The mitochondrial genome: mutation, selection and recombination

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Within an individual, mitochondria must function in a range of tissue specific environments that are largely governed by expression of a particular suite of nuclear genes. Furthermore, mitochondrial proteins form large complexes with nuclear-encoded proteins to form the electron-transport system. These dynamics between mitochondrial and nuclear genomes have important implications in studies of within and among species genetic variation, and interpretation of disease phenotypes. Experimentally disrupting naturally occurring combinations of nuclear and mitochondrial genomes should provide insights into the coevolutionary dynamics among genomes.

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Abbreviations
COX cytochrome *c* oxidase
mtDNA mitochondrial DNA

Introduction

Mitochondrial DNA (mtDNA) has been widely employed as an evolutionary marker to study the processes of molecular evolution and to infer phylogeographic and phylogenetic patterns. Researchers have also linked disease to mitochondrial mutations and physiological senescence to the cumulative effects of oxidative injury to mitochondria. Here we review recent findings regarding the patterns of mutation, selection, and recombination in mtDNA within individuals, within species and among species. Within individuals, we consider somatic and genetic changes. Somatic changes may be expected to give insight into cell and tissue specific selection and may have a significant impact on disease, empirical evidence for population genetic variation, and phylogenetic inference. Evolutionarily, however, it is the changes in the female germline that are significant. Within species, selection may act directly on the mtDNA and indirectly through proteins encoded by the nuclear genome. Each mtDNA gene product must interact with nuclear-encoded proteins to perform oxidative phosphorylation. Mixing nuclei and mtDNA among closely related species results in reduced function, however, the molecular basis of these interactions is only beginning to be unraveled.

In this review, we restrict our discussion to metazoans where mitochondria are inherited maternally, and to intrinsic forces that influence the evolution of mtDNA. Intrinsic forces include mitochondrial and nucleomitochondrial interactions that may vary spatially. We do not consider the influence of any extrinsic factors. One extrinsic factor that has been shown to influence mtDNA evolution in invertebrates in theory [1], in population cages [2], and in natural populations [3,4] is *Wolbachia* [5,6]. *Wolbachia* may influence the evolutionary dynamics of mtDNA by the process of genetic hitchhiking. However, it also seems quite possible that mitochondria and *Wolbachia* are interacting at the cellular level [7]. If this is true, *Wolbachia* may be more than "influential passengers" [8] — they may be more like 'species navigators'.

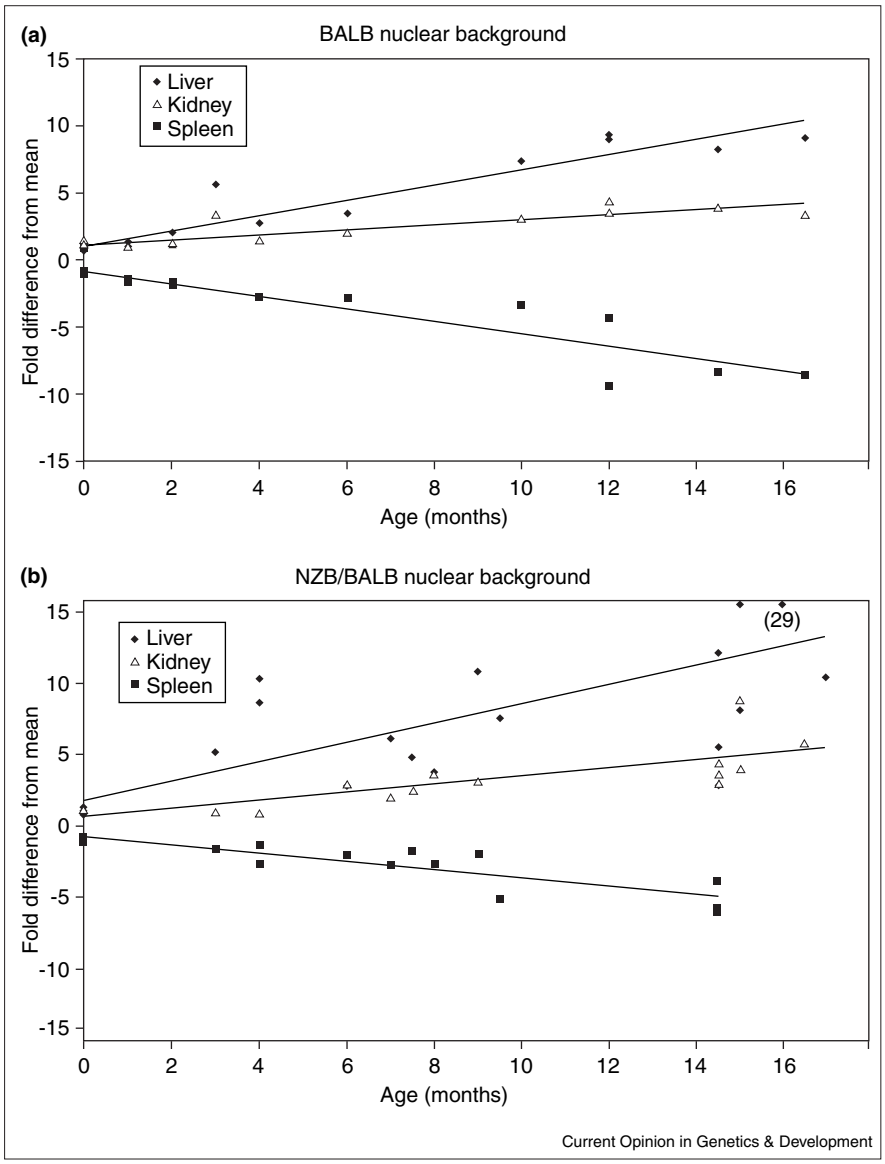
Mutation

MtDNA commonly undergoes higher rates of evolutionary change than the nuclear genome, although this may not be the case in insects. The higher rate of evolution is probably caused by the relative lack of repair mechanisms, and to the more frequent exposure of mtDNA to reactive oxygen metabolites. This high mutation rate may pose a significant selection pressure on mitochondrion-encoded proteins. Berg and Kurland [9••] show that as the mutation rate of mtDNA increases, relative to the mutation rate of nuclear DNA, there is an inherent bias towards gene movement from the mitochondrion into the nucleus. Their models predict that after transfer to the nucleus, mutation will eventually lead to irreversible deletion of redundant genes, thus leading to a reduction in the original genome size.

The mitochondrial molecule must function in a wide range of cellular environments and distinct selective forces may be operating in specific tissues and cells. As a consequence, the molecule may be viewed as a 'best-fit' sequence to the nuclear genome in a specific environment. We suggest that one strategy to investigate tissue specific selective forces, potentially generating locally optimal mtDNA sequences, is to study somatic mutations. There are few good estimates at present of the somatic point mutation rate in the mtDNA of healthy individuals although deletions of mtDNA have been shown to accumulate with age in a variety of species regardless of mean or maximal lifespan. This implies that such mutations are either a molecular biomarker of senescence or that they are more causally linked to senescence itself [10].

Early evidence that somatic point mutations occurred in humans came indirectly through studies tracing the frequency of mutant mtDNAs in the ancestors of people with a diagnosable clinical condition. Tulinius *et al.* [11] identified a mutation in the ATP synthase subunit 6 gene in a male who died of Leigh syndrome at age 15 months. He had 94% mutated mtDNA in muscle and 92% in lymphocytes. His mother was healthy but had 37% mutated mtDNA in muscle and 38% in lymphocytes. The infant's brother, who was also healthy, had 44% mutated mtDNA in lymphocytes. No mutated mtDNA was detected

Figure 1



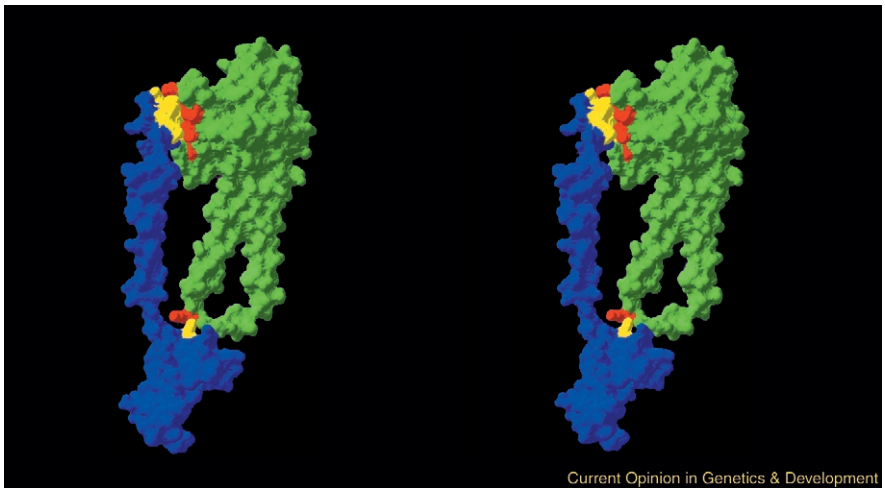
Selection for two mtDNA genotypes (BALB, NZB) in spleen versus liver and kidney of heteroplasmic animals with increasing age. The mean level of heteroplasmy for each animal was determined from the following tissues: colon, cerebral cortex, gastrocnemius muscle, testes, tail, tongue and cardiac ventricle. Each point corresponds to the value obtained in an individual animal. Tissues from four newborn animals were sampled at postnatal day 3–4 for both the **(a)** BALB and **(b)** mixed BALB/NZB nuclear backgrounds. (Reproduced with permission from [22].)

in muscle and lymphocytes from the maternal grandmother or in lymphocytes from 15 other maternal relatives, showing that the first carrier of the mutation in this family was the mother. This study shows that the point mutation occurred at intermediate frequency in a carrier of a *de novo* mutation and segregated over two generations, causing neurodegenerative disease.

Parsons *et al.* [12] examined the rate and pattern of sequence substitutions in the mtDNA control region of humans. They compared mtDNA sequences of two hypervariable segments from close maternal relatives, from 134 independent mtDNA lineages spanning 327 generational events. Ten substitutions were observed, resulting in an empirical rate of 1/33 generations, or 2.5/site/million years. This is roughly 20 times higher than estimates derived from phylogenetic analyses. The authors suggested the disparity cannot be accounted for simply by substitutions at mutational hot spots, but rather additional factors produce the discrepancy between short-term and phylogenetic rates of sequence divergence. An alternate hypothesis to explain these intriguing results is that the aged individuals in this study accumulated somatic point mutations and the observed mutation rate is a product of both somatic and genetic mutations. A second alternate hypothesis is that nuclear copies of mtDNA pseudogenes or Numts [13] were sequenced in some cases. In fact, an early study of mtDNA heteroplasmy in Alzheimer's patients later rejected as the original authors apparently sequenced a Numt [14]. Bensasson *et al.* [15] have reported that Numts have been isolated in 53 species including humans (also see 'Update').

Among species, a prominent form of mutation is deletion of mtDNA protein coding genes [9••]. From a phylogenetic

Figure 2



Stereo view of the mtDNA-encoded COX II (green) and nDNA-encoded COX IV (blue) subunits. Residues of COX II that are in close contact with COX IV are red; those of COX IV in close contact with COX II are yellow. Distances between atoms of residues were calculated with a domain interface program. Close contact between residues was defined as ≤4 Å. (Reproduced with permission from [24].)

perspective, it is not always clear how these deletions and recombinational events should be scored [16–18]; however, it is clear that they are significant evolutionary markers. Mitochondrial genomes show varying degrees of reduction, ranging from just three protein-coding genes in the malarial parasite *Plasmodium falciparum* to 97-protein coding genes of the protozoan *Reclinomas americana*. Different species of *Rickettsia* show varying degrees of 'mutational meltdown', suggesting this process is ongoing on an evolutionary time scale [19]. Studies of simple rearrangements are likely to provide insight into the mechanism of mtDNA rearrangement. For example, *Drosophila yakuba* and *Ceratitis capitata* share the gene order ND3, $tRNA_{ala}$, $tRNA_{arg}$, while the more distant *Anopheles* fly shows the order ND3, $tRNA_{arg}$, $tRNA_{ala}$.

Selection

Ultimately, it is only the changes in the female germline that are evolutionarily significant. Bergstrom and Pritchard [20] emphasized the importance of the bottleneck in the number of mitochondria during oogenesis, and posited that this may serve as a ratchet that removes mutations. Barritt *et al.* [21••] provided statistical support for this hypothesis when they observed a significant reduction in the percentage of mtDNA rearrangements in human early embryos (n = 137) and oocytes (n = 74); however, tissue-specific physiology may pose unique selective environments. In an intriguing and well controlled study, Jenuth *et al.* [22] found evidence for tissue-specific and age-related directional selection on distinct mtDNA genotypes present in heteroplasmic mice (Figure 1). These surprising data suggest that the coding sequence of mtDNA may represent a compromise between the competing demands of different tissues and point to the existence of unknown, tissue-specific expression of nuclear genes important in the interaction between the nuclear and mitochondrial genomes.

Selection may act directly on the mtDNA and indirectly through proteins encoded by nuclear DNA. In the electron-transport system of the inner membrane of the mitochondria, only 13 proteins are encoded by the mtDNA genome as compared to >100 encoded by the nuclear genome [23]. Schmidt *et al.* [24••] studied the functional interactions between mtDNA- and nuclear DNA-encoded proteins in the multisubunit respiratory complex cytochrome *c* oxidase (COX). In this complex, nuclear DNA-encoded residues in close physical proximity to mtDNA-encoded residues evolved more slowly than the other nuclear-encoded residues. In contrast, mtDNA-encoded residues in close physical proximity to nuclear DNA-encoded residues evolved more rapidly than the other mitochondrion-encoded residues, indicating positive selection (Figure 2).

Within a species, distinct mtDNA haplotypes often exist in a non-random spatial or temporal distribution in nature, but few studies have attempted to experimentally investigate the fitness consequences of distinct mtDNA haplotypes. de Stordeur *et al.* [25] conducted cytoplasm microinjections among eggs from each of the three *D. simulans* haplotypes (*si*I, -II, and -III) [26,27,28•] and detected heteroplasmic flies when *si*II or *si*III mitochondria were injected into any cytoplasm. In the majority of cases, the percentage of *si*II mitochondria increased whereas *si*III mitochondria tended to increase in the *si*I nuclear background. Analysis of the number of heteroplasmic lines and the rate of fixation of each haplotype lead de Stordeur *et al.* to rank the fitness of the haplotypes as *si*II > *si*III > *si*I. This is a particularly intriguing result as it suggests that there are very strong nucleomitochondrial interactions and/or mtDNA haplotype fitness differences. This tractable system is a fertile arena for further study.

Among species, the first experiments considering the coevolution of nuclear and mitochondrial genomes were reported in 1971 [29]. In a classic experiment, elongated cells from hominoid apes — chimpanzee, pigmy chimpanzee,

gorilla, and orangutan — were fused with mtDNA-less human cells [30] thereby creating cells with ape mtDNA and human nuclear DNA. Only the combinations of human nuclear DNA with mitochondria from the most closely related species (chimpanzee, gorilla) yielded cells even capable of oxidative phosphorylation. More recently, it was shown that mice with introgressed interspecific and intersubspecific mtDNA exhibit reduced physical performance as measured by running time on a treadmill [31]. Also, species-specific rates of mtDNA mutation have been observed that associate with other nuclear-encoded factors. A rate increase in the evolution of many mitochondrion-encoded proteins in the anthropoid lineage shows a pattern consistent with positive selection correlated with expansion of the energy-demanding neocortex [32••].

Recombination

The degree of genetic hitchhiking is proportional to the frequency of recombination along a mtDNA region. In the absence of recombination, selection on any mutation in the mtDNA will lead to the concomitant fixation of all variants in that genome by a process of genetic hitchhiking [33]. Within a mitochondrial genome, a selective sweep is most likely to occur if a nonsynonymous mutation in a protein-coding gene causes an advantageous amino acid substitution. Alternatively, polymorphism within a mitochondrial genome may be depressed through selection against linked deleterious mutations, a process known as 'background selection' [34–36]. Even the hypervariable noncoding origin of replication region cannot be assumed to have neutral allele frequencies because of its linkage to the rest of the genome. However, it should be noted that the once accepted dogma that mtDNA does not recombine is increasingly questionable [37–41,42••,43••].

Within an individual, recombination may create novel variants that are dependent on the specific physiology of tissues. Andersson and Kurland [44] offered a mechanism for intrachromosomal recombination that includes illegitimate homologous pairing facilitated by repeated sequences within the genome. An alternate mechanism of recombination has been proposed [18]. Kajander *et al.* [42••] have analyzed the pattern and distribution of sublimons — small circular portions of mtDNA that most likely arise through intrachromosomal recombination and excision — in the various tissues of individual humans. These results support the hypothesis that tissue-specific nuclear genes are important in nucleus–mitochondrion interactions. The pattern of products representing rearranged mtDNAs was different. For example, a very prominent product of 3.75 kb was evident in both heart and skeletal muscle, but the same product was either much less abundant or not evident at all in other tissues. Furthermore, sublimons accumulated in different tissues at different rates. The relative abundance of sublimons was in the order heart > muscle > brain ≈ kidney > skin ≈ liver.

Paternal leakage of mtDNA may provide a mechanism for establishing heteroplasmic individuals and experimentally testing for recombination. Most often, paternal mtDNA within a species is quickly degraded following recognition of a ubiquitin tag [45], but recognition of paternal mtDNA apparently depends on phylogenetic relatedness. In intraspecific hybrids of *Mus musculus*, paternal mtDNA in a single mouse embryo was detected only through the early pronucleus stage, and its disappearance coincided with loss of membrane potential in sperm-derived mitochondria. By contrast, in interspecific hybrids between *M. musculus* and *M. spretus*, paternal mtDNA was detected throughout development from pronucleus stage to neonates [46]. If heteroplasmic lines can be constructed [22,47,48], it may be possible to test experimentally for recombination following bombardment with X-rays or a chemical mutagen. Mammalian mitochondria possess the enzymes necessary for recombination [49].

Conclusions

We have considered the processes acting on mtDNA within an individual, within a species and among species. Within an individual, we discuss both somatic and genetic changes. Study of intra-individual changes will help us develop a better understanding of the heterogeneous selective forces that act on mtDNA in distinct cellular environments. From perspective, nucleomitochondrial interactions are likely to be one of the most important arenas for future research, although somatic mutations and Numts may confound estimates of genetic diversity and phylogeny [50]. We suggest that researchers studying genetic variation control for tissue-specific selection by sampling the same tissue from younger individuals. If this is not possible, multiple tissues should be sampled. Among species, the deletion of mitochondrial proteins and the rearrangement of genomes provides a continuing challenge for robust phylogenetic reconstruction.

Update

Rand *et al.* [51] recently developed a model of joint transmission of X chromosomes and mitochondria. They then test aspects of the model by analyzing fitness interactions between pairwise combinations of X chromosomes and cytoplasm from wild strains of *D. melanogaster*. The results from both the simulations and the experimental study suggest that fitness interactions with the sex chromosomes may account for a proportion of mitochondrial variation in natural populations. These results have important implications for mitochondrial disease phenotypes that differ between the sexes and highlight the need for increased attention on nucleomitochondrial interactions more generally.

An extensive examination of Numts in the human nuclear genome [52] found 296 Numts ranging in size from 106→14,654 bp. All parts of the mtDNA genome are represented in the nuclear genome with four of the fragments >10,000 bp. Four Numts also covered the complete control region, suggesting that they are the result of DNA-based transfer [53].

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