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# 23. ENDOSYMBIOSIS

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Eukaryotic biology is replete with cases in which cells are inhabited by descendants of other species, often bacterial in origin, with the latter frequently having relinquished a capacity for free-living. The most celebrated examples involve the mitochondrion and the plastid (called a chloroplast in photosynthesizing tissues). Almost all eukaryotes contain a mitochondrion or modified version of one, ultimately derived from an $\alpha$-proteobacterium, and all photosynthetic eukaryotes contain chloroplasts with cyanobacterial ancestry. Although the vast majority of the proteomes within these organelles is encoded in the nuclear genome, mitochondria and chloroplasts contain diminutive remnant genomes, and it is the sequence information within these that confirms their bacterial roots. Speculation that other eukaryotic features, including the mitotic apparatus and the eukaryotic flagellum, owe their origins to endosymbiosis (Sagan 1967; Margulis 1970), has garnered no support. There are, however, numerous other examples of lineage-specific endosymbionts in eukaryotes, ranging from bacteria residing within specialized organs in sap-feeding insects and tube worms to those within ciliates, amoebozoans, and certain algae.

Various words have been used to describe such intracellular occupants, e.g., endosymbionts, endocytobionts, and proto-organelles. However, the degree to which the interactants derive a benefit varies and is often unclear. Endosymbiosis implies the living of one type of cell within another, but the interaction may be jointly favorable (mutualism), beneficial for one member to the detriment of the other (parasitism), or essentially neutral (commensalism). Moreover, depending on the environmental context, the same consortium may switch from one of these conditions to another. One operational distinction between an organelle and an endosymbiont is that the former relies on protein import from the host cell for at least some cell functions other than nutrition (Cavalier-Smith and Lee 1985), and mitochondria and chloroplasts clearly meet this criterion. Both are locked into obligate mutualisms with their host cells, but even here there can be significant uncertainty regarding costs and benefits depending on one's point of reference (McCutcheon et al. 2019). For example, although today's mitochondria cannot survive without their host cells, and vice versa, this does not necessarily mean that the interdependency started from a mutually beneficial situation.

Given their wide phylogenetic distributions, the primary focus of this chapter will be on the origins of mitochondria and chloroplasts and the subsequent remodeling of their functional operations following integration into the cellular environments of their host cells. Special attention will be given to bioenergetic consequences, as there has been considerable debate regarding the benefits that host cells derive from

endosymbiosis (Chapter 8).

The comparative biology of organelles provides an ideal platform for integrating evolutionary theory with cell biology for two reasons. First, the population-genetic environments of organelles is often dramatically different from that experienced by genes residing within the nucleus. Unlike the latter, the former are typically inherited uniparentally and without recombination, and often exhibit dramatically altered mutation rates, sometimes elevated and other times reduced. Second, most of the protein-coding genes in organelle genomes produce products that coassemble with nuclear-encoded subunits. This raises unique issues with respect to intermolecular coevolution between participants residing in the same cell but experiencing different population-genetic constraints.

## Mitochondria

One of the grandest events in the history of the biosphere was the emergence of the mitochondrion, which ultimately became associated with an entire domain of eukaryotic life. Often referred to as the "powerhouse of the cell," the mitochondrion is the location of ATP production by oxidative phosphorylation. Via the tricarboxylic-acid (or Krebs) cycle, the mitochondrion also fuels pathways for amino-acid and lipid biosynthesis, and is the site of synthesis of iron-sulfur clusters that are incorporated into numerous biomolecules, including those involved in the electron-transport chain.

Although a few eukaryotes harbor "mitochondrion-related organelles," phylogenetic evidence indicates that all such variants are simply modifications descendent from the same stock as the more familiar mitochondrion (Figure 23.1). For example, some anaerobic ciliates and parabasalids (e.g., *Trichomonas*) contain independently evolved organelles called hydrogenosomes, which are incapable of oxidative phosphorylation, and instead regenerate ATP from ADP by substrate-level phosphorylation and generate molecular hydrogen as a by-product of the conversion of pyruvate to acetyl-CoA (Lewis et al. 2020). At least three other biochemically modified forms of mitochondria are known across various unicellular lineages, including the mitosomes of diplomonads (e.g., *Giardia*) and microsporidians (parasitic fungi), again independently evolved, which do not generate ATP at all but retain the ancestral trait of synthesizing iron-sulfur clusters (Zimorski et al. 2019). As noted below, a few eukaryotes are completely devoid of of any form of mitochondrion.

**Origins.** The debate as to whether mitochondria were derived from endosymbiotic bacteria or instead somehow arose endogenously concluded as the realization that mitochondria contain their own genomes provided the gold standard for the determination of phylogenetic relationships by DNA-sequence comparisons. Multiple analyses of this sort point to a single origin of the mitochondrion from an $\alpha$-proteobacterium, but this leaves many questions unanswered (Archibald 2015; López-Garcia et al. 2017; Martin et al. 2017). From what specific $\alpha$-proteobacterial lineage did the mitochondrion emerge, and what might this tell us about the nature of the initial colonizer? From what microbial lineage was the host cell derived – bacterial, archaeal, or eukaryotic, and is the eukaryotic nucleus a descendant of that cell? Did the mitochondrion evolve after the establishment of the many other

eukaryotic-specific attributes, or did it come first, with its presence somehow facilitating the origin of the latter? What, if anything, did the original host cell gain from the presence of its colonist and vice versa?

There are three central challenges to achieving definitive answers to these questions. First, all of today's mitochondrial genomes contain $< 100$ protein-coding genes (Figure 23.2), greatly reducing any remaining phylogenetic signal. Second, the potentially long time span between the establishment of the primordial mitochondrion and the most recent common ancestor of today's eukaryotes (LECA) blurs the signal from the few genes that remain. Third, although a large number of genes from the primordial mitochondrion were preserved by transfer to the nuclear genome on the branch to LECA, expanding the range of informative sequence, additional nuclear host-cell acquisitions of genes from other bacteria further cloud the issue.

As new lineages of the diverse $\alpha$-proteobacteria phylum continue to be evaluated by genome sequencing, the picture remains murky as to which particular branch of the group gave rise to the mitochondrion (Rochette et al. 2014; Gray 2015; Martijn et al. 2018; Muñoz-Gómez et al. 2019). The initial view was that the base of the mitochondrial lineage resides near the order Rickettsiales (Andersson et al. 1998; Emelyanov 2001). As all members of this and closely related groups (e.g., *Rickettsia, Wolbachia, Anaplasma,* and *Orientia*) are intracellular parasites of eukaryotic cells, this raises the possibility that the primordial mitochondrion was an energy parasite (Andersson et al. 2003; Sassera et al. 2011; Amiri et al. 2003; Wang and Wu 2014), contrary to the common assertion that the mitochondrion gave a major energetic boost to its virgin host (Lane and Martin 2010).

If this hypothesis is correct, the genome contents of the above species imply that the founder mitochondrion initially harbored $\sim 1200$ genes, contained an ATP/ADP antiporter that enabled ATP import from the host cell, and had a flagellum. In terms of metabolism, it was likely capable of driving a TCA cycle, had an electron-transport chain allowing for oxidative phosphorylation, and carried out ribosomal biogenesis and fatty-acid synthesis. A limited capacity of amino-acid biosynthesis would have been accommodated by the presence of transporters for acquiring amino acids from the host cell.

An alternative hypothesis, based primarily on morphological observations, is that the mitochondrion arose from an anaerobic autotroph (Martin and Muller 1998; Cavalier-Smith 2006; Mũnoz-Gómez et al. 2015, 2017). A hallmark feature of mitochondria is their internal network of invaginated membranes (cristae) upon which the ETS complexes and ATP synthase reside (Figure 23.1). Such structures appear to be homologous to intracellular membranes used in bioenergetic transactions by members of a large $\alpha$-proteobacterial clade containing anaerobic photosynthesizers (purple nonsulfur bacteria), methanotrophs, and nitrite-oxidizing bacteria. The argument for inheritance of such features (as opposed to establishment after the origin of mitochondria) is strengthened by the observation that the development of mitochondrial cristae junctions is organized by a protein orthologous to the one used for similar functions in these bacteria. Under this view, the primordial mitochondrion would have had a capacity for free-living (Martijn et al. 2018; Mũnoz-Gómez et al. 2019), although it may still have derived more benefits from its host cell than it provided in return, i.e., have been a facultative parasite.

Adding to the uncertainties about mitochondrial ancestry are an enormous num-

ber of hypotheses for the mechanism of mitochondrial establishment and the nature of the host cell. A historical compendium of proposed hypotheses assembled by Martin et al. (2015) outlines how these ideas vary in terms of timing relative to the origin of other eukaryote-specific traits, the types of metabolic transactions between host and endosymbiont, and the downstream evolutionary consequences for eukaryotes. Only a few of the more broadly embraced ideas are presented here.

One idea, called the hydrogen hypothesis, is that a methanogenic host cell consumed fuel (waste products) provided by an associated hydrogen-producing bacterium, which eventually became integrated as the primordial mitochondrion (or hydrogenosome) (Martin and Muller 1998). In this and related models, the host cell is generally assumed to be a member of the archaea (Rivera and Lake 1992; Vellai et al. 1998) and likely an anaerobe, an idea that is consistent with the emerging consensus that eukaryotes are derived archaea (Chapter 3). An alternative idea, the oxygen-scavenging hypothesis, proposes that the mitochondrion arose as a mechanism to remove toxic oxygen from an anaerobic host cell (Sagan 1967; Andersson et al. 2003). One thing is clear – assuming the host cell was indeed a member of the archaea, its metabolic features must have been largely displaced by those derived from the mitochondrion (or perhaps other bacteria by horizontal gene transfer), as most genes associated with eukaryotic metabolism are bacterial derived (Chapter 3).

A final matter of concern is the morphological nature of the primordial host cell. Under the mitochondria-early view, the hydrogen hypothesis being one example, the initial host was prokaryotic in form. The strongest variant of this argument is that eukaryogenesis was impossible without the presumed energetic boost provided by the endosymbiont (Lane and Martin 2010). This then raises the question as to how host-cell invasion could occur in the absence of phagocytotic engulfment, which is thought to require a well-developed cytoskeleton. There are examples of bacteria living inside of other bacteria. These include *Bdellovibrio,* which burrows into host cells (Davidov and Jurkevitch 2009; Martin et al. 2017), and a $\beta$-proteobacterium *Tremblaya* that serves as an endosymbiont in insects and is itself inhabited by a $\gamma$-proteobacterium *Moranella* (von Dohlen et al. 2001; Husnik et al. 2013; Husnik and McCutcheon 2016). However, *Bdellovibrio* is a predator, and the cell envelope of *Tremblaya* has been modified in ways that are more like eukaryotic than prokaryotic membranes. Although the cyanobacterium *Pleurocapsa* has been reported to be occupied by other bacteria (Wujek 1979), the latter may have been confused with internal reproductive structures called baeocytes, a normal part of the *Pleurocapsa* life cycle. Thus, there are as yet no definitive examples of bacteria stably coexisting in cells with bacterial membranes.

In contrast, under the mitochondria-late view, many of the early stages in eukaryogenesis, including the origin of a nucleus and internal membranes, are assumed to be present prior to mitochondrial entry (de Duve 2007; Cavalier-Smith 2009). This view was initially championed by Cavalier-Smith (1987) at a time when basal-branching lineages of amitochondriate eukaryotes were thought to exist, a position that is no longer tenable. However, in a comparative analysis focused on the timing of origin of various classes of eukaryotic genes, Pittis and Gabaldón (2016) concluded that the mitochondrion arose subsequent to the establishment of a number of eukaryotic-specific features (including aspects of internal membrane systems), po-

tentially derived from genes acquired from lineages outside of the $\alpha$-proteobacteria. Although interpretation of the genomic data has been debated (Esposti 2016; Martin et al. 2017; Gabaldón 2018), the same can be said for the majority of hypotheses on the roots of the mitochondrion.

Most discussion of the origin of the mitochondrion starts (and often ends) with a consideration of the benefits gained by the host cell. However, the eukaryotic consortium consists of two participants, and evolutionarily stability of a mutualism demands that both partners acquire more resources than would be possible by living alone. How might this condition have been achieved, particularly given that one member of the original consortium may have been a parasite rather than a benevolent partner? Despite its disadvantages to the host, such a system would have been rendered stable if the host lost a key function that was complemented by the presence of the endosymbiont, and if the emerging mitochondrion relocated just a single self-essential gene to the host genome. This scenario almost certainly played out in the lineage leading to LECA, as all mitochondria have forfeited nearly all genes for biosynthesis, replication, and maintenance to the nuclear genome, and as a consequence depend entirely on the gene products of their host cells for these essential metabolic functions. In contrast, the host cell abandoned key metabolic functions, such as membrane bioenergetics and iron-sulfur cluster biosynthesis, to the endosymbiont.

Such an outcome represents a grand example of the preservation of two ancestral components by complementary degenerative mutations (Force et al. 1999). Notably, this process of subfunctionalization (Chapter 6) is most likely to proceed in relatively small populations because the end state is slightly deleterious (both mutationally and bioenergetically) owing to the additional investment required to carry out individual tasks (Lynch et al. 2001). Thus, a plausible scenario is that the full eukaryotic cell plan emerged at least in part by initially nonadaptive processes made possible by a very strong and prolonged population bottleneck (Lynch 2007; Koonin 2015).

This type of functional partitioning is the rule in endosymbiont evolution. The many origins of bacterial endosymbiosis in various lineages sap-feeding insects (e.g., aphids, mealy bugs, whiteflies, and psyllids) provide another case in point (McCutcheon et al. 2019). One of the more dramatic examples of genome reduction concerns the mealy-bug endosymbiont *Tremblaya* noted above, whose genome is just 139 kb in length and contains only 120 protein-coding genes, whereas its own inhabitant *Moranella* has a somewhat larger genome containing 406 protein-coding genes (Husnik and McCutcheon 2016). Remarkably, *Tremblaya* has a number of metabolic pathways that appear to be assembled piecemeal, with some components imported from *Moranella* and others from nuclear-encoded host genes derived from prior horizontal transfers from still other bacteria. Such metabolic-pathway mosaicisms are consistent the principles of intergenomic subfunctionalization just noted. They also provide further evidence in support of the concept of serial endosymbiosis, and resonate well with the theory of remodeling of metabolic pathways by nonorthologous gene replacement (Chapter 19).

**Energetic boost or burden.** The preceding section highlights numerous uncertainties regarding even the most basic features of mitochondrial evolution. We have

a vague understanding of the phylogenetic roots of the mitochondrion, but not so refined as to be certain of the metabolic nature of the original foundress. Likewise, most aspects of the host-cell's biology remain unclear. However, these voids in our knowledge need not constrain our understanding of the evolutionary biology of modern mitochondria. In particular, observations of today's eukaryotes raise considerable doubts about a long-engrained assumption in cell biology – the enduring belief that mitochondria endow eukaryotes with exceptional bioenergetic capacity.

Dating to Sagan (1967), this idea has been pushed most intensively by Lane and Martin (Lane 2006; Lane and Martin 2010; Martin 2017; Martin et al. 2017). They argue that the primordial mitochondrion-bearing host was not only promoted by positive selection on the basis of its enhanced energetic capabilities, but that this boost was essential for the emergence of essentially all eukaryotic features, including increased cell volume and genome size, capacity for phagocytosis, and many other elaborations of morphological and behavioral complexity. This belief persists despite diverse sources of contrary evidence (Lynch and Marinov 2015, 2017; Hampl et al. 2018).

First, as discussed in several prior chapters (7-9, and 17), direct measures of metabolic rates and growth potential provide no evidence that eukaryotic cells are superior relative to prokaryotes. For all well-studied eukaryotic groups, maximum specific growth rates decline with cell size and are no greater than those for similar-sized prokaryotes. Moreover, in contrast to the negative scaling in eukaryotes, growth rates of bacterial species increase with cell size.

Second, the idea that complex internal structures cannot be sustained in the absence of mitochondria is contradicted by the presence of intracytoplasmic membranes in several members of the $\alpha$-proteobacteria (noted above) and in other bacterial lineages discussed in Chapter 3. It has been suggested that phagotrophy (and by extension, complex internal cell structure) by a mitochondrion-free archaebacterium would be selectively disadvantageous relative to a higher energetic yield achieved by the absorption of small dissolved metabolites (Martin et al. 2017). However, this argument assumes that such resources are available in unlimited supply. Natural selection operates on features in the context of realized environments, and as there are very few settings in which resources are unlimited, there will always be a premium on moving into new ecological niches that minimize competition for prevailing resources. Bamboo and eucalyptus are not particularly nutritious, but pandas and koalas have found unique ways to exploit such food sources. Moreover, contrary to the supposed impossibility of phagocytosis without mitochondria, members of the bacterial planktomycete group do ingest and digest bacterial and eukaryotic cells (Shiratori et al. 2019).

Third, eukaryotic species with reduced mitochondria or none at all still have elaborate internal and external complexities. The most extreme case is the oxymonad *Monocercomonoides exilis*, an excavate that lives in the guts of chinchillas, which not only retains the standard internal cellular structure of eukaryotes, but uses four energy-consuming flagella for motility (Karnkowska et al. 2016, 2019). *M. exilis* consumes bacteria and is not a parasite, although it has no TCA cycle and instead makes ATP by glycolysis. *Henneguya salminicola,* a multicellular member of the Cnidaria (related to hydras), which parasitizes salmon, also appears to be completely free of oxidative phosphorylation, although it does retain a genome-

free mitochondrion-related organelle of unknown function (Yahalomi et al. 2020). Many other parasitic eukaryotes with highly modified mitochondria, such as *Giardia* and *Trichomonas,* generate their ATP by substrate-level phosphorylation. Thus, membrane-bound mitochondrial energetics is not a requirement for the maintenance of the complex morphological features of eukaryotic cells.

Fourth, the expansion of genome size in eukaryotes, thought by some to be essential to eukaryogenesis (Lane and Martin 2010), is readily explained by the increased power of random genetic drift in such lineages relative to prokaryotes (Lynch 2007). Moreover, although genome sizes increase by factors of $10^2$ to $10^3$ from prokaryotes to unicellular eukaryotes to multicellular species, the vast majority of this increase is a consequence of the proliferation of noncoding DNA, in particular the expansion of introns and mobile-element insertions, rather than an increase in gene number.

Finally, aside from these direct lines of evidence against the quantum boost in energetic capacity engendered by the mitochondrion, a more fundamental issue is the basic premise that an increase in energy availability per gene drives evolutionary diversification and a natural progression towards complexity (Lane and Martin 2010). Energy is a requirement for life, but no convincing argument has been offered as to why increased access to energy should promote evolutionary change by either adaptive or nonadaptive processes. Long-term rates of evolution are a function of the rate of introduction of variation by mutation, but whereas the mutation rate increases with organism size, this is not a function of energy, but of an increase in the power of random genetic drift (Chapter 4). Recombination rates decline with increases in organism size, but again this has nothing to do with energy, but with the growth of chromosome sizes by nonadaptive processes (Chapter 4). Increased rates of adaptive evolution require increases in directional selection pressure, and there is no obvious reason why organisms with greater energetic capacity would burden themselves by inhabiting environments imposing stronger selection pressures.

To sum up, the idea that more energy allows evolution the freedom to do more tinkering and diversification (Martin 2017; Lane 2020), with apparently no harmful side effects, remains to be explained in terms of known evolutionary mechanisms. Moreover, there is no evidence of a relentless push by natural selection towards complexity, and given that simpler structures are energetically less expensive, it is these that should be promoted by selection. If there is a causal connection between the establishment of the mitochondrion and the radiation of eukaryotes, it does not appear to involve a revolution in bioenergetic potential.

**Functional remodeling.** Although almost all of the genes in the primordial mitochondrial genome were lost prior to LECA, either by outright deletion or transfer to the nuclear genome, mitochondria generally have proteomes consisting of $\sim 1000$ nuclear-encoded proteins. In yeast (Karlberg et al. 2000) and the ciliate *Tetrahymena* (Smith et al. 2007), about half of these proteins appear to have bacterial affinities, although only a small fraction of these are clearly $\alpha$-proteobacterial in origin (Gray 2015; Ku et al. 2015), which may or may not have originated with the primordial mitochondrion. Such observations are consistent with the idea that the road from FECA to LECA experienced serial endosymbiosis, with successive cycles of endosymbiont colonization, extinction, and transfer of genetic material

to the nucleus (Pittis and Gabaldón 2016), as has been documented with insect endosymbionts (Husnik and McCutcheon 2016). Under this view, prior bacterial inhabitants may have contributed to host-cell modifications that in turn paved the way for the later arrival of the mitochondrion. Likewise, the emergence of the mitochondrion would have altered the context in which all other eukaryotic cellular features evolved, potentially opening up new ways of living and access to resources not previously available to prokaryotes.

The genetic accommodation of the mitochondrion had manifest consequences. For example, numerous mitochondrially derived genes residing in the nucleus address their products to organelles other than the mitochondrion, and some original host genes evolved new functions in the mitochondrial proteome (Sloan et al. 2018). The key points are that the establishment of the mitochondrion was followed by the emergence of a diversity of cellular functions with no precedent in the host or endosymbiont, and that much of this remodeling occurred pre-LECA (Huynen et al. 2013; Ku et al. 2015). One of the more astounding such alterations is the origin of editing of transcript sequences that has independently evolved in multiple lineages, often by remarkably complex mechanisms (Foundations 23.1). Here, we briefly describe four additional functional modifications that followed the genesis of the mitochondrion.

First, some of the key morphological innovations of mitochondria involve the entry mechanisms for protein import from nuclear-encoded genes, which necessitated the evolution of novel molecular-recognition systems. For example, the SAM (sorting and assembly machinery) complex, which resides in the outer mitochondrial membrane plays a central role in incorporating outer-membrane proteins, all of which are nuclear-encoded. SAM appears to be related to a similar outer membrane protein in bacteria called Omp85, and therefore likely was contained in the original endosymbiont, but other mitochondrial translocases have less certain provenances.

For proteins that function within the lumen of the mitochondrion, the situation is more challenging, as two mitochondrial membranes must be traversed. The TOM and TIM (translocases of the outer and inner membranes) import hundreds of cytoplasmic proteins marked with specific N-terminal localization signals, which are cleaved upon translocation. As in the case of SAM, the TIM and TOM proteins are nuclear-encoded, but their origins remain unclear and are not obviously derived from prokaryotes (Dolezal et al. 2006).

Second, critical to the maintenance of a stable endosymbiosis are mechanisms for preventing uncontrolled organelle proliferation and for reliably promoting organelle fission and genome inheritance at appropriate times. Within the lifespan of a host cell, mitochondria can undergo multiple rounds of fission and fusion. Whereas bacterial division relies on an inner constriction produced by the GTPase protein FtsZ (Chapter 10), and this seems also to be true of mitochondrial division in a number of eukaryotic lineages (Leger et al. 2015), other eukaryotes appear to have independently evolved a mitochondrial division mechanism that also requires dynamin-related proteins (Friedman and Nunnari 2014; Leger et al. 2015), which pinch from the outside while FtsZ pulls from the inside. Phylogenetic analysis suggests that the ancestral mitochondrial dynamins had dual functions of mitochondrial division and vesicle scission (Purkanti and Thattai 2015), suggesting that internal vesicles

preceded the origin of the mitochondrion. On at least three occasions (ancestors to alveolates, green algae, and opisthokonts), the dynamin gene was duplicated and then subfunctionalized into the two separate functions, and in each case, FtsZ was lost.

Third, a number of novel relationships have evolved between mitochondria and non-endosymbiont derived organelles. For example, endoplasmic reticulum-mitochondrion contact sites serve to coordinate mitochondrial division (Friedman et al. 2011; Wideman et al. 2013; Lewis et al. 2016).

Fourth, while mitochondria are critical to the growth and maintenance of virtually all eukaryotic cells, in a wide range of species they also play a central role in targeting certain cells for death. In particular, the loss of mitochondrial membrane potential often elicits a cascade of molecular events resulting in either homeostatic rebalancing, or if beyond recovery, signaling a death sentence for the cell by autophagy (Galluzzi et al. 2012). Eukaryotic cells also have an internal mechanism called mitophagy that enables the selective recognition and removal of individual mitochondria containing defective proteins (Youle 2019). Such a mechanism also occurs during inheritance, at least in metazoans, as a means by which maternal gametes induce paternal mitochondrial elimination after fusion with sperm (Satoh and Kuroiwa 1991; Zhou et al. 2016). Although all of the molecules participating in such signaling cascades are nuclear encoded in today's eukaryotes, the capacity for inducing cell death might have been carried by an ancestral mitochondrion that killed cells not containing it (Kobayashi 1998); as will be discussed at the close of the chapter, bacteria with exactly these properties exist in *Paramecium* (Preer et al. 1971), demonstrating the potential for some endosymbionts to promote their own existence without providing any advantages to their host cells.

## The Extreme Population-genetic Environments of Mitochondria

As emphasized in previous chapters, the population-genetic environment (defined by the power of mutation, recombination, and random genetic drift) is a critical determinant of the ways in which phenotypes respond to imposed selective pressures. The matter is of particular interest with respect to the evolution of mitochondria, which exhibit dramatic shifts in population-genetic features relative to both their extracellular ancestors and their adopted hosts. The historical consequences of such shifts are reflected in a wide array of changes in genomic architecture (Lynch et al. 2006; Lynch 2007), and as described further below, extend to multiple aspects of organelle proteome integrity. To provide the setting for such discussion, we first consider the three major population-genetic alterations experienced by organelle genomes: mutation-rate modifications, reductions in population size, and loss of recombinational activity, all of which alter the efficiency of natural selection (Chapter 4).

**Mutation rates.** Despite the fact that the replication and repair of organelle genomes is almost exclusively carried out by nuclear-encoded gene products, the mutation rates of organelle genomes often diverge substantially from those in the nucleus. Two molecular factors may contribute to unusual patterns of organelle-

genome mutation. First, as sites of metabolic activity, organelles generate high levels of free-oxygen radicals, which encourage DNA damage via the deamination of cytosine to uracil and the oxidative modification of guanine to 8-oxoG. If not repaired, these two types of premutation respectively cause C:G → T:A transitions and C:G → A:T transversions, and likely are responsible for the near universal A/T bias in organelle genomes (Lynch 2007). Second, in contrast to nuclear DNA, organellar DNA generally replicates within nondividing cells, magnifying the opportunities for replication errors per cell cycle.

The realized mutation rate is a function of the accuracy of the replication machinery and the ability of repair enzymes to correct pre-replication damage, but information on these matters in mitochondria is largely derived from studies of budding yeast and human cells, a small fraction of overall biodiversity (Bohr et al. 2002; Kang and Hamasaki 2002; Mason and Lightowlers 2003). Even here, there is a mix of observations. Base-misincorporation rates of organelle DNA polymerases may be lower than those for polymerases deployed in the nucleus (Kunkel and Alexander 1986; Johnson and Johnson 2001), while proof-reading accuracy may be less efficient in the mitochondrion (Anderson et al. 2020). Although base-excision repair may often replace damaged bases with incorrect nucleotides in mitochondria (Phadnis et al. 2006; Stein and Sia 2017), nucleotide-excision repair may be entirely absent, and the lack of strand-specificity in mismatch-repair implies that correct and incorrect bases are equally likely to be altered (Mason et al. 2003).

The key issue is the net effect of this diversity of factors on mitochondrial mutation rates, the most reliable estimates of which derive from mutation-accumulation experiments (Chapter 4). For metazoans, these estimates are extraordinarily high. In the nematode *Caenorhabditis elegans,* the mitochondrial base-substitution mutation rate is $9.7 \times 10^{-8}$/nucleotide site/generation (Denver et al. 2000), which is $\sim 70\times$ the directly observed nuclear rate for this species (Denver et al. 2004, 2009). In the fly *Drosophila melanogaster,* the mitochondrial rate of $4.4 \times 10^{-8}$ (Haag-Liautard et al. 2008) is $\sim 9\times$ that for the nuclear genome. For two species of the microcrustacean *Daphnia,* mitochondrial rates of $1.6 \times 10^{-7}$ (Xu et al. 2012) and $8.7 \times 10^{-7}$/site/generation (Ho et al. 2020), are respectively $40\times$ and $97\times$ those in the nuclear genomes. Using pedigree data, the average mutation-rate estimate for humans, $3.6 \times 10^{-5}$, is $\sim 2700\times$ the nuclear rate (Howell et al. 1996; Santos et al. 2005). Unfortunately, the only data of this sort outside of metazoans are for the diatom *Phaeodactylum tricornutum,* where the mitochondrial rate of $1.1 \times 10^{-9}$ is just $2.3\times$ the nuclear rate (Krasovec et al. 2019).

All other attempts to estimate organelle mutation rates have relied upon the enumeration of substitutions at silent sites in pairs of species with geologically based divergence-time estimates (see Chapter 4), and these yield results that are not fully concordant with the direct observations noted above. Averaging over a wide range of vertebrates and invertebrates, the ratio of indirect mitochondrial to nuclear rates falls mostly in the range of 2 to 20, whereas the average for a range of unicellular species is $\simeq 1.5$ (Lynch 2007; Popescu and Lee 2007; Smith et al. 2014; Smith 2015). One concern here is that phylogenetically based mutation-rate estimates can be biased by factors such as selection (Stewart et al. 2008), although the overall qualitative interpretation is that, relative to rates in the nuclear genome, mitochondrial mutation rates are inflated to a greater extent in metazoans than in unicellular

species..

However, in striking contrast to all of these results, mitochondrial mutation rates in land plants are typically $\sim 5\%$ of nuclear rates, and at least $100\times$ lower than in metazoan mitochondria (Wolfe et al. 1987; Lynch 2007). Although the mechanisms responsible for the extraordinary mutational quiescence of land-plant mitochondria are unclear, they are not invariant features, as a number of plant genera are known to have mitochondrial silent-site substitution rates up to $5000\times$ greater than the usual background rate (Palmer et al. 2000; Cho et al. 2004; Richardson et al. 2013).

From the standpoint of evolutionary theory, explaining this diversity of organelle mutation rates is a fundamental challenge. As discussed in Chapter 4, the mutation rate is expected to be driven down by selection to the lowest level compatible with the power of random genetic drift, owing to the association of mutator alleles with the linked deleterious mutations that they create. However, as the replicative and DNA-repair machinery associated with mitochondria is nuclear encoded, in a sexually reproducing species a mutator allele will be quickly dissociated from its instigated damage. The elevated mutation rates in animal and some land-plant mitochondria are consistent with this argument. In addition, the reduced deviation between mutation rates in organelle and nuclear genomes of unicellular eukaryotes may be explained by the fact that such organisms reproduce in a predominantly clonal fashion, ensuring a long-term one-to-one relationship between nuclear and organelle genomes.

Less clear is the mechanism by which land-plant organelles are able to maintain some of the lowest mutation rates known in eukaryotes, while experiencing random segregation between organelles and nuclear autosomes, a small effective population size (relative to unicellular species), and a $\sim 100$-fold in reduction in the gene-number target size for mutations relative to the nuclear genome. One potential explanation for this apparent puzzle is that key enzymes involved in nuclear replication and/or repair are also utilized in the organelles, which would result in the organelle mutation rates being a by-product of selection on the nuclear rate. Wu et al. (2020) find that a mismatch-repair pathway shared between mitochondrial and plastid genomes in *Arabidopsis* reduces the mutation rate 10- to 100-fold, but this is no greater than the efficacy of such systems in nuclear genomes, and so taken alone cannot fully explain the reduced rate in plant organelle genomes. Reconciliation of such an elevation in genome stability with conventional theory would be possible if land plants have acquired a mechanism for substantial enough improvement of replication fidelity or DNA repair to make a quantum leap beyond the typical location of the drift barrier, but no such land-plant-specific mechanisms have yet been revealed.

**Modes of inheritance.** Unlike nuclear genomes, all organellar genomes are replicated ameiotically (as in bacteria). In most species, they are also inherited uniparentally, usually through the mother in multicellular species. This raises significant questions about the genetic effective population sizes of genes within organelles relative to those in the nucleus. The simplest view is that with uniparental inheritance the mitochondrial effective population size ($N_e$) would be one-quarter that of nuclear genes in a diploid species, as there is one of the former for each of the four alleles of a nuclear-encoded locus in a mating pair (Palumbi et al. 2001). However, as emphasized in Chapter 4, when population sizes are even moderately large, a ma-

jor determinant of $N_e$ is the influence of background selection operating on linked genes. If there is essentially no recombination among organelle genomes, this effect could be quite pronounced.

Might such effects be compensated by the presence of multiple organelles, each containing multiple genome copies, within individual hosts cells? Although hundreds to thousands of mitochondrial-genome copies may exist in some growing cells, strong transmission bottlenecks typically occur during progeny production. The issue has been addressed on several occasions through the serendipitous discovery of heteroplasmic females (carrying two distinct mitochondrial types). Letting $p$ and $(1-p)$ denote the frequencies of two haplotypes in a mother, assuming random assortment, the variance in frequencies among progeny follows from simple binomial sampling, $p(1-p)/n_o$, where $n_o$ (the effective number of mitochondrial genomes per individual) can be ascertained from the degree of dispersion of the haplotype frequencies among progeny. (This formula is identical in form to that for genetic drift of two autosomal alleles within a population; Chapter 4). Using this approach, or a close variant of it, the effective number of mitochondrial genomes per female transmission is estimated to be $\sim 2$ to 10 in mammals (Ashley et al. 1989; Jenuth et al. 1996; Marchington et al. 1997), and 30 to 300 in insects (Solignac et al. 1983; Rand and Harrison 1986; Haag-Liautard et al. 2008). Less is known on the matter for unicellular organisms, although one might surmise that for small-celled species, $n_o$ would be reduced even further, as the number of mitochondria/cell approaches just one in the smallest cells. Consistent with this view, $n_o$ is on the order of 5 in the slime mold *Physarum* (Meland et al. 1991) and in the yeast *S. cerevisiae* (Birky et al. 1978).

Although the mechanisms responsible for transmission bottlenecks are unclear (candidates include direct organelle destruction, differential replication, and random partitioning of cytoplasm; Burt and Trivers 2006), rapid sorting of variants bears on the issue of organelle $N_e$ in two significant ways if inheritance is primarily uniparental. First, all genomic copies within an individual will coalesce genealogically to a single ancestral molecule in just a few generations (Birky et al. 1983). Second, even though organelle genomes are physically capable of recombination (Thyagarajan et al. 1996; Kazak et al. 2012), the opportunities for generating novel recombinant genotypes are restricted, as this requires the participation of two molecules differing at a minimum of two nucleotide sites, an unlikely mutational scenario with rapid within-individual sorting.

Thus, genetically effective recombination between organelles will generally require biparental transmission to bring divergent genomes into contact. The degree to which such situations arise has been debated considerably (Eyre-Walker and Awadalla 2001; McVean 2001; Piganeau et al. 2004), although most of the discussion has revolved around multicellular species. Although sperm mitochondria are generally targeted for destruction upon delivery, the process is not perfect, and low levels of biparental inheritance have been revealed in nematodes (Lunt and Hyman 1997) and humans (Luo et al. 2018). Mitochondrial inheritance in fungi is often biparental, and recombination does occur (Wilkie and Thomas 1973; Silliker et al. 1996; MacAlpine et al. 1998; Saville et al. 1998; Ling et al. 2000; Anderson et al. 2001), but little is known on the matter in other unicellular species.

The presence of genomically semi-independent mitochondria within host cells

raises significant "levels of selection" issues, most notably the potential for within-host selection among mutant organelle genomes, with those with a replicative advantage having the capacity to expand selfishly within host cells despite the disadvantage to the latter (Havird et al. 2019). The most dramatic examples of such expansions involve mitochondrial deletion mutants, which despite having lost key genes, proceed through replication more rapidly than ancestral molecules (Clark et al. 2012; Jasmin and Zeyl 2014; Phillips et al. 2015). In principle, these very processes may elicit counter-adaptations on the part of the host species to prevent its own loss of fitness due to the competing interests of the endosymbiont. Uniparental inheritance and selective mitophagy are potential cases in point, as both minimize the chances for competition between divergent mitochondrial genomes.

Although the fixation of deletion mutants for essential genes is ordinarily not possible, when combined with processes involving gene transfer to the nucleus, the proliferative advantage of mitochondrial genomes of reduced size may have cascading effects. Once a gene transfer to the nucleus has been integrated to the extent that its products are fed back to the mitochondrion in an efficient manner, deletions of the mitochondrial gene will be free to advance, as the cytonuclear conflict will have been eliminated. This perhaps explains why inter-genomic transfer between mitochondrial and nuclear genomes has been essentially unidirectional, and provides an extension to Doolittle's (1998) suggestion that sheer mutational pressure created a ratchet-like mechanism that ultimately ensured the relocation of organelle genes to the nucleus.

Finally, uniparental inheritance further alters the selective environment for the endosymbiont, by favoring features in the latter that enhance the fitness in the transmitting sex, while severing the selective connection with the non-transmitting sex (Cosmides and Tooby 1981; Frank and Hurst 1996; Gemmell et al. 2004). In the case of maternal inheritance, for example, a mitochondrial genome with beneficial female effects can be promoted through mothers even if it has severe negative effects on males, as males with superior mitochondria do not pass them on to offspring (assuming no paternal leakage). The presumed outcomes of such a process, sometimes referred to as the "mother's curse," are commonly observed in land plants with cytoplasmic male sterility. Of course, such a situation will also select for mutations in nuclear genes that suppress the male-fitness reducing effects female-driven mutations, setting up a sort of coevolutionary arms race between the sexes. Not surprisingly mutations that restore male fertility are commonly found (Fujii and Toriyama 2009; Gaborieau et al. 2016; Yamauchi et al. 2019).

**Muller's ratchet.** The magnitude of reduction of mitochondrial $N_e$ is a key determinant of the evolutionary limitations of mitochondrial genomes, as mutations with selective effects smaller than the inverse of $N_e$ are essentially immune to selection (Chapter 4). Given the multitude of effects that define $N_e$, the combination of low (to no) recombination and uniparental inheritance being of particular relevance to mitochondria, resolving this issue requires empirical analysis. Recall from Chapter 4 that the usual approach is to estimate standing levels of genetic of variation at neutral genomic sites (generally third-positions in redundant codons), setting this equal to the drift-mutation equilibrium expectation $2N_eu$ for haploids, and factoring out the mutation rate $u$ to obtain $N_e$. Our interest here is in the depression of $N_e$

in mitochondrial relative to nuclear genomes in the same species.

Unfortunately, there are very few species with the diversity and mutation-rate data necessary for such a computation. Consider, however, the situation in humans where the ratio of silent-site diversity in the mitochondrial vs. nuclear genome is $\simeq 5.5$ (Lynch et al. 2006). Letting $2N_{gn}u_n$ be the expected nucleotide diversity per silent site in the nuclear genome at mutation-drift equilibrium, with $N_{gn}$ and $u_n$ being, respectively, the effective number of chromosomes and mutation rate per nuclear genomic site, and $2N_{gm}u_m$ be the similar expression for the mitochondrion, then 5.5 provides an estimate of $(N_{gm}u_m)/(N_{gn}u_n)$. If, as suggested above, $u_m/u_n \simeq 2700$, this implies a ratio of effective sizes of just $N_{gm}/N_{gn} \simeq 0.002$. For arthropods, the average ratio of diversities is close to 1.0 (Lynch et al. 2006), but as noted above, the ratio of mutation rates is $\simeq 40$, suggesting $N_{gm}/N_{gn} \simeq 0.025$. On the other hand, the mean ratio of diversities for the few unicellular species with available data is $\simeq 0.5$ (Lynch et al. 2006), and using a mutation-rate ratio of 1.5 (from above) implies $N_{gm}/N_{gn} \simeq 0.3$. Thus, based on the few systems for which data are available, the power of drift operating on mitochondrial genes can be as much as $500\times$ that in nuclear genes in some metazoans, but may be much closer to parity in unicellular species.

The inability to shed mutations by recombination has inspired the idea that organelle genomes are uniquely susceptible to mutational degradation by a process known as Muller's ratchet (Muller 1964; Felsenstein 1974). In the absence of recombination, parent molecules cannot produce offspring with a reduced number of deleterious mutations, except in the rare case of back or compensatory mutations. Thus, in an asexual population, when by chance the best class of individuals produces either no surviving offspring or only offspring with at least one new deleterious mutation, a nearly irreversible decline in fitness is experienced. Each generation, there is an appreciable chance of such an event because recurrent mutation pressure generally reduces the best-fit class to just a small fraction of the total population (Haigh 1978). Moreover, each time the currently best-fit class is lost from the population, the previously second-best class becomes subject to the same stochastic process, eventually suffering an identical fate. Once this process has proceeded to the point at which the mutation load is so high that the average individual cannot replace itself, the population size must begin to decline. This further enhances the magnitude of random genetic drift, promoting increasingly higher rates of deleterious-mutation accumulation and ultimately culminating in population extinction by mutational meltdown (Lynch and Gabriel 1990; Lynch et al. 1993, 1995a,b).

Aside from recombination, which enables pairs of parental genomes to produce progeny molecules with reduced numbers of deleterious mutations (Maynard Smith 1978; Charlesworth et al. 1993; Lynch et al. 1995a,b), the only remedy to this problem is back- or compensatory-mutation (Wagner and Gabriel 1990; Poon and Otto 2000; Goyal et al. 2012). However, even then, one expects the long-term mean phenotype to deviate from the optimum to a degree that depends on the power of random genetic drift (Lynch 2020). Given that LECA dates to $> 1.5$ million years ago, it is clear that mitochondrial mutational meltdowns have been avoided on this time scale in a large number of eukaryotic lineages. Nonetheless, it remains possible that individual lineages have succumbed to such decay and that others may still be

predisposed to do so (Takahata and Slatkin 1983; Hastings 1992; Gabriel et al. 1993; Reboud and Zeyl 1994; Loewe 2006). Moreover, as outlined in the next section, it is clear that organelle genomes have commonly gone down paths of degradative evolution in ways never seen in nuclear genomes.

## Organelle Genome Degradation

The central point of the preceding discussion is that the peculiar population-genetic environment of the mitochondrion, combined with the asymmetry of interests of organelle and nuclear genomes, have played a key role in the remodeling of eukaryotic cell functions, with a reach that goes far beyond cellular energetic performance, e.g., the evolution of mutation rates and uniparental inheritance. Here, we further explore how the resultant reduction in mitochondrial $N_e$ and the associated decline in the efficiency of selection against deleterious mutations have had cascading effects across key genes in both the mitochondrial and nuclear genomes. Despite the centrality of the mitochondrion for cellular energetics, multiple lines of evidence support the view that mitochondria have become compromised by mildly deleterious-mutation accumulation since their inception.

The most obvious manifestation of mitochondrial decay has been noted above – the massive reductive evolution in genome size that occurred on the road from FECA to LECA (Figure 23.1). Most mitochondrial genomes are $< 100$ kb in length, with the exception of those of land plants, which can reach 1 Mb. They are typically $> 10\times$ smaller than their partner nuclear genomes, retaining $< 100$ of the probably $> 1000$ protein-coding genes harbored in the ancestral mitochondrion. A number of genes in the primordial mitochondrion were simply lost, presumably owing to any fitness advantages being reduced to the point of effective neutrality, and perhaps in some cases owing to disadvantages in the host-cell environment. Nearly all of the few protein-coding genes that remain in mitochondrial genomes are involved in energy metabolism, with nearly all genes involved in DNA replication and repair, transcription, and translation residing in the nucleus.

The presence of fragments of mitochondrial DNA in the nuclear genomes of nearly all eukaryotes highlight the ample opportunities that exist for such transfer even today (Hazkani-Covo et al. 2010). However, physical relocation need not lead to functional transfer, which requires some form of positive and stabilizing selection for gene relocation. As noted above, selection on host cells to resist the expansion of rogue mitochondria with deletion mutations is one potential source of such selection, and the need to escape from the consequences of Muller's ratchet provides another long-term advantage for transfer to a recombining nuclear genome. Kelly (2020) also makes the case for a short-term advantage – a substantial reduction in the energetic cost of organelle genes residing in nuclear genomes. The large number of organelle genomes typically present in cells greatly magnifies the DNA-level cost relative to that of a nuclear gene present in just one (haploid) to two (diploid) copies.

Several indirect lines of evidence are consistent with the idea that the few genes retained in organelle genomes are vulnerable to deleterious-mutation accumulation. For example, in a wide variety of animals and land plants, within-population surveys of nucleotide-sequence variation in organelles consistently reveal that ratios of non-

synonymous (amino-acid replacement) to synonymous (silent-site) polymorphisms are two to ten times greater than those for divergence between closely related species (Ballard and Kreitman 1994; Nachman et al. 1994, 1996; Rand et al. 1994; Templeton 1996; Hasegawa et al. 1998; Wise et al. 1998; Fry 1999; Städler and Delph 2002; James et al. 2015). These patterns are dramatically different than those seen in nuclear genomes, where there is often an excess of replacement substitution at the level of divergence, which is a presumed reflection of fixation of adaptive mutations (Chapter 4). There is need to extend this sort of work to unicellular eukaryotes, but the most reasonable interpretation of the existing patterns is that a significant numbers of deleterious mitochondrial mutations are able to expand to high enough frequencies to be observed in population surveys but not so high as to go to fixation. If this is correct, because mildly deleterious mutations have a wide range of selective effects (Chapter 5), it follows that some mutations with very mild individual effects are vulnerable to advancing all the way to fixation in organelle genomes with small $N_e$.

**Animal mitochondrial tRNAs.** More direct insight into the matter of mutation accumulation can be obtained by examining the evolutionary fates of parallel sets of mitochondrial and nuclear genes with identical functions in the same species. Transfer RNA (tRNA) and ribosomal RNA (rRNA) genes provide an ideal venue for such analysis, as many organelle genomes contain full sets of both, with parallel nuclear-encoded sets operating in the cytosol. In particular, the extreme conservation of the primary, secondary, and tertiary structure of tRNAs (Kimura 1983; Söll and RajBhandary 1995) across the entire Tree of Life testifies to the power of natural selection at maintaining the optimal molecular architecture of these genes. Transfer RNAs have a standard cloverleaf secondary structure, with their $\sim 70$ bases being contained mostly in three loops and four stems, with 13 of these bases being essentially invariant across the tRNAs for all amino acids in all prokaryotes and all nuclear genomes (Lynch 1997, 1998; Lynch and Blanchard 1998). Because this extraordinary degree of constancy must have been present in the tRNAs within the primordial mitochondrial genome, any deviations from the canonical architecture are likely to reflect a reduction in the efficiency of selection imposed by mitochondrial population-genetic environments. The evidence for such a shift is compelling.

First, contrary to the situation in the nuclear genome, there are no invariant sites in organelle tRNAs, and every region of such molecules evolves at a higher rate than the homologous region in nuclear tRNAs. This is not simply a consequence of elevated mitochondrial mutation rates, as the ratio of the observed substitution rate to the neutral expectation is elevated several-fold in mitochondrial tRNAs of all phylogenetic groups. Second, animal mitochondrial tRNAs exhibit a wide array of structural deviations from the canonical form of the prokaryotic/nuclear tRNAs, including losses of entire arms in some cases (Wolstenholme 1992). Third, for animals, plants, and fungi, the average binding strength of mitochondrial tRNA stems is 40 to 90% of that for those in the nucleus, largely due to the higher incidence of A:U vs. G:C bonds (two vs. three hydrogen bonds) in the former (Lynch 1997, 1998).

Although compensatory mutations in tRNA molecules, such as the restoration of Watson-Crick base pairs in stem positions, may eventually mitigate some negative

effects of single-base changes (Steinberg and Cedergren 1994; Steinberg et al. 1994; Watanabe et al. 1994; Wolstenholme et al. 1994; Kern and Kondrashov 2004), experimental evidence suggests that the structural modifications noted above compromise the efficiency of protein synthesis. For example, bovine mitochondrial tRNAs have been shown to have an unusually low rates of amino-acid loading (Kumazawa et al. 1989, 1991; Hanada et al. 2001). Thus, although the bizarre architectures of animal mitochondrial tRNAs have been accompanied by dramatic changes in the recognition mechanisms used by their nuclear-encoded cognate tRNA amino-acyl synthetases (Kuhle et al. 2020), the compensating effects are less than perfect.

Could the increased width of the selective sieve for organelle-encoded genes be a simple consequence of the relaxation of selection in organelles (i.e., smaller selection coefficients), as suggested by some (Brown et al. 1982; Kumazawa and Nishida 1993), rather than an outcome of a reduction in the efficiency of selection owing to a smaller effective population size? Analyses laid out in Lynch (2007) and Popadin et al. (2012) suggest that the absolute strength of selection (i.e., the selection coefficient) against deleterious mutations in the mitochondrion is equivalent, if not higher, than in the nuclear genome.

**Coevolutionary drive and compensatory mutations.** The conclusion that mitochondrial genomes harbor a reduced ability to purge deleterious mutations further motivates the idea that such mutations secondarily drive the fixation of compensatory mutations (Rand et al. 2004). In principle, such fitness-restoring mutations may arise in the organelle genes themselves (Oliveira et al. 2008; Meer et al. 2010; James et al. 2016), although most attention has been given to alterations in key nuclear-encoded genes. Three types of nuclear genes with intimate connections with organelle partners are of particular interest: 1) the sets of tRNA amino-acyl synthetases (noted above), each of which attaches a specific amino acid to its cognate tRNA, either in the mitochondrion or in the cytosol; 2) the ribosomal protein-coding genes designated for cytosolic vs. mitochondrial ribosomes; and 3) the nuclear-encoded components of the complexes in the mitochondrial oxidative phosphorylation (OXPHOS) pathway.

As noted in Chapter 6, relative to the bacterial ancestral state, mitochondrial ribosomes have experienced a dramatic increase in the number of protein subunits, all encoded in the nuclear genome. Early in eukaryotic evolution, on the order of 75 new subunits were added to the mitochondrial ribosome, and this was then followed by multiple lineage-specific gains and reductive evolution in some cases, with patchy additions suggesting recruitment to ameliorate pre-existing structural instabilities (van der Sluis et al. 2015; Petrov et al. 2019). Thus, as in the case of tRNAs, the diversification of the structural features of mitochondrial ribosomes contrasts dramatically with the high degree of phylogenetic stasis in cytosolic ribosomes. As with tRNAs, many of the rRNA stem pairs that are G:C in bacterial ribosomal RNAs are A:U in mitochondrial rRNAs, resulting in a loss of $\sim 260$ hydrogen bonds and hence a substantial reduction in stability (van der Sluis et al. 2015).

These kinds of observations on structural modifications generalize to the mitochondrial OXPHOS complexes. Despite the overall mass migration of organelle genes to the nuclear genome, several large mitochondrial complexes, such as those involved in the electron-transport chain, retain a few mitochondrially-encoded sub-

units. However, in the path from FECA to LECA, the individual complexes acquired multiple novel protein subunits (in addition to the nuclear-transferred units), nearly tripling the numbers of components relative to the ancestral state (Hirst 2011; Huynen et al. 2013; van der Sluis et al. 2015). For example, mitochondrial complex I has 40 to 50 protein subunits in most eukaryotic lineages, whereas the orthologous structure in bacteria has only 14 (Gabaldón et al. 2005; Cardol 2011). As with ribosomes, an early (pre-LECA) phase of expansion was followed by smaller numbers of lineage-specific gains and losses, such that not all eukaryotic lineages have the same sets of supernumerary proteins. Although there is evidence that such proteins play important roles in maintaining enzyme stability (Angerer et al. 2011; Stroud et al. 2016), this is expected for an evolved compensatory modification and need not imply improved overall enzyme performance. Indeed, there is no evidence that the simpler bacterial complexes are less stable or inferior in any way (Hirst 2011).

Finally, several studies at the amino-acid sequence level have shown that lineages with rapidly evolving mitochondrial-encoded proteins show parallel elevations in the rate of evolution of nuclear-encoded subunits (Osada and Akashi 2012; Zhang and Broughton 2013; Sloan et al. 2014; Adrion et al. 2015; Havird et al. 2017). For example, studies in animals and yeast indicate that, even after accounting for mutation-rate differences, mitochondrial ribosomal-protein sequences evolve $> 10\times$ more rapidly than those for cytoplasmic ribosomes, despite both being encoded in the nuclear genome (Pietromonaco et al. 1986; Barreto and Burton 2013; Barreto et al. 2018). Notably, components of OXPHOS complexes that are fully encoded in the nuclear genome do not exhibit such elevated rates of evolution (Havird et al. 2015). Although it has been suggested that elevated rates of amino-acid sequence evolution in nuclear-encoded mitochondrial vs. cytosolic proteins may simply be due to lower expression levels of the former (and hence potentially weaker purifying selection against deleterious mutations), this conclusion does not have wide support (Osada and Akashi 2012; Barreto and Burton 2013; Barreto et al. 2018).

While observations like these are qualitatively consistent with the hypothesis that mutation-driven changes in mitochondrial proteins accelerate the coevolutionary accumulation of compensatory changes in their nuclear-encoded partners, the degree to which this verbal model is consistent with the population-genetic conditions experienced by interacting genes remains unclear. For the presumed coevolutionary loop to be sustained, the selective disadvantages of mutations in the organelle genes must, on the one hand, be sufficiently mild relative to the power of random genetic drift to enable to them to become frequent enough to impose reliable selection on the associated nuclear-encoded loci. On the other hand, the deleterious effects of organelle mutations must also be sufficiently large to impose effective selection on the nuclear-encoded loci. The key theoretical work essential to disentangling these issues remains to be done.

## Plastid Evolution

Long after the establishment of the mitochondrion, on the order of 1.0 BYA (Parfrey et al. 2011; Keeling 2013; Eme et al. 2014), another endosymbiotic event forever changed the eukaryotic world – the colonization of a lineage that would go on to

form the base of the Archaeplastida, which subsequently diversified into the red algae, green plants (including green algae), and glaucophytes (a basal group of unicellular algae). Giving rise to the chloroplast, this brought photosynthesis into the eukaryotic domain.

As with the mitochondrion, the search for the ancestral roots of this event is made possible by the presence of a genome within the plastid. Although the plastid origin undoubtedly has affinities with cyanobacteria, this a large phylogenetic group with lineages with diverse properties (including multicellular forms, and those capable of fixing nitrogen). From comparative genomics, an emerging consensus is that the closest living relative is *Gloeomargarita lithophora,* a non-nitrogen-fixing species (Ponce-Toledo et al. 2017; Sánchez-Baracaldo et al. 2017). The fact that this species and its relatives are restricted to freshwater environments, as is the basal plastid-containing lineage (the glaucophytes) (Price et al. 2012), suggests that photosynthetic eukaryotes arose in a terrestrial freshwater environment before colonizing the oceans.

Unlike the universal spread of the mitochondrion across the entire eukaryotic phylogeny by simple vertical inheritance, photosynthesis acquired a punctate phylogenetic distribution by horizontal transfer. Secondary plastids have arisen on multiple occasions as heterotrophic eukaryotes from one lineage engulfed photosynthetic species from another and then retained them in a permanent endosymbiotic state. For example, the basal ancestors of euglenoids and chlorarachniophytic algae independently acquired photosynthesis via the capture and domestication of green algae. Morphological support for such transfer derives from the presence of four membranes surrounding secondary plastids – two from the primary plastid, a third from the plasma membrane of the donating eukaryotic cell, and a fourth putatively from the phagosomal membrane of the host cell. Thus, whereas primary plastids float freely in the cytoplasm, secondary plastids are topologically integrated into the endomembrane system.

Secondary plastids have also arisen on multiple occasions via engulfment of members of the red-algal lineage. Although uncertainty remains as to how many independent events have occurred, such horizontal transfers have led to the spread of photosynthesis across a wide array of eukaryotic groups, including the stramenopiles (including diatoms), cryptomonads, haptophytes, and dinoflagellates (Keeling 2013). A few cases are even known in which dinoflagellate species absorbed another cell containing secondary plastids, endowing them with a tertiary plastid.

Cases of secondary and tertiary endosymbiosis must have initiated with an endosymbiont containing three genomes – plastid, mitochondrion, and nucleus, but in all known cases, the mitochondrion has been lost. In a few cases, however, a remnant of the nuclear genome has been retained in the form of a nucleomorph. These include the cryptophytes, chlorarachniophytes, and some dinoflagellates (Sarai et al. 2020). As a result of endosymbiotic gene transfer, the nuclear genomes of the host cells in these lineages are substantially chimeric.

Aside from the primary plastid colonization at the base of the Archaeplastida and its secondary and tertiary spreads, additional introductions of photosynthesis into eukaryotes occurred by independent primary events. For example, dating back to $< 200$ MYA, a freshwater amoeba called *Paulinella chromatophora* became colonized by another cyanobacterium (related to *Synechococcus*) (Nowack and Gross-

man 2012; Nowack 2014). Given that about 30 genes of cyanobacterial origin have been relocated to the nuclear genome and produce products addressed back to the endosymbiont, *P. chromatophora* can be regarded as a legitimate organelle. An even more recent establishment involves the colonization of the diatom *Rhopalodia,* probably $< 20$ MYA, by a nitrogen-fixer related to the cyanobacterium *Cyanothece,* which is no longer photosynthetic in its host (which itself is photosynthetic via a secondary plastid) (Nakayama et al. 2011, 2014).

Examples also exist in which cyanobacteria are less fully integrated with eukaryotic partners. For example, *Atelocyanobacterium thalassa,* a cyanobacterium lacking both oxygen-producing photosystem II and the citric-acid cycle, associates extracellularly with a marine prymnesiophyte, to which it provides fixed nitrogen while gaining fixed carbon in return (Thompson et al. 2012). A dinoflagellate called *Ornithocercus* carries a load of cyanobacteria, distantly related to marine *Prochlorococcus / Synechococcus,* in an extracellular chamber, apparently periodically digesting them in a farming-like process (Nakayama et al. 2019). In both of these cases, the cyanobacterial genome is highly reduced in size, consistent with genome-reduction following a long-term association. Finally, kleptoplasty, wherein a heterotrophic consumer ingests a photosynthetic prey item and then retains its chloroplasts, is found in a number of lineages, dinoflagellates in particular (Hehenberger et al. 2019).

Conventional chloroplasts, derived from the ancestral archaeplastid event, provide dramatic examples of parallel evolution with the types of remodeling observed in mitochondria, just a few of which will be mentioned here. First, as in mitochondria, chloroplast division typically proceeds with a cyanobacterial-derived FtsZ protein operating on the inside and a eukaryotic dynamin protein on the outside of the membrane (Miyagishima et al. 2014). However, glaucophyte chloroplasts, which branched off from the Archaeplastida prior to the red and green algae, are unusual in having a peptidoglycan layer between the inner and outer membranes and divide using only FtsZ. Thus, the integration of dynamin into chloroplast division in the latter two groups apparently occurred after the loss of the peptidoglycan layer.

Second, nuclear-encoded chloroplast genes require the presence of terminal targeting sequences for localization to the plastid, with the situation being even more extreme in the case of secondary plastids, which must carry dual targeting messages, one to the external endosymbiont membrane and the other to its internal organelle membrane. Independent of mitochondrial TIM and TOM, plastid TIC and TOC evolved as inner- and outer-membrane chloroplast channels tethered together to control protein import from the cytoplasm (Chen et al. 2018).

Third, although there are unique features in individual lineages, for the most part genome evolution has proceeded down parallel pathways in mitochondria and plastids (Lynch et al. 2006; Smith and Keeling 2015), including substantial genome-size reduction (Figure 23.2), the gravitation towards AT richness, and the emergence of uniparental inheritance. Plastid genomes are highly diminished relative to those of free-living cyanobacteria, generally containing just 30 to 230 protein-coding genes. Many of the original genes were transferred to the nucleus, some of which have taken on entirely novel functions (Martin et al. 2002). Some land plants have multichromosomal plastid genomes, but the same is true for a number of bilaterian metazoan lineages (Lavrov and Pett 2016).

One of the most unique features of plastid evolution concerns the degree to

which the mutation rate has been reduced – commonly 5 to 10× lower than that of the nuclear genome in land plants (Gaut et al. 1996), although less extreme than in plant mitochondria and less diminished in algal lineages (Smith and Keeling 2012; Ness et al. 2016). Despite the lower mutation rates than in metazoan mitochondria, the plastid proteome has evolved in a number of ways that suggest an influence from deleterious-mutation accumulation, similar to that observed in mitochondria. For example, new protein subunits have been recruited to plastid ribosomes, although not as extensively as in the case of mitochondria (Yamaguchi et al. 2000; Sharma et al. 2007). Notably, a few land-plant lineages have evolved dramatic increases in plastid mutation rates, and these exhibit the kinds of alterations in protein-sequence evolution noted above for mitochondria, including enhanced rates of amino-acid substitutions in nuclear-encoded subunits of plastid molecular complexes (Sloan et al. 2014; Zhang et al. 2015; Rockenbach et al. 2016; Weng et al. 2016).

## Addiction to Endosymbionts

Given their roles in energy production, it remains seductive to think of mitochondria and other endosymbionts as having been driven to fixation by adaptive mechanisms, most notably by magnifying the growth potential of the host species. As this view ignores any adaptive losses by the endosymbiont, it is not entirely consistent with the general postulates of evolution by natural selection. A more fundamental issue is whether even the host species achieves a net advantage in the long run. To be sure, well-embedded endosymbiotic systems are essential to their host species – once locked in by processes of reciprocal subfunctionalization, reversion is no longer possible. However, although such relationships are often viewed as cooperative mutualisms, it need not follow that the total productivity of the pair (or just the host cell) exceeds the pre-mutualism condition. For this reason, McCutcheon et al. (2019) advocate the use of the label "host-beneficial endosymbiont" to describe the internal inhabitant.

Further insights into these issues are provided by attempts to establish microbial mutualisms in experimental systems, and these consistently show that such systems have bidirectional costs. Although a symbiosis may start out with a recipient simply benefiting from a waste product of a donor, the recipient then responds evolutionarily to feed the donor so as to create more by-product, with the donor then becoming more enslaved by the original recipient and provisioning it even more (Harcombe et al. 2018). Thus, the establishment of a stable mutualism incurs bidirectional costs, which can only be revealed in the early stages of establishment when the participants can still be grown alone. Moreover, once established, functional interdependence enhances the likelihood of loss of genes essential to independent living but no longer required (Hillesland et al. 2014).

In this sense, mutualisms represent a sort of reciprocal addiction. In some cases, this may enable the system to survive in a novel nutritional environment, e.g., with species A providing B with a critical supply of carbon, and B being an essential source of nitrogen for A (Hom and Murray 2014; Fritts et al. 2020), but it remains unclear whether evolved mutualisms can ever outcompete virgin host cells in ancestral environments. Of course, to the extent that endosymbioses initiate as

host-pathogen systems, an idea with considerable support (Sachs et al. 2011, 2014), then the eventual taming of the pathogen by the host cell can be viewed as beneficial. Again, however, this need not mean that the host has become better off than in the complete absence of the pathogen.

We close with a brief overview of some of the more dramatic examples of addiction of host cells to externally acquired agents, the toxin-antitoxin systems in bacteria. Many bacteria produce toxins that are excreted into the environment, and in doing so eliminate susceptible competing species or other innocent bystanders. However, thousands systems are known in which a toxin is released intracellularly along with an antitoxin molecule, which prevents autotoxicity. Nearly all bacterial species harbor one or more such systems, frequently carried as linked toxin-antitoxin (TA) cassettes on extrachromosomal DNAs called plasmids (Hayes and Van Melderen 2011; Goeders and Van Melderen 2014). Generally, TA phylogenies are incongruent with host-cell phylogenies, implying horizontal transfer of the plasmids. Moreover, the toxicity mechanisms associated with TA cassettes are highly diverse, ranging from transcription / translation inhibition to transcript destruction to interference with membranes or cell division, and the antitoxins can be either proteins or RNAs, implying the independent evolution of such systems.

TA systems are exquisitely constructed to ensure self-proliferation. As the antitoxin is less stable than the toxin, unless the plasmid is retained after host-cell division, death will rapidly ensue as the toxin is freed from inhibition. Thus, the host becomes addicted to the TA-carrying plasmid. Over evolutionary time, many TA systems become incorporated into bacterial chromosomes, in effect serving as an "anti-addiction" solution and allowing the loss of the plasmid, although not fully relieving the host from carrying the cassette.

The key point is that plasmid-born TA systems provide a compelling example of highly successful parasites that ensure their own selfish proliferation by selectively eliminating non-cooperating host cells. In principle, such systems can be stable for a long time, provided a subpopulation of plasmid free host cells does not emerge (e.g., by fortuitous deletion of the toxin gene). Moreover, a TA system can become an essentially permanent fixture if the toxin and/or antitoxin provides enough additional side-benefit to the host to offset the occasional loss by post-segregation killing (Rankin et al. 2012). In some case, such secondary benefits are acquired through alterations of the host-cell response to additional stresses (Van Melderen 2010; Yamaguchi et al. 2011).

Although the mechanisms are not fully understood, similar sorts of system seem exist in ciliated protozoans. Numerous *Paramecium* strains are carriers of so-called "killer bacteria" that when released into the environment are lethal to naive (bacteria-free) host cells (Görtz and Fokin 2009; Schrallhammer and Schweikert 2009). These bacteria are phylogenetically diverse, distributed over multiple $\alpha$-proteobacterial lineages and exhibit a wide array of killing mechanisms, including paralysis, osmotic imbalance, mate killing, and out-of-control spinning. Thus, as in the case of bacterial TA systems, there appear to be multiple independent origins of killer-bacterial systems.

Under certain physiological conditions, killer bacteria produce a huge ribbon-like inclusion called an R body, which unwinds into a spear-like structure at low pH, carrying defective phage particles that might be the carriers of toxins (Pond et al.

1989). The four small proteins that polymerize to form R bodies are often carried on plasmids. But in some cases, they have moved to the bacterial chromosome (Jeblick and Kusch 2005), reminiscent of the fates of TA systems. Moreover, genomic surveys suggest that R bodies are widely distributed across the bacterial phylogeny, although their functions are generally unknown (Raymann et al. 2013). It remains unclear how the *Paramecium* carriers of killer bacteria acquire an immunity to toxicity. However, it is known that if the host cells are grown at maximum rates, the bacterial cells are gradually lost owing to their inability to proliferate as fast as the *Paramecium,* i.e., the host cells can be progressively weaned from their addiction. Upon loss of the bacteria, resistance to the toxin is lost (Grosser et al. 2018), as expected if the endocytobiont is a carrier of a toxin-antitoxin system.

**Summary**

- The eukaryotic phylogeny is replete with endosymbiotic mutualisms, the most famous being the mitochondrion, derived from an $\alpha$-proteobacterium, and the younger plastid, derived from a cyanobacterium. The mitochondrion led to the establishment of internal membrane bioenergetics throughout all eukaryotes by vertical transmission. The plastid, which brought photosynthesis into the eukaryotic domain, acquired a more punctate phylogenetic distribution by spreading horizontally into previously heterotrophic lineages.

- It remains unclear whether the establishment of the mitochondrion preceded the emergence of other eukaryotic-specific traits such as internal membranes and cytoskeletons, but numerous observations are consistent with this hypothesis.

- It is commonly argued that the establishment of mitochondrial membrane bioenergetics led to a massive increase in the bioenergetic capacity of eukaryotes relative to prokaryotes, and that this boost was an essential pre-requisite for all things related to eukaryogenesis. However, multiple lines of evidence are inconsistent with this view, including direct bioenergetic measurements, the bidirectional costs and conflicts expected when mutualisms evolve, and the accumulation of mildly deleterious mutations in nonrecombining genomes.

- Substantial intracellular remodeling followed the establishment of the mitochondrion, demonstrating the power of the cellular environment at directing the course of evolution. The vast majority of surviving genes of mitochondrial origin were relocated to the nuclear genome; mechanisms for targeting the nuclear-encoded products of these genes back to the mitochondria evolved; host mechanisms for regulating mitochondrial proliferation emerged; and mitochondria became progressively intertwined in entirely new functions, such as targeted cell death.

- Relative to the situation for nuclear genes, organelle genes often experience a

substantial reduction in effective population size, an absence of effective recombination, and altered mutation rates. Taken together, these conditions have arguably led to the accumulation of excess deleterious mutations in organelle genomes, generating as coevolutionary side-effects compensatory mutations in interacting nuclear-encoded genes. As a result, the structural features of mitochondrial proteins and RNAs have sometimes acquired dramatic alterations never seen in nuclear-encoded genes or in bacterial genomes.

**Foundations 23.1. Messenger RNA editing.** A long history of research in molecular biology inspires confidence that coding information at the DNA level provides a reliable prediction of protein sequences. However, some organelles use post-transcriptional editing to modify mRNA, tRNA, and/or rRNA sequences. The most spectacular display of editing occurs in the mitochondrial genes of kinetoplastids (e.g., *Trypanosoma*), where insertion and deletion of Us (uridines) affects about 90% of all codons (Simpson et al. 2000; Horton and Landweber 2002). With editing at such a massive scale, the underlying genomic sequences for genes are literally nonsensical. Editing in such species relies on the baroque structure of the kinetoplastid mitochondrial genome – a vast network of intertwined molecules, including several 20-40 kb maxicircles carrying the cryptic gene sequences, and thousands of 0.5-3.0 kb minicircles carrying guide RNA templates for the addition/removal of Us in the immature maxicircle-derived mRNAs (Koslowsky et al. 1992). Although no other mitochondrial lineage engages in editing as extensively as kinetoplastids, some slime molds insert nucleotides every 25 to 40 nucleotides in mRNAs, rRNAs, and tRNAs (Horton and Landweber 2000; Cheng et al. 2001; Byrne and Gott 2004), and 2 to 4% of the amino-acid replacement sites in dinoflagellate mitochondrial genomes are edited (Lin et al. 2002; Zhang and Lin 2005). Multiple animals, rhizopod amoebae, and basal fungi use editing to restore base-mismatches in the stems of mitochondrial tRNAs (Janke and Pääbo 1993; Lonergan and Gray 1993; Yokobori and Pääbo 1995; Tomita et al. 1996; Paquin et al. 1997; Lavrov et al. 2000; Laforest et al. 2004).

This sporadic distribution of diverse forms of editing strongly suggests that such processes have evolved independently in different lineages. To date, no compelling explanation has been promoted as to how such substantial investments in editing may have arisen by adaptive mechanisms. However, a plausible case has been made that such seemingly superfluous systems can in some cases be inadvertently promoted by effectively neutral processes (Stoltzfus 1999; Gray 2012). Given one of the central themes of the book – that neutral evolutionary mechanisms can drive modifications at the cellular level, further exploration of the matter of editing is warranted. Here, the focus will be primarily on the organelle genomes of plants, where the phenomenon has been studied most intensely.

Messenger RNA editing is used extensively in land-plant organelles. In *Arabidopsis* mitochondria, for example, 441 editing sites are present in coding regions along with smaller numbers in introns and intergenic DNA, nearly all of them changing C to U (Giegé and Brennicke 1999). Similar levels of C→U mRNA editing are found in the mitochondria of other land plants, including some liverworts (Malek et al. 1996; Freyer 1997). Although editing is less extensive in land-plant plastids, there are still commonly 25 to 30 editing sites per genome in angiosperms (Tsudzuki et al. 2001) and up to several hundred sites in ferns and hornworts (Kugita et al. 2003; Wolf et al. 2004). These observations, combined with the absence of mRNA editing in the organelles of green algae (Rüdinger et al. 2012), suggest a dramatic expansion of organelle editing with the origin of multicellular plants (Hiesel et al. 1994).

The vast majority of mRNA editing in land-plant organelles occurs at amino-acid replacement (rather than silent) sites, often ensuring the preservation of amino acids that are highly conserved across distantly related species (Maier et al. 1996; Tsudzuki et al. 2001). Although this type of observation motivates the idea that editing provides a genomic buffer against the accumulation of deleterious mutations (Cavalier-Smith 1997; Horton and Landweber 2002; Smith 2006), several observations raise significant doubts about this adaptive interpretation. First, if the buffering hypothesis were correct, we would expect editing to be most common in genomes with high mutation rates, which as discussed below is exactly the opposite of the pattern actually seen.

Second, the buffering hypothesis ignores the complexities of the editing process, which necessarily relies on the sequence stability of both the recognition sites

in the organelle and the significant investment in the editing apparatus itself. The *cis*-recognition sites for *trans*-acting editing factors span at least 23 bp (Choury et al. 2004; Miyamoto et al. 2004), and as further discussed below, the *trans*-acting factors minimally involve a large family of nuclear-encoded proteins, each devoted to just one or two specific editing sites. To be promoted by positive selection, any advantage to editing a particular site would have to exceed the prices paid.

Third, editing in plant organelles is quite noisy, resulting in the production of a heterogeneous pool of transcripts, some being incompletely edited and containing erroneous editorial changes (Phreaner et al. 1996; Inada et al. 2004; Guo et al. 2015). In many cases, completely edited transcripts are the exception rather than the rule (Schuster et al. 1990).

Perhaps the most significant challenge to the hypothesis that mRNA editing is maintained by selection derives from the following observation. Recall that the vast majority of mRNA editing in plant organelles involves conversions of C to U. Because C→T mutations at these sites eliminate the need for editing, such mutations are expected to accumulate at the neutral rate under the buffering hypothesis, as an allele with an encoded T should be selectively equivalent to one that simply acquires a C→U replacement by editing. Editing sites do turn over frequently between species (Tsudzuki et al. 2001; Shields and Wolfe 1997), and the rate of conversion of C→U editing sites to nonedited Ts is 4× greater than the neutral expectation (Shields and Wolfe 1997; Fujii and Small 2011). Thus, if anything, editing sites in land-plant organelles are at least mildly deleterious.

In addition to the problem of inaccuracies of editing and the energetic burden of maintaining the editing apparatus, this disadvantage may relate to the excess degenerative mutation rate for alleles bearing editing sites (Lynch 2007). The intrinsic mutational disadvantage of an editing site will be approximately equal to the total mutation rate over the nucleotide sites reserved for editing-site recognition, i.e., $\sim 23$ times the mutation rate per site. Thus, the reduced level of editing in land-plant plastids vs. mitochondria is consistent with this mutational-hazard hypothesis, as the former have higher mutation rates than the later. The dramatic reduction in the incidence of editing in the organelle genomes of plants that have experienced massive increases in the mutation rate (Palmer et al. 2000; Parkinson et al. 2005; Fan et al. 2019), is also consistent with the hypothesis. In contrast, the mitochondrion of the tulip tree, which has the one of the most mutationally quiescent genomes known, is heavily edited (Richardson et al. 2013).

The preceding arguments lead to a reasonably satisfying hypothesis for the phylogenetic distribution of editing, but substantial questions remain as to how such processes initially become established. To account for such a system, a mechanism must exist for the establishment of dozens to hundreds of nuclear-encoded editing factors, each specialized to recognize a small number (perhaps even single) of organelle sites. Moreover, although gene duplication can plausibly allow the expansion and specialization of a well-operating system, its initial establishment requires the existence of factors with latent editing potential prior to the origin of mRNA editing, presumably as a by-product of some other essential cellular function (Covello and Gray 1993). In addition, the emergence of site-specific refinements by natural selection is difficult with nuclear-encoded factors, which would have to remain in tight linkage disequilibrium with their serviced organelle sites while both are en route to fixation. Uniparental inheritance of organelles facilitates such associations, as half of the gametes of the transmitting parent will contain the appropriate nuclear-cytoplasmic combination, but the probability of dissociation is still considerable.

Although the precise mechanism of C→U mRNA editing remains unclear, the process intimately involves one of the largest nuclear-encoded gene families in land plants – the PPR (pentatricopeptide) proteins. Containing up to 600 copies per genome, many of the members of this family are targeted to specific mRNAs within organelles, where they likely recruit another enzyme to complete the editing step

(Schmitz-Linneweber and Small 2008; Fujii and Small 2011). Some PPR genes have functions unassociated with editing (e.g., RNA folding and translation), but one subclass in particular, the DYW-domain containing PPR genes is largely restricted to land plants that carry out editing. Species such as *Marchantia* that have lost editing have also lost the DYW family, and others with small number of editing sites have greatly diminished numbers of PPR genes, e.g., the moss *Physcomitrella* has only 10 organellar editing sites and just a single DYW PPR gene. Unicellular lineages of green algae without editing lack members of this family, whereas the amoeboid protist *Naegleria,* which has C→U editing, also has DYW PPRs (Fritz-Laylin et al. 2010).

In summary, the origin of mRNA editing is one of more enigmatic aspects of genome evolution. There is no evidence that such processes have originated to buffer mutational damage. In addition, the hypothesis that editing promotes the generation of adaptive variation at the RNA level (Tillich et al. 2006) is entirely without support, as is the idea that editing arises as a resolution of a nucleo-cytoplasmic conflict (Castandet and Araya 2011). Whereas it is difficult to reject the hypothesis that mRNA editing in organelles has arisen by nearly neutral processes, the mechanisms by which editing factors acquire their apparent site-specificity remain unclear. Although a really creative selfish editor might inflict the organelle genomic change necessary to ensure its own survival, no such element is known to exist. It is, however, intriguing that some proteins involved in nuclear-mRNA editing are capable of inducing site-specific mutations (Smith 2006; Iyer et al. 2011).

## Literature Cited

Adrion, J. R., P. S. White PS, and K. L. Montooth. 2016. The roles of compensatory evolution and constraint in aminoacyl tRNA synthetase evolution. Mol. Biol. Evol. 33: 152-161.

Amiri, H., O. Karlberg, and S. G. Andersson. 2003. Deep origin of plastid/parasite ATP/ADP translocases. J. Mol. Evol. 56: 137-150.

Anderson, A. P., X. Luo, W. Russell, and Y. W. Yin. 2020. Oxidative damage diminishes mitochondrial DNA polymerase replication fidelity. Nucleic Acids Res. 48: 817-829.

Anderson, J. B., C. Wickens, M. Khan, L. E. Cowen, N. Federspiel, T. Jones, and L. M. Kohn. 2001. Infrequent genetic exchange and recombination in the mitochondrial genome of *Candida albicans.* J. Bacteriol. 183: 865-872.

Andersson S. G, O. Karlberg, B. Canback, C. G. Kurland. 2003. On the origin of mitochondria: a genomics perspective. Phil. Trans. Roy. Soc. Lond. B Biol. Sci. 358: 165-177.

Andersson S. G., A. Zomorodipour, J. O. Andersson, T. Sicheritz-Ponten, U. C. Alsmark, R. M. Podowski, A. K. Naslund, A. S. Eriksson, H. H. Winkler, and C. G. Kurland. 1998. The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. Nature 396: 133-140.

Angerer, H., K. Zwicker, Z. Wumaier, L. Sokolova, H. Heide, M. Steger, S. Kaiser, E. Nübel, B. Brutschy, M. Radermacher, et al. 2011. A scaffold of accessory subunits links the peripheral arm and the distal proton-pumping module of mitochondrial complex I. Biochem. J. 437: 279-288.

Archibald, J. 2015. One Plus One Equals One: Symbiosis and the Evolution of Complex Life. Oxford Univ. Press, Oxford, UK.

Ashley, M. V., P. J. Laipis, and W. W. Hauswirth. 1989. Rapid segregation of heteroplasmic bovine mitochondria. Nucleic Acids Res. 17: 7325-7331.

Ballard, J. W., and M. Kreitman. 1994 Unraveling selection in the mitochondrial genome of *Drosophila.* Genetics 138: 757-772.

Barreto, F. S., and R. S. Burton. 2013. Evidence for compensatory evolution of ribosomal proteins in response to rapid divergence of mitochondrial rRNA. Mol. Biol. Evol. 30: 310-314.

Barreto, F. S., E. T. Watson, T. G. Lima, C. S. Willett, S. Edmands, W. Li, and R. S. Burton. 2018. Genomic signatures of mitonuclear coevolution across populations of *Tigriopus californicus.* Nat. Ecol. Evol. 2: 1250-1257.

Birky, C. W. Jr., C. A. Demko, P. S. Perlman, and R. Strausberg. 1978. Uniparental inheritance of mitochondrial genes in yeast: dependence on input bias of mitochondrial DNA and preliminary investigations of the mechanism. Genetics 89: 615-651.

Birky, C. W. Jr., T. Maruyama, and P. Fuerst. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103: 513-527.

Bohr, V. A., T. Stevnsner, and N. C. de Souza-Pinto. 2002. Mitochondrial DNA repair of oxidative damage in mammalian cells. Gene 286: 127-134.

Brown, M. D., and D. C. Wallace. 1994. Molecular basis of mitochondrial DNA disease. J. Bioenerg. Biomembr. 26: 273-289.

Brown, W. M., E. M. Prager, A. Wang, and A. C. Wilson. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. J. Mol. Evol. 18: 225-239.

Burki, F. 2016. Ditochondrial evolution: going, going, gone. Curr. Biol. 26: R410-R412.

Burt, A., and R. Trivers. 2006. Genes in Conflict. Harvard Univ. Press, Cambridge, MA.

Byrne, E. M., and J. M. Gott. 2004. Unexpectedly complex editing patterns at dinucleotide insertion sites in *Physarum* mitochondria. Mol. Cell. Biol. 24: 7821-7828.

Cardol, P. 2011. Mitochondrial NADH: ubiquinone oxidoreductase (complex I) in eukaryotes: a highly conserved subunit composition highlighted by mining of protein databases. Biochim. Biophys. Acta 1807: 1390-1397.

Castandet, B., and A. Araya. 2011. RNA editing in plant organelles. Why make it easy? Biochemistry (Mosc.) 76: 924-931.

Cavalier-Smith, T. 1987. The origin of eukaryotic and archaebacterial cells. Ann. N. Y. Acad. Sci. 503: 17-54.

Cavalier-Smith, T. 1997. Cell and genome coevolution: facultative anaerobiosis, glycosomes and kinetoplastan RNA editing. Trends Genet. 13: 6-9.

Cavalier-Smith, T. 2006. Origin of mitochondria by intracellular enslavement of a photosynthetic purple bacterium. Proc. Roy. Soc. Lond. B 273: 1943-1952.

Cavalier-Smith, T. 2009. Predation and eukaryote cell origins: a coevolutionary perspective. Int. J. Biochem. Cell Biol. 41: 307-322.

Cavalier-Smith, T., and J. J. Lee. 1985. Protozoa as hosts for endosymbioses and the conversion of symbionts into organelles. J. Euk. Microbiol. 32: 371-558.

Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1993. The effect of deleterious mutations on neutral molecular variation. Genetics 134: 1289-303.

Chen, Y. L., L. J. Chen, C. C. Chu, P. K. Huang, J. R. Wen, and H. M. Li. 2018. TIC236 links the outer and inner membrane translocons of the chloroplast. Nature 564: 125-129.

Cheng, Y. W., L. M. Visomirski-Robic, and J. M. Gott. 2001. Non-templated addition of nucleotides to the $3'$ end of nascent RNA during RNA editing in *Physarum.* EMBO J. 20: 1405-1414.

Cho, Y., J. P. Mower, Y. L. Qiu, and J. D. Palmer. 2004. Mitochondrial substitution rates are extraordinarily elevated and variable in a genus of flowering plants. Proc. Natl. Acad. Sci. USA 101: 17741-17746.

Choury, D., J. C. Farre, X. Jordana, and A. Araya. 2004. Different patterns in the recognition of editing sites in plant mitochondria. Nucleic Acids Res. 32: 6397-6406.

Clark, K. A., D. K. Howe, K. Gafner, D. Kusuma, S. Ping, S. Estes, and Denver DR. 2012. Selfish little circles: transmission bias and evolution of large deletion-bearing mitochondrial DNA in *Caenorhabditis briggsae* nematodes. PLoS One 7: e41433.

Cosmides, L. M., and J. Tooby. 1981. Cytoplasmic inheritance and intragenomic conflict. J. Theor. Biol. 89: 83-129.

Covello, P. S., and M. W. Gray. 1993. On the evolution of RNA editing. Trends Genet. 9: 265-268.

Davidov, Y., and E. Jurkevitch. 2009. Predation between prokaryotes and the origin of eukaryotes. Bioessays 31: 748-757.

de Duve, C. 2007. The origin of eukaryotes: a reappraisal. Nat. Rev. Genet. 8: 395-403.

Denver, D. D., P. C. Dolan, L. J. Wilhelm, W. Sung, J. I. Lucas-Lledó, D. K. Howe, S. C. Lewis, K. Okamoto, M. Lynch, W. K. Thomas, et al. 2009. A genome-wide view of *Caenorhabditis elegans* base-substitution mutation processes. Proc. Natl. Acad. Sci. USA 106: 16310-16314.

Denver, D. R., K. Morris, M. Lynch, and W. K. Thomas. 2004. High mutation rate and predominance of insertions in the Caenorhabditis elegans nuclear genome. Nature 430: 679-682.

Denver, D. R., K. Morris, M. Lynch, L. L. Vassilieva, and W. K. Thomas. 2000. High direct estimate of the mutation rate in the mitochondrial genome of *Caenorhabditis elegans.* Science 289: 2342-2344.

Dolezal, P., V. Likic, J. Tachezy, and T. Lithgow. 2006. Evolution of the molecular machines for protein import into mitochondria. Science 313: 314-318.

Doolittle, W. F. 1998. You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. Trends Genet. 14: 307-311.

Eme, L., S. C. Sharpe, M. W. Brown, and A. J. Roger. 2014. On the age of eukaryotes: evaluating evidence from fossils and molecular clocks. Cold Spring Harb. Perspect. Biol. 6: a016139.

Emelyanov, V. V. 2001. Evolutionary relationship of Rickettsiae and mitochondria. FEBS Lett. 501: 11-18.

Esposti, M. D. 2016. Late mitochondrial acquisition, really? Genome Biol. Evol. 8: 2031-2035.

Eyre-Walker, A., and P. Awadalla. 2001. Does human mtDNA recombine? J. Mol. Evol. 53: 430-435.

Fan, W., W. Guo, L. Funk, J. P. Mower, and A. Zhu. 2019. Complete loss of RNA editing from the plastid genome and most highly expressed mitochondrial genes of *Welwitschia mirabilis.* Sci. China Life Sci. 62: 498-506.

Felsenstein, J. 1974. The evolutionary advantage of recombination. Genetics 78: 737-756.

Force, A., M. Lynch, B. Pickett, A. Amores, Y.-L. Yan, and J. Postlethwait. 1999. Preservation of duplicate genes by complementary, degenerative mutations. Genetics 151: 1531-1545.

Frank, S. A., and L. D. Hurst. 1996. Mitochondria and male disease. Nature 383: 224.

Freyer, R., M. C. Kiefer-Meyer, and H. Kossel. 1997. Occurrence of plastid RNA editing in all major lineages of land plants. Proc. Natl. Acad. Sci. USA 94: 6285-6290.

Friedman, J. R., L. L. Lackner, M. West, J. R. DiBenedetto, J. Nunnari, and G. K. Voeltz. 2011. ER tubules mark sites of mitochondrial division. Science 334: 358-362.

Friedman, J. R., and J. Nunnari. 2014. Mitochondrial form and function. Nature 505: 335-343.

Fritts, R. K., J. T. Bird, M. G. Behringer, A. Lipzen, J. Martin, M. Lynch, and J. B. McKinlay. 2020. Enhanced nutrient uptake is sufficient to drive emergent cross-feeding between bacteria in a synthetic community. ISME J. 14: 2816-2828.

Fritz-Laylin, L. K., S. E. Prochnik, M. L. Ginger, J. B. Dacks, M. L. Carpenter, M. C. Field, A. Kuo, A. Paredez, J. Chapman, J. Pham, et al. 2010. The genome of *Naegleria gruberi* illuminates early eukaryotic versatility. Cell 140: 631-642.

Fry, A. J. 1999. Mildly deleterious mutations in avian mitochondrial DNA: evidence from neutrality tests. Evolution 53: 1617-1620.

Fujii, S., and I. Small. 2011. The evolution of RNA editing and pentatricopeptide repeat genes. New Phytol. 191: 37-47.

Fujii, S., and K. Toriyama. 2009. Suppressed expression of retrograde-regulated male sterility restores pollen fertility in cytoplasmic male sterile rice plants. Proc. Natl. Acad. Sci. USA 106: 9513-9518.

Gabaldón, T. 2018. Relative timing of mitochondrial endosymbiosis and the "pre-mitochondrial symbioses" hypothesis. IUBMB Life 70: 1188-1196.

Gabaldón, T., D. Rainey, and M. A. Huynen. 2005. Tracing the evolution of a large protein complex in the eukaryotes, NADH: ubiquinone oxidoreductase (Complex I). J. Mol. Biol. 348: 857-870.

Gaborieau, L., G. G. Brown, and H. Mireau. 2016. The propensity of pentatricopeptide repeat genes to evolve into restorers of cytoplasmic male sterility. Front. Plant Sci. 7: 1816.

Gabriel, W., M. Lynch, and R. Bürger. 1993. Muller's ratchet and mutational meltdowns. Evolution 47: 1744-1757.

Galluzzi, L., O. Kepp, and G. Kroemer. 2012. Mitochondria: master regulators of danger signalling. Nat. Rev. Mol. Cell Biol. 13: 780-788.

Gaut, B. S., B. R. Morton, B. C. McCaig, and M. T. Clegg. 1996. Substitution rate comparisons between grasses and palms: synonymous rate differences at the nuclear gene Adh parallel rate differences at the plastid gene rbcL. Proc. Natl. Acad. Sci. USA 93: 10274-10279.

Gemmell, N. J., V. J. Metcalf, and F. W. Allendorf. 2004. Mother's curse: the effect of mtDNA on individual fitness and population viability. Trends Ecol. Evol. 19: 238-244.

Giegé, P., and A. Brennicke. 1999. RNA editing in *Arabidopsis* mitochondria effects 441 C to U changes in ORFs. Proc. Natl. Acad. Sci. USA 96: 15324-15329.

Goeders, N., and L. Van Melderen. 2014. Toxin-antitoxin systems as multilevel interaction systems. Toxins (Basel) 6: 304-324.

Görtz, H.-D., and S. I. Fokin. 2009. Diversity of endosymbiotic bacteria in *Paramecium,* pp. 131-160. In M. Fujishima (ed.) Endosymbionts in *Paramecium.* Springer-Verlag, Berlin, Germany.

Goyal, S., D. J. Balick, E. R. Jerison, R. A. Neher, B. I. Shraiman, and M. M. Desai. 2012. Dynamic mutation-selection balance as an evolutionary attractor. Genetics 191: 1309-1319.

Gray, M. W. 2012. Mitochondrial evolution. Cold Spring Harb. Perspect. Biol. 4: a011403.

Gray, M. W. 2015. Mosaic nature of the mitochondrial proteome: implications for the origin and evolution of mitochondria. Proc. Natl. Acad. Sci. USA 112: 10133-10138.

Grosser, K., P. Ramasamy, A. D. Amirabad, M. H. Schulz, G. Gasparoni, M. Simon, and M. Schrallhammer. 2018. More than the "killer trait": infection with the bacterial endosymbiont *Caedibacter taeniospiralis* causes transcriptomic modulation in *Paramecium* host. Genome Biol. Evol. 10: 646-656.

Guo, W., F. Grewe, and J. P. Mower. 2015. Variable frequency of plastid RNA editing among ferns and repeated loss of uridine-to-cytidine editing from vascular plants. PLoS One 10: e0117075.

Haag-Liautard, C., N. Coffey, D. Houle, M. Lynch, B. Charlesworth, and P. D. Keightley. 2008.

Direct estimation of the mitochondrial DNA mutation rate in *D. melanogaster*. PLoS Biology 6: 1706-1714.

Haigh, J. 1978. The accumulation of deleterious genes in a population – Muller's ratchet. Theor. Pop. Biol. 14: 251-267.

Hampl, V., I. Čepicka, and M. Eliaš. 2019. Was the mitochondrion necessary to start eukaryogenesis? Trends Microbiol. 27: 96-104.

Hanada, T., T. Suzuki, T. Yokogawa, C. Takemoto-Hori, M. Sprinzl, and K. Watanabe. 2001. Translation ability of mitochondrial tRNAsSer with unusual secondary structures in an *in vitro* translation system of bovine mitochondria. Genes Cells 6: 1019-1030.

Harcombe, W. R., J. M. Chacón, E. M. Adamowicz, L. M. Chubiz, and C. J. Marx. 2018. Evolution of bidirectional costly mutualism from byproduct consumption. Proc. Natl. Acad. Sci. USA 115: 12000-12004.

Hasegawa, M., Y. Cao, and Z. Yang. 1998. Preponderance of slightly deleterious polymorphism in mitochondrial DNA: nonsynonymous/synonymous rate ratio is much higher within species than between species. Mol. Biol. Evol. 15: 1499-1505.

Hastings, I. M. 1992. Population genetic aspects of deleterious cytoplasmic genomes and their effect on the evolution of sexual reproduction. Genet. Res. 59: 215-225.

Havird, J. C., E. S. Forsythe, A. M. Williams, J. H. Werren, D. K. Dowling, and D. B. Sloan. 2019. Selfish mitonuclear conflict. Curr. Biol. 29: R496-R511.

Havird, J. C., P. Trapp, C. M. Miller, I. Bazos, and D. B. Sloan. 2017. Causes and consequences of rapidly evolving mtDNA in a plant lineage. Genome Biol. Evol. 9: 323-336.

Havird, J. C., N. S. Whitehill, C. D. Snow, and D. B. Sloan. 2015. Conservative and compensatory evolution in oxidative phosphorylation complexes of angiosperms with highly divergent rates of mitochondrial genome evolution. Evolution 69: 3069-3081.

Hazkani-Covo, E., R. M. Zeller, and W. Martin. 2010. Molecular poltergeists: mitochondrial DNA copies (numts) in sequenced nuclear genomes. PLoS Genet. 6: e1000834.

Hayes, F., and L. Van Melderen. 2011. Toxins-antitoxins: diversity, evolution and function. Crit. Rev. Biochem. Mol. Biol. 46: 386-408.

Hehenberger, E., R. J. Gast, and P. J. Keeling. 2019. A kleptoplastidic dinoflagellate and the tipping point between transient and fully integrated plastid endosymbiosis. Proc. Natl. Acad. Sci. USA 116: 17934-17942.

Hiesel, R., B. Combettes, and A. Brennicke. 1994. Evidence for RNA editing in mitochondria of all major groups of land plants except the Bryophyta. Proc. Natl. Acad. Sci. USA 91: 629-633.

Hillesland, K. L., S. Lim, J. H. J. Flowers, S. Turkarslan, N. Pinel, G. M. Zane, N. Elliott, Y. Qin, L. Wu, N. S. Baliga, et al. 2014. Erosion of functional independence early in the evolution of a microbial mutualism. Proc. Natl. Acad. Sci. USA 111: 14822-14827.

Hirst, J. 2011. Why does mitochondrial complex I have so many subunits? Biochem. J. 437: e1-3.

Ho, E. K. H., F. Macrae, L. C. Latta, P. McIlroy, D. Ebert, P. D. Fields, M. J. Benner, and S. Schaack. 2020. High and highly variable spontaneous mutation rates in *Daphnia*. Mol. Biol. Evol. 36: 1942-1954.

Hom, E. F., and A. W. Murray. 2014. Niche engineering demonstrates a latent capacity for fungal-algal mutualism. Science 345: 94-98.

Horton, T. L., and L. F. Landweber. 2000. Evolution of four types of RNA editing in myxomycetes. RNA 6: 1339-1346.

Horton, T. L., and L. F. Landweber. 2002. Rewriting the information in DNA: RNA editing in kinetoplastids and myxomycetes. Curr. Opin. Microbiol. 5: 620-626.

Howell, N. 1996. Mutational analysis of the human mitochondrial genome branches into the realm of bacterial genetics. Am. J. Hum. Genet. 59: 749-755.

Husnik, F., N. Nikoh, R. Koga, L. Ross, R. P. Duncan, M. Fujie, M. Tanaka, N. Satoh, D. Bachtrog, A. C. Wilson, et al. 2013. Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis. Cell 153: 1567-1578.

Husnik, F., and J. P. McCutcheon. 2016. Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. Proc. Natl. Acad. Sci. USA 113: E5416-E5424.

Huynen, M. A., I. Duarte, and R. Szklarczyk. 2013. Loss, replacement and gain of proteins at the origin of the mitochondria. Biochim. Biophys. Acta 1827: 224-231.

Inada, M., T. Sasaki, M. Yukawa, T. Tsudzuki, and M. Sugiura. 2004. A systematic search for RNA editing sites in pea chloroplasts: an editing event causes diversification from the evolutionarily conserved amino acid sequence. Plant Cell Physiol. 45: 1615-1622.

Iyer, L. M., D. Zhang, I. B. Rogozin, and L. Aravind. 2011. Evolution of the deaminase fold and multiple origins of eukaryotic editing and mutagenic nucleic acid deaminases from bacterial toxin systems. Nucleic Acids Res. 39: 9473-9497.

James, J. E, G. Piganeau, and A. Eyre-Walker. 2016. The rate of adaptive evolution in animal mitochondria. Mol. Ecol. 25: 67-78.

Janke, A., and S. Pääbo. 1993. Editing of a tRNA anticodon in marsupial mitochondria changes its codon recognition. Nucleic Acids Res. 21: 1523-1525.

Jasmin, J. N., and C. Zeyl. 2014. Rapid evolution of cheating mitochondrial genomes in small yeast populations. Evolution 68: 269-275.

Jeblick, J., and J. Kusch. 2005. Sequence, transcription activity, and evolutionary origin of the R-body coding plasmid pKAP298 from the intracellular parasitic bacterium *Caedibacter taeniospiralis.* J. Mol. Evol. 60: 164-173.

Jenuth, J. P., A. C. Peterson, K. Fu, and E. A. Shoubridge. 1996. Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. Nature Genet. 14: 146-151.

Johnson, A. A., and K. A. Johnson. 2001. Fidelity of nucleotide incorporation by human mitochondrial DNA polymerase. J. Biol. Chem. 276: 38090-38096.

Kang, D., and N. Hamasaki. 2002. Maintenance of mitochondrial DNA integrity: repair and degradation. Curr. Genet. 41: 311-322.

Karlberg, O., B. Canbäck, C. G. Kurland, and S. G. Andersson. 2000. The dual origin of the yeast mitochondrial proteome. Yeast 17: 170-187.

Karnkowska, A., V. Vacek, Z. Zubáčová, S. C. Treitli, R. Petrželková, L. Eme, L. Novák, V. Žárský,

L. D. Barlow, E. K. Herman, et al,. 2016. A eukaryote without a mitochondrial organelle. Curr. Biol. 26: 1274-1284.

Karnkowska, A., S. C. Treitli, O. Brzoň, L. Novák, V. Vacek, P. Soukal, L. D. Barlow, E. K. Herman, S. V. Pipaliya, T. Pánek, et al.. 2019. The oxymonad genome displays canonical eukaryotic complexity in the absence of a mitochondrion. Mol. Biol. Evol. 36: 2292-2312.

Kazak, L., A. Reyes, and I. J. Holt. 2012. Minimizing the damage: repair pathways keep mitochondrial DNA intact. Nat. Rev. Mol. Cell Biol. 13: 659-671.

Keeling, P. J. 2013. The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. Annu. Rev. Plant Biol. 64: 583-607.

Kelly, S. 2020. The economics of endosymbiotic gene transfer and the evolution of organellar genomes. bioRxiv doi: https://doi.org/10.1101/2020.10.01.322487.

Kern, A. D., and F. A. Kondrashov. 2004. Mechanisms and convergence of compensatory evolution in mammalian mitochondrial tRNAs. Nature Genet. 36: 1207-1212.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge, U.K.

Kobayashi, I. 1998. Selfishness and death: raison detre of restriction, recombination and mitochondria. Trends Genet. 14: 368-374.

Koonin, E. V. 2015. Origin of eukaryotes from within archaea, archaeal eukaryome and bursts of gene gain: eukaryogenesis just made easier? Philos. Trans. R. Soc. Lond. B Biol. Sci. 370: 20140333.

Koslowsky, D. J., H. U. Goringer, T. H. Morales, and K. Stuart. 1992. *In vitro* guide RNA/mRNA chimaera formation in *Trypanosoma brucei* RNA editing. Nature 356: 807-809.

Krasovec, M., S. Sanchez-Brosseau, and G. Piganeau. 2019. First estimation of the spontaneous mutation rate in diatoms. Genome Biol. Evol. 11: 1829-1837.

Ku, C., S. Nelson-Sathi, M. Roettger, F. L. Sousa, P. J. Lockhart, D. Bryant, E. Hazkani-Covo, J. O. McInerney, G. Landan, and W. F. Martin. 2015. Endosymbiotic origin and differential loss of eukaryotic genes. Nature 524: 427-432.

Kugita, M., Y. Yamamoto, T. Fujikawa, T. Matsumoto, and K. Yoshinaga. 2003. RNA editing in hornwort chloroplasts makes more than half the genes functional. Nucleic Acids Res. 31: 2417-2423.

Kuhle, B., J. Chihade, and P. Schimmel. 2020. Relaxed sequence constraints favor mutational freedom in idiosyncratic metazoan mitochondrial tRNAs. Nat. Commun. 11: 969.

Kumazawa, Y., and M. Nishida. 1993. Sequence evolution of mitochondrial tRNA genes and deep-branch animal phylogenetics. J. Mol. Evol. 37: 380-398.

Kumazawa, Y., C. J. Schwartzbach, H. X. Liao, K. Mizumoto, Y. Kaziro, K. Miura, K. Watanabe, and L. L. Spremulli. 1991. Interactions of bovine mitochondrial phenylalanyl-tRNA with ribosomes and elongation factors from mitochondria and bacteria. Biochim. Biophys. Acta 1090: 167-172.

Kumazawa, Y., T. Yokogawa, E. Hasegawa, K. Miura, and K. Watanabe. 1989. The aminoacylation of structurally variant phenylalanine tRNAs from mitochondria and various nonmitochondrial

sources by bovine mitochondrial phenylalanyl-tRNA synthetase. J. Biol. Chem. 264: 13005-13011.

Kunkel, T. A., and P. S. Alexander. 1986. The base substitution fidelity of eucaryotic DNA polymerases. Mispairing frequencies, site preferences, insertion preferences, and base substitution by dislocation. J. Biol. Chem. 261: 160-166.

Laforest, M. J., C. E. Bullerwell, L. Forget, and B. F. Lang. 2004. Origin, evolution, and mechanism of 5′ tRNA editing in chytridiomycete fungi. RNA 10: 1191-1199.

Lane, N. 2006. Power, Sex, Suicide: Mitochondria and the Meaning of Life. Oxford Univ. Press, Oxford, UK.

Lane N. 2020. How energy flow shapes cell evolution. Curr. Biol. 30: R471-R476.

Lane, N., and W. Martin. 2010. The energetics of genome complexity. Nature 467: 929-934.

Lavrov, D. V., W. M. Brown, and J. L. Boore. 2000. A novel type of RNA editing occurs in the mitochondrial tRNAs of the centipede *Lithobius forficatus.* Proc. Natl. Acad. Sci. USA 97: 13738-13742.

Lavrov, D. V., and W. Pett. 2016. Animal mitochondrial DNA as we do not know it: mt-genome organization and evolution in nonbilaterian lineages. Genome Biol. Evol. 8: 2896-2913.

Leger, M. M., M. Petru, V. Žárský, L. Eme, Č. Vlček, T. Harding, B. F. Lang, M. Eliáš, P. Doležal, and A. J. Roger. 2015. An ancestral bacterial division system is widespread in eukaryotic mitochondria. Proc. Natl. Acad. Sci. USA 112: 10239-10246.

Lewis, S. C., L. F. Uchiyama, and J. Nunnari. 2016. ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells. Science 353: aaf5549.

Lewis, W. H., A. E. Lind, K. M. Sendra, H. Onsbring, T. A. Williams, G. F. Esteban, R. P. Hirt, T. J. G. Ettema, and T. M. Embley. 2020. Convergent evolution of hydrogenosomes from mitochondria by gene transfer and loss. Mol. Biol. Evol. 37: 524-539.

Lin, S., H. Zhang, D. F. Spencer, J. E. Norman, and M. W. Gray. 2002. Widespread and extensive editing of mitochondrial mRNAS in dinoflagellates. J. Mol. Biol. 320: 727-739.

Ling, F., H. Morioka, E. Ohtsuka, and T. Shibata. 2000. A role for MHR1, a gene required for mitochondrial genetic recombination, in the repair of damage spontaneously introduced in yeast mtDNA. Nucleic Acids Res. 28: 4956-4963.

Loewe, L. 2006. Quantifying the genomic decay paradox due to Muller's ratchet in human mitochondrial DNA. Genet. Res. 87: 133-159.

Lonergan, K. M., and M. W. Gray. 1993. Editing of transfer RNAs in *Acanthamoeba castellanii* mitochondria. Science 259: 812-816.

López-Garc´a, P., L. Eme, and D. Moreira. 2017. Symbiosis in eukaryotic evolution. J. Theor. Biol. 434: 20-33.

Lunt, D. H., and B. C. Hyman. 1997. Animal mitochondrial DNA recombination. Nature 387: 247.

Luo, S., C. A. Valencia, J. Zhang, N. C. Lee, J. Slone, B. Gui, X. Wang, Z. Li, S. Dell, J. Brown, et al. 2018. Biparental inheritance of mitochondrial DNA in humans. Proc. Natl. Acad. Sci. USA 115: 13039-13044.

Lynch, M. 1996. Mutation accumulation in nuclear, organelle, and prokaryotic transfer RNA genes. Mol. Biol. Evol. 13: 209-220.

Lynch, M. 1997. Mutation accumulation in transfer RNAs: molecular evidence for Muller's ratchet in mitochondrial genomes. Mol. Biol. Evol. 14: 914-925.

Lynch, M. 2007. The Origins of Genome Architecture. Sinauer Assocs., Inc., Sunderland, MA.

Lynch, M. 2020. The evolutionary scaling of cellular traits imposed by the drift barrier. Proc. Natl. Acad. Sci. USA 117: 10435-10444.

Lynch, M., and J. L. Blanchard. 1998. Deleterious mutation accumulation in organelle genomes. Genetica 102-103: 29-39.

Lynch, R. Bürger, D. Butcher, and W. Gabriel. 1993. Mutational meltdowns in asexual populations. J. Heredity 84: 339-344.

Lynch, M., J. Conery, and R. Bürger. 1995a. Mutational meltdowns in sexual populations. Evolution 49: 1067-1080.

Lynch, M., J. Conery, and R. Bürger. 1995b. Mutation accumulation and the extinction of small populations. Amer. Natur. 146: 489-518.

Lynch, M., and W. Gabriel. 1990. Mutation load and the survival of small populations. Evolution 44: 1725-1737.

Lynch, M., B. Koskella, and S. Schaack. 2006. Mutation pressure and the evolution of organelle genomic architecture. Science 311: 1727-1730.

Lynch, M., and G. K. Marinov. 2015. The bioenergetic costs of a gene. Proc. Natl. Acad. Sci. USA 112: 15690-15695.

Lynch, M., and G. K. Marinov. 2017. Membranes, energetics, and evolution across the prokaryote-eukaryote divide. ELife 6: e20437.

Lynch, M., M. O'Hely, B. Walsh, and A. Force. 2001. The probability of preservation of a newly arisen gene duplicate. Genetics 159: 1789-1804.

MacAlpine, D. M., P. S. Perlman, and R. A. Butow. 1998. The high mobility group protein Abf2p influences the level of yeast mitochondrial DNA recombination intermediates *in vivo*. Proc. Natl. Acad. Sci. USA 95: 6739-6743.

Maier, R. M., P. Zeltz, H. Kossel, G. Bonnard, J. M. Gualberto, and J. M. Grienenberger. 1996. RNA editing in plant mitochondria and chloroplasts. Plant Mol. Biol. 32: 343-365.

Malek, O., K. Lattig, R. Hiesel, A. Brennicke, and V. Knoop. 1996. RNA editing in bryophytes and a molecular phylogeny of land plants. EMBO J. 15: 1403-1411.

Marchington, D. R., G. M. Hartshorne, D. Barlow, and J. Poulton. 1997. Homopolymeric tract heteroplasmy in mtDNA from tissues and single oocytes: support for a genetic bottleneck. Amer. J. Hum. Genet. 60: 408-416.

Margulis, L. 1970. Origin of Eukaryotic Cells: Evidence and Research Implications for a Theory of the Origin and Evolution of Microbial, Plant, and Animal Cells on the Precambrian Earth. Yale Univ. Press, New Haven, CT.

Martijn, J., J. Vosseberg, L. Guy, P. Offre, and T. J. G. Ettema. 2018. Deep mitochondrial origin outside the sampled alphaproteobacteria. Nature 557: 101-105.

Martin, W. F. 2017. Physiology, anaerobes, and the origin of mitosing cells 50 years on. J. Theor. Biol. 434: 2-10.

Martin, W. F., S. Garg, and V. Zimorski. 2015. Endosymbiotic theories for eukaryote origin. Philos. Trans. R. Soc. Lond. B Biol. Sci. 370: 20140330.

Martin, W. F., and M. Muller. 1998. The hydrogen hypothesis for the first eukaryote. Nature 392: 37-41.

Martin, W., T. Rujan, E. Richly, A. Hansen, S. Cornelsen, T. Lins, D. Leister, B. Stoebe, M. Hasegawa, and D. Penny. 2002. Evolutionary analysis of *Arabidopsis,* cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. Proc. Natl. Acad. Sci. USA 99: 12246-12251.

Martin, W. F., A. G. M. Tielens, M. Mentel, S. G. Garg, and S. B. Gould. 2017. The physiology of phagocytosis in the context of mitochondrial origin. Microbiol. Mol. Biol. Rev. 81: e00008-17.

Mason, P. A., and R. N. Lightowlers. 2003. Why do mammalian mitochondria possess a mismatch repair activity? FEBS Lett. 554: 6-9.

Mason, P. A., E. C. Matheson, A. G. Hall, and R. N. Lightowlers. 2003. Mismatch repair activity in mammalian mitochondria. Nucleic Acids Res. 31: 1052-1058.

Maynard Smith, J. M. 1978. The Evolution of Sex. Cambridge University Press, Cambridge, UK.

McCutcheon, J. P., B. M. Boyd, and C. Dale. 2019. The life of an insect endosymbiont from the cradle to the grave. Curr. Biol. 29: R485-R495.

McVean, G. A. 2001. What do patterns of genetic variability reveal about mitochondrial recombination? Heredity 87: 613-620.

Meer, M. V., A. S. Kondrashov, Y. Artzy-Randrup, and F. A. Kondrashov. 2010. Compensatory evolution in mitochondrial tRNAs navigates valleys of low fitness. Nature 464: 279-282.

Meland, S., S. Johansen, T. Johansen, K. Haugli, and F. Haugli. 1991. Rapid disappearance of one parental mitochondrial genotype after isogamous mating in the myxomycete *Physarum polycephalum.* Curr. Genet. 19: 55-59.

Miyagishima, S. Y., M. Nakamura, A. Uzuka, and A. Era. 2014. FtsZ-less prokaryotic cell division as well as FtsZ- and dynamin-less chloroplast and non-photosynthetic plastid division. Front Plant Sci. 5: 459.

Miyamoto, T., J. Obokata, and M. Sugiura. 2004. A site-specific factor interacts directly with its cognate RNA editing site in chloroplast transcripts. Proc. Natl. Acad. Sci. USA 101: 48-52.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutat. Res. 106: 2-9.

Muñoz-Gómez, S. A., S. Hess, G. Burger, B. F. Lang, E. Susko, C. H. Slamovits, and A. J. Roger. 2019. An updated phylogeny of the *Alphaproteobacteria* reveals that the parasitic *Rickettsiales* and *Holosporales* have independent origins. Elife 8: e42535.

Muñoz-Gómez, S. A., C. H. Slamovits, J. B. Dacks, K. A. Baier, K. D. Spencer, and J. G. Wideman. 2015. Ancient homology of the mitochondrial contact site and cristae organizing system points to an endosymbiotic origin of mitochondrial cristae. Curr. Biol. 25: 1489-1495.

Muñoz-Gómez, S. A., J. G. Wideman, A. J. Roger, and C. H. Slamovits. 2017. The origin of mitochondrial cristae from Alphaproteobacteria. Mol. Biol. Evol. 34: 943-956.

Nachman, M. W., S. N. Boye, and C. F. Aquadro. 1994. Nonneutral evolution at the mitochondrial NADH dehydrogenase subunit 3 gene in mice. Proc. Natl. Acad. Sci. USA 91: 6364-6368.

Nachman, M. W., W. M. Brown, M. Stoneking, and C. F. Aquadro. 1996. Nonneutral mitochondrial DNA variation in humans and chimpanzees. Genetics 142: 953-963.

Nakayama, T., Y. Ikegami, T. Nakayama, K. Ishida, Y. Inagaki, and I. Inouye. 2011. Spheroid bodies in rhopalodiacean diatoms were derived from a single endosymbiotic cyanobacterium. J. Plant Res. 124: 93-97.

Nakayama, T., R. Kamikawa, G. Tanifuji, Y. Kashiyama, N. Ohkouchi, J. M. Archibald, and Y. Inagaki. 2014. Complete genome of a nonphotosynthetic cyanobacterium in a diatom reveals recent adaptations to an intracellular lifestyle. Proc. Natl. Acad. Sci. USA 111: 11407-11412.

Nakayama, T., M. Nomura, Y. Takano, G. Tanifuji, K. Shiba, K. Inaba, Y. Inagaki, and M. Kawata. 2019. Single-cell genomics unveiled a cryptic cyanobacterial lineage with a worldwide distribution hidden by a dinoflagellate host. Proc. Natl. Acad. Sci. USA 116: 15973-15978.

Ness, R. W., A. D. Morgan, R. B. Vasanthakrishnan, N. Colegrave, and P. D. Keightley. 2015. Extensive *de novo* mutation rate variation between individuals and across the genome of *Chlamydomonas reinhardtii.* Genome Res. 25: 1739-1749.

Nowack, E. C. M. 2014. *Paulinella chromatophora* – rethinking the transition from endosymbiont to organelle. Acta Soc. Bot. Pol. 83: 387-397.

Nowack, E. C., and A. R. Grossman. 2012. Trafficking of protein into the recently established photosynthetic organelles of *Paulinella chromatophora.* Proc. Natl. Acad. Sci. USA 109: 5340-5345.

Oliveira, D. C., R. Raychoudhury, D. V. Lavrov, and J. H. Werren. 2008. Rapidly evolving mitochondrial genome and directional selection in mitochondrial genes in the parasitic wasp *Nasonia* (hymenoptera: pteromalidae). Mol. Biol. Evol. 25: 2167-2180.

Osada, N., and H. Akashi. 2012. Mitochondrial-nuclear interactions and accelerated compensatory evolution: evidence from the primate cytochrome C oxidase complex. Mol. Biol. Evol. 29: 337-346.

Palmer, J. D., K. L. Adams, Y. Cho, C. L. Parkinson, Y. L. Qiu, and K. Song. 2000. Dynamic evolution of plant mitochondrial genomes: mobile genes and introns and highly variable mutation rates. Proc. Natl. Acad. Sci. USA 97: 6960-6966.

Palumbi, S. R., F. Cipriano, and M. P. Hare. 2001. Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. Evolution 55: 859-68.

Paquin, B., M. J. Laforest, L. Forget, I. Roewer, Z. Wang, J. Longcore, and B. F. Lang. 1997. The fungal mitochondrial genome project: evolution of fungal mitochondrial genomes and their gene expression. Curr. Genet. 31: 380-395.

Parfrey, L. W., D. J. Lahr, A. H. Knoll, and L. A. Katz. 2011. Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proc. Natl. Acad. Sci. USA 108: 13624-13629.

Parkinson, C. L., J. P. Mower, Y. L. Qiu, A. J. Shirk, K. Song, N. D. Young, C. W. DePamphilis, and J. D. Palmer. 2005. Multiple major increases and decreases in mitochondrial substitution rates in the plant family Geraniaceae. BMC Evol. Biol. 5: 73.

Petrov, A. S., E. C. Wood, C. R. Bernier, A. M. Norris, A. Brown, and A. Amunts. 2019. Structural patching fosters divergence of mitochondrial ribosomes. Mol. Biol. Evol. 36: 207-219.

Phadnis, N., R. Mehta, N. Meednu, and E. A. Sia. 2006. Ntg1p, the base excision repair protein, generates mutagenic intermediates in yeast mitochondrial DNA. DNA Repair 5: 829-839.

Phillips, W. S., A. L. Coleman-Hulbert, E. S. Weiss, D. K. Howe, S. Ping, R. I. Wernick, S. Estes, and D. R. Denver. 2015. Selfish mitochondrial DNA proliferates and diversifies in small, but not large, experimental populations of *Caenorhabditis briggsae.* Genome Biol. Evol. 7: 2023-2037.

Phreaner, C. G., M. A. Williams, and R. M. Mulligan. 1996. Incomplete editing of rps12 transcripts results in the synthesis of polymorphic polypeptides in plant mitochondria. Plant Cell 8: 107-117.

Pietromonaco, S. F., R. A. Hessler, and T. W. O'Brien. 1986. Evolution of proteins in mammalian cytoplasmic and mitochondrial ribosomes. J. Mol. Evol. 24: 110-117.

Piganeau, G., M. Gardner, and A. Eyre-Walker. 2004. A broad survey of recombination in animal mitochondria. Mol. Biol. Evol. 21: 2319-2325.

Pittis, A. A., and T. Gabaldón. 2016. Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry. Nature 531: 101-104.

Pond, F. R., I. Gibson, J. Lalucat, and R. L. Quackenbush. 1989. R-body-producing bacteria. Microbiol. Rev. 53: 25-67.

Poon, A., and S. P. Otto. 2000. Compensating for our load of mutations: freezing the meltdown of small populations. Evolution 54: 1467-1479.

Ponce-Toledo, R. I., P. Deschamps, P. López-Garc´a, Y. Zivanovic, K. Benzerara, and D. Moreira. 2017. An early-branching freshwater cyanobacterium at the origin of plastids. Curr. Biol. 27: 386-391.

Popadin, K. Y., S. I. Nikolaev, T. Junier, M. Baranova, and S. E. Antonarakis. 2013. Purifying selection in mammalian mitochondrial protein-coding genes is highly effective and congruent with evolution of nuclear genes. Mol. Biol. Evol. 30: 347-355.

Popescu, C. E., and R. W. Lee. 2007. Mitochondrial genome sequence evolution in *Chlamydomonas.* Genetics 175: 819-26.

Preer, J. R., Jr., L. B. Preer, B. Rudman, and A. Jurand. 1971. Isolation and composition of bacteriophage-like particles from kappa of killer Paramecia. Mol. Gen. Genet. 111: 202-208.

Price, D. C., C. X. Chan, H. S. Yoon, E. C. Yang, H. Qiu, A. P. Weber, R. Schwacke, J. Gross, N. A. Blouin, C. Lane, et al. 2012. *Cyanophora paradoxa* genome elucidates origin of photosynthesis in algae and plants. Science 3: 843-847.

Purkanti, R., and M. Thattai. 2015. Ancient dynamin segments capture early stages of host-mitochondrial integration. Proc. Natl. Acad. Sci. USA 112: 2800-2805.

Rand, D. M., M. Dorfsman, and L. M. Kann. 1994. Neutral and non-neutral evolution of *Drosophila* mitochondrial DNA. Genetics 138: 741-756.

Rand, D. M., R. A. Haney, and A. J. Fry. 2004. Cytonuclear coevolution: the genomics of cooperation. Trends Ecol. Evol. 19: 645-653.

Rand, D. M., and R. G. Harrison. 1986. Mitochondrial DNA transmission genetics in crickets.

Genetics 114: 955-970.

Rankin, D. J., L. A. Turner, J. A. Heinemann, and S. P. Brown. 2012. The coevolution of toxin and antitoxin genes drives the dynamics of bacterial addiction complexes and intragenomic conflict. Proc. Biol. Sci. 279: 3706-3715.

Raymann, K., L. M. Bobay, T. G. Doak, M. Lynch, and S. Gribaldo. 2013. A genomic survey of Reb homologs suggests widespread occurrence of R-bodies in proteobacteria. G3 (Bethesda) 3: 505-516.

Reboud, X., and C. Zeyl 1994. Organelle inheritance in plants Heredity 72: 132-140.

Richardson, A. O., D. W. Rice, G. J. Young, A. J. Alverson, and J. D. Palmer. 2013. The "fossilized" mitochondrial genome of *Liriodendron tulipifera:* ancestral gene content and order, ancestral editing sites, and extraordinarily low mutation rate. BMC Biol. 11: 29.

Rivera, M. C., and J. A. Lake. 1992. Evidence that eukaryotes and eocyte prokaryotes are immediate relatives. Science 257: 74-76.

Rochette, N. C., C. Brochier-Armanet, and M. Gouy. 2014. Phylogenomic test of the hypotheses for the evolutionary origin of eukaryotes. Mol. Biol. Evol. 31: 832-845.

Rockenbach, K., J. C. Havird, J. G. Monroe, D. A. Triant, D. R. Taylor, and D. B. Sloan. 2016. Positive selection in rapidly evolving plastid-nuclear enzyme complexes. Genetics 204: 1507-1522.

Rüdinger, M., U. Volkmar, H. Lenz, M. Groth-Malonek, and V. Knoop. 2012. Nuclear DYW-type PPR gene families diversify with increasing RNA editing frequencies in liverwort and moss mitochondria. J. Mol. Evol. 74: 37-51.

Sachs, J. L., R. G. Skophammer, and J. U. Regus. 2011. Evolutionary transitions in bacterial symbiosis. Proc. Natl. Acad. Sci. USA 108 (Suppl. 2): 10800-10807.

Sachs, J. L., R. G. Skophammer, N. Bansal, and J. E. Stajich. 2013. Evolutionary origins and diversification of proteobacterial mutualists. Proc. Biol. Sci. 281: 20132146.

Sagan, L. 1967. On the origin of mitosing cells. J. Theor. Biol. 14: 255-274.

Sánchez-Baracaldo, P., J. A. Raven, D. Pisani, and A. H. Knoll. 2017. Early photosynthetic eukaryotes inhabited low-salinity habitats. Proc. Natl. Acad. Sci. USA 114: E7737-E7745.

Santos, C., R. Montiel, B. Sierra, C. Bettencourt, E. Fernandez, L. Alvarez, M. Lima, A. Abade, and M. P. Aluja. 2005. Understanding differences between phylogenetic and pedigree-derived mtDNA mutation rate: a model using families from the Azores Islands (Portugal). Mol. Biol. Evol. 22: 1490-1505.

Sarai C., G. Tanifuji, T. Nakayama, R. Kamikawa, K. Takahashi, E. Yazaki, E. Matsuo, H. Miyashita, K. I. Ishida, M. Iwataki, et al. 2020. Dinoflagellates with relic endosymbiont nuclei as models for elucidating organellogenesis. Proc. Natl. Acad. Sci. USA 117: 5364-5375.

Sassera, D., N. Lo, S. Epis, G. D'Auria, M. Montagna, F. Comandatore, D. Horner, J. Peretó, A. M. Luciano, F. Franciosi, et al. 2011. Phylogenomic evidence for the presence of a flagellum and cbb(3) oxidase in the free-living mitochondrial ancestor. Mol. Biol. Evol. 28: 3285-3296.

Satoh, M., and T. Kuroiwa. 1991. Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. Exp. Cell Res. 196: 137-140.

Saville, B. J., Y. Kohli, and J. B. Anderson. 1998. mtDNA recombination in a natural population. Proc. Natl. Acad. Sci. USA 95: 1331-1335.

Schmitz-Linneweber, C., and I. Small. 2008. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. Trends Plant Sci. 13: 663-670.

Schrallhammer, M., and M. Schweikert. 2009. The killer effect of *Paramecium* and its causative agents, pp. 227-246. In M. Fujishima (ed.) Endosymbionts in *Paramecium.* Springer-Verlag, Berlin, Germany.

Schuster, W., B. Wissinger, M. Unseld, and A. Brennicke. 1990. Transcripts of the NADH-dehydrogenase subunit 3 gene are differentially edited in *Oenothera* mitochondria. EMBO J. 9: 263-269.

Shields, D. C., and K. H. Wolfe. 1997. Accelerated evolution of sites undergoing mRNA editing in plant mitochondria and chloroplasts. Mol. Biol. Evol. 14: 344-349.

Sharma, M. R., D. N. Wilson, P. P. Datta, C. Barat, F. Schluenzen, P. Fucini, and R. K. Agrawal. 2007. Cryo-EM study of the spinach chloroplast ribosome reveals the structural and functional roles of plastid-specific ribosomal proteins. Proc. Natl. Acad. Sci. USA 104: 19315-19320.

Shiratori, T., S. Suzuki, Y. Kakizawa, and K. I. Ishida. 2019. Phagocytosis-like cell engulfment by a planctomycete bacterium. Nat. Commun. 10: 5529.

Silliker, M. E., M. R. Liotta, and D. J. Cummings. 1996. Elimination of mitochondrial mutations by sexual reproduction: two *Podospora anserina* mitochondrial mutants yield only wild-type progeny when mated. Curr. Genet. 30: 318-324.

Simpson, L., O. H. Thiemann, N. J. Savill, J. D. Alfonzo, and D. A. Maslov. 2000. Evolution of RNA editing in trypanosome mitochondria. Proc. Natl. Acad. Sci. USA 97: 6986-6993.

Sloan, D. B., D. A. Triant, M. Wu, and D. R. Taylor. 2014. Cytonuclear interactions and relaxed selection accelerate sequence evolution in organelle ribosomes. Mol. Biol. Evol. 31: 673-682.

Sloan, D. B., J. M. Warren, A. M. Williams, Z. Wu, S. E. Abdel-Ghany, A. J. Chicco, and J. C. Havird. 2018. Cytonuclear integration and co-evolution. Nat. Rev. Genet. 19: 635-648.

Smith, D. G., R. M. Gawryluk, D. F. Spencer, R. E. Pearlman, K. W. Siu, and M. W. Gray. 2007. Exploring the mitochondrial proteome of the ciliate protozoon *Tetrahymena thermophila:* direct analysis by tandem mass spectrometry. J. Mol. Biol. 374: 837-863.

Smith, D. R. 2015. Mutation rates in plastid genomes: they are lower than you might think. Genome Biol. Evol. 7: 1227-1234.

Smith, D. R., C. J. Jackson, and A. Reyes-Prieto. 2014. Nucleotide substitution analyses of the glaucophyte *Cyanophora* suggest an ancestrally lower mutation rate in plastid vs. mitochondrial DNA for the Archaeplastida. Mol. Phylogenet. Evol. 79: 380-384.

Smith, D. R., and P. J. Keeling. 2012. Twenty-fold difference in evolutionary rates between the mitochondrial and plastid genomes of species with secondary red plastids. J. Eukaryot. Microbiol. 59: 181-184.

Smith, D. R., and P. J. Keeling. 2015. Mitochondrial and plastid genome architecture: reoccurring themes, but significant differences at the extremes. Proc. Natl. Acad. Sci. USA 112: 10177-10184.

Smith, H. C. 2006. Editing informational content of expressed DNA sequences and their transcripts,

pp. 248-265. In L. H. Caporale (ed.) The Implicit Genome. Oxford Univ. Press, Oxford, UK.

Solignac, M., M. Monnerot, and J. C. Mounolou. 1983. Mitochondrial DNA heteroplasmy in *Drosophila mauritiana.* Proc. Natl. Acad. Sci. USA 80: 6942-6946.

Söll, D., and U. L. RajBhandary. 1995. tRNA: Structure, Biosynthesis, and Function. ASM Press, Washington, DC.

Städler, T., and L. F. Delph. 2002. Ancient mitochondrial haplotypes and evidence for intragenic recombination in a gynodioecious plant. Proc. Natl. Acad. Sci. USA 99: 11730-11735.

Stein, A., and E. A. Sia. 2017. Mitochondrial DNA repair and damage tolerance. Front. Biosci. 22: 920-943.

Steinberg, S., and R. Cedergren. 1994. Structural compensation in atypical mitochondrial tRNAs. Nature Struct. Biol. 1: 507-510.

Steinberg, S., D. Gautheret, and R. Cedergren. 1994. Fitting the structurally diverse animal mitochondrial tRNAs(Ser) to common three-dimensional constraints. J. Mol. Biol. 236: 982-989.

Stewart, J. B., C. Freyer, J. L. Elson, A. Wredenberg, Z. Cansu, A. Trifunovic, and N. G. Larsson. 200. Strong purifying selection in transmission of mammalian mitochondrial DNA. PLoS Biol. 6: e10.

Stoltzfus, A. 1999. On the possibility of constructive neutral evolution. J. Mol. Evol. 49: 169-181.

Stroud, D. A., E. E. Surgenor, L. E. Formosa, B. Reljic, A. E. Frazier, M. G. Dibley, L. D. Osellame, T. Stait, T. H. Beilharz, D. R. Thorburn, et al. 2016. Accessory subunits are integral for assembly and function of human mitochondrial complex I. Nature 538: 123-126.

Takahata, N., and M. Slatkin. 1984. Mitochondrial gene flow. Proc. Natl. Acad. Sci. USA 81: 1764-1767.

Templeton, A. R. 1996. Gene lineages and human evolution. Science 272: 1363-1364.

Thompson, A. W., R. A. Foster, A. Krupke, B. J. Carter, N. Musat, D. Vaulot, M. M. Kuypers, and J. P. Zehr. 2012. Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. Science 337: 1546-1550.

Thyagarajan, B., R. A. Padua, and C. Campbell. 1996. Mammalian mitochondria possess homologous DNA recombination activity. J. Biol. Chem. 271: 27536-27543.

Tillich, M., P. Lewahrk, B. R. Morton, and U. G. Maier. 2006. The evolution of chloroplast RNA editing. Mol. Biol. Evol. 23: 19121921.

Tomita, K., T. Ueda, and K. Watanabe. 1996. RNA editing in the acceptor stem of squid mitochondrial tRNA(Tyr). Nucleic Acids Res. 24: 4987-4991.

Tsudzuki, T., T. Wakasugi, and M. Sugiura. 2001. Comparative analysis of RNA editing sites in higher plant chloroplasts. J. Mol. Evol. 53: 327-332.

van der Sluis, E. O., H. Bauerschmitt, T. Becker, T. Mielke, J. Frauenfeld, O. Berninghausen, W. Neupert, J. M. Herrmann, and R. E. Beckmann. 2015. Parallel structural evolution of mitochondrial ribosomes and OXPHOS complexes. Genome Biol. Evol. 7: 1235-1251.

Van Melderen, L. 2010. Toxin-antitoxin systems: why so many, what for? Curr. Opin. Microbiol. 13: 781-785.

Vellai, T., K. Takács, and G. Vida. 1998. A new aspect to the origin and evolution of eukaryotes. J. Mol. Evol. 46: 499-507.

von Dohlen, C. D., S. Kohler, S. T. Alsop, and W. R. McManus. 2001. Mealybug beta-proteobacterial endosymbionts contain gamma-proteobacterial symbionts. Nature 412: 433-436.

Wagner, G. P., and W. Gabriel. 1990. Quantitative variation in finite parthenogenetic populations: what stops Muller's ratchet in the absence of recombination? Evolution 44: 715-731.

Wang, Z., and M. Wu. 2014. Phylogenomic reconstruction indicates mitochondrial ancestor was an energy parasite. PLoS One 9: e110685.

Watanabe, Y., H. Tsurui, T. Ueda, R. Furushima, S. Takamiya, K. Kita, K. Nishikawa, and K. Watanabe. 1994. Primary and higher order structures of nematode (*Ascaris suum*) mitochondrial tRNAs lacking either the T or D stem. J. Biol. Chem. 269: 22902-22906.

Weng, M. L., T. A. Ruhlman, and R. K. Jansen. 2016. Plastid-nuclear interaction and accelerated coevolution in plastid ribosomal genes in Geraniaceae. Genome Biol. Evol. 8: 1824-1838.

Wideman, J. G., R. M. Gawryluk, M. W. Gray, and J. B. Dacks. 2013. The ancient and widespread nature of the ER-mitochondria encounter structure. Mol. Biol. Evol. 30: 2044-2049.

Wilkie, D., and D. Y. Thomas. 1973. Mitochondrial genetic analysis by zygote cell lineages in *Saccharomyces cerevisiae.* Genetics 73: 367-377.

Wise, C. A., M. Sraml, and S. Easteal. 1998. Departure from neutrality at the mitochondrial NADH dehydrogenase subunit 2 gene in humans, but not in chimpanzees. Genetics 148: 409-421.

Wolf, P. G., C. A. Rowe, and M. Hasebe. 2004. High levels of RNA editing in a vascular plant chloroplast genome: analysis of transcripts from the fern *Adiantum capillus-veneris.* Gene 339: 89-97.

Wolfe, K. H., W. H. Li, and P. M. Sharp. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proc. Natl. Acad. Sci. USA 84: 9054-9058.

Wolstenholme, D. R. 1992. Animal mitochondrial DNA: structure and evolution, pp. 173-216. In D. R. Wolstenholme and K. W. Jeon (eds.) Mitochondrial Genomes. Academic Press, New York, NY.

Wolstenholme, D. R., R. Okimoto, and J. L. Macfarlane. 1994. Nucleotide correlations that suggest tertiary interactions in the TV-replacement loop-containing mitochondrial tRNAs of the nematodes, *Caenorhabditis elegans* and *Ascaris suum.* Nucleic Acids Res. 22: 4300-4306.

Wu, Z., G. Waneka, A. K. Broz, C. R. King, and D. B. Sloan. 2020. *MSH1* is required for maintenance of the low mutation rates in plant mitochondrial and plastid genomes. Proc. Natl. Acad. Sci. USA 117: 16448-16455.

Wujek, D. E., and B. C. Asmund. 1978. *Mallomonas cyathellata* sp. nov. and *Mallomonas cyathellata* var. *kenyana* var. nov. (Chrysophyceae) studied by means of scanning and transmission electron microscopy. Phycologia 18: 115-119.

Xu, S., S. Schaack, A. Seyfert, E. Choi, M. Lynch, and M. E. Cristescu. 2012. High mutation rates in the mitochondrial genomes of *Daphnia pulex.* Mol. Biol. Evol. 29: 763-769.

Yahalomi, D., S. D. Atkinson, M. Neuhof, E. S. Chang, H. Philippe, P. Cartwright, J. L. Bartholomew, and D. Huchon. 2020. A cnidarian parasite of salmon (Myxozoa: Henneguya) lacks a mito-

chondrial genome. Proc. Natl. Acad. Sci. USA 117: 5358-5363.

Yamaguchi, K., K. von Knoblauch, and A. R. Subramanian. 2000. The plastid ribosomal proteins. Identification of all the proteins in the 30 S subunit of an organelle ribosome (chloroplast). J. Biol. Chem. 275: 28455-28465.

Yamaguchi, Y., J. H. Park, and M. Inouye. 2011. Toxin-antitoxin systems in bacteria and archaea. Annu. Rev. Genet. 45: 61-79.

Yamauchi, A., T. Yamagishi, R. Booton, A. Telschow, and G. Kudo. 2019. Theory of coevolution of cytoplasmic male-sterility, nuclear restorer and selfing. J. Theor. Biol. 477: 96-107.

Yokobori, S, and S. Pääbo. 1995. Transfer RNA editing in land snail mitochondria. Proc. Natl. Acad. Sci. USA 92: 10432-10435.

Youle, R. J. 2019. Mitochondria – striking a balance between host and endosymbiont. Science 365: eaaw9855.

Zhang, F., and R. E. Broughton. 2013. Mitochondrial-nuclear interactions: compensatory evolution or variable functional constraint among vertebrate oxidative phosphorylation genes? Genome Biol. Evol. 5: 1781-1791.

Zhang, H., and S. Lin. 2005. Mitochondrial cytochrome b mRNA editing in dinoflagellates: possible ecological and evolutionary associations? J. Eukaryot. Microbiol. 52: 538-545.

Zhang, J., T. A. Ruhlman, J. Sabir, J. C. Blazier, and R. K. Jansen. 2015. Coordinated rates of evolution between interacting plastid and nuclear genes in Geraniaceae. Plant Cell 27: 563-573.

Zhou, Q., H. Li, H. Li, A. Nakagawa, J. L. Lin, E. S. Lee, B. L. Harry, R. R. Skeen-Gaar, Y. Suehiro, D. William, et al. 2016. Mitochondrial endonuclease G mediates breakdown of paternal mitochondria upon fertilization. Science 353: 394-399.

Zimorski, V., M. Mentel, A. G. M. Tielens, and W. F. Martin. 2019. Energy metabolism in anaerobic eukaryotes and Earth's late oxygenation. Free Radic. Biol. Med. 140: 279-294.

**Figure 23.1.** Idealized variants of classical mitochondria, and a few of their key modifications, including the presence/absence of a genome (pink rings), an electron-transport chain (red membrane-bound complexes), and ability to produce ATP. All three variants are sites of production of iron-sulfur clusters (yellow). The dark surrounding lines denote the double membrane, and the internal invaginations are called cristae. From Burki (2016).

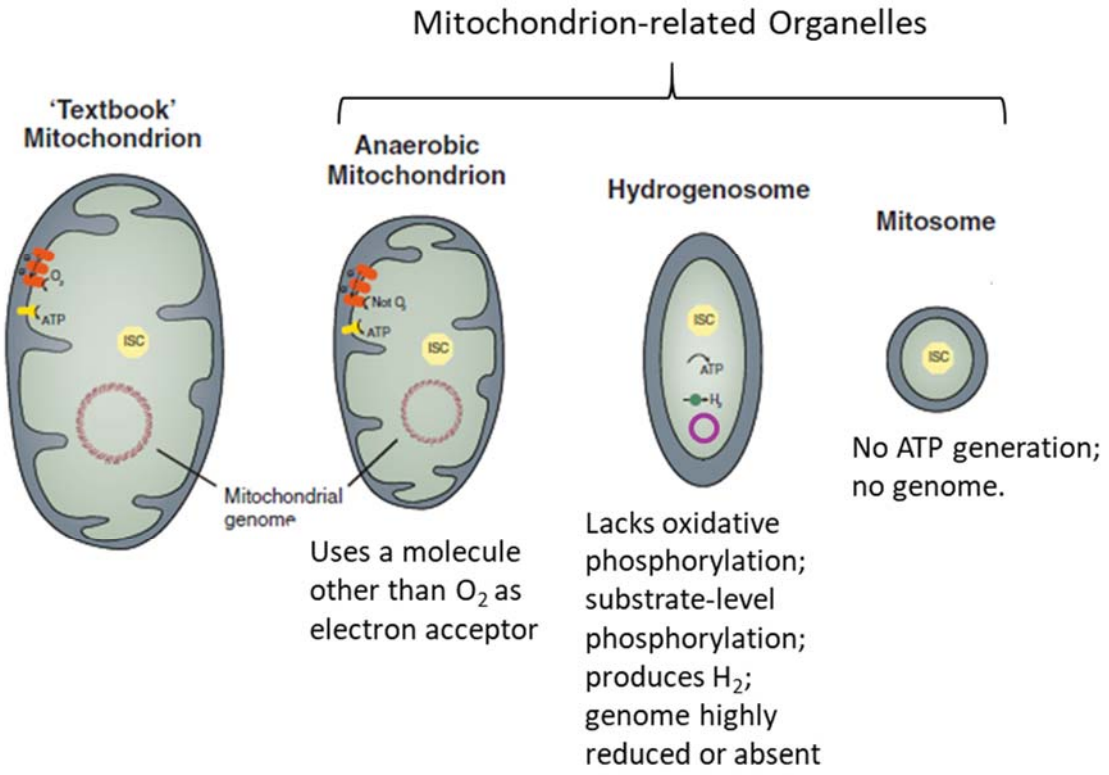

**Figure 23.2.** The number of protein-coding genes and genome sizes of fully sequenced organelle genomes and of the bacterial lineages containing the putative ancestors of the mitochondrion ($\alpha$-proteobacteria) and of the chloroplast (cyanobacteria). Data are from the NCBI Genomes Database (October 2020). Upper and lower diagonal dashed lines denote scalings of 1 and 0.1 genes per kilobase of genomic sequence, which is closely adhered to by bacterial species.

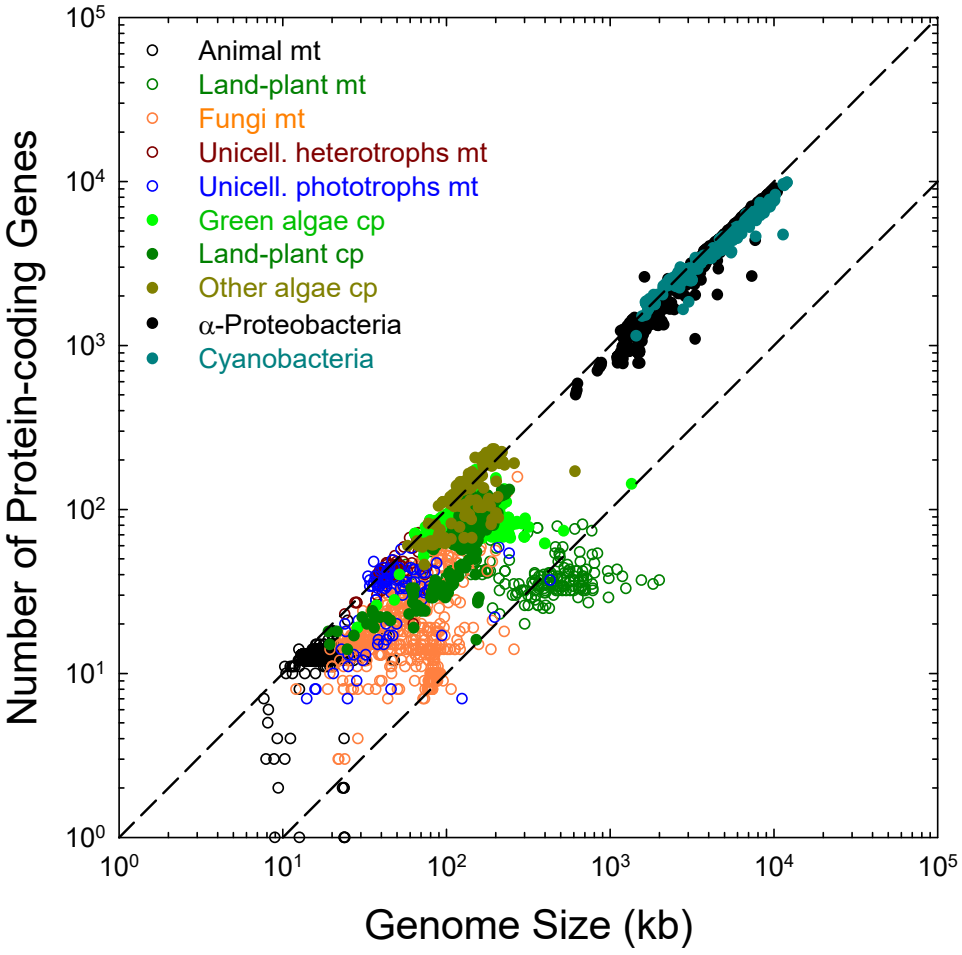

**Figure 23.3.** Muller's ratchet, as illustrated by the stochastic, but progressive movement of the distribution of the numbers of deleterious mutations among members of the population. The red vertical line denotes the initial mean number, whereas the first number on the $x$ axis denotes the class of individuals with the minimum number of mutations.