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Cytonuclear coevolution: the genomics of cooperation

David M. Rand, Robert A. Haney and Adam J. Fry

Department of Ecology and Evolutionary Biology, Box G-W, 80 Waterman Street, Brown University, Providence, RI 02912, USA

Without mitochondria we would be in big trouble, and there would be a global biological energy crisis if it were not for chloroplasts. Fortunately, genomic evolution over the past two billion years has ensured that the functions of these key organelles are with us to stay. Whole-genome analyses have not only proven that mitochondria and chloroplasts are descended from formerly free-living bacteria, but have also shown that it is difficult to define eukaryotes without reference to the fusion and coevolution of host and endosymbiont genomes. Here, we review how the macro- and micro-evolutionary insights that follow from the genomics of cytonuclear interactions are uniting molecular evolution, structural proteomics, population genetics and problems in aging and disease. Our goals are to clarify the coevolutionary events that have governed nuclear and organelle evolution, and to encourage further critical analyses of these interactions as problems in the study of co-adapted gene complexes.

Why is it that the two most important energy-processing functions among the dominant organisms today are housed in organelles that were acquired from 'outside'? What can we learn about organelle evolution from the gene content in bacterial endosymbionts of insects? Endosymbionts, such as *Wolbachia* and *Buchnera*, show patterns of origin, transmission, genomic evolution and biochemical necessity that have striking parallels with organelle evolution [1–4]. Moreover, endosymbiont life styles range from parasitic to mutualistic, making the coordinated evolution of cytoplasmic and nuclear genomes a grand experiment in the genomics of cooperation.

Comparative genomics of organelles, bacteria and eukaryotes has begun to unravel the relationships among genome content, biochemical pathways and ecological novelty. Reviews in this area have focused on organelle origins, gene transfer from organelle to the nucleus, and the emergence of eukaryotic cells [1–3,5,6]. Our aim here is to examine how these findings might illustrate the problem of coevolution. We begin at a macroevolutionary scale, examining the origin and modification of nuclear–organelle associations, and addressing endosymbiont transmission dynamics, as this creates a context for compensatory coevolution. We then consider the population genetics of cytonuclear interactions and close by encouraging ecologists and evolutionists to apply model systems to rigorous tests of the hypothesis that nuclear and organelle genomes are indeed co-adapted gene complexes.

Futuyma defines coevolution as 'Reciprocal genetic change in interacting species owing to natural selection imposed by each on the other.' [7]. We argue that much of nuclear–organelle evolution fits this definition (with 'genomes' in place of 'species'). However, coordinated evolution between nuclear and organelle genomes can occur by reciprocal changes in the functional constraints on interacting proteins. Such changes might alter the selection coefficients without driving positive darwinian evolution on proteins. Thus, coevolution could occur without the coordinated adaptive changes typically recognized as co-adaptation. Elucidating how (and whether) the evolution of such intergenomic cooperation has lead to co-adapted cytonuclear complexes provides rich material for new research (e.g. fitness studies of organisms with mitochondrial 'transplants' across divergent nuclear backgrounds, or statistical tests of coordinated amino acid substitutions in interacting nuclear and mitochondrial proteins).

Phylogenomics, gene transfer to the nucleus and reductive evolution

Phylogenetic analyses of organelle, endosymbiont and bacterial genomes have established that mitochondria are derived from free-living α-proteobacteria, whereas chloroplasts are derived from free-living cyanobacteria [8–10]. Likewise, bacterial endosymbionts of insects, such as *Wolbachia* in *Drosophila* or *Buchnera* in aphids, are descendants of α-, β- or γ-proteobacteria [3,4,11]. These studies have ended the controversy about organelle origins [12] and have provided the material with which to explore the coevolutionary nature of these genomic fusions. The most consistent consequence of endosymbiosis is the reduction in endosymbiont genome size, either by gene transfer from the endosymbiont to the host genome, or by outright gene loss. During the evolution of mitochondria and chloroplasts, there was substantial gene transfer from the early endosymbionts to the host genome [9]. For bacterial endosymbionts of insects, the reductive evolution has been more from gene loss, owing to redundant functions provided by the more-derived host genome [4].

Loss of redundant or nonfunctional organelle genes is a weak example of reciprocal coevolution (such as the use-it-or-lose-it case of reduced chloroplast genomes in

Corresponding author: David M. Rand (David_Rand@brown.edu).

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nonphotosynthetic plants [13]). However, the loss of genes transferred to the nucleus from organelle genomes (e.g. mitochondrial ribosomal proteins [14,15]) does fit the strict definition of coevolution. Mitochondrial ribosomes perform protein synthesis in the mitochondrial matrix, independent of the cytoplasmic ribosomes that lie outside the mitochondria. All ribosomes have RNA and protein subunits, but, despite their eubacterial ancestry, mitochondrial ribosomes are unusual because their protein:RNA ratios (69:31) are the inverse of those of prokaryotic ribosomes (33:67) [16,17]. Mitochondrial genomes encode the two RNA genes, but lack a full complement of the ribosomal proteins [plant mitochondrial genomes (mtDNA) encode 14–16 of these proteins [15], whereas animal mtDNAs encode none [18]]. Thus, functional mitochondrial ribosomes require import of nuclear-encoded proteins that were originally encoded in the (proto) mitochondrion but have been transferred to the nuclear genome.

The transfer of genes encoding such proteins from mitochondria to the nucleus requires that a mitochondrial nucleic acid (i) incorporates into a chromosomal location of the host; (ii) acquires expression signals to drive its transcription; (iii) acquires protein-targeting sequences to ensure that the protein is moved to the mitochondria, once it is translated; and, in some cases, (iv) obtains point mutations to accommodate different genetic codes for organelle and nuclear genes (the UGA stop codon codes for tryptophan in animal mitochondria). In angiosperms, these functional transfer events occur frequently on genetic and evolutionary time frames (one functional chloroplast gene transfer per 16 000 gametes [19]; 26 independent mitochondrial gene transfers in 169 angiosperm families [15]). At such rates, the history of cytonuclear fusion involved a 'bombardment' of the nucleus by organelle genes [6]. This imposed mutational pressure on the nuclear genome and will have generated fitness variation that probably influenced natural selection on the evolving cytonuclear genomic association. This is an example of the (proto) organelle imposing genetic changes on the nuclear host that has generated novel selective pressures.

What about the reciprocal component of coevolution? If organelle gene transfer (and protein re-targeting to the organelle) renders the organelle gene redundant, selection for gene loss in the organelle is established. This selection involves an important units-of-selection component [20]: selection favors full functionality at the level of the organism, and an intracellular race for replication in mixed ('heteroplasmic') cytoplasms favors smaller organelle genomes with deletions of the redundant gene [21]. When intracellular organelle competition leads to the fixation of the deleted genome in all (germ) cells, this represents a case of a genetic change imposed on the organelle genome by the nuclear-encoded selection to remove redundant functions. This is fully reciprocal coevolution given that this redundancy was itself induced by organelle gene transfer to the host.

This kind of cytonuclear coevolution has been rampant in the evolution of mitochondria and chloroplasts, because significant fractions of nuclear genomes are derived from organelle genes [10,22,23]. This has been inferred from joint analyses of the functional role and phylogenetic affinity of each well conserved gene in nuclear genomes [10,24]. In *Arabidopsis*, 18% of the nuclear proteome is of cyanobacterial (i.e. chloroplast) origin [10]. A new analysis in yeast indicates that ~75% of the yeast proteome is of eubacterial origin [25], but it is not certain that this fraction was due to gene transfers from the eubacterial ancestor of mitochondria. Nevertheless, organelle gene transfer events have played a defining role in the origin of nuclear genomes [2,26], and the targeting of these transferred gene products back to the organelle has provided the selective context for reductive evolution of organelle genomes.

Co-adaptation or co-exaptation?

Functional and phylogenomic analyses have indicated that some proteins imported to the organelle are newly recruited nuclear proteins with no ancestral function in the organelle. Subunit recruitment of nuclear proteins has been a factor in the evolution of mitochondrial OXIDATIVE PHOSPHORYLATION (OXPHOS; see Glossary) enzyme complexes [27], mitochondrial ribosomes [16] and many aspects of organelle function [6,10]. This adds a creative role for nuclear proteins in cytonuclear co-adaptation. To the extent that some nuclear proteins have been co-opted for novel function and modified by natural selection to increase the contribution of cytonuclear interactions to overall fitness, this process is exaptation rather than adaptation [28]. We submit that a substantial proportion of cytonuclear coevolution has been a case of co-exaptation rather than co-adaptation. Quantitative validation of this hypothesis must await rigorous analysis of the evolutionary origin of proteins targeted to different cellular compartments.

However, why do such different patterns of gene transfer occur in different lineages? Among early-branching eukaryotes, *Giardia* has no mtDNA but has highly modified mitochondria [29,30], whereas other lineages have large mitochondrial genomes [31]. Transfer of functional organelle genes to the nucleus has stopped in animals [18], but continues in plants [14,15,32], which must reflect the biochemical demands of the cell; however, much uncertainty remains. Ongoing nuclear genome

Glossary

Cytochrome c oxidase: complex IV of the electron transport chain that passes electrons to molecular oxygen; comprises mitochondrial and nuclear subunits and provides a model enzyme for studies of cytonuclear co-adaptation.

Electron transport chain: protein complexes in the inner mitochondrial membrane that carry electrons from organic compounds (e.g. NADH or succinate) to molecular oxygen and pump protons into the inner mitochondrial membrane space to establish an energy gradient for ATP synthesis.

Muller's Ratchet: the accumulation of deleterious mutations on chromosomes that are transmitted with little or no recombination, due to the random loss of less–mutated chromosomes by genetic drift.

Nonsynonymous:synonymous ratio: a measure of selection on a protein-encoding gene. Because synonymous changes (at the redundant third position of a codon) are less constrained than are nonsynonymous changes that alter the amino acid, genes under functional constraint should have dN:dS <1, whereas genes experiencing adaptive evolution should have dN:dS >1.

Oxidative phosphorylation: the coupling of the energy gradient established by the electron transport chain and the phosphorylation of ADP to make ATP, the primary energy-carrying molecule of the cell

projects in diverse, basal eukaryotes might shed light on this problem [31].

Biochemical pathways and contingencies in cytonuclear coevolution

What processes determine whether genes are transferred or eventually lost? One general answer is the inventory of essential biochemical pathways. Because endosymbiotic associations are unions between distantly related taxa (e.g. proteobacteria and archaebacteria; bacteria and insects), each player provides some novel pathways, whereas the other pathways will be redundant [2,4]. Moreover, the history of cytonuclear coevolution between initial associates should affect which pathways are novel versus redundant in any subsequent endosymbiotic associations.

For example, under the hydrogen hypothesis of mitochondrial origins [33], the waste from a eubacterial heterotroph (proto-mitochondrion) provided the nutrients for an archaebacterial methanogen 'host' (proto-nucleus). In eukaryotic cells, genes with eubacterial ancestry are largely responsible for metabolic and biosynthetic (operational) functions, whereas genes descended from archaebacterial methanogens perform transcription and translation (informational tasks) [25,34]. Mitochondria tend to retain genes for ribosomal RNAs and components of the CYTOCHROME OXIDASE COMPLEX (COX) [9], implying that the expression of these genes within the organelle is a biochemical necessity for replication in the intracellular niche of eukaryotic cells. But the predominance of eubacterial genes in operational functions [25] indicates that evolving eukaryotes were better able to colonize diverse ecological niches with these biochemical pathways transferred to the nucleus.

Because endosymbiosis has occurred repeatedly through the history of life, the biochemical repertoires resulting from initial cytonuclear coevolution established evolutionary contingencies for subsequent metabolic and genomic evolution. Mitochondrial origins predate chloroplast origins [~2 billion years ago (BYA) versus 1.5 BYA, respectively [5,6]], so the cyanobacterial endosymbiont had to adapt to an existing mitochondrial-dominated metabolic system. But that system was not the typical aerobic eukaryote that we know today. Marine sediments harboring early eukaryotic lineages were sulfur rich and anaerobic until as late as 0.6 BYA [35]. Interestingly, this coincides with the diversification of major animal lineages during the late Precambrian. The evolution of aerobic, oxidative phosphorylation functions of extant mitochondria are probably a derived state that occurred late in the game of endosymbiosis [36]. The history of the genes for these pathways in mitochondria and chloroplasts has been contingent on coevolutionary interactions between these genomes (and their nuclear host), which, in turn, were driven by changes in environmental oxygen levels.

Phylogenomic analyses of chloroplasts reveal that dinoflagellates have acquired plastids by secondary endosymbiosis of a red alga [37,38]. This 'Russian-doll' model (W. Gehring, pers. commun.) of sequential, nested endosymbioses establishes multiple contingencies for biochemical coevolution; however, these are not restricted to energy-processing pathways. In *Arabidopsis*, genes for mitochondrial DNA and RNA maintenance are clustered on chromosome III of the nuclear genome [39]. These genes have either rickettsial (i.e. mitochondrial) or cyanobacterial (i.e. chloroplast) ancestry. Remarkably, some of these gene products are targeted to both the mitochondrion and the plastid [39]. Selection on nucleic acid metabolism in both mitochondria and chloroplasts might have led to a shared function of the nuclear gene despite independent acquisition from the two divergent endosymbionts. This example of the genomics of cooperation illustrates the resourcefulness of cytonuclear co-adaptation.

Similarly, bacterial endosymbionts of insects evolve in the context of a eukaryotic biochemistry of OXPHOS and heterotrophy. As a result of gene redundancy, much of the evolution of bacterial endosymbiont genomes is reductive gene loss [4,11]. However, the ecological niche of the particular insect host has an impact on the nature of this loss. The *Buchnera aphidicola* endosymbiont of aphids retains specific genes for the synthesis of amino acids that are rare in the plant sap consumed by the aphid [4]. Thus, organelle and endosymbiont genomes are coevolutionary products of genetic complementation and redundancy dictated by the biochemical pathways provided by each player.

Cytoplasmic inheritance, recombination and deleterious mutations

Organelle and endosymbiont genomes generally have uniparental inheritance and lack normal recombination [4,40]. As a result, the effective population size of these genomes is reduced relative to both the nuclear loci of the host, and the free-living ancestor of the endosymbiont. These transmission dynamics establish several contexts for coevolutionary phenomena.

In obligate bacterial endosymbionts of insects, specialized tissues are set aside in the germ line to ensure bacterial inheritance. These bacteriocytes in aphids harbor the population of the endosymbiont *Buchnera* and mediate the transmission of a single *Buchnera* to the oocyte during oogenesis [4]. This represents a true case of reciprocal coevolution: the endosymbiont has induced genetic changes on the host by triggering the evolution of a novel tissue dedicated to the transmission of the endosymbiont [41]. The host, in turn, has imposed genetic changes on the endosymbiont by virtue of the nutritional environment that it provides for the endosymbiont.

A consequence of maternal inheritance, lack of recombination and small effective population size for organelle and endosymbiont genomes is a weakening of natural selection. As a result, deleterious mutations accumulate faster in endosymbiont genomes than in biparental, recombining nuclear genes or free-living relatives. This evolution under MULLER'S RATCHET is evident in mitochondrial [42] and insect endosymbiont genomes [43]. Moreover, the genome-wide pattern of reductive evolution might be triggered by the accumulation of deleterious mutations if individual genes are rendered nonfunctional and can be complemented by gene products from the host [4]. Population genetic analyses

of mitochondrial [20,44,45] and *Buchnera* genes [46] have documented a history of negative selection coefficients in the evolution of these genomes.

Compensatory co-adaptation

One model of co-adaptation invokes compensatory mutations between interacting genes (or nucleotide positions within a gene). A deleterious mutation in one locus establishes a positive selection pressure for the compensatory mutation in the interacting locus. Given the Muller's ratchet environment of endosymbiont genomes, cytonuclear co-adaptation should be inherently asymmetric. Because positive selection is facilitated by recombination and large effective population sizes, the nuclear genes are more likely to contribute the adaptive compensatory mutations that maintain co-adapted states between interacting genomes. Here, we review evidence in support of this pattern.

OXPHOS complexes: model systems for analyses of co-adaptation

The enzyme complexes of ELECTRON TRANSPORT CHAINS and OXPHOS (Figure 1) are attractive models for the analysis of cytonuclear co-adaptation [47]. OXPHOS proteins are jointly encoded by nuclear and mitochondrial genes, structural models for complexes II–V are available that increase the power of molecular evolutionary analyses, and phenotypes can be studied with the use of enzyme assays. A simple prediction of co-adaptation is that an experimental 'transplant' of interacting partners should result in diminished performance, and this disruption should increase as the level of evolutionary divergence increases (Figure 2a). At a molecular level, co-adaptation should be evident from coordinated amino acid changes on gene trees of interacting OXPHOS proteins (Figure 2b).

Cytonuclear transplant experiments: mammalian cell culture models

Cell cultures have been established in which mitochondria from one species are placed in a cell with a 'foreign' nucleus. In primate models, cells carrying a human nucleus with mitochondria from chimpanzee or gorilla showed normal cellular respiration, but mitochondria from the orangutan or more distant primate species did not restore respiration [48]. In a mouse model, *Mus musculus domesticus* cell lines carrying mitochondria from six different murid species spanning 2–12 million years of divergence revealed a near-linear disruption of respiratory chain function with evolutionary distance [49]; complex II, which lacks mtDNA subunits, failed to show disruption (Figure 3a). These results are consistent with the 'transplant' prediction of cytonuclear co-adaptation (Figure 2a), and the complex II result provides an internal control: the disruption is only seen in those complexes involving both nuclear and mitochondrial subunits.

Backcross analyses in whole organisms

Maternal inheritance of organelle DNA enables one to 'transplant' the cytoplasmic genome from one strain or species onto the nuclear background of the paternal line. With control backcrosses to the maternal line, one can compare phenotypes of 'disrupted' (mtDNA on foreign nuclear background) and 'reconstituted' (mtDNA on original nuclear background) genotypes. Studies of the intertidal copepod *Tigriopus californicus* by Ron Burton and colleagues provide evidence for cytonuclear co-adaptation using enzyme assays of cytochrome c oxidase (COX; complex IV [50–52]). In backcross genotypes between different geographical populations, COX activity is significantly reduced relative to control backcrosses. A similar mtDNA introgression study of *Drosophila* showed a more pronounced COX disruption

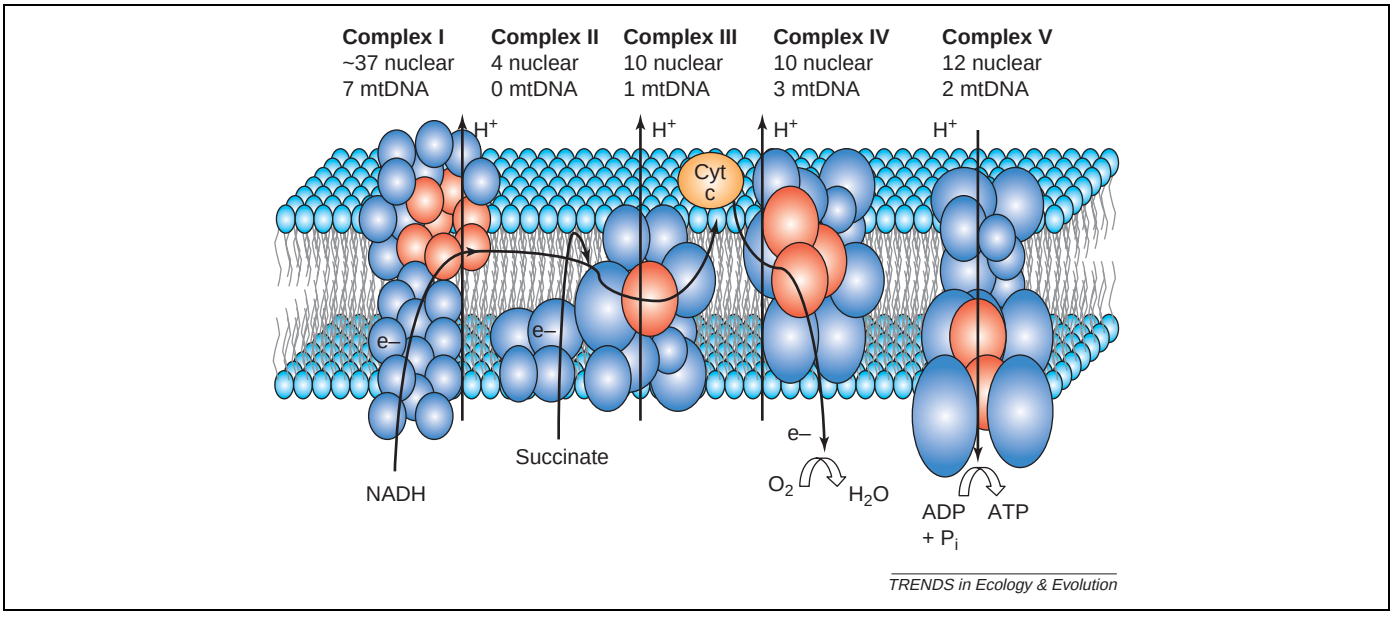

Figure 1. Enzyme complexes of oxidative phosphorylation (OXPHOS). Five multi-subunit enzyme complexes (I–V) embedded in the inner mitochondrial membrane carry out OXPHOS in mitochondria. Complexes I–IV (NADH dehydrogenase, succinate dehydrogenase, bc1, and cytochrome c oxidase [84], respectively) transport electrons (e−) from NADH or succinate to molecular oxygen and establish a proton gradient (H^+) across the inner mitochondrial membrane. This energy gradient is used by complex V (ATP synthase) to drive ATP synthesis from ADP and inorganic phosphate. The subunit composition is listed above each complex (nuclear in blue; mitochondrial in red). The joint nuclear and mitochondrial composition of these complexes provide a model system for the study of cytonuclear co-adaptation.

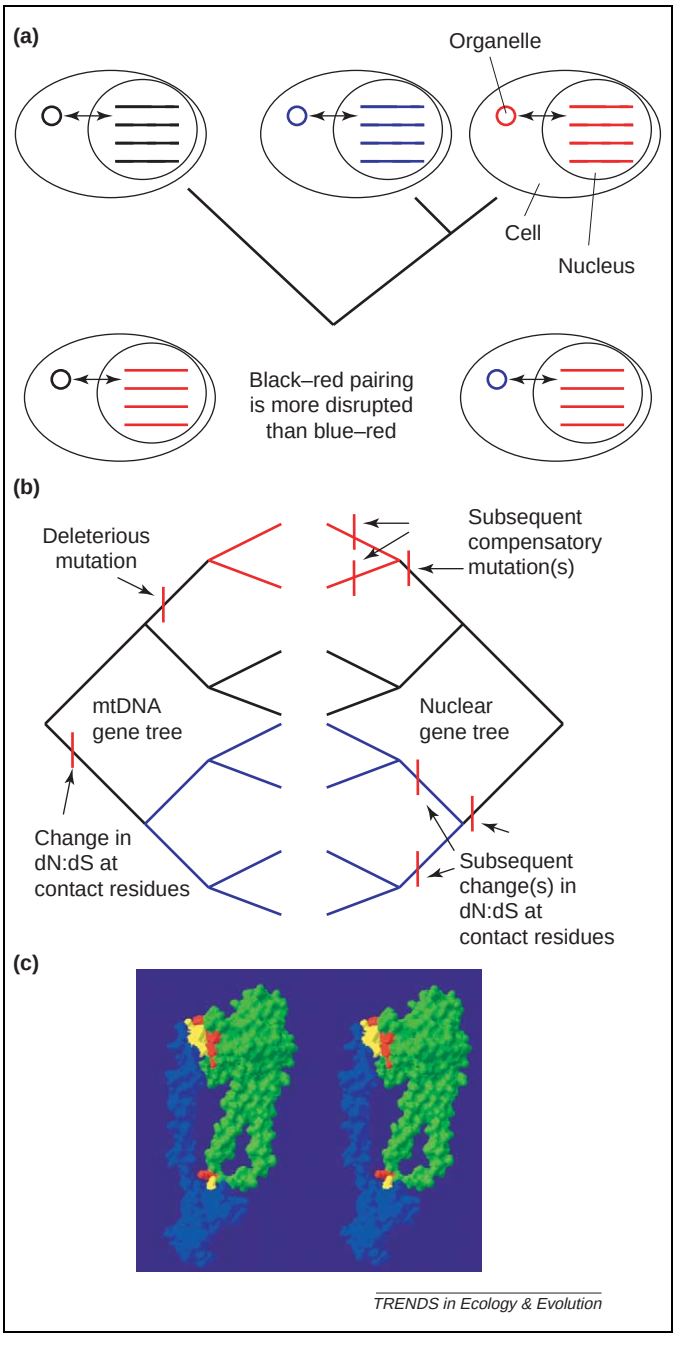

Figure 2. Predictions of cytonuclear co-adaptation. Co-adaptation occurs when natural selection affects the evolution of interacting genes in independent lineages. **(a)** Co-adapted gene complexes should be disrupted when an organelle from one lineage is transplanted onto the nuclear background of a distinct lineage, and the degree of disruption should scale with time of divergence. **(b)** At the molecular level, co-adaptation predicts that mutations in one interacting gene will be compensated by mutations in its interacting partner. Nonsynonymous:synonymous substitution ratios (dN:dS) can be mapped onto concordant nuclear and mitochondrial branches, enabling specific tests of deleterious versus positive selection in mediating coevolutionary interactions (compensatory changes should occur after deleterious mutations on the trees). **(c)** Structural properties of interacting nuclear and mitochondrial-encoded subunits of the cytochrome oxidase complex (COX) protein show different amino acid substitution patterns for contact (red) versus non-contact amino acid residues (blue or green surfaces). Adapted, with permission, from [60].

effect in interspecific versus intraspecific backcrosses, as predicted from the co-adaptation hypothesis [53] (Figure 2).

In the *Tigriopus* system, a significant portion of this co-adaptation lies between the COX enzyme and cytochrome c, the nuclear-encoded protein that is oxidized by COX in electron transport from complex III [54]. When mitochondria were used to oxidize different population variants of cyt c, COX activity was significantly lower in heterologous combinations (e.g. Santa Cruz cyt c+ San Diego mitochondria) than in the native pairings [52] (e.g. Santa Cruz cyt c+ Santa Cruz mitochondria; Figure 3b). Sequence polymorphism surveys among *Tigriopus* populations show evidence for positive selection at cyt c but negative selection at mitochondrial and nuclear genes of complex III (plus other enzymes) [55]. This is consistent with the model of asymmetric compensatory co-adaptation discussed above. Similar analyses among strains and species of *Drosophila* [53,56] provide important contrasts for the outcome of co-adaptation. Deleterious mutations should accumulate more rapidly in small (*Tigriopus*) than in large (*Drosophila*) populations; hence, disruption of co-adapted gene complexes might be detected among geographical populations in *Tigriopus*, but not *Drosophila*. More studies are needed to establish the generality of this population size effect on cytonuclear co-adaptation

Molecular evolutionary approach to co-adaptation

There is considerable interest in testing the hypothesis that co-adaptation occurred among nuclear and mitochondrial subunits of OXPHOS proteins during primate evolution driven by the metabolic demands associated with an expanding cortex [57,58]. If the nuclear and mitochondrial proteins are co-adapted, then nuclear and mtDNA amino acid substitutions should show coordinated changes between species at positions of structural importance to enzyme function (e.g. sites of physical contact among subunits; Figure 2c). Patterns of amino acid substitutions at sites in close physical contact between nuclear and mitochondrial subunits of COX are distinct from substitution patterns at non-contact residues, indicating coordinated evolution [59,60]. In primates, there are duplicate genes for some of the nuclear-encoded subunits of COX and changes in the tissue-specific expression of different isoforms are associated with changes in nuclear–mitochondrial coevolution [58]. In old-world monkeys and apes, two evolutionary events are correlated: the heart isoform of COX subunit VIII (VIII-H) is not expressed and the liver isoform (COX VIII-L) shows accelerated amino acid substitution and elevated NONSYNONYMOUS:SYNONYMOUS SUBSTITUTIONS RATIOS (dN:dS). The fastest evolving part of COX VIII-L is that which contacts mtDNA-encoded subunit COX I, strongly implicating structurally mediated nuclear–mitochondrial coevolution driven by tissue-specific shifts in subunit expression [58].

These analyses illustrate a distinction between coevolution and co-adaptation. The most compelling examples of coordinated substitutions between nuclear and mitochondrial subunits only involve increased rates of amino acid substitution or elevated dN:dS, but significant evidence for coordinated positive selection (dN:dS (1) is limited [58–60]. Thus, nuclear–mitochondrial coevolution might occur through coordinated substitutions that change the functional constraint on interacting proteins by modulating

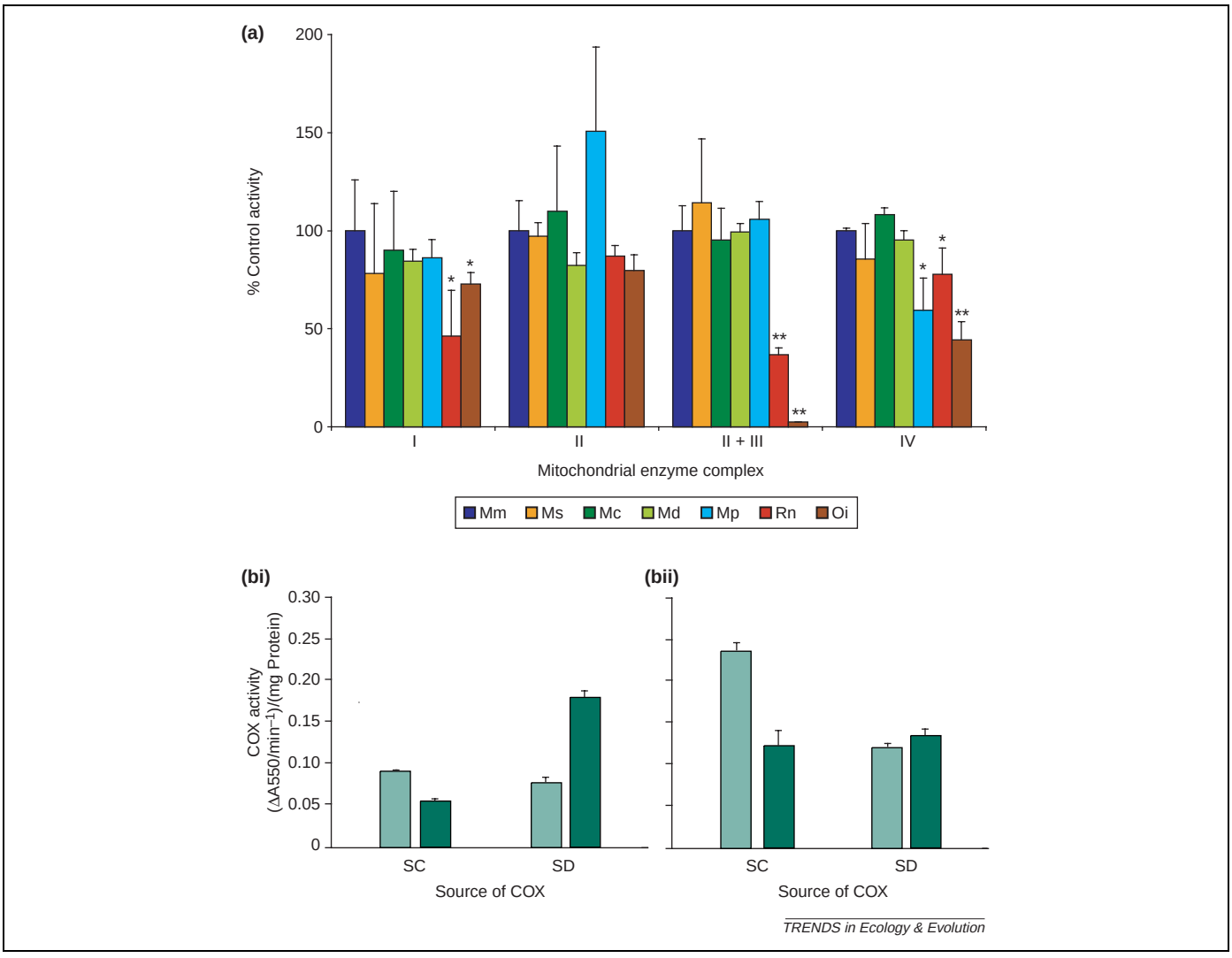

Figure 3. Mito–nuclear co-adaptation from functional assays. **(a)** Activities of oxidative phosphorylation (OXPHOS) enzymes in cultured cells with a *Mus musculus* (Mm) nucleus and mitochondria from other species (i.e. cytonuclear hybrid, or 'cybrid' cells). Species names, abbreviations and divergence times in millions of years (my) are as follows: *Mus spretus* (Ms) ~2 my, *Mus caroli* (Mc) ~3 my, *Mus dunni* (Md) ~4 my, *Mus pahari* (Mp) ~6 my, *Rattus norvegicus* (Rn) and *Otomys irroratus* (Oi) ~12 my. OXPHOS enzyme activities were normalized to control activity (Mm, 100%±s.d.), and show normal levels with mitochondria from Ms, Mc and Md. The Mp cybrids showed normal complex I, II and III activity, but a deficiency in complex IV activity. The Rn cybrid showed deficiencies of complex I and III and a partial defect of complex IV, whereas the Oi cybrid showed a marked complex I and IV defect, and a severe complex III defect. (*$P<0.05$; **$P<0.005$; reproduced, with permission, from [49].) **(b)** Cytochrome oxidase complex (COX) activities in the copepod *Tigriopus californicus* using mitochondria isolated from Santa Cruz (SC) or San Diego (SD) individuals and cytochrome c isolated from either SC (light-green bars) or SD (dark-green bars). Assays at 18°C (bi) or 25°C (bii). (Error bars=s.e.; $P<0.0001$ for COX-by-CYTC, COX-by-Temperature, and CYTC-by Temperature interactions. Reproduced, with permission, from [54].)

purifying (negative) selection. If these changes do not drive adaptive positive selection, this would be coevolution without a strictly adaptive component.

A related issue is the temporal association between nuclear and mitochondrial substitutions and the phenotypic traits driving these changes. If the evolution of an expanded cortex drove co-adaptation of COX subunits in the apes, then these events should be correlated most clearly on the human lineage since it split from the common ancestor with chimpanzees. Positive selection (dN:dS (1) is inferred at two COX subunits (COX VIIc and COX VIII-L) on two of the branches leading from the common ancestor of orangutans [58,61], which predates the expansion of the neocortex by millions of years. How tightly correlated must these events be to provide support for the adaptive nature coevolution of nuclear and mitochondria OXPHOS proteins? By combining site-specific tests of positive selection [62] with phylogenetic tests of coordinated evolution [63] more rigorous tests of the co-adaptation hypothesis could be developed.

Despite uncertainties about evidence for strict co-adaptation, most of the adaptive components come from nuclear-encoded subunits that interact with mitochondrial proteins. This supports the compensatory model of coevolution discussed above. One notable exception is positive selection on some mtDNA variants in arctic populations of humans [64]. MtDNA mutations that confer higher thermal output from an uncoupling of electron transport and ATP synthesis in mitochondria were probably favored in colder climates. Because the uncoupling proteins (UCPs) modulate this balance between proton leakage and heat generation versus ATP synthesis, UCPs might be important players in adaptive aspects of cytonuclear coevolution (e.g. [65]).

Sex and cytonuclear conflicts in aging and disease

The maternal inheritance of most cytoplasmic genomes creates a sexual asymmetry for selection on cytoplasmic genes and on their interactions with nuclear genes. MtDNA mutations with deleterious effects in males can still persist in populations if they are effectively neutral in females. The lack of mtDNA transmission in males prevents direct selection on mtDNA haplotypes with male-specific phenotypes, and further stifles the spread of nuclear modifier mutations that might suppress male-specific mitochondrial defects [66]. Consistent with this genetic argument is evidence that mitochondrial diseases can be more severe – or more prevalent – in males than in females [66,67]. A stronger disruption of COX activity was observed in male versus female *Drosophila* carrying foreign mtDNA [53]. These sex-specific mtDNA phenotypes are similar to cytoplasmic male sterility factors in plants. In plants, mutations in mtDNA cause pollen failure, but nuclear restorer loci can effectively suppress the deleterious mtDNA effect [68]. This is usually interpreted in the context of genomic conflict owing to the differential transmission of chromosomes [69,70].

Insects carrying *Wolbachia* provide the best understood example of host–endosymbiont conflicts. Crosses between an uninfected female and a male carrying *Wolbachia* results in cytoplasmic incompatibility (CI, which is expressed as one of several phenotypes, the most common being reduced egg hatching or male killing [71]). *Wolbachia* is a superb example of the coevolutionary continuum from parasite to mutualist [72]. Although *Wolbachia* commonly promote their own transmission by inducing negative effects on uninfected hosts, obligate *Wolbachia*–host associations indicate the coevolutionary nature of these interactions. These transitions can occur over relatively short evolutionary time periods. In *Drosophila simulans*, *Wolbachia*-induced CI is widespread, but in *D. melanogaster* CI is weak or absent and *Wolbachia* can increase longevity and fecundity in this species [73,74]. These transitions must drive genetic changes in the nuclear host genome and in the endosymbiont (*cf.* [75]). If positive interactions are to evolve, rules of chromosomal transmission suggest that these interactions involve the X chromosome [76] (Box 1). Perhaps consistent with this is the observation that a segment of the *Wolbachia* genome has been transferred to the X chromosome in beetles [77].

Given that the Y chromosome and mtDNA are both haploid nonrecombining chromosomes, they should accumulate deleterious mutations. As such, coevolutionary dynamics of these two chromosomes should be complex. Sperm function is an important arena for Y–mtDNA fitness interactions because fertility factors are Y inked and the energy for motility is provided by mitochondria. MtDNA polymorphisms have been associated with reduced sperm function [78], but there are few data to partition these defects between Y versus chromosomes. As phylogeographical data sets expand, tests of association could be performed to examine if one chromosome is driving variation in the other [79]. The notion that joint Y-chromosome–mtDNA interactions might play more general roles in aging and disease is compelling [80], but few direct genetic tests have been carried out. Sex-chromosome–cytoplasm interactions in female-heterogametic species are further contexts for examination of these chromosomal interactions [81]. These reciprocal rules of transmission provide an opportunity to test the generality of cytonuclear coevolution from the context of transmission conflicts.

Box 1. Sex-specific cytonuclear interactions

Several predictions about the nature of cytonuclear conflicts follow from the patterns of chromosomal inheritance (Table I). In a mated pair of animals, mtDNA is co-transmitted with half of the autosomal genes, two-thirds of the X-linked genes and none of the Y-linked genes [76]. This predicts that, relative to the autosomal case, positive nuclear–mitochondrial interactions are more likely to evolve for X-linked loci whereas deleterious interactions between Y-linked genes and mtDNA should accumulate (or cannot be purged efficiently).

These transmission patterns have implications for haplodiploid species [85,86], sex-ratio evolution and the evolution of conflicts between endosymbionts and nuclear host genomes [87]. In cases where there is sex-specific disruption of cytonuclear co-adaptation [53], there are parallels with the chromosomal incompatibilities that might underlie Haldane's rule [the reduced fitness (viability or fertility) of the heterogametic sex in hybrids between species or divergent populations] [88].

Table I. Patterns of chromosomal transmission

	Chromosomes			
Sex	MtDNA[a]	Y	X	Autosomes
Female (homogametic)	1	0	2	2
Male (heterogametic)	0	1	1	2
Total copies	1	1	3	4
Proportion co-transmitted with mtDNA	–	0	0.66	0.50

[a]Assuming strict maternal transmission of mtDNA.

Prospects

On a macroevolutionary scale, new insights into the history of nuclear–organelle coevolution will emerge from genome projects looking at microbial eukaryotes [31]. The distribution of eubacterial and archaebacterial genes in species living in different extreme environments will tell us much about how cytonuclear coevolution has been driven by biochemical opportunities in distinct ecological contexts. We need more studies in systems where OXPHOS functional assays can be linked to amino acid substitutions in the interacting proteins encoded by mitochondrial and nuclear genes. Plants offer promising material given the ongoing nature of gene transfer from organelles to nucleus, and the opportunity to manipulate both respiration and photosynthesis through backcross analyses among populations [82] and divergent (but crossable) species [83]. Sex-specific cytonuclear interactions are poorly understood, but offer material that could link population genetic models to phenotypes of medical relevance. As more genomic sequences are deciphered, it will become increasingly clear how important cytonuclear coevolution has been in the history of diversity, and these data will drive a diversity of approaches that will provide us with a more-complete understanding of the genomics of cooperation.

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