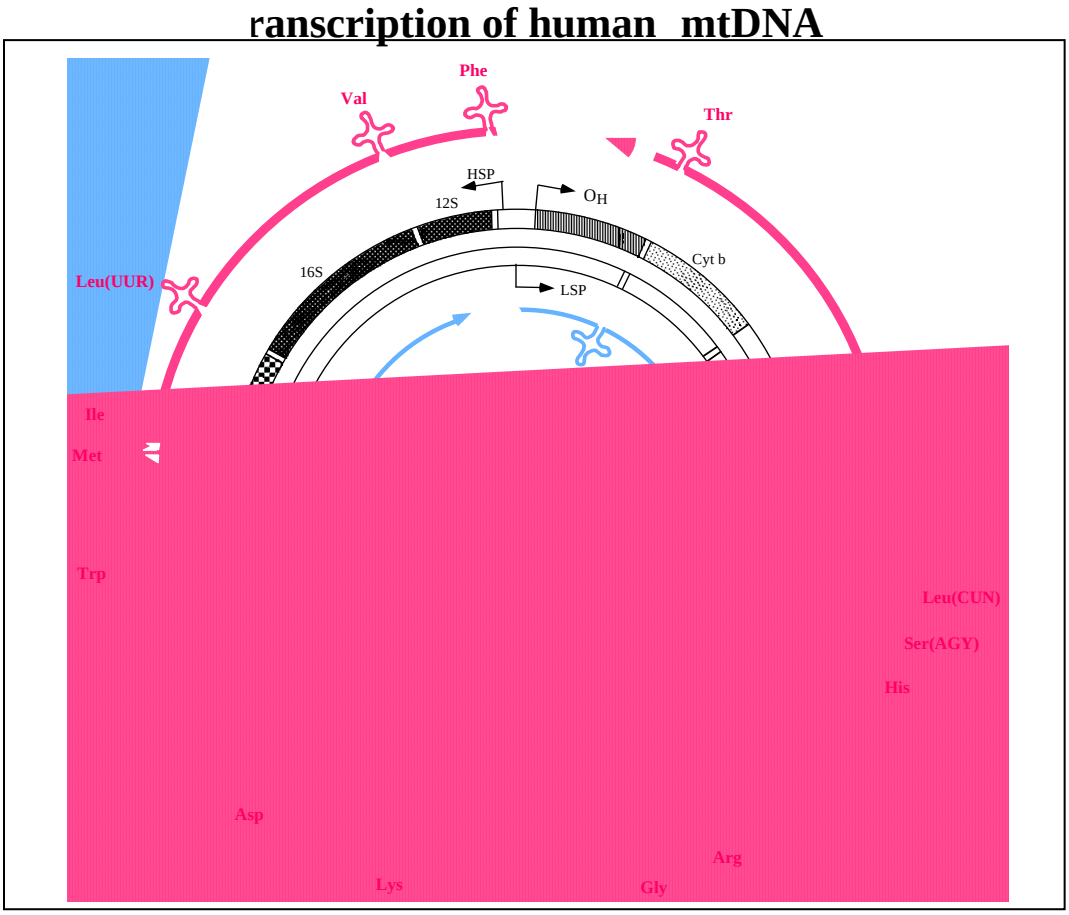

Figure 10. Transcription of mtDNA. Courtesy of Carlos Moraes (Columbia University).

Mitochondrial translation

Translation of the mitochondrial mRNAs takes place on mitochondrial ribosomes, consisting of the mtDNA-encoded 12S and 16S rRNAs plus imported ribosomal proteins. The initiation codon for translation (AUG or AUA, both of which specify methionine) is located at the very beginning of the mature message (i.e. there is almost no 5'-untranslated region [5'-UTR]); in the absence of a 5'-UTR, it is unclear how the ribosome recognizes and binds to the message. The downstream end of the message, at the 3' end, is also quite short. In fact, the codon specifying the last amino acid is usually located within one or two nucleotides of the end of the message, which often end with a U or a UA. Addition of the poly(A) tail to the mRNA converts these last nucleotides to UAA, which is a translational stop codon. Two mRNAs contain overlapping messages, that is, one contiguous piece of mRNA specifies two different polypeptides: one message encodes subunits ND4 and ND4L of complex I, while the other encodes subunits 6 and 8 of complex V.

As noted above, human mitochondria have their own genetic code **(Fig. 11)**. It differs from the "universal" code at four of the 64 triplet positions (AUA specifies methionine instead of isoleucine; UGA specifies tryptophan instead of "stop"; and AGA and AGG specify "stop" instead of arginine.

UUU F Phe	UCU S Ser	UAU Y Tyr	UGU C Cys
UUC F Phe	UCC S Ser	UAC Y Tyr	UGC C Cys
UUA L Leu	UCA S Ser	UAA * Stop	UGA * Stop→Trp
UUG L Leu	UCG S Ser	UAG * Stop	UGG W Trp
CUU L Leu	CCU P Pro	CAU H His	CGU R Arg
CUC L Leu	CCC P Pro	CAC H His	CGC R Arg
CUA L Leu	CCA P Pro	CAA Q Gln	CGA R Arg
CUG L Leu	CCG P Pro	CAG Q Gln	CGG R Arg
AUU I Ile	ACU T Thr	AAU N Asn	AGU S Ser
AUC I Ile	ACC T Thr	AAC N Asn	AGC S Ser
AUA I Ile→Met	ACA T Thr	AAA K Lys	AGA R Arg→Stop
AUG M Met	ACG T Thr	AAG K Lys	AGG R Arg→Stop
GUU V Val	GCU A Ala	GAU D Asp	GGU G Gly
GUC V Val	GCC A Ala	GAC D Asp	GGC G Gly
GUA V Val	GCA A Ala	GAA E Glu	GGA G Gly
GUG V Val	GCG A Ala	GAG E Glu	GGG G Gly

Figure 11. The human mitochondrial genetic code.

Mitochondrial inheritance

Human mitochondria and mtDNAs are maternally-inherited. A mother transmits mitochondria to all of her children - both boys and girls - but only her daughters will transmit their mitochondria to their children **(Fig. 12)**. Only ~1% of the maternal mitochondria present at fertilization - those in the inner cell mass; - eventually repopulate the embryo; the remainder become part of the extraembryonic tissues. It is known that paternal mitochondria enter the ovum at fertilization, but they are destroyed selectively by some unknown mechanism. Recently, however, a patient was reported to have a mitochondrial myopathy in which the mutation was paternally-inherited, but this seems to be an exception that "proves" the maternal-inheritance rule.

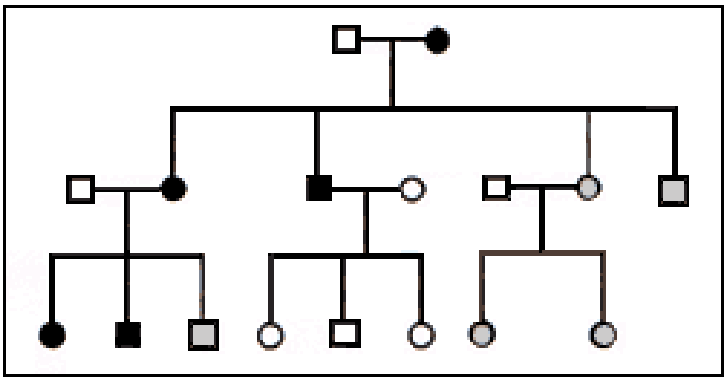

Figure 12. A maternal pedigree. Black, affected; gray, partially affected.

Segregation and heteroplasmy

Somatic cells contain ~5 mtDNAs per organelle, whereas germ-line cells (e.g. oocytes) appear to contain only one mtDNA/organelle. The number of mitochondria in a cell is related to its dependence on oxidative energy: cells in tissues with high oxidative energy demands, such as skeletal muscle, heart, and brain (including the eye), may contain thousands of mitochondria, whereas skin fibroblasts may contain only a few hundred.

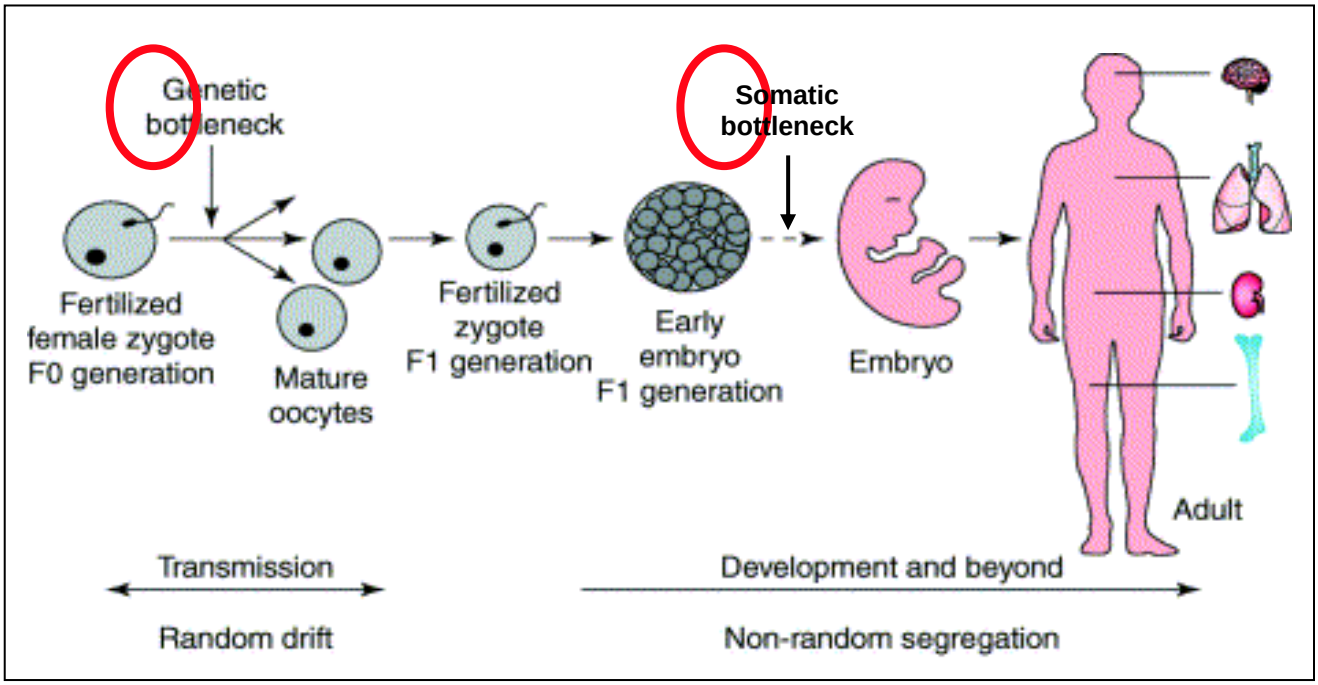

Figure 13. Mitochondrial segregation during germline development.

Thus, mitochondrial genetics is *population* genetics. In nuclear mendelian genetics, a somatic cell contains only two alleles for each autosomal gene, one derived from the mother (e.g. allele "A") and the other from the father (e.g. allele "B"), and a person may be heterozygous (A/B), homozygous (A/A or B/B), or even hemizygous (A/null or B/null) at any particular gene locus. In mitochondrial genetics, however, the number of potential alleles for a mtDNA-encoded gene is equal to the number of mtDNAs in that cell (e.g. many thousands of possible alleles, not just 1 or 2). To all intents and purposes, however, all of the mtDNAs in an individual are identical, i.e. they individual is *homoplasmic*. This is because all of the mtDNAs in a person are derived from a relatively small

number of mitochondrial genomes passing from mother to child. Of the ~100,000 mtDNAs in an oocyte, a surprisingly small number - estimated to be between 5 and 50 - actually repopulate the cells of the developing embryo. The "bottleneck" in mtDNA transmission actually occurs at two points in developemnt **(Fig. 13)**. Initially, there is a "germiline" bottleneck between the primordial germ cells (i.e. oogonia) and the mature oocyte. Both mitochondrial division and mtDNA replication are arrested until the blastocyst stage of development (64-128 cells), around the time of implantation. Thus, the original 100,000 mitochondria in the zygote are "diluted out" to about 1,000 mitochondria/cell in the blastocyst. Since oinly a few cellsof the inner cell mass actually populate the fetus, there is a second, "somatic"' bottleneck at implantation. The "bottleneck" would therefore explain why there is a very low probability of a mother passing a mutation in any single mitochondrial genome to her child.

Mutations in mtDNA can arise randomly in single molecules, probably during DNA replication. If a mutation occurs in the germline and is in one of those few mtDNAs that is passed on to the fetus, the child will be born with two types of mtDNA. In this case, the child will be *heteroplasmic* (i.e. more than one mtDNA genotype present). Usually these mutations are non-deleterious and neutral, but on rare occasions they can be pathogenic and cause disease, as will be discussed by Dr. Hirano. Because of the combination of the bottleneck and genetic drift, eventually only one of the genotypes becomes fixed in a pedigree. This is how random mutations eventually result in different mitochondrial genotypes in different populations. Note that heteroplasmy can exist at any level of organization in an individial: within mitochondria, among mitochondria, among cells, among tissues, and ultimately, among individuals **(Fig. 14)**.

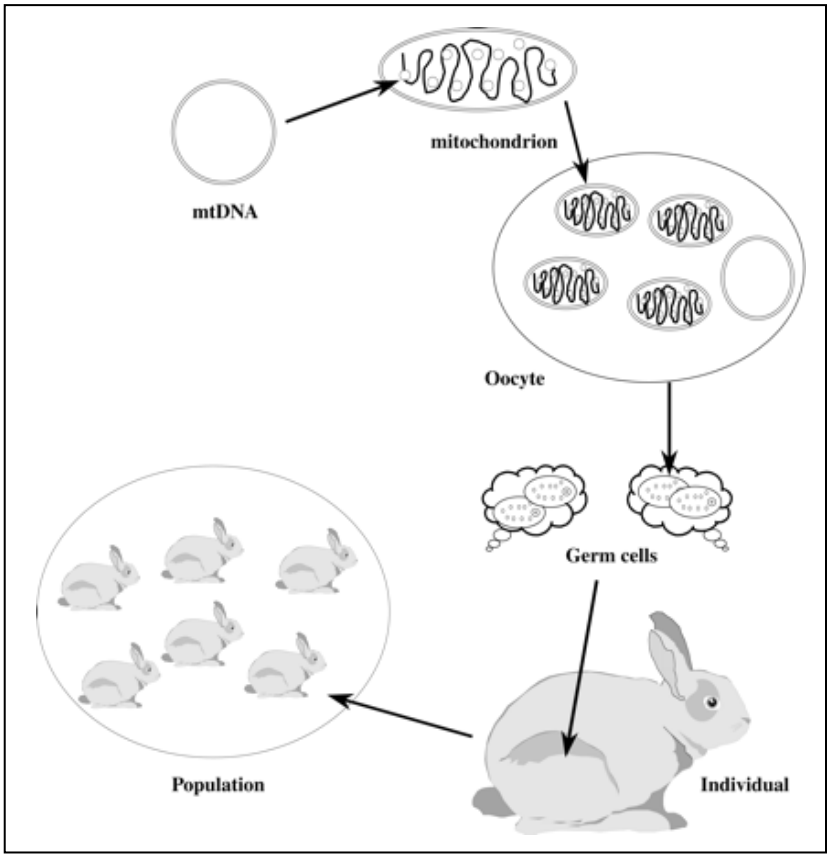

Figure 14. mtDNA exists in a nested heirarchy of populations. From Rand (2001) Annu. Rev. Ecol. Syst. 32:415.

Two ethnically unrelated individuals differ at approximately 50 of the 16,569 bases in their mtDNAs, almost always as neutral polymorphisms. The exclusive maternal inheritance of different mtDNA genotypes has become the basis of the analysis of migrations of populations, and has been used in many other areas, including linguistics and forensics .

The timing of organellar division and mtDNA replication have no obvious relationship to the timing of cell division. Thus, the numbers of organelles and mtDNAs in a cell vary both in space (i.e. among cells and tissues) and in time (i.e. during development and aging). The dynamic aspect of mitochondrial transmission from cell to cell is termed *mitotic segregation* **(Fig. 15)**. Moreover, mitochondrial numbers can vary based on the oxidative energy requirements of the moment (e.g. after prolonged training in athletes, or after acclimation at high altitude).

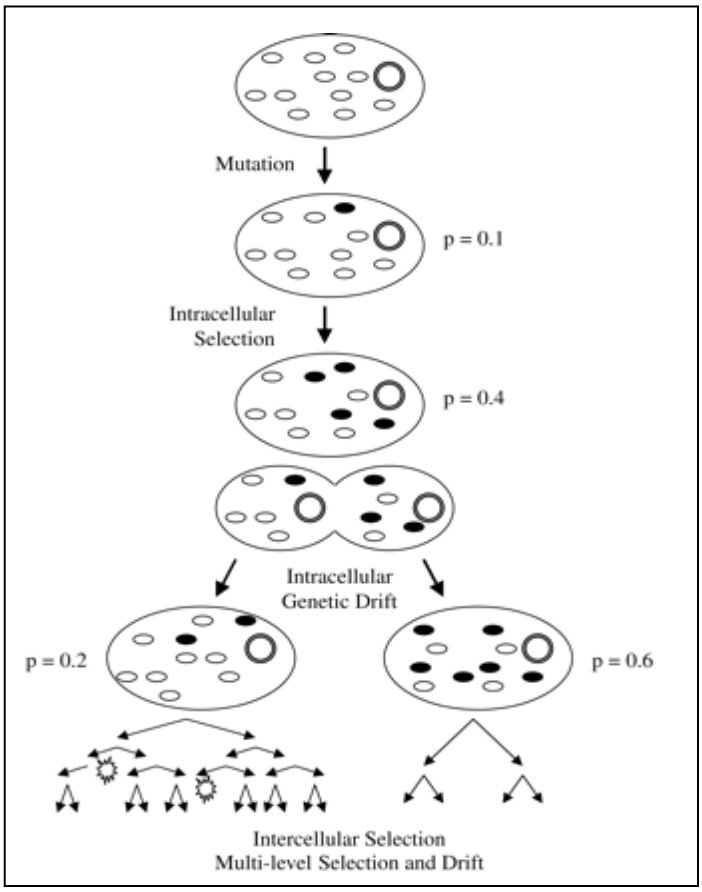

Figure 15. Population genetics in the cytoplasm. From Rand (2001) Annu. Rev. Ecol. Syst. 32:415

A tissue culture system to study defects in mtDNA

As an introduction to Dr. Hirano's lecture on pathogenesis and Dr. DiMauro's lecture on treatment, it is useful to review one technology that is unique to the study of mitochondrial genetics, both in normal and diseased states, namely, *cybrid technology* **(Fig. 16)**.

Because no methods are available to transfect DNA into mammalian mitochondria, a "backdoor" approach has been developed to introduce exogenous mitochondria (and their mtDNAs) into transformed human cell lines devoid of endogenous mtDNAs (called ρ^0, using the nomenclature established for yeast). Specifically, a ρ^0 human osteosarcoma cell line that was also deficient in thymidine kinase activity (TK^-) by growing the cells in ethidium bromide, an inhibitor of mtDNA replication. Due to the loss of a functional respiratory chain, the ρ^0 cells require pyrimidines and pyruvate in order to grow, thus providing two selection schemes for the repopulation of these cells by exogenous mtDNA-containing mitochondria. The ρ^0 cells are repopulated by forming *cytoplasmic hybrids*, or *cybrids*, between the host ρ^0 cells and cytoplasts (enucleated cells) from an mtDNA donor cell line (e.g. from a patient with a pathogenic mtDNA mutation). After cell fusion, cells are plated in medium containing bromodeoxyuridine (BrdU) and lacking either pyruvate or uridine. These selective media permit only the growth of ρ^0 cells which had fused with cytoplasts containing functional mitochondria, because ρ^0 cells are not able to grow in the absence of uridine or pyruvate, and TK^+ donor cells are not able to grow in the presence of BrdU. Cybrid technology has been used successfully by many groups to study pathogenic mtDNA point mutations.

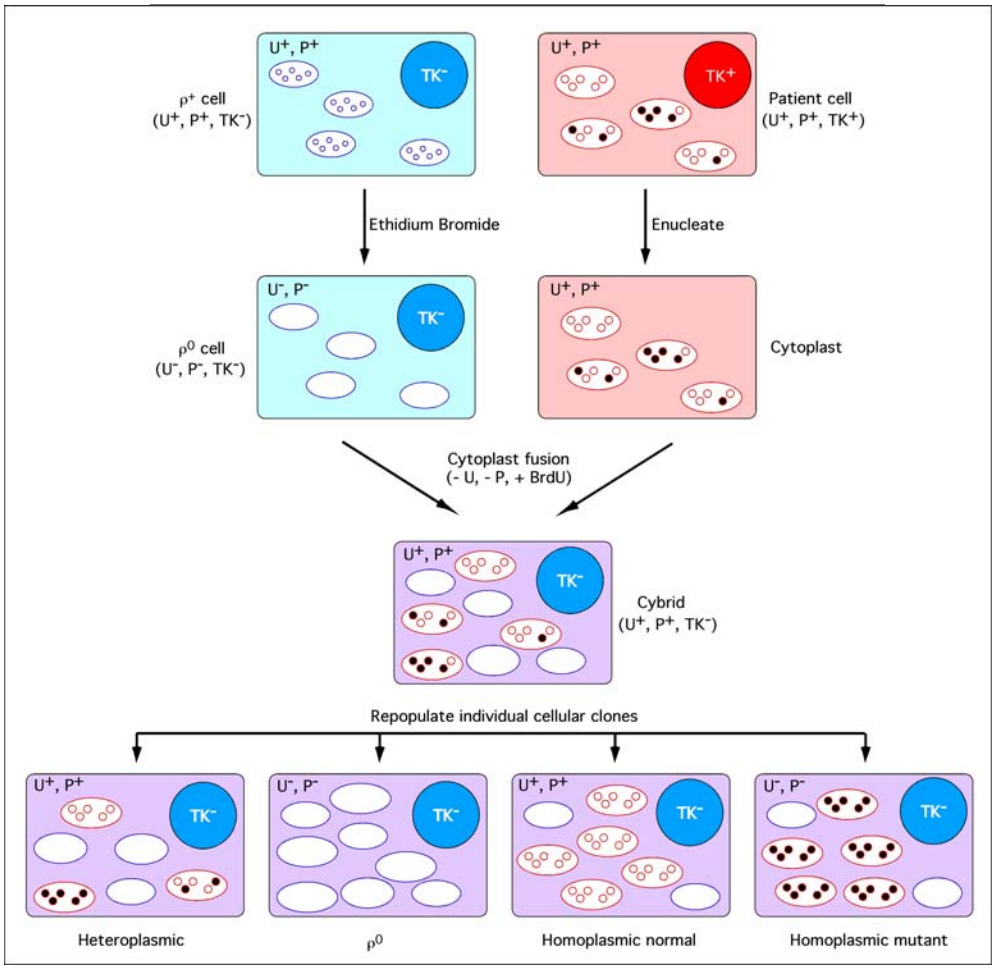

Figure 16. Cybrid technology.

Further readings

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Selected glossary

Bottleneck hypothesis. The hypothesis that only a tiny minority of mitochondria (and mtDNAs) pass from mother to child, due to a "filtering" process in the maternal germline cells

Endosymbiont theory. The theory that mitochondria are actually derived from bacteria that were "captured' by proto-eukaryotic cells early in evolution

Heteroplasmy. The condition in which the mitochondrial genomes in a particular cell, organ, or individual have different DNA sequences; heteroplasmy usually implies only 2 mtDNA genotypes, wild-type and mutated

Homoplasmy. The condition in which all the mitochondrial genomes in a particular cell, organ, or individual have the identical DNA sequence

Maternal inheritance. Transfer of genetic information (e.g. mitochondria and mtDNAs) from a mother to all of her children (both sons and daughters); only the daughters will pass on that information to the following generation.

Mitotic segregation. The stochastic and dynamic nature of the passage of mitochondria to daughter cells after cell division

Oxidative phosphorylation. The conversion of ADP to ATP by the action of ATP synthetase, in which a proton gradient across the mitochondrial inner membrane (generated by the respiratory chain) drives ATP synthesis. Oxygen is required for this process to occur.

Respiratory chain. The overall name given to the multisubunit complexes and other electron carriers located in the mitochondrial inner membrane responsible for the transfer of reducing equivalents (protons) from the matrix into the intermembrane space. These protons are then re-pumped back into the matrix during the process of oxidative phosphorylation.

Threshold effect. The idea that there is a minimum requirement for oxidative energy, below which pathology can occur; the threshold is low in tissues that are highly aerobic, such as muscle and brain.