

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, and frequently with the latter 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 alphaproteobacterium, and nearly all eukaryotic photosynthesis involves chloroplasts with cyanobacterial ancestry. Although the bulk 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 single-celled ciliates and amoebozoans.

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). Mitochondria and plastids come close to meeting this criterion, as both are generally locked into obligate mutualisms with their host cells (with a few exceptions noted below). But even here, there can be significant uncertainty regarding costs and benefits depending on one's point of reference (Keeling and McCutcheon 2017; 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 host cellular environments. Special attention will be given to bioenergetic consequences, as there has been considerable debate regarding the benefits that host cells derive from endosym-

biosis.

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 the 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, driven by the electron transport chain. 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 modifications descendent from the same stock as the mitochondrion itself (Figure 23.1). For example, some anaerobic ciliates and parabasalids (e.g., *Trichomonas*) independently evolved mitochondrial derivatives 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 any form of mitochondrion, having lost them secondarily.

Origins. The early debate as to whether mitochondria were derived from endosymbiotic bacteria or instead somehow arose endogenously was put to rest with the realization that mitochondria contain their own genomes (generally circular as in bacteria), as this 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 alphaproteobacterium, but this leaves many questions unanswered (Archibald 2015; López-García et al. 2017; Martin et al. 2017). From what specific lineage of the large alphaproteobacterial group 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 mitochondrion and the most recent common ancestor of today’s eukaryotes (LECA) blurs the signal from the few genes that remain. Third, although the large number of genes from the primordial mitochondrion transferred to the nuclear genome on the branch to LECA expands the range of informative sequence, background nuclear host-cell acquisitions of genes from other bacteria further cloud the issue.

Although numerous lineages of the Alphaproteobacteria phylum have been 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 (Amiri et al. 2003; Andersson et al. 2003; Sassera et al. 2011; Wang and Wu 2014).

If this hypothesis is correct, the genome contents of the Rickettsiales and related species imply that the founder mitochondrion may have initially harbored ~ 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. One caveat here is provided by a recent analysis that suggests that the mitochondrion may have emerged from an unknown clade that is sister to the entire Alphaproteobacteria, which leaves the nature of the ancestral state more ambiguous (Muñoz-Gómez et al. 2022).

An alternative hypothesis, based primarily on morphological observations, is that the mitochondrion arose from an anaerobic autotroph (Martin and Muller 1998; Cavalier-Smith 2006; Muñoz-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 alphaproteobacterial clade containing anaerobic photosynthesizers (purple nonsulfur bacteria), methanotrophs, and nitrite-oxidizing bacteria. The argument for inheritance of such features (as opposed to independent establishment after the origin of mitochondria) is strengthened by the observation that mitochon-

drial cristae junctions are organized by a protein orthologous to the one used for similar functions in these bacteria. Under this view, the primordial mitochondrion would have been capable of free-living (Martijn et al. 2018; Muñoz-Gómez et al. 2019), although it may still have derived more benefits from its host cell than it provided in return, i.e., it may have been a facultative parasite.

Adding to the uncertainties about mitochondrial ancestry are an enormous number 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 the postulated 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 frequently invoked narratives 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 (initially, a hydrogenosome) (Martin and Muller 1998). In this and related models, the host cell is generally assumed to be an anaerobic member of the archaea (Rivera and Lake 1992; Vellai et al. 1998), 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 machinery must have been largely displaced by that derived from the mitochondrion (or perhaps other bacteria by horizontal gene transfer), as most genes associated with metabolism in today's eukaryotes 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, e.g., lacking internal membranes. The strongest variant of this argument is that eukaryogenesis was impossible without the presumed energetic boost provided by the endosymbiont (Lane and Martin 2010; Raval et al. 2022). 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 betaproteobacterium *Tremblaya* that serves as an endosymbiont in insects and is itself inhabited by a gammaproteobacterium *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 so heavily modified that its ancestral bacterial nature is no longer apparent. 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 free-living cells with bacterial membranes.

In contrast, under the mitochondria-late view, many of the early stages in eu-

karyogenesis, including the origin of a nucleus and internal membranes, are assumed to have been 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, comparative analyses focused on the estimated times of origin of various classes of eukaryotic genes, support the idea that the mitochondrion arose subsequent to the establishment of a number of eukaryotic-specific features (including aspects of internal membrane systems), potentially derived from genes acquired from lineages outside of the alphaproteobacteria (Pittis and Gabaldón 2016; Vosseberg et al. 2021). Although interpretation of the genomic data has been debated (Esposti 2016; Martin et al. 2017; Gabaldón 2018; Tria et al. 2021), this is true for the majority of hypotheses on the roots of the mitochondrion.

Most discussion of the origin of the mitochondrion starts (and often ends) with imagined benefits gained by the host cell, e.g., the oxygen-scavenging and energy-production hypotheses noted above. However, the eukaryotic consortium consists of two participants, and evolutionary stability of a mutualism usually 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: 1) the host lost a key function that was complemented by the presence of the endosymbiont; and 2) the emerging mitochondrion relocated just a single self-essential gene to the host genome, cementing its dependence on the latter.

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 hence depend entirely on their host cells for these essential metabolic functions. Likewise, the host cell abandoned key metabolic functions, such as membrane bioenergetics and iron-sulfur cluster biosynthesis, to the endosymbiont, thereby establishing reciprocal interdependence. Such an outcome represents a grand example of the preservation of two ancestral components by complementary degenerative mutations (Force et al. 1999). Notably, the 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 appears to be the rule in endosymbiont evolution. The many origins of bacterial endosymbiosis in various lineages sap-feeding insects (e.g., aphids, mealybugs, whiteflies, and psyllids) provide a case in point (McCutcheon et al. 2019). One of the more dramatic examples of genome reduction concerns the mealybug 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 (e.g., Bublitz et al. 2019). Such metabolic-pathway mosaicisms are consistent with the principles of intergenomic subfunctionalization just noted, resonate well with the theory of remodeling of metabolic pathways by nonorthologous gene replacement (Chapter 19), and provide further evidence in support of the concept of serial endosymbiosis (below).

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, as touched upon in Chapter 17, observations of today's eukaryotes raise considerable doubts about the enduring belief among some cell biologists 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 downstream 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 numerous lines of evidence to the contrary (Lynch and Marinov 2015, 2017; Hampl et al. 2018; Schavemaker and Muñoz-Gómez 2022).

First, as discussed in several prior chapters (7, 8, 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 less 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 alphaproteobacterial species (noted above) and in other bacterial lineages discussed in Chapters 3 and 15. It has been suggested that phagotrophy (and by extension, complex internal cell structure) by a mitochondrion-free archaeal species 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 the latter resources are available in unlimited supply, an unlikely scenario. 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 them. Moreover, contrary to the supposed impossibility of phagocytosis without mitochondria, members of the bacterial planctomycete 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; the former 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. *Heneguya 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; Chapters 17 and 24). 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 expansion is a consequence of the proliferation of noncoding DNA, in particular the colonization of introns and mobile-element insertions, rather than an increase in gene number and functional diversification.

Finally, aside from these direct lines of evidence against the quantum boost in energetic capacity engendered by the mitochondrion, a more fundamental problem 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; Raval et al. 2022). Energy is a requirement for life, but no convincing argument has been offered as to why increased access to energy should promote phenotypic evolutionary divergence 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 and the resultant inability to retain high replication fidelity (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. 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. An alternative is the passive establishment

of new reproductively isolated lineages by subfunctionalization of the genomes of hosts and endosymbionts (Chapter 3).

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 ~ 1000 nuclear-encoded proteins. In yeast (Karlberg et al. 2000) and the ciliate *Tetrahymena* (Smith et al. 2007), about half of the nuclear-encoded proteins appearing in mitochondria have bacterial affinities, although only a small fraction of these are clearly alphaproteobacterial in origin (Gray 2015; Ku et al. 2015), which themselves 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), a well-documented feature of 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, energetic issues aside, the emergence of the mitochondrion would have altered the context in which all other eukaryotic cellular features evolved downstream. 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; Raval et al. 2022). 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 mechanisms by which proteins from nuclear-encoded genes are imported. Such orchestrations involve the evolution of organelle-localization signals on the front ends of the traveling proteins and of novel molecular-recognition systems on organelle membranes. For example, the SAM (sorting and assembly machinery) complex, which resides in the outer mitochondrial membrane plays a central role in incorporating other 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 may have resided in the original endosymbiont. However, for proteins that function within the central chamber 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 prokaryotic (Dolezal et al. 2006).

Second, critical to the maintenance of a stable endosymbiosis are mechanisms

for preventing uncontrolled organelle proliferation while reliably promoting organelle division and inheritance at appropriate times. Within the lifespan of a host cell, mitochondria can undergo multiple rounds of fission and fusion. Mitochondrial division in a number of eukaryotic lineages relies on an inner constriction produced by the GTPase protein FtsZ as in most bacteria (Chapter 10; Leger et al. 2015). However, 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), raising the possibility 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 endogenously evolved organelles. For example, contact sites between the endoplasmic reticulum and mitochondria serve to coordinate mitochondrial division and play important roles in lipid and ion exchange (Friedman et al. 2011; Wideman et al. 2013; Lewis et al. 2016; Murley and Nunnari 2016; Prinz et al. 2020).

Fourth, while mitochondria are critical to the growth and maintenance of nearly 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 to the cell (Galluzzi et al. 2012). Eukaryotic cells also have an internal mechanism called mitophagy that enables the selective recognition and removal of mitochondria containing defective proteins (Youle 2019). Such a mechanism also occurs during inheritance, at least in metazoans, where maternal gametes induce the elimination of paternal mitochondrial transmitted by fused sperm (Sato and Kuroiwa 1991; Zhou et al. 2016).

All of the molecules participating in these signaling cascades for cell death are nuclear encoded in today's eukaryotes. However, the capacity for inducing cell death might have been carried by a selfish ancestral mitochondrion that killed cells not containing it, ensuring its permanent residence (Kobayashi 1998). As will be discussed at the close of this chapter, bacteria with exactly these properties exist in *Paramecium* (Preer et al. 1971), further 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 genotypes 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 transcriptome and 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 in fundamental ways (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 rates. 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 \rightarrow T:A transitions and C:G \rightarrow 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, which replicates only once per cell division, organellar DNA can replicate within nondividing cells, magnifying the opportunities for replication errors per cell cycle.

The realized mutation rate is also a function of the accuracy of the replication machinery and the ability of repair enzymes to correct pre-replication damage. Unfortunately, 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), but proofreading may be less accurate in the mitochondrion (Anderson et al. 2020). Base-excision repair may often replace damaged bases with incorrect nucleotides in mitochondria (Phadnis et al. 2006; Stein and Sia 2017), and nucleotide-excision repair may be entirely absent. In addition, 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 central question concerns the net effect of this diversity of factors on overall mitochondrial mutation rates, the most reliable estimates of which derive from mutation-accumulation experiments (Chapter 4). For metazoans, these rates are extraordinarily high. In the nematode *Caenorhabditis elegans*, the mitochondrial base-substitution mutation rate is 9.7×10^{-8} /nucleotide site/generation (Denver et al. 2000), $\sim 70\times$ the nuclear rate for this species (Denver et al. 2004, 2009). In the fly *Drosophila melanogaster*, the mitochondrial rate of 4.4×10^{-8} (Haag-Liautard et al. 2008) is $\sim 9\times$ that for the nuclear genome. For two species of the microcrustacean *Daphnia*, the average mitochondrial mutation rates is 5.2×10^{-7} /site/generation (Xu et al. 2012; Ho et al. 2020), $68\times$ that in the nuclear genome. Using pedigree data, the average mutation-rate estimate for humans, 3.6×10^{-5} , is $\sim 2700\times$ the nuclear rate (Howell 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 1.1×10^{-9} is just $2.3\times$ the nuclear rate (Krasovec et al. 2019), and for the yeast *S. cerevisiae*, where the rate in the mitochondrial genome is $\sim 10\times$ that in the nuclear genome (Liu and Zhang 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). These estimates 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). Aside from the inaccuracy of fossil-based estimates of divergence times, one concern here is that phylogenetically based mutation-rate estimates can be biased by factors such as selection (Stewart et al. 2008). However, the data do uphold the interpretation that, relative to rates in the nuclear genome, mitochondrial mutation rates are inflated to a greater extent in metazoans than in unicellular species.

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 some plant species 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 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, minimizing the power of linked selection to modulate the mutation rate. 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 mitochondria are able to maintain some of the lowest mutation rates known in eukaryotes, while presumably experiencing random segregation between organelles and nuclear autosomes, a small effective population size (relative to unicellular species), and a ~ 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 plant 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) found that a mismatch-repair pathway shared between mitochondrial and plastid genomes in *Arabidopsis* reduces the organelle mutation rate 10- to 100-fold, but this is no greater than the efficacy of such systems in nu-

clear genomes, and so taken alone cannot fully explain the further reduced rate in plant organelle genomes. Reconciliation of such an elevation in genome stability with conventional theory would be possible if land plants 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 (Chapter 20), but no such land-plant-specific mechanisms have been revealed.

Modes of inheritance. Unlike nuclear genomes, all organellar genomes are replicated asexually (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 for 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 major 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 host 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 ~ 2 to 10 in mammals (Ashley et al. 1989; Jenuth et al. 1996; Marchington et al. 1997), 30 to 300 in insects (Solignac et al. 1983; Rand and Harrison 1986; Haag-Liautard et al. 2008), and 4 in the plant *Arabidopsis* (Broz et al. 2022). 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, $n_o \simeq 5$ 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), if inheritance is primarily uniparental, rapid sorting of variants bears on the issue of organelle N_e in two significant ways. 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 will be 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. Sperm mitochondria are generally targeted for destruction upon delivery, but the process is not perfect, and low levels of biparental inheritance have been revealed in nematodes (Lunt and Hyman 1997), *Daphnia* (Ye et al. 2022), 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 autonomous mitochondria within host cells raises significant “levels of selection” issues. Most notably, within-host selection has the potential for the promotion of mutant organelle genomes with a replicative advantage despite the disadvantage to the host cells (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 same 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 ameliorating factors, 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 efficiently fed back to the mitochondrion, deletions of the mitochondrial gene will be free to advance. 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 male offspring, 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 of female-driven mutations, setting up a sort of coevolutionary arms race between the sexes. Not surprisingly mutations that restore male fertility are commonly found in plants (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). The combination of low (to no) recombination and uniparental inheritance is of particular relevance to mitochondria, but given the multitude of additional effects that define N_e , resolving the totality of the issues requires empirical analysis. Recall from Chapter 4 that the usual approach here is to estimate standing levels of genetic variation at neutral genomic sites (generally third positions in redundant codons), setting this equal to the drift-mutation equilibrium expectation $2N_e u$ 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 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 $N_{gm}/N_{gn} \simeq 0.1$. 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, with the situation being much less extreme 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 molecules 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. If this process proceeds 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). It has been argued that random segregation of mitochondrial variants at the intracellular level can reduce the rate of the ratchet by creating more variation upon which natural selection can act (Takahata and Slatkin 1983; Bergstrom and Pritchard 1998; Radzvilavicius et al. 2017; Edwards et al. 2021), but this alone cannot prevent the ultimate accumulation of deleterious mutations.

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, 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. The reach goes far beyond cellular energetic performance, 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 eukaryotic cell integrity, mitochondria have likely been 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 23.2). 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 ~ 1000 protein-coding genes likely harbored in the ancestral mitochondrion. A number of genes in the primordial mitochondrion were simply lost, presumably because the fitness advantages were reduced to the point of effective neutrality, and perhaps in

some cases owing to disadvantages in the host-cell environment. Most of the few protein-coding genes remaining in mitochondrial genomes are involved in energy metabolism. Nearly all genes involved in mitochondrial DNA replication and repair, transcription, and translation reside in the nucleus.

The presence of recent fragments of mitochondrial DNA in the nuclear genomes of most eukaryotes highlight the ample opportunities that exist for such transfer even today (Rho et al. 2009; 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. The need to escape from the consequences of Muller's ratchet provides another long-term advantage for transfer to a recombining nuclear genome. Kelly (2021) 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 per cell.

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. 2016). 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 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 also follows that some deleterious 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 producing RNAs that operate 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 of prokaryotes and eukaryotic nuclear genes 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 ~ 70 bases being contained mostly in three loops and four stems. Thirteen of these base positions are essentially invariant across tRNAs for all amino acids in all prokaryotes and all nuclear genomes (Lynch 1996, 1997; Lynch and Blanchard 1998).

Given this extraordinary degree of phylogenetic constancy, the same features must also have been present in the tRNAs within the primordial mitochondrial genome. Thus, any deviations from the canonical tRNA 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 strengths of mitochondrial tRNA stems are 40 to 90% of those in nuclear tRNAs, largely due to the higher incidence of A:U vs. G:C bonds (two vs. three hydrogen bonds) in the former (Lynch 1996, 1997).

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 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), rather than an outcome of a reduction in the efficiency of selection owing to a smaller effective population size (Brown et al. 1982; Kumazawa and Nishida 1993)? Analyses laid out in Lynch (2007) and Popadin et al. (2013) 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 motivates the additional 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 interacting 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 losses 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. In addition, 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 ~ 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 involved in the electron-transport chain retain a few mitochondrially-encoded subunits. However, as with ribosomal proteins, along the path from FECA to LECA, the individual OXPHOS 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 64 protein subunits in most eukaryotic lineages, whereas the orthologous structure in bacteria has only 14 (Gabaldón et al. 2005; Cardol 2011; Huynen and Elurbe 2022). 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 rates of evolution of nuclear-encoded subunits (Osada and Akashi 2012; Zhang and Broughton 2013; Sloan et al. 2014; Adrion et al. 2016; 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 idea does not have wide support (Osada and Akashi 2012; Barreto and Burton 2013; Barreto et al. 2018).

Plastid Evolution

Long after the establishment of the mitochondrion, on the order of 1.0 BYA, 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) (Parfrey et al. 2011; Keeling 2013; Eme et al. 2014). Giving rise to the chloroplast, this brought photosynthesis into the eukaryotic domain.

As with the mitochondrion, the search for the historical roots of this event is made possible by the presence of a genome within the plastid. Although the plastid has clear affinities with cyanobacteria, this large phylogenetic group contains 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 of the plastid 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, as well as the basal glaucophytes, are restricted to freshwater environments (Price et al. 2012), suggests that photosynthetic eukaryotes arose in a terrestrial freshwater environment, with colonization of the oceans occurring secondarily.

Unlike the universal spread of the mitochondrion across the entire eukaryotic phylogeny by simple vertical inheritance, eukaryotic photosynthesis acquired a punctate phylogenetic distribution by horizontal transfer among a small number of lineages (Keeling 2013). 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 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 transfer must initiate 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, still more introductions of photosynthesis into eukaryotes have occurred by independent primary events. For example, about 200 MYA, a freshwater amoeba called *Paulinella chromatophora* became colonized by a cyanobacterium related to *Synechococcus* (Nowack and Grossman 2012; Nowack 2014; Singer et al. 2017). Given that about 30 genes of cyanobacterial origin have been relocated to the nuclear genome and produce products addressed back to the endosymbiont, the latter can be regarded as a legitimate organelle. An even more recent establishment, probably < 20 MYA, involves the colonization of the diatom *Rhopalodia* 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 in an extracellular chamber a load of cyanobacteria, distantly related to marine *Prochlorococcus* / *Synechococcus*, 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 these 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 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 mitochondrial genomes in a number of 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. 2015). 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).

As with plant mitochondria, it remains a mystery as to how selection has been able to promote the extremely low mutation rates of plastid genomes. However, 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 is common 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. However, as this view ignores the apparent adaptive losses incurred by endosymbionts, it is not entirely consistent with the general postulates of evolution by natural selection. A more fundamental issue, emphasized above, is whether the host species achieves a net advantage even 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 have been gained through attempts to establish microbial mutualisms in experimental systems. Although a symbiosis may start out with a recipient simply benefiting from a waste product of a donor, the recipient can then respond 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. 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, 2013), 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 of bacterial 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 Melderren 2011; Goeders and Van Melderren 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. In this sense, the host becomes addicted to the TA-carrying plasmid. Over evolutionary time, many TA systems become incorporated into bacterial chromosomes, in effect serving as partial cure to addiction, allowing the loss of the plasmid, although not fully relieving the host from carrying the TA cassette.

The key point here 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 cases, such secondary benefits involve alterations of the host-cell response to additional stresses (Van Melderen 2010; Yamaguchi et al. 2011).

Finally, although the mechanisms are not fully understood, TA-like systems of a different nature seem to 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 alphaproteobacterial 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 in ciliates.

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, and appears to carry 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 prominent being the mitochondrion, derived from an alphaproteobacterium that colonized the stem eukaryote, and the younger plastid, derived from a cyanobacterium. The basal mitochondrion led to the establishment of internal-membrane bioenergetics throughout all eukaryotes. The plastid, which brought photosynthesis into the eukaryotic domain, acquired a more punctate phylogenetic distribution by spreading horizontally into preexisting 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 from comparative genomics are consistent

with this hypothesis.

- It is commonly argued that the establishment of mitochondrial membrane bioenergetics led to a massive increase in the bioenergetic and evolutionary capacity of eukaryotes relative to prokaryotes, and that this boost was an essential prerequisite 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.
- These caveats aside, substantial intracellular remodeling did follow the establishment of the mitochondrion. 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. This diverse set of observations demonstrates the power of the intracellular environment at directing the course of evolution.
- 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 been modified in dramatic ways never seen in nuclear-encoded genes or in bacterial genomes.
- Beyond the mitochondrion and plastid, there are many additional examples of intracellular inhabitants within unicellular eukaryotes. The advantages of such consortia to the host cell are generally unknown, and in many cases may not exist, with the endocytobiont simply ensuring its own existence by use of a toxin-antitoxin mechanism that serves as an addictive leash on the host.

Foundations 23.1. Messenger RNA editing. A long history of research in molecular biology inspires confidence that coding-sequence 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 and, with very few minor exceptions, always confined to organelle genomes. 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. For example, in *Arabidopsis* mitochondria, 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 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 doubts about this adaptive interpretation. First and most notably, 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 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/or 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).

A fourth 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. However, the rate of conversion of C→U editing sites to nonedited Ts is 4× greater than the neutral expectation (Shields and Wolfe 1997; Tsudzuki et al. 2001; Fujii and Small 2011). Thus, if anything, editing sites in land-plant organelles are at least mildly deleterious.

As just noted, the disadvantages associated with editing a site include the inaccuracies of editing and the energetic burden of maintaining the editing apparatus. An additional issue is 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., ~ 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 latter. 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. 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 full dissociation becomes almost certain beyond two generations of sexual reproduction.

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 gene numbers per land-plant 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. A really creative selfish editor might inflict the organelle genomic change necessary to ensure its own survival, but 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).

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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).

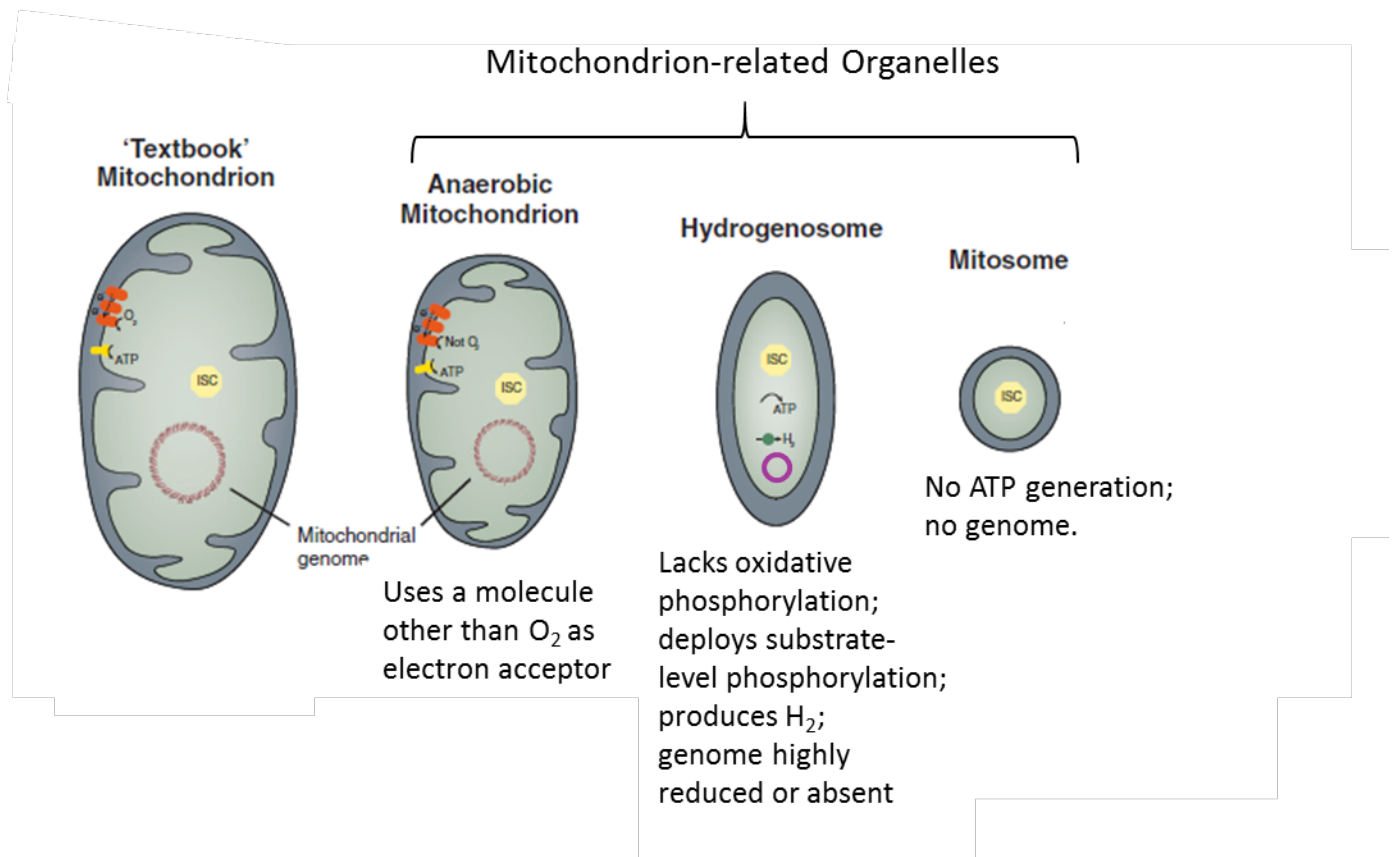


Figure 23.2. The number of protein-coding genes and genome sizes of fully sequenced organelle genomes and of the bacterial lineages containing the putative ancestors of the mitochondrion (Alphaproteobacteria) and of the chloroplast (cyanobacteria). Data are from the NCBI Genomes Database (October 2020); mt and cp denote mitochondria and chloroplasts, respectively. 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.

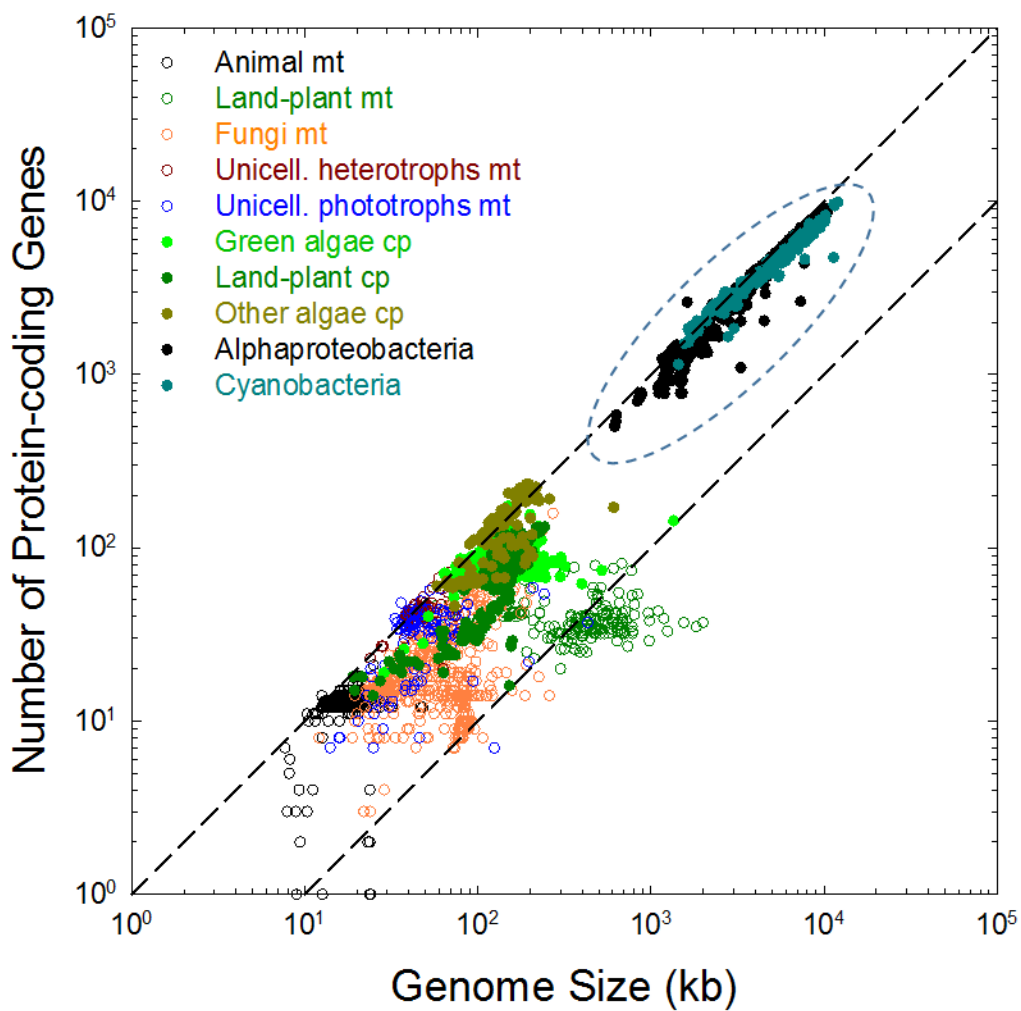


Figure 23.3. Muller's ratchet, as illustrated by the stochastic, but progressive movement of the distribution of the numbers of deleterious mutations among members of the population. The red vertical line denotes the initial mean number, whereas the first number on the x axis denotes the class of individuals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

