Trends in Genetics

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Review

Mitonuclear Compensatory Coevolution

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In bilaterian animals, the mitochondrial genome is small, haploid, does not typically recombine, and is subject to accumulation of deleterious alleles via Muller's ratchet. These basic features of the genomic architecture present a paradox: mutational erosion of these genomes should lead to decline in mitochondrial function over time, yet no such decline is observed. Compensatory coevolution, whereby the nuclear genome evolves to compensate for the deleterious alleles in the mitochondrial genome, presents a potential solution to the paradox of Muller's ratchet without loss of function. Here, I review different proposed forms of mitonuclear compensatory coevolution. Empirical evidence from diverse eukaryotic taxa supports the mitonuclear compensatory coevolution hypothesis, but the ubiquity and importance of such compensatory coevolution remains a topic of debate.

Highlights

Because they reproduce asexually, mitochondrial genomes are predicted to accumulate deleterious mutations and show functional decline. Paradoxically, no such decline is observed.

Via compensatory coevolution, nuclear genes evolve to 'fix' problems created by deleterious mitochondrial alleles.

Across diverse taxa, nuclear genes that have functional interaction with mitochondrial genes evolve faster than other nuclear genes and the pace of evolution is fastest when mitochondrial DNA mutation rates are highest.

Accessory proteins that have been recruited to electron transport system enzymes and mitoribosome stabilize the enzymes and may compensate for potential dysfunctions of mitochondrial genes, but these proteins may not have evolved as compensatory mechanisms.

The Paradox of Muller's Ratchet without Loss of Mitochondrial (mt) Function

Mitochondria have been described as ticking time-bombs, destined to annihilate species when they ignite [1]. The basis for such a sensationalized view of mitochondria is that, because mt genomes replicate asexually and typically without recombination (Box 1), mt DNA is vulnerable to the accumulation of deleterious mutations via **Muller's ratchet** (see Glossary) [2,3]. As a consequence, theory predicts that **mutational erosion** will undercut the function of mt gene products, which play essential roles in cellular respiration, leading to the demise of the organism. Despite the clear theoretical expectation of decline in mt function over time, no such decline is observed as a general feature of eukaryotes [4,5]. Strong **purifying selection**, which effectively removes functional mutations on the mt genome each generation [6–8], provides a partial explanation for how mt genomes might resist mutational erosion; however, purifying selection cannot be a complete explanation because empirical studies indicate that deleterious mutations do accumulate in mt genomes [9–12].

Compensatory coevolution has been proposed as an explanation to reconcile the observations of accumulation of deleterious mutations in the mt genome but no loss of function of the **electron transport system (ETS)** [13–19]. This mitonuclear compensatory coevolution hypothesis proposes that products of the nuclear (N) genome evolve novel features, enabling them to alleviate or nullify the dysfunctions that would otherwise be caused by deleterious alleles in the mt genome. Such mitonuclear compensatory coevolution has been proposed to come in two fundamentally different forms: (i) the evolution of novel N-encoded amino acid sequences that reverse or alleviate specific dysfunctions caused by deleterious changes in the mt genome [13,20], and (ii) the recruitment of novel N-encoded proteins to serve as accessory subunits of ETS complexes or **mitoribosomes** that help to stabilize the enzymes and alleviate enzyme dysfunctions caused by deleterious alleles in the mt genome [9] (Figure 1). A growing number of studies have documented evidence in support of evolution of N genes that compensate for dysfunctions caused by mt genes, but the importance of mitonuclear compensatory coevolution in eukaryotic evolution remains a topic of debate and discussion.

Mitonuclear Coadaptation and Coevolution

Out of the roughly 20 000 genes in the N genome of a vertebrate [21], the products of approximately 1200 genes are transported to the mitochondrion (hereafter **N-mt genes**) [22]. However,

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Box 1. Biparental Inheritance, Recombination, and Muller's Ratchet

Muller's ratchet describes the irreversible accumulation of deleterious alleles in genomes that do not engage in recombination, which can lead to mutational erosion of the mt genome and, theoretically, to mutational meltdown [2,63]. Nuclear genomes avoid Muller's ratchet by, at least periodically, engaging in sexual reproduction with reciprocal recombination [64]. Recombination breaks genetic linkages and allows good genes and bad genes to be acted upon independently by natural selection [65]. Mitochondrial genomes are generally described as nonrecombining genomes (hence the expectation is that they will be subject to Muller's ratchet [66]) but rates of recombination of mitochondrial genes vary widely among groups of eukaryotes [67]. Because compensatory coevolution is proposed as a means to reverse the effects of deleterious mutations in the mt genome, whether or not mitochondria engage in recombination becomes a key consideration for predicting in which taxa compensatory coevolution is most likely to be important. When mt genomes are transmitted by only one sex and there is strong purifying selection in the germ line, then rates of heteroplasmy are low and recombination becomes ineffective [68]. Conversely, when there is biparental transmission of mitochondria, heteroplasmy becomes common and recombination can become effective as a means to reveal deleterious alleles to natural selection [69]. It follows that there should be less need for compensatory coevolution if recombination enables natural selection to eliminate deleterious mutations in the mt genome. A key prediction, therefore, is that there should be less compensatory coevolution in eukaryotic taxa that have biparental transmission of mitochondria [70].

only about 150 of these N-mt genes code for proteins that engage in close functional association with mt DNA products and thus have the opportunity to engage in compensatory coevolution. The foundation of the mitonuclear compensatory coevolution hypothesis lies in the intricate functional interactions between the products of the mt genes and N-mt genes in enabling aerobic respiration and core energy production in eukaryotes [6]. The large and complex enzymes that constitute the ETS are composed of multiple protein subunits, with most subunits encoded by the N genome and a few core subunits encoded by the mt genome [23]. These mt- and N-encoded subunits must fit together with angstrom-scale precision to enable unimpeded flow of electrons and pumping of protons [24,25]. The function of chloroplasts is also a product

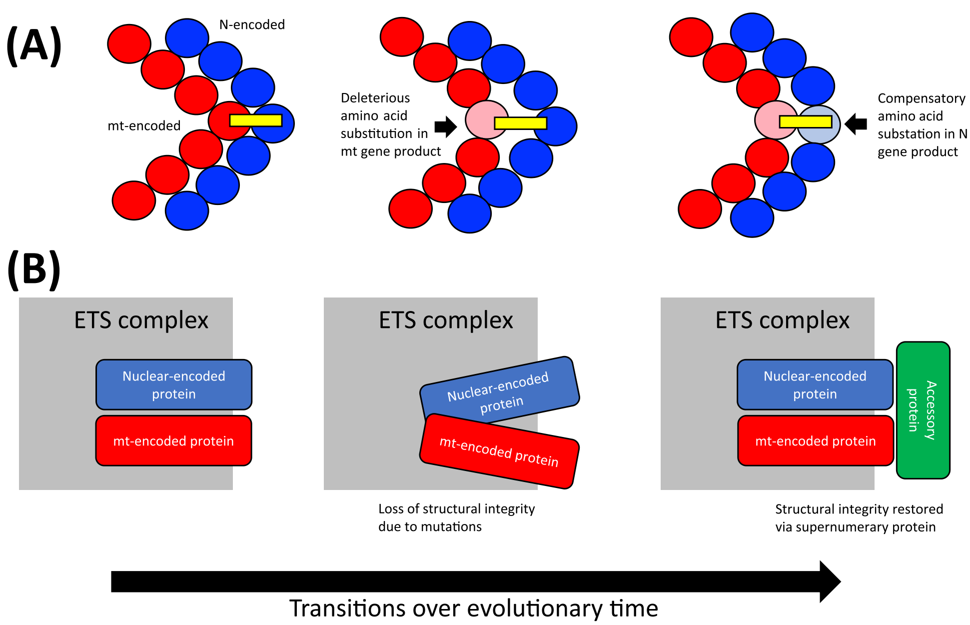

Figure 1. Illustrations of Proposed Mechanisms by Which Eukaryotes Might Escape the Negative Effects of Mutational Erosion. (A) A mutation in a mitochondrial (mt) gene affects the amino acid sequence in an electron transport system (ETS) complex shifting the spacing in a site of an important mitonuclear interaction (yellow bar). A compensatory change in the complementary nuclear (N) protein restores proper spacing. (B) Mutational erosion of mt gene products destabilizes an ETS complex and leads to reduced function. A newly recruited N-encoded protein restores complex stability and function.

Glossary

Accessory (supernumerary) subunits: protein subunits of multicomponent enzymes that appear to have been added to an original, core functional set of protein subunits.
Aminoacyl tRNA synthases (ARS): a nuclear-encoded protein that loads amino acids onto tRNAs.
Anterograde signaling: signaling from the nucleus to the mitochondrion.
Compensatory coevolution: an evolutionary change in one gene or set of genes specifically to reduce or remove (compensate for) a negative effect of another gene.
Constructive neutral model: the proposition that initial recruitment of ETS and mitoribosomal accessory proteins was neutral, but once incorporated into these enzymes, accessory proteins provided an important platform for subsequent evolution, including compensatory coevolution.
Electron transport system (ETS): five enzymes (Complex I, II, III, IV, V) embedded in the inner membrane of the mitochondrion that use energy from electrons to create a membrane potential that is used to produce ATP as a useable form of energy. Complexes I, III, IV, and V of the ETS of animals are partly encoded by N genes and partly by mt genes.
Epistasis: the process in which the expression of a gene is affected by the expression of other genes.
Mitonuclear coadaptation: the coordination of function by the products of the mitochondrial genome and the nuclear genome to achieve oxidative phosphorylation.
Mitoribosome: ribosomes that function in the mitochondrion in the translation of mt genes. Components of mitoribosomes are partly encoded by N genes and partly by mt genes.
Muller's ratchet: the process by which the genomes of an asexual population accumulate deleterious mutations in an irreversible, ratchet-like manner.
Mutational erosion: loss of fitness through the accumulation of deleterious mutations in a genome.
N-mt genes: nuclear genes whose products function in the mitochondrion.
Oxidative phosphorylation (OXPHOS): the primary energy production mechanism in eukaryotic cells whereby enzymes use energy from electrons to pump protons across the inner mitochondrial membrane and then

of the interaction of N-encoded and plastid-encoded proteins such that there is the potential for plastid–nuclear compensatory coevolution, but plastid–nuclear compensatory coevolution is predicted to be less common than mitonuclear compensatory coevolution and is not included as a focal topic in this review (Box 2). Along with protein–protein interactions, there are also key functional interactions between N-encoded **aminoacyl tRNA synthases (ARS)** and mt-encoded tRNAs and between mt-encoded rRNAs and N-encoded proteins in the mitoribosome [26,27]. These protein–RNA interactions involve recognition of nucleotide sequence codes that, although critical to the function of mitochondria, can perhaps accommodate more variability within species than protein–protein interactions [28]. There are also key interactions between N-encoded polymerase enzymes and DNA sequences that initiate transcription and replication of mt DNA [29]. And finally, there is growing evidence for extensive and functionally important interactions between N and mt gene products in **retrograde** and **anterograde signaling** between the nucleus and mitochondria [30]. For each of these interactions, changes to mt DNA sequence could affect the functional interaction with N gene products and reduce the efficiency of cellular respiration (reviewed in [6]). And for each such dysfunction caused by a change in mt DNA, there is potential for the evolution of a novel N DNA sequence to reduce or eliminate the dysfunction.

use the energy in the membrane potential to phosphorylate ADP into ATP.
Purifying selection: natural selection to maintain a current gene product.
Retrograde signaling: signaling from the mitochondrion to the nucleus.

Changes in the Rates of Evolution among N Genes

The list of vertebrate genes whose products directly interact with the products of mt genes include about 73 N-mt genes that code for subunits of respiratory chain complexes to enable **oxidative phosphorylation (OXPHOS)** (N_{OXPHOS} genes), 20 N-mt genes that code for ARS ($N_{mt\text{-}ARS}$ genes) that load amino acids onto tRNAs in the mitochondrion, and about 80 N-mt genes that are subunits of the mitoribosome ($N_{mt\text{-}ribo}$) and cofunction with mt-encoded rRNA [31] (Figure 2). Each of these three classes of N-mt genes has been used in comparisons of rates of evolution as a test of the mitonuclear compensatory coevolution hypothesis. The remaining N-mt genes whose products are known or suspected to engage in functional interactions with the products of mt genes serve as transcriptional or replication promoters, assembly factors, and processing endonucleases and have been little studied in the context of **mitonuclear coadaptation** [6].

In a comparative study of mammals and fish, the rates of evolution of N_{OXPHOS} was compared with that of N housekeeping genes [32]. As predicted by the mitonuclear compensatory coevolution hypothesis, N_{OXPHOS} genes evolved significantly faster than N genes not involved in aerobic respiration. However, among the N_{OXPHOS} genes, those that coded for subunits involved in the catalytic activity of a complex showed rates of evolutionary change that were lower than the rates of evolutionary change of N_{OXPHOS} genes that coded for more peripheral subunits. Subsequent comparative studies focused on the prediction that there should be greater need for

Box 2. Plastid–Nuclear Compensatory Coevolution

This review is focused exclusively on the epistatic interactions of mt and N-mt genes, but plastids also carry their own genomes and, like the products of mt genes, the products of plastid genes must cofunction with N genes to enable core function, including photosynthesis [71,72]. Thus, there is a potential for compensatory coevolution wherein N genes evolve to compensate for deleterious mutation in chloroplast genes. In contrast to the mutation rates of the mt genomes of animals that have been the focus of studies of compensatory coevolution, the mutation rates of plastid genomes are typically much lower than mutation rates of N DNA [73,74]. As a general rule (with exceptions), plastid genomes have low rates of amino acid substitutions [75,76]. Slow evolution of plastid genes should result in slower accumulation of deleterious alleles, slower mutational erosion, and presumably less need for compensatory coevolution [72]. Nevertheless, in plant taxa showing accelerated evolution of plastid-encoded proteins, there is also accelerated evolution of cofunctioning N genes, suggesting that there may be compensatory coevolution [18,77]. Comparative studies also provide some evidence that there may be compensatory coevolution between N and plastid genomes. Accelerated rates of evolution of nuclear proteins associated with plastid ribosomes compared with cystolic ribosomes supported the hypothesis of compensatory coevolution in plastid–nuclear evolution [71]. Compensatory coevolution of N and plastid genes may not be as ubiquitous as mitonuclear compensatory coevolution but nevertheless, it may play an important role in some lineages and is certainly worthy of additional study.

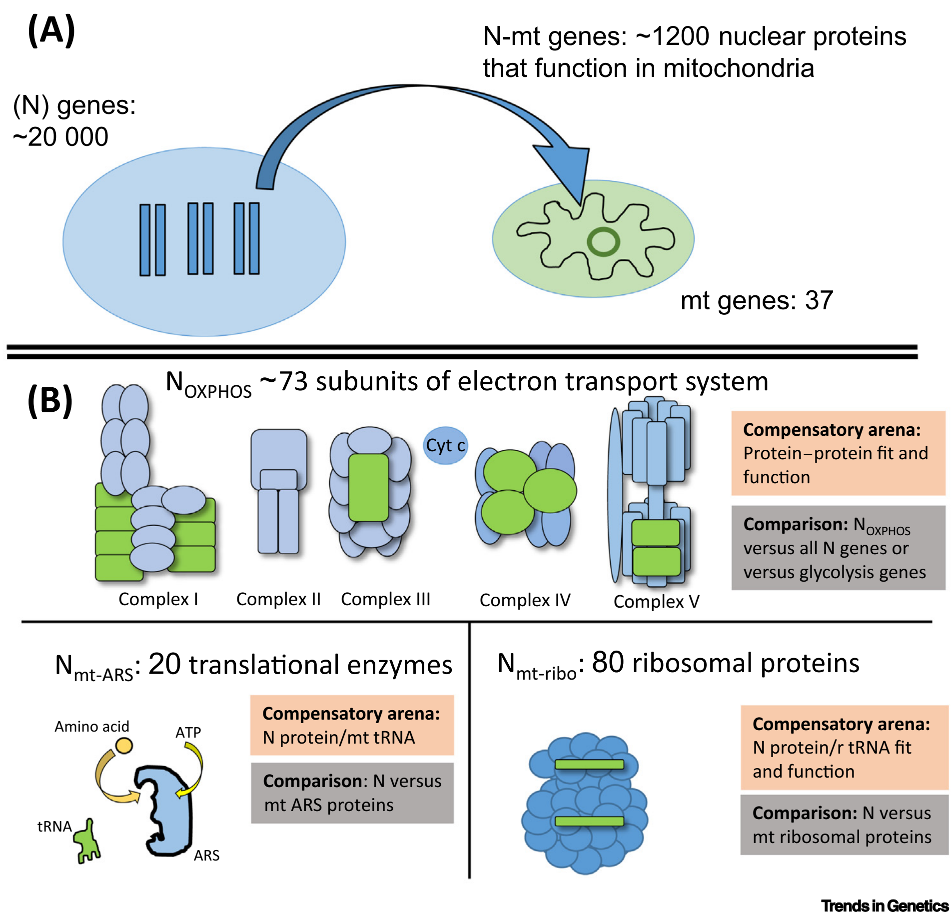

Figure 2. The Products of N-encoded Genes that have Close Functional Interaction with the Products of mt-Encoded Genes. (A) The approximate numbers of genes in the nucleus and mitochondrion of a mammal as well as the number of nuclear (N)-mitochondrial (mt) genes. (B) Classes of N-mt genes that have been used in comparative tests of the mitonuclear compensatory coevolution hypothesis. Genes are grouped according to the compensatory arena in which mitonuclear coevolution might occur as well as by the type of comparative analysis conducted with that class of protein. Classes of N-mt genes that engage in interactions with mt genes but have not been used in comparative studies are not shown. N components are coded blue and mt components coded green. Abbreviations: ARS, aminoacyl tRNA synthase; OXPHOS, oxidative phosphorylation.

compensatory coevolution of N genes when the mt DNA of a lineage is subjected to elevated mutation rates relative to other taxa in the lineage and, hence, to faster accumulation of deleterious alleles (but see Box 3). It follows that taxa with higher rates of mt DNA mutation should have higher rates of evolutionary change in N-mt genes. This basic hypothesis cannot be tested in most eukaryotic taxa because mutation rates tend to be conserved within taxonomic groups. In the angiosperm genus *Silene*, however, there are substantial differences in the mutation rates of mt DNA among closely related taxa and, as predicted by the mitonuclear compensatory coevolution hypothesis, the rates of amino acid sequence divergence are accelerated in N-mt genes but not in other N genes in species with higher rates of mt DNA mutations [18]. In a similar comparison, researchers took advantage of the exceptionally high rate of nonsynonymous changes in mt OXPHOS genes of ants, wasps, and bees (Hymenoptera) compared with other insects [33]. They predicted that if mitonuclear compensatory coevolution was an important process, then in the Hymenoptera lineage there would be accelerated rates of change in the N-encoded genes that cofunction with mt OXPHOS genes (N_{OXPHOS} genes). As predicted by the mitonuclear

Box 3. Estimating mt DNA Mutation Rates

This review is predicated on the assumption that mt genomes are experiencing ongoing decay due to accumulation of deleterious mutations. While mutational erosion of mt genomes is a widely held view in evolutionary biology, as documented by the empirical and theoretical studies cited throughout this review, the inevitability of mutation erosion in asexually propagating genomes remains a topic of discussion. Assessing the likelihood of accumulation of deleterious alleles depends on rates of mutation of mt DNA, but there are few direct measures of such mutation rates [78]. Most estimates of rates of change of mt DNA are actually estimates of the rate of nucleotide substitutions at neutral sites in the mt genome [79]. For rates of nucleotide substitution to be equal to rates of mutation, there can be no selection on mt DNA. In practice, because selection is nearly ubiquitous, substitution rates of mt DNA are much lower than mutation rates [80]. Moreover, based on available estimates of mt DNA mutation rates, it is clear that mutation rates vary markedly among eukaryotic taxa [81]. The evolution of mt DNA mutation rates is affected by population size, genome size, and whether or not mt DNA engages in recombination [57]. Improved estimates of mutation rates of mt DNA in diverse eukaryotic taxa will better frame the potential for mitonuclear compensatory coevolution to shape genome evolution.

compensatory coevolution hypothesis, they observed a higher rate of evolutionary change in N_{OXPHOS} genes in Hymenoptera compared with other insects. In a taxonomically broader comparative study that included plants, fungi, and animals, there were positive relationships between the mutation rate of mt DNA and the rate of evolution of N_{OXPHOS} genes but not glycolysis genes (the latter consisting of N genes whose products function in the mitochondria but do not cofunction with mt genes) [34]. The patterns observed in these comparative studies all support the mitonuclear compensatory coevolution hypothesis.

Comparisons involving rates of evolution of ARS and ribosomal proteins can be particularly powerful tests of relative rates of evolution of N genes that may be engaging in compensatory coevolution. N-encoded ARS proteins enable aminoacylation of N-encoded tRNAs to amino acids in the cytosol while different N-encoded ARS proteins ($N_{mt\text{-}ARS}$) enable aminoacylation of mt-encoded tRNAs in mitochondria. Likewise, N-encoded ribosomal proteins cofunction with N-encoded rRNA to create ribosomes in the cytosol, while different N-encoded ribosomal proteins ($N_{mt\text{-}ribo}$) cofunction with mt-encoded rRNA to create mitoribosomes in mitochondria. For both of these sets of N-encoded proteins, the mitonuclear compensatory coevolution hypothesis predicts more rapid evolutionary change in the mt-functioning proteins, where there is a potential for compensatory coevolution, than in the cytosolic-functioning proteins, where all of the interacting gene products are encoded by the N genome, subject to recombination, and hence resistant to the accumulation of deleterious mutations that might initiate compensatory coevolution. Indeed, Kuhle *et al*. [35] presented data showing that the rapid accumulation of mutation in the mt tRNAs of bilaterian animals resulted in shortened and more fragile structures. They proposed that $N_{mt\text{-}ARS}$ evolved altered rules for codon recognition, an interesting form of compensatory coevolution, to maintain translational function. The prediction for more rapid evolution of N products that cofunction with mt products also was supported for ribosomal proteins in copepods [17] and for ARS proteins in birds, mammals, and fruit flies [36]. The prediction was also supported in the most detailed comparison of the relative rates of evolution of sets of N genes in a study that took advantage of the complete sequencing of mt and N genomes of 18 genetically diverse lineages of the copepod *Tigriopus californicus* [12]. Despite a strong signature of purifying selection across mt genes of the copepods from all populations, there was evidence for change in allele frequency, including evidence for directional selection, on mt-encoded proteins, particularly protein subunits of Complex I [12]. Moreover, as predicted by the mitonuclear compensatory coevolution hypothesis, the rate of evolution of N proteins with close functional interaction with mt gene products was significantly and substantially greater than the rate of evolution of N proteins targeted to the mitochondria but not engaging in functional interactions with mt gene products. This study included separate comparative analysis of N_{OXPHOS}, $N_{mt\text{-}ARS}$, and $N_{mt\text{-}ribo}$ genes (Figure 3). This line of investigation bears not only

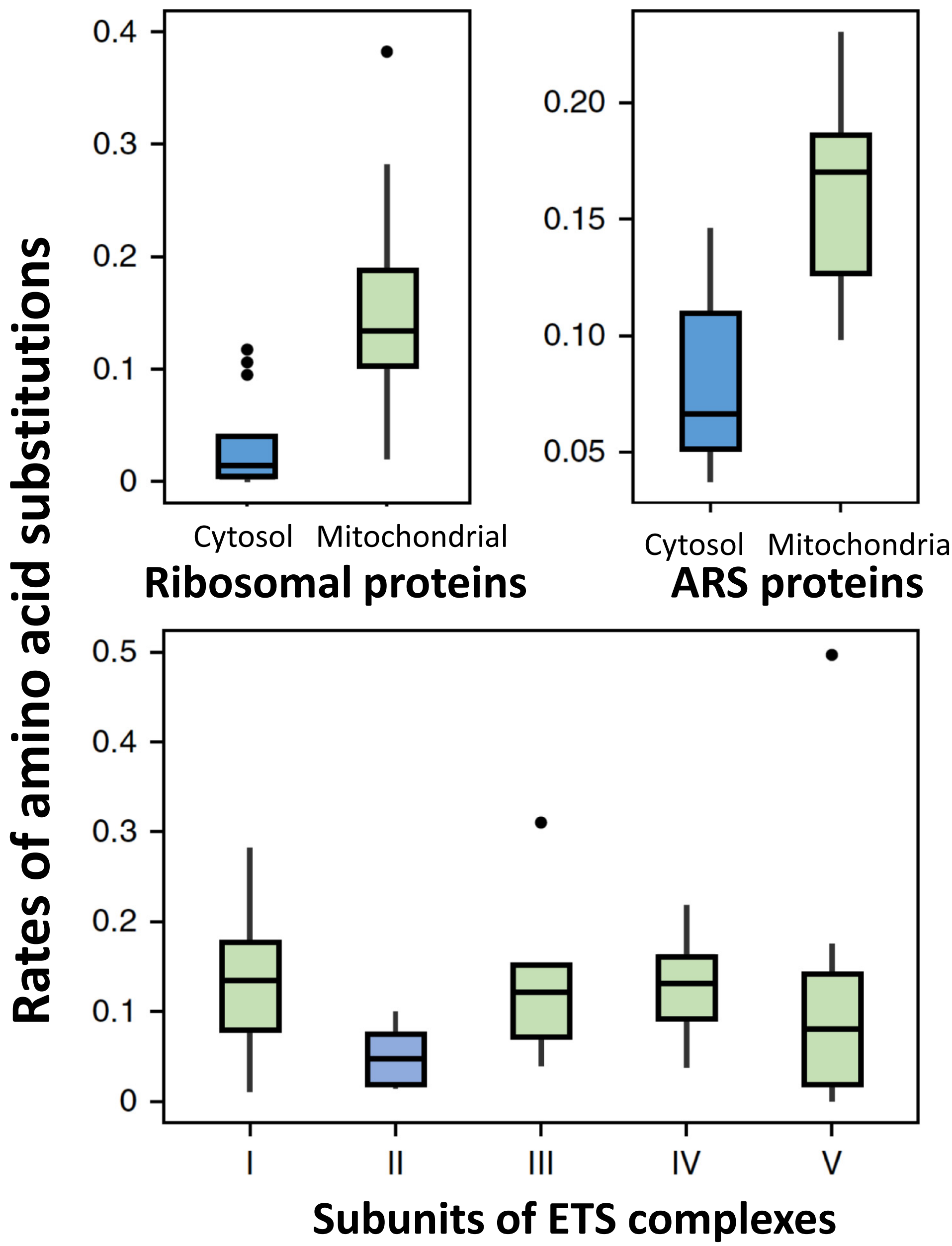

Figure 3. Comparisons of Rates of Evolution of Different Classes of Nuclear (N)-Encoded Genes in Divergent Populations of *Tigriopus californicus* as Tests of the Mitonuclear Compensatory Coevolution Hypothesis. In comparisons of rates of evolution of ribosomal proteins, aminoacyl tRNA synthase (ARS) proteins, and electron transport system (ETS) proteins, N-encoded proteins with close functional interaction with mitochondrial (mt)-encoded products (shaded green) show higher rates of evolution, estimated as the ratio of non-synonymous to synonymous substitutions, than N-encoded proteins that are not directly affiliated with mt genes (shaded blue). Modified from Barreto *et al*. [12].

on the relevance of mitonuclear compensatory coevolution to patterns of evolution within a species but also on the mechanism that might disrupt gene flow and lead to cladogenesis (Box 4).

Box 4. Compensatory Coevolution in Speciation Theory

Speciation requires the disruption of gene flow and, among the best-documented genetic mechanisms for the disruption of the flow of genes between closely related populations of eukaryotes, is incompatibility in coadapted sets of mt and N-mt genes [82,83]. While the existence of mitonuclear incompatibilities leading to hybrid dysfunction is well established [6], the origin of uniquely coadapted sets of mt and N-mt genes and the importance of such incompatibilities as a widespread mechanism for speciation is a focus of current debate [20,84]. Studies of bilaterian animals indicate that the rate of coalescence of mt genes is faster than predicted by neutral theory [85,86] and that the rate of evolution of mt genes is independent of population size, violating a key assumption of neutral evolution [87,88]. These observations suggest that adaptive evolution drives the divergence of coadapted sets of mitonuclear genes in the process of speciation. Adaptation to the internal genetic environment has been invoked as a potentially key mechanism in animal speciation [89,90] and mitonuclear compensatory coevolution is frequently invoked as potentially the key mechanism for the evolution of Dobzhansky-Muller incompatibilities in the speciation process [82,84]. However, any mechanism that resulted in divergent sets of codependent mt and N genes, including a constructive neutral model, could underlie a speciation process.

Comparative studies of the rates of evolution of N genes whose products engage in functional interaction with the products of mt genes versus rates of evolution of N genes whose products do not provides confirmation of a key prediction of the mitonuclear compensatory coevolution hypothesis. However, alternative explanations for these patterns have been proposed. With regard to rates of change of N_{OXPHOS} versus other N genes, it has been argued that compared with mt-encoded OXPHOS genes, the N_{OXPHOS} occupy peripheral (noncritical) positions in ETS complexes and, therefore, may be subjected to relaxed selection relative to average N genes, leading to their more rapid evolution [32]. This same argument cannot be applied to comparisons of rates of evolution of ARS and ribosomal proteins that function in the mitochondria or the cytosol. In the case of these paired comparisons, an alternative explanation to the mitonuclear compensatory coevolution hypothesis for increased rates of evolution of $N_{mt\text{-}ARS}$, and $N_{mt\text{-}ribo}$ is that there is less need for efficiency in the translation of proteins in the mitochondrion compared with the cytosol [18,36].

Evidence for the Evolution of Specific Compensations

Compensation for a dysfunction caused by a deleterious mt allele via a change to an N gene is a form of **epistasis**, and there is a growing focus in the genetics literature on epistatic interactions as major drivers of protein evolution [37,38]. In a comparative study of diverse metazoan taxa, it was estimated that 90% of all neutral or beneficial amino acid substitutions in both N and mt-encoded proteins that exist as the fixed state in a species would be deleterious if they were expressed in the genome of another closely related species [39]. In other words, the native genetic environment provides compensation for potentially deleterious effects. More recently, Levin and Mishmar [40] undertook a broad-scale assessment of functional nodal mutations (lineage-defining genetic changes with significant functional effects) in birds, mammals, and reptiles. Such functional changes could arise from selection if they are adaptations to the internal genomic environment (i.e., mitonuclear coevolution) or they could contribute to adaptations to the external environment (e.g., for thermal adaptation). These authors found that 37% of the nodal mutations in mt DNA and 27% of the nodal mutations in N DNA were the result of compensatory changes in those genomes, changes in response to the internal genomic environment. These whole-genome comparative studies document the pervasiveness of epistatic effects that compensate for otherwise deleterious nucleotide sequences, but they do not directly assess whether N genes evolve to compensate for dysfunctions caused by changes to mt genes.

Studies in a variety of eukaryotes have documented evidence for specific epistatic effects between mt and N genes wherein potential dysfunctions caused by a deleterious mutation to an mt gene appeared to be compensated by a change in an N gene [6,23]. In studies of plants in the genus *Silene*, structural modeling indicated that substitutions in N-encoded OXPHOS proteins could potentially offset structural instability introduced by mt mutations [41]. In mammals, researchers documented evidence for similar changes to N OXPHOS proteins that compensated for potentially deleterious changes to mt OXPHOS proteins [42]. The potential for N genetic background to affect the function of an mt gene product was documented in detail in a study of the functional consequences of different combinations of N-encoded ARS proteins and mt-encoded tRNA molecules from two species of fruit flies [43]. Moreover, the outcomes of mt gene × N gene interactions were highly dependent on thermal environment [44].

These studies hint at the sort of change and counterchange between N and mt genes that is predicted by the mitonuclear compensatory coevolution hypothesis, but in most cases the chronology of changes could not be deduced. Indeed, the most common sequence was proposed to be for the solution (genetic compensation) to appear before the problem (mt mutation that otherwise would cause dysfunction) [42] and such a sequence is not what the mitonuclear compensatory coevolution hypothesis is intended to explain. The foundational assumption of the mitonuclear compensatory coevolution hypothesis is that Muller's ratchet leads to the accumulation of deleterious genes in the mt genome and then the N genome evolves so as to counteract the negative effects of the mt genes. Among the most convincing examples of true mitonuclear compensatory coevolution comes from a study of primates by Osada and Akashi [16], who documented that mt-encoded and N-encoded ETS subunits in close physical proximity in Complex IV showed a strong tendency for correlated evolution. Moreover, by using phylogenetic reconstructions, these authors presented evidence that deleterious changes to mt DNA evolved before the compensatory N-encoded change, as predicted by the mitonuclear compensatory coevolution hypothesis.

Discussions of mitonuclear compensatory coevolution typically consider epistatic interactions limited to a single mt-encoded and N-encoded gene product, but each of the enzymes of the ETS involve multiple and complex interactions among the subunits of the enzyme. Protein modeling and experiments with human cell culture and yeast were used to document functional interactions among two N-encoded Complex I subunits (NDUFC2 and NDUFA1) and the seven mt-encoded Complex I subunits. Models predicted and experiments confirmed functionally significant mt–mt as well and mt–N interactions with very likely mitonuclear compensatory coevolution events in the evolution of the complex [45,46].

Recruitment of Novel N Protein Subunits

A second proposed form of compensation for overall loss of stability of ETS complexes and mitoribosomes resulting from the mutational erosion of the mt genome is the recruitment of novel N-encoded proteins to shore up complex integrity (Figure 1). The OXPHOS complexes in the mitochondria of eukaryotes are much larger and more complex than equivalent OXPHOS complexes of the prokaryotic lineages from which mitochondria evolved [47]. For instance, although the Complex I of bacteria and mammals share a common set of 14 core subunits that are required for enzymatic function, the Complex I of mammals (the taxon for which a eukaryotic Complex I is best characterized) have an additional ~30 **accessory (or supernumerary) subunits** that are not found in α-proteobacteria [48,49]. Similarly, eukaryotes have evolved novel subunits in Complexes III, IV, and V as well as in the mitoribosome that are not found in comparable protein complexes of bacteria [47].

The accessory proteins of both ETS complexes and mitoribosomes do not participate in core enzymatic functions; rather, they play key roles in the assembly of the complex subunits and in the stability of the enzymes once they are assembled [50–52]. For instance, each of the 31 accessory proteins have been added to the enzymatic core 14 subunits of the mammalian Complex 1 enzyme and each of these accessory subunits affects the stability of surrounding subunits and adds to the overall stability of the enzyme [53]. In a similar manner, the mitoribosomes of eukaryotes have recruited accessory proteins to the 55 ribosomal proteins that compromise the ribosomes of alpha-proteobacteria. For example, yeast have 70 protein subunits in their mitoribosomes, while mammals have 80 subunits [54]. In addition, many RNA–RNA interactions in the bacterial ribosome and in cytoplasmic ribosomes have been replaced by protein–protein and protein–RNA interactions in the mammalian mitoribosome [55]. In other words, key molecular interactions that had involved exclusively mt gene products have evolved so that they now include interactions with nuclear gene products, increasing the potential for N compensation of mt dysfunction. As predicted by the mitonuclear compensatory coevolution hypothesis, ribosomal accessory proteins stabilize the structure of mitoribosomes [9,56].

An explanation for the recruitment of novel protein subunits to the ETS complexes and mitoribosomes of eukaryotes that does not invoke compensatory coevolution as a selective force is the drift barrier hypothesis [57]. This hypothesis proposes that the rate of accumulation of deleterious mutations via genetic drift in a genome scales with genome size and population size. By this hypothesis, the much smaller effective population sizes of eukaryotes relative to prokaryotes may have promoted a genome-wide increase in the rates of accumulation of neutral and deleterious mutations during the evolution of eukaryotes [57]. The drift barrier hypothesis proposes that increased genetic drift in eukaryotic lineages not only led to changes in the nucleotide sequences but also enabled the recruitment of novel protein subunits whose fitness effects on complexes are neutral or nearly neutral [58,59]. Once in place, however, the novel protein subunits were shaped by natural selection to take on the currently observed roles of stabilizing ETS complexes and mitoribosomes [58].

Van der Sluis *et al*. [9] proposed that the evolution of both ETS complexes and the mitoribosome in eukaryotes is characterized by an initial constructive phase that occurred early in eukaryotic evolution, during which novel N-encoded subunits were recruited to aid in complex assembly and to stabilize complexes once they were assembled. This constructive phase was followed by a destructive phase, which occurred primarily in metazoans, during which the length of mt-encoded functional RNAs and proteins was significantly reduced. The key idea is that mutational erosion of the mt genome was countered by the compensatory coevolution whereby the functional contribution of mt DNA products was reduced and entirely new N-encoded products were recruited to stabilize key structures that were destabilized by mutational erosion of the mt genome. Van der Sluis *et al*. [9] emphasized that their analysis strongly supported the hypothesis that accessory proteins recruited to ETS complexes and mitoribosomes now function as compensatory mechanisms that counter the negative effects of mutational erosion of the mt gene products but that accessory proteins appeared to have not evolved initially as a compensatory mechanism. An evolutionary analysis aimed at reconstructing the timing of key events in the recruitment of novel N-encoded proteins to ETS complexes and mitoribosomes also concluded that there was little evidence that novel OXPHOS proteins evolved as compensatory mechanisms in response to deleterious changes in mt DNA [49]. Based on patterns of coevolution of the mt genome and the recruitment of novel N proteins, however, these authors concluded that novel RNA-interacting proteins of mitoribosomes had likely evolved as compensatory responses to changes to mt DNA [49].

One telling aspect of ETS accessory proteins is that these proteins were recruited to respiratory complexes very early in mt evolution [47]. Based on an extensive comparative search for putative Complex I homologous subunits among all major lineages of eukaryotes, Cardol [60] concluded ‘that all conserved accessory subunits might originate from events that happened in the stem branch leading to the ancestor of all extant eukaryotes'. The ancient recruitment of accessory proteins, followed by relative stasis through the diversification of eukaryotes, could be taken as evidence that compensatory coevolution has not been a major factor in the recent evolution of ETS complexes or mitoribosomes.

An alternative to the hypothesis that accessory subunits were recruited to ETS complexes and mitoribosomes as a compensation to deleterious alleles in mt genes is the idea that novel proteins were first incorporated into complexes for no functional outcome, the initial recruitment was functionally neutral. By this **constructive neutral model**, which presents an alternative hypothesis to the mitonuclear compensatory coevolution hypothesis as an explanation for the evolution of accessory proteins, once a set of N-encoded proteins of little functional significance were part of the ETS or ribosomal complexes, they provided a key platform for subsequent evolution of both mt and N genes [61]. As we learn more about the functions of accessory proteins in both the ETS and in mitoribosomes, a better understanding of the role of compensatory coevolution is certain to emerge.

Concluding Remarks

The close functional interactions of mt and N gene products and the species-specific coadaptation of sets of mitonuclear genes are undeniable features of complex eukaryotic life [24,62]. However, the mechanisms that maintain mitonuclear coadaptation across evolutionary time remain poorly understood. At present, there is substantial circumstantial evidence, but little direct evidence, that compensatory evolution by N genes to counteract mutational erosion of mt genomes is a widespread and important process in eukaryotic evolution. As rapid advances are made in sequencing techniques, structural biology, biophysics, and reconstructions of the evolutionary histories of specific genes, more definitive tests of the mitonuclear compensatory coevolution hypothesis will be possible (see Outstanding Questions). The data at hand continue to make this an intriguing hypothesis for the coevolution of mt and N genes.

A theme that emerges repeatedly in reflections on the evolution of the eukaryotic cell is that challenges to the function of a cell with two genomes led to improbable solutions that then created opportunities for truly novel evolutionary pathways. If the mitonuclear compensatory coevolution hypothesis is correct, then in solving the lineage-threatening challenge of mutational erosion of mt genomes, ETS complexes and mitoribosomes evolved to have more components. These complexes, in turn, may have provided targets for selection not only for compensatory coevolution as a means to counteract mutational erosion but also for functional adaptions for improved energy production in different environments.

Outstanding Questions

What is the order of evolutionary changes in epistatically interacting genes? Do compensations evolve after deleterious mutations are fixed or do the solutions evolve before the potential problems arise? While a high mutation rate and accelerated rate of evolutionary change has been documented for mt genes compared with N genes in many eukaryotes, the prediction of mutational erosion remains to be convincingly tested.

Through what process and for what purpose were the numerous accessory proteins of the electron transport system and mitoribosomes recruited? Why are equivalent subunits lacking in prokaryotic electron transport system enzymes? Once they were recruited, how important were accessory proteins to the adaptive evolution of core respiratory processes of eukaryotes?

What is the role of compensatory coevolution in the process of speciation? Does compensatory coevolution lead to the divergence in coadapted sets of mt and N genes and promote the disruption of gene flow between populations?

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