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Mitochondrial DNA, nuclear context and the risk for carcinogenesis

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Abstract

The inheritance of mitochondrial DNA (mtDNA) from mother to child is complicated by differences in the stability of the mitochondrial genome. Although the germ line mtDNA is protected through the minimization of replication between generations, sequence variation can occur either through mutation or due to changes in the ratio between distinct genomes that are present in the mother (known as heteroplasmy). Thus, the unpredictability in transgenerational inheritance of mtDNA may cause the emergence of pathogenic mitochondrial and cellular phenotypes in offspring. Studies of the role of mitochondrial metabolism in cancer have a long and rich history, but recent evidence strongly suggests that changes in mitochondrial genotype and phenotype play a significant role in the initiation, progression and treatment of cancer. At the intersection of these two fields lies the potential for emerging mtDNA mutations to drive carcinogenesis in the offspring. In this review, we suggest that this facet of transgenerational carcinogenesis remains underexplored and is a potentially important contributor to cancer.

Keywords

transplacental; mitochondrion; mutagenesis; heteroplasmy; cancer

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Statement of Author Contributions

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Introduction to mitochondria and its genome

Mitochondria are thought to have arisen from an endosymbiotic event to form the first organelle in the cell [Margulis 1970]. Over millennia, the double membraned organelle has become the site of many key biosynthetic and metabolic processes that require compartmentalization. In this partnership with the cell, most of the mitochondrial-localized gene products have become encoded in the nucleus, where they are transcribed, cytoplasmically expressed, and then imported into the mitochondria. The most well known of the mitochondrial functions is oxidative phosphorylation, a process of efficient conversion of carbohydrates and fats with oxygen into energy in the form of ATP and carbon dioxide.

Oxidative phosphorylation (OXPHOS) complexes are unique in that subunits are encoded by either the nuclear or mitochondrial genome, making this essential function bigenomic in origin [Scarpulla 2008]. The mitochondrial DNA (mtDNA) is a small 16.5kb, multi-copy genome that encodes 37 genes and is located in the mitochondrial matrix (Figure 1). The mitochondrial genes include 13 protein-coding genes, plus two ribosomal RNAs and 22 tRNA required for protein translation. All of the protein machinery required for genome replication, transcription, RNA processing and translation are nuclear encoded. The proteins encoded by the mtDNA are core, hydrophobic subunits of four of the five OXPHOS complexes, and the sequences have been potentially retained due to complications of encoding these proteins in the nucleus. The bigenomic nature also creates the potential for interplay between the nuclear and mitochondrial compartments to affect mitochondrial respiratory function [Lane 2011].

Mitochondrial genome stability

The mitochondrial genome is in close proximity to the OXPHOS complexes, which are major sites of reactive oxygen species (ROS) generation in the form of superoxide. Superoxide is converted by dismutases to hydrogen peroxide, which is membrane diffusible. However, through Fenton chemistry, dependent upon the iron which is present at high levels in mitochondria [Seo et al. 2008], hydrogen peroxide can be converted to hydroxide ions [Imlay et al. 1988], which cause single or double strand breaks in DNA [Cassano et al. 2002]. This proximity to the source of reactive oxygen species is thought to contribute to the increased rates of mtDNA damage [Yakes and Van Houten 1997] and potentially mutation [Shokolenko et al. 2009] relative to the nucleus. Furthermore, mtDNA lacks many of the nuclear DNA repair pathways, and is slower to recover from the insult [Yakes and Van Houten 1997; Liu and Demple 2010]. The accumulation of damage and mutations negatively impacts mtDNA sequence integrity, primarily with age [Li et al. 2017; Neuhaus et al. 2017; Sondheimer et al. 2011], but the inheritance of existing mutations or the acquisition of mutations during oocyte maturation and embryogenesis is the primary cause of mtDNA-borne diseases [Alston et al. 2017].

Unlike nuclear DNA, which is contributed to equally by both mother and father, mtDNA is exclusively transferred from the mother [Giles et al. 1980]. The prevention of paternal transmission occurs through several mechanisms, including low incorporation of sperm mitochondria into the zygote, ubiquitination and destruction of the sperm mitochondria, and

paternal mtDNA destruction by nucleases [Sato and Sato 2013]. Direct evidence as to the advantage for this clonal transmission has recently been suggested in model organisms. Paternal mitochondrial elimination is thought to aid in maintaining a single mitochondrial population of normal sequence, as interfering with paternal mtDNA destruction interferes with embryo survival in *C. elegans* [Wang et al. 2016]. Although the cause and consequence of this process is an area of on-going research, it is clear that the matrilineal transmission drives clonal mtDNA transmission.

The selective transmission of mtDNA provides stability to the genome sequence, which is extremely useful for tracking ancestry and migration. The evolutionary clock of mtDNA is fast enough to detect changes during recent human evolution, along the time scale of the emergence from Africa [Rito et al. 2013]. The co-inherited polymorphisms that help define modern day populations and their origins are called “haplogroups”. These variations have been thought to be stable, neutral changes in the genome, but increasingly, evidence suggests that these sequences interact with the sequences of the nuclear genome to modify overall mitochondrial function [Suisa et al. 2009; Cohen et al. 2016].

Heteroplasmy and its consequences

Maternal transmission from an ostensibly healthy parent does not eliminate the risk of disease because of heteroplasmy. Heteroplasmy is common feature of organellar genomes. It refers to the state that occurs when more than one allelic variant of the mitochondrial sequence is present. Because of their variable copy number, the nuclear concept of heterozygosity is inapplicable. Heteroplasmy is very commonly observed in human mtDNA samples, with progressively greater levels present with increasing age [Sondheimer et al. 2011].

When mitochondrial mutations have functional consequences, the degree of heteroplasmy (often referred to as the “load” of the variant or pathogenic allele) is one of the factors that determine disease phenotype. This has been extensively demonstrated in studies of mitochondrial mutations causing classical mitochondrial pathologies [Chinnery et al. 1997]. For example, the m.3243A>G mutation, most often associated with mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS), is associated with earlier ages of onset at higher loads of heteroplasmy. Furthermore, the phenotype expressed can be different at different loads of mutation, possibly resulting from differences in nuclear gene expression responses with increasing mutation load [Picard et al. 2014]. Lower loads might be associated with maternally inherited diabetes, whereas higher loads cause the more lethal MELAS phenotype.

Heteroplasmy is also challenging to evaluate at an organismal level because it is unstable. If the tissues of a heteroplasmic individual are separately evaluated, it is apparent that the load of mutation can be widely variable [He et al. 2010]. This is particularly evident in pre-cancerous and cancerous tissues [Sui et al. 2006]. Heteroplasmy is also variable as a function of time. Multiple studies have demonstrated increasing heteroplasmy in individuals as they age [Barker and Murthy 2009; Sondheimer et al. 2011; Murdock et al. 2000].

Perhaps the most perplexing aspect of heteroplasmy (and indeed mitochondrial genetics) is the shifts in heteroplasmy between generations. The birth of children with mtDNA-dependent mitochondrial disorders from mothers with no evidence of disease implies that changes in heteroplasmy or novel mutations can and do occur. In a series of three children born with fatal and infantile-onset Leigh syndrome due to m.10158T>C mutation, the mutation was only detectable in only one of the three mothers [McFarland et al. 2004]. In the mother where it was observed, the level of mutation had progressed from 7% in her fibroblasts to >98% in tissues tested in her offspring.

Thus, although mutations in mtDNA can arise *de novo* during oocyte generation, observed shifts in heteroplasmy between mothers and their children are a variance due to sampling of maternal mtDNA through the “bottleneck” of oocyte formation, leading to altered distribution of heteroplasmy in the offspring (Figure 2). This bottleneck occurs because of a reduction in the number of mitochondrial genomes that are transmitted between generations through the oocyte line. Despite the fact that there can be $\sim 10^6$ copies of mtDNA in some cell types, the effective number of genomes that makes it through this bottleneck is orders of magnitude smaller [Marchington et al. 1998], although strikingly difficult to calculate and even distinct for specific mutations [Wilson et al. 2016].

The existence of the bottleneck was most elegantly proven in studies of oocytes obtained at oophorectomy from a known carrier of the m.3243A>G mutation [Brown et al. 2001] and another of a woman carrying a mitochondrial deletion [Marchington et al. 1998]. These studies demonstrated that even for pathogenic mutations was no bias present in the selection process and that the derived heteroplasmies in the oocytes were effectively a normal distribution of the mother’s own heteroplasmy. Work in animal models has suggested that only very severe and high heteroplasmy mutations can be counter-selected by purifying selection over generations [Fan et al. 2008]. These studies and others also suggest that the number of effective mitochondrial genomes transmitted across the bottleneck is extremely small compared to the number of mitochondrial genomes present in the oocyte at fertilization, allowing considerable shifts in heteroplasmic ratios from mother to child.

Furthermore, once inherited, certain pathogenic mtDNA mutation levels can exhibit vast differences in the degree of observed heteroplasmy by adulthood. For example, pairs of monozygotic twins were found to carry very similar m.3243A>G mutation levels across multiple tissues, where some tissues like blood contained <10% heteroplasmy whereas the heart and brain contained a mutational load >80% [Maeda et al. 2016]. Together with the bottleneck influencing maternal transmission of mtDNA mutations, tissue-specific factors during development can also influence the dynamics of heteroplasmy across the lifespan [Picard and Hirano 2016].

Mitochondrial DNA operates within a nuclear context

Although mtDNA is derived from an organism once capable of independent life, the mtDNA now works cooperatively with the nuclear genome. All of the mtDNA-encoded polypeptides function within the electron transport chain or as a component of the F_0F_1 -ATPase (Complex V). In all cases, the encoded mitochondrial proteins physically interact with nuclear-encoded

partners. Furthermore, the regulation of mtDNA transcription and replication are entirely under the control of nuclear proteins [Falkenberg et al. 2007].

The interaction between mitochondrial and nuclear encoded gene products, and the use of nuclear-encoded proteins in genome regulation, creates the potential for loss of fitness when the mitochondrial and nuclear genomes are not well co-adapted. Many of the polymorphic variants that define the human haplogroups have an impact on functions like mitochondrial transcription [Suissa et al. 2009; Cohen et al. 2016]. If these variants have negative interactions with poorly co-adapted nuclear variation, overall mitochondrial activity could be impacted.

This loss of fitness due to differing ancestry of nuclear and mtDNA has been shown in studies of conplastic animals. A conplastic animal is created by transferring the mitochondrial genome of one strain into the nuclear context of another by repeatedly backcrossing to males. Conplastic mice have been found to have a range of problems including defects in behavior, susceptibility to autoimmune disorders, susceptibility to metabolic syndrome and changes in longevity [Yu et al. 2009; Latorre-Pellicer et al. 2016; Betancourt et al. 2014]. Many of these defects may be initiated by changes in the production of free radicals by mitochondrial with poorly aligned genetic input [Fetterman et al. 2013].

It is uncertain whether differences between human haplogroup and human nuclear variation will have a similar effect. One of us has recently developed a system for quantifying the discrete ancestry of mitochondrial and nuclear information, based upon genotyping performed on genome wide single-nucleotide polymorphism arrays. These studies showed that spontaneous preterm birth was more likely in infants where discrete mitochondrial ancestry was present [Crawford et al. 2017]. Future studies will be required to identify the range of phenotypes that may be associated with larger evolutionary distance between inherited nuclear and mitochondrial DNA.

The role of the mitochondrion in the cancer cell

A hallmark of cancer cell is a shift away from oxidative phosphorylation as major source of energy, in favor of glycolysis [Hanahan and Weinberg 2011]. This phenomenon was first recognized by Otto Warburg [Warburg 1956] and thereafter named the Warburg effect. Although the initial assumption from this observation was that mitochondria became “unimportant” or dispensable in cancer cells, this view has changed substantially in recent years due to evidence that mitochondria – even if not the powerhouse of the cancer cell – contribute significantly to growth [Ward and Thompson 2012]. Indeed, the loss of respiratory chain function causes proliferative failure of cancer cells [Birsoy et al. 2015; Dong et al. 2017]. Together, these data demonstrate that even if mitochondria are no longer providing most of the cellular ATP in cancer cells, mitochondria are generally essential for oncogenesis.

One reason for this phenomenon is that in the transformed cell, mitochondria are actively involved in anabolic pathways required for rapid cell growth [Ward and Thompson 2012]. Glucose and glutamine oxidation generate citrate and acetyl-CoA for the synthesis of lipids [Vander Heiden et al. 2009], a process termed reductive carboxylation that favors cell growth

[Mullen et al. 2011]. Mitochondria also release soluble chemical signals, including reactive oxygen species, that contribute to the activation of hypoxia-inducible genes (i.e., pseudohypoxic state) [Al-Mehdi et al. 2012] and act as mitogenic signals [Weinberg et al. 2010]. The antineoplastic effects of certain drugs, such as metformin, may also act in part by preventing reductive carboxylation, citrate production, and de novo lipid biosynthesis [Griss et al. 2015]. The production of oncometabolites by cells with mutations in genes encoding the Krebs cycle enzymes succinate dehydrogenase subunits B, C, and D (*SDHB*, *SDHC*, *SDHD*) [Ishii et al. 2005; van Gisbergen et al. 2015], and isocitrate dehydrogenase (*IDH1/2*) may also favor oncogenic transformation and be responsible for a small group of central nervous system tumors including astrocytomas, oligodendrogliomas, and secondary glioblastomas [M Gagné et al. 2017; Wallace 2012]. Thus, overall, clinical and pre-clinical models suggest that mitochondria actively contribute to tumorigenesis via intermediate metabolism and metabolic reprogramming.

The mitochondrion, genetics and cancer

Three major lines of evidence implicate the mitochondrial genome in cancer. The least understood is the integration of nuclear mitochondrial DNA segments (NUMTs) into the nuclear genome [Hazkani-Covo et al. 2010]. The first report to bring attention to this issue identified large mtDNA sequences – some almost the entire mtDNA sequence (16,556 bp) integrated into the tumor nuclear genome [Ju et al. 2015]. The presence of mtDNA-derived DNA sequences was extensively validated by PCR across the nuclear-mitochondrial genome junction, and sequencing of the NUMT to confirm concordance of the mtDNA sequence polymorphisms with those of blood cells from the same donor. In another study, compared to blood DNA, colorectal adenocarcinoma tumor DNA of the same individuals showed a 4-fold elevation in NUMTs [Srinivasainagendra et al. 2017]. It will be informative in future studies to quantify and compare the NUMT status of tumors with surrounding tissues rather than blood. The cause of increased NUMT insertions is unclear, as is their impact on oncogenesis, although NUMTs may increase genomic instability. Little is known about the regulation of these clandestine retrotransposed genetic elements and their functional significance in human cancer.

The second line of evidence suggests that mtDNA sequence variants can confer susceptibility to certain cancers [Seyfried 2015]. Controversial evidence suggests that mtDNA haplogroups can predispose to cancer. In epidemiological studies, it has been reported that mtDNA haplogroup U is associated with a ~2–2.5-fold higher risk for renal and prostate cancer [Canter et al. 2006] (see [van Gisbergen et al. 2015] for a review). However, some limitations must be noted: some studies have reported negative findings, we cannot rule out publication bias (studies with null results are not published, but positive findings are consistently published), and cross-sectional observational study designs can be confounded in many ways precluding causal inference. More work is thus required to overcome these caveats. Nevertheless, converging evidence from experimental studies indicate that mild mtDNA mutations may also promote tumor cell growth [Cruz-Bermúdez et al. 2015]. This implies that functional mtDNA variants (i.e., mutations) could drive and/or favor oncogenic transformation, although this point remains contentious and in need of further validation in humans.

Finally, a body of evidence has demonstrated an increased prevalence of mtDNA mutations in tumors. mtDNA sequence analysis of 1,675 tumors revealed a mutational pattern with heavy strand bias, predominantly C>T and A>G mutations [Ju et al. 2014]. Whereas synonymous mutations were often observed in homoplasmy, indicative of clonal expansion without selection, deleterious mutations causing protein truncation showed evidence of negative selection in this and another study [Strakova et al. 2016], suggesting that even in cancer cells mutations that affect the electron transport chain have a deleterious effects on cell growth, consistent with the notion that functional mitochondria are required for cancerous cell growth.

Cancer susceptibility (or lack thereof) in primary disorders of mtDNA

If mtDNA mutations that cause primary mitochondrial disorders could also trigger oncogenic transformation or support the progression, one would expect a higher prevalence of cancer among patients with inherited mtDNA mutations. However, there is no clinical or epidemiological evidence to support this possibility. To our knowledge, there has not been a formal study of the incidence of cancer in patients with mitochondrial disease. One exception is the identification of a rare form of lipoma in patients with the m.8344A>G mutation [Holme et al. 1993]. The general dissociation of these disease states could reflect a biological incompatibility between cancer and highly pathological forms of mitochondrial dysfunction.

Alternatively, this apparent clinical dissociation may arise from the fragmented medical architecture that has traditionally treated these diseases quite distinctly. Except for rare severe pediatric cases, mitochondrial disease is likely under diagnosed [Gorman et al. 2015]. Moreover, there is substantially greater medical and general public awareness around cancer than primary mitochondrial disorders. Thus it is possible that adults with both mitochondrial diseases are not uncommon, but fail to be recognized. On the other hand, some adult patients with confirmed and pre-existing mtDNA disease have been followed for many years, with few reports of malignancy. However, this represents a fairly small number of individuals and sampling bias could be at the origin of this apparent dichotomous state between cancer and mitochondrial disease. More research is needed in this area.

Hypothesis: Transgenerational Alterations in Mitochondrial Genetic Potential May Predispose to Cancer

We suggest that transgenerational alterations in mtDNA are a potentially under-recognized contributor to pro-oncogenic states. It is now well recognized that mitochondrial metabolism plays a critical role in supporting many types of cancer, and that adaptations to typical mitochondrial function play roles in both transformation and metastasis. A growing body of evidence suggests that these changes in mitochondrial metabolism are not bystanders to the cancer phenotype, but are rather essential aspects of the transformed state [Mullen et al. 2011; Wang et al. 2013; Spinelli et al. 2017]. Nuclear mutations in genes encoding mitochondrial function are known to be a potential oncogenic “hit,” and it is reasonable that mtDNA mutations could act in the same fashion.

Changes in mtDNA between generations, either as shifts in heteroplasmy or through the generation of new mutations, are not rare. Because of the dense coding of the mitochondrial genome, random mutations have a high potential to impact mitochondrial function. Additionally, the highly reactive environment of mitochondrial matrix creates the potential for positive feedback loops. Mutations that lead to higher free radical burden may create a more mutagenic milieu, a process with the potential to accelerate as the mutational burden grows. Even non-mutated transmission of mtDNA has the potential to result in altered mitochondrial capacity in offspring if the nuclear-encoded repertoire of the developing embryo is sufficiently distinct from the mother. We have seen in animal models [Betancourt et al. 2014; Latorre-Pellicer et al. 2016] and now in some human studies [Crawford et al. 2017] that the nuclear context of the mtDNA, and the interactions between mitochondrial and nuclear proteins, ultimately determine how effectively the mitochondrial functions.

What type of mutations would we expect to act as drivers for mutation? With few anecdotal exceptions, it is not likely that highly pathogenic, disease-causing mutations will end up with strong associations with cancer. Were this the case, the links between mitochondrial disorders and the risk for cancer would be well established, as these patients are highly medically involved. Instead we find only scattered reports of patients with obvious mitochondrial syndromes and cancer, potentially below the expected level due to random association, even given the shortened life span associated with these disorders that could reduce the window for observing cancer initiation and progression. This suggests that highly disruptive mutations may actually act as suppressors of cancer by compromising the reprogramming of mitochondrial function. Likewise, how would we consider the risk imposed by mtDNA operating in a distinct nuclear context within offspring as compared to their mothers?

If we hypothesize that transplacental mutagenesis or change in nuclear context can create the opportunity for elevated cancer risk, how would this be observable? An ideal data set would include high-resolution genome-level sequencing with accurate heteroplasmy detection and quantification, encompassing mothers and their children, obtained on DNA samples prior to the detection of cancer. It is clear that the use of mitochondrial sequencing from cancers themselves cannot be used because it will be impossible to determine whether individual mutations are drivers of cancer initiation and progression or merely passenger mutations that developed within the mutagenic environment of the cancer and have been clonally expanded. The population under study would need to be unselected and of sufficient size so that the study would be powered to detect differences between affected individuals with specific cancer types and controls who were free from cancer at similar ages. Clearly, a data set of this magnitude does not currently exist, although it might potentially be collected through mass population screening approaches, such as the newborn screen or other widespread population based sequencing approaches [Sondheimer 2013; Sankar and Parker 2017]. Alternative approaches may be possible with some large, well-genotyped cancer pedigrees, since mitochondrial sequence data is often inadvertently collected and available for analysis.

The determination that transplacental changes in mtDNA sequence were associated with the potential for cancer development would place a new emphasis on understanding mtDNA sequence changes in newborns. As we approach an era where sequencing is universally

applied, this information could be used to guide screening approaches and improve health by managing cancer susceptibility risk.

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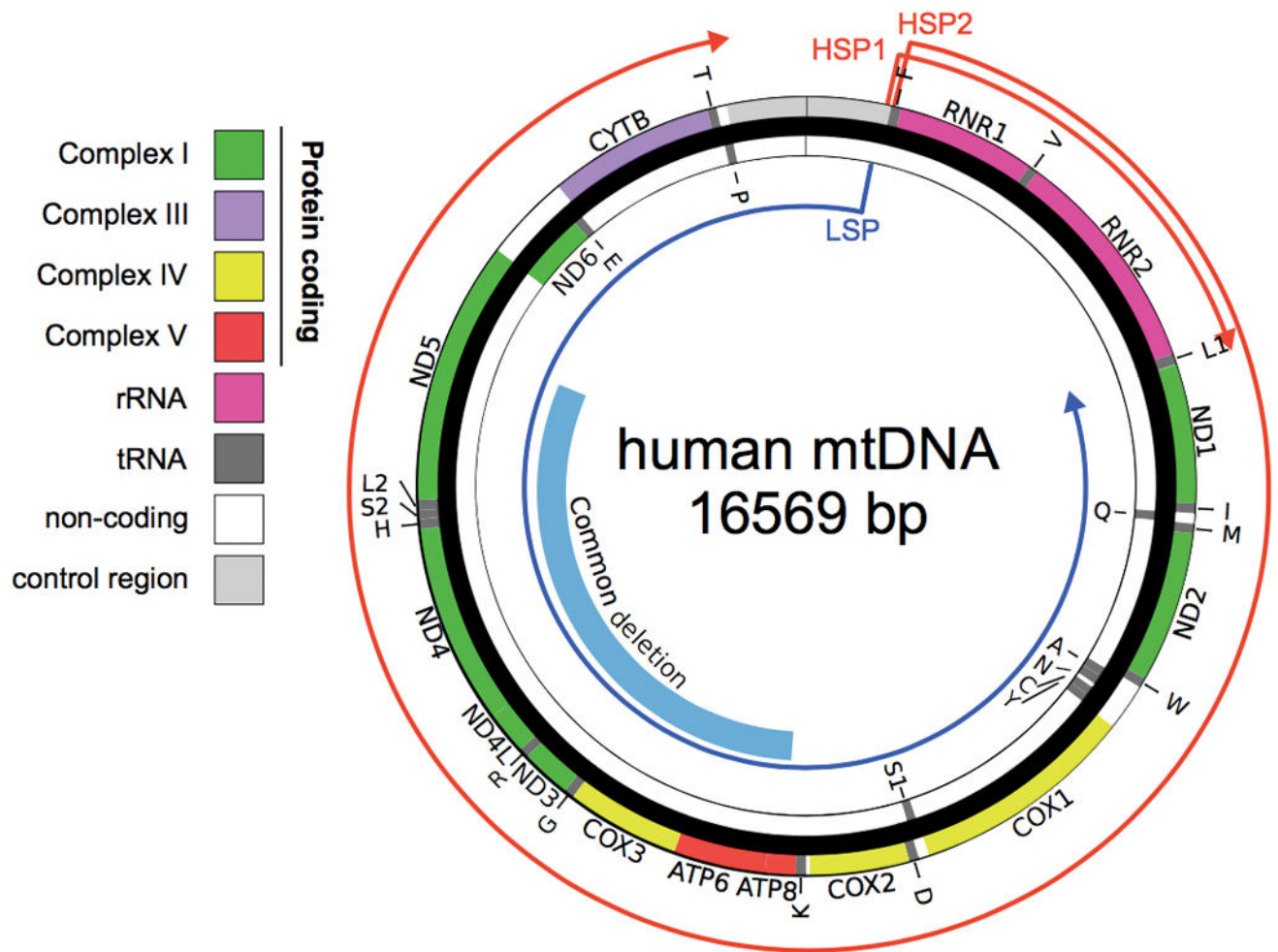


Figure 1.

Map of the human mtDNA with genes and transcripts marked. The mitochondrial genome is a 16,569 b.p. circular DNA. The region eliminated in the “common deletion” is shown in blue. Genes are encoded on either strand, shown separated into inner and out strand for clarity. The nascent transcript originating from heavy strand promoters (HSP) 1 and 2 are shown in red, from the light strand promoter (LSP) in blue. Regions of each strand are color coded by oxidative phosphorylation complex (protein coding), ribosomal RNA (rRNA), transfer RNA (tRNA), non-coding, and control region.

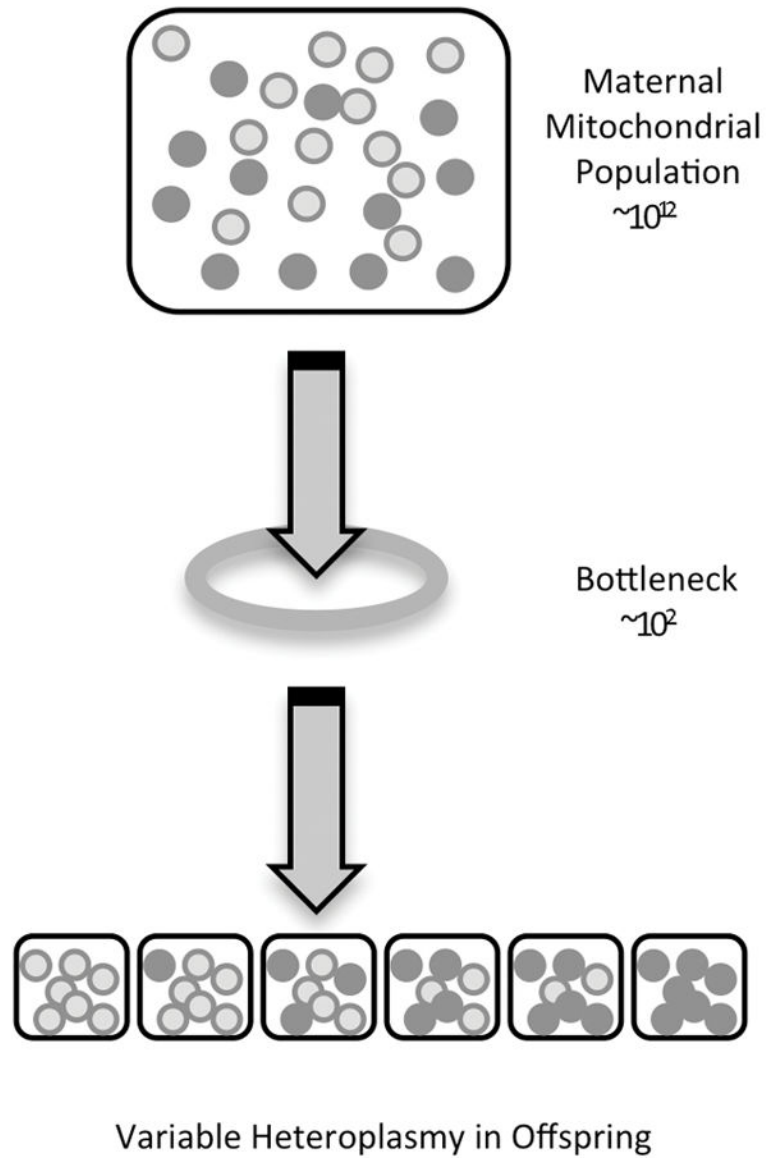


Figure 2. The mitochondrial bottleneck between generations allows for a distribution of heteroplasmy in offspring. The small number of molecules at the bottleneck limits the number maternal mtDNA inherited to produce varied heteroplasmy outcomes.