replication and transfer of the plasmid to related bacteria through a complex macromolecular tube called a pilus. These plasmids, called R factors (for drug resistance), can contain multiple drug resistance genes introduced by transposition and selected in environments where antibiotics are used to sterilize surfaces, such as hospitals. These have led to the rapid spread of resistance to multiple antibiotics between pathogenic bacteria. Coping with the spread of R factors is a major challenge for modern medicine. ■

KEY CONCEPTS of Section 6.3

Transposable (Mobile) DNA Elements

- Transposable DNA elements are moderately repeated sequences interspersed at multiple sites throughout the genomes of higher eukaryotes. They are present less frequently in prokaryotic genomes.
- DNA transposons move to new sites directly as DNA; retrotransposons are first transcribed into an RNA copy of the element, which then is reverse-transcribed into DNA (see Figure 6-8).
- A common feature of all mobile elements is the presence of short direct repeats flanking the sequence.
- Enzymes encoded by transposons themselves catalyze insertion of these sequences at new sites in genomic DNA.
- Although DNA transposons, similar in structure to bacterial IS elements, occur in eukaryotes (e.g., the *Drosophila* P element), retrotransposons generally are much more abundant, especially in vertebrates.
- LTR retrotransposons are flanked by long terminal repeats (LTRs), similar to those in retroviral DNA; like retroviruses, they encode reverse transcriptase and integrase. They move in the genome by being transcribed into RNA, which then undergoes reverse transcription in the cytosol, nuclear import of the resulting DNA with LTRs, and integration into a host-cell chromosome (see Figure 6-14).
- Non-LTR retrotransposons, including long interspersed elements (LINEs) and short interspersed elements (SINEs), lack LTRs and have an A/T-rich stretch at one end. They are thought to move by a nonviral retrotransposition mechanism mediated by LINE-encoded proteins involving priming of reverse transcription by chromosomal DNA (see Figure 6-17).
- SINE sequences exhibit extensive homology with small cellular RNAs and are transcribed by the same RNA polymerase. *Alu* elements, the most common SINEs in humans, are ≈300-bp sequences found scattered throughout the human genome.
- Some interspersed repeats are derived from cellular RNAs that were reverse-transcribed and inserted into genomic DNA at some time in evolutionary history. Processed pseudogenes derived from mRNAs lack introns, a feature that distinguishes them from pseudogenes, which arose by sequence drift of duplicated genes.
- Mobile DNA elements most likely influenced evolution significantly by serving as recombination sites and by mobilizing adjacent DNA sequences.

6.4 Organelle DNAs

Although the vast majority of DNA in most eukaryotes is found in the nucleus, some DNA is present within the mitochondria of animals, plants, and fungi, and within the chloroplasts of plants. These organelles are the main cellular sites for ATP formation, during oxidative phosphorylation in mitochondria and photosynthesis in chloroplasts (Chapter 12). Many lines of evidence indicate that mitochondria and chloroplasts evolved from eubacteria that were engulfed into ancestral cells containing a eukaryotic nucleus, forming **endosymbiotes** (Figure 6-20). Over evolutionary time, most of the bacterial genes were lost from organellar DNAs. Some, such as genes encoding proteins involved in nucleotide, lipid, and amino acid biosynthesis, were lost because their functions were provided by genes in the nucleus of the host cell. Other genes encoding components of the present-day organelles were transferred to the nucleus. However, mitochondria and chloroplasts in today's eukaryotes retain DNAs encoding some proteins essential for organellar function, as well as the ribosomal and transfer RNAs required for synthesis of these proteins. Thus eukaryotic cells have multiple genetic systems: a predominant nuclear system and secondary systems with their own DNA, ribosomes, and tRNAs in mitochondria and chloroplasts.

Mitochondria Contain Multiple mtDNA Molecules

Individual mitochondria are large enough to be seen under the light microscope, and even the mitochondrial DNA (mtDNA) can be detected by fluorescence microscopy. The mtDNA is located in the interior of the mitochondrion, the region known as the matrix (see Figure 12-6). As judged by the number of yellow fluorescent "dots" of mtDNA, a *Euglena gracilis* cell contains at least 30 mtDNA molecules (Figure 6-21).

Replication of mtDNA and division of the mitochondrial network can be followed in living cells using time-lapse microscopy. Such studies show that, in most organisms, mtDNA replicates throughout interphase. At mitosis, each daughter cell receives approximately the same number of mitochondria, but since there is no mechanism for apportioning exactly equal numbers of mitochondria to the daughter cells, some cells contain more mtDNA than others. By isolating mitochondria from cells and analyzing the DNA extracted from them, it can be seen that each mitochondrion contains

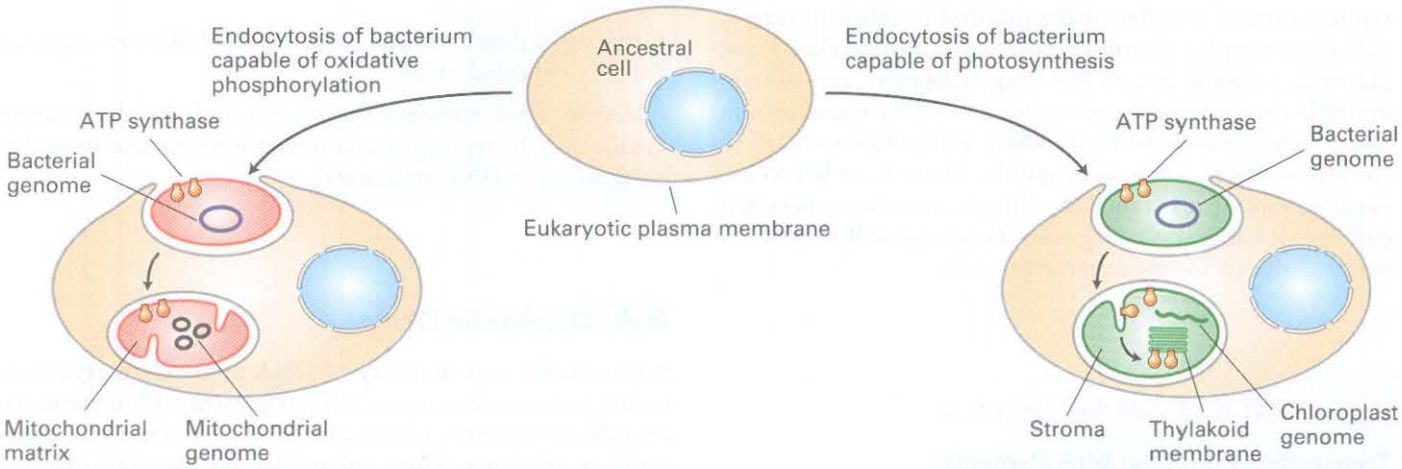
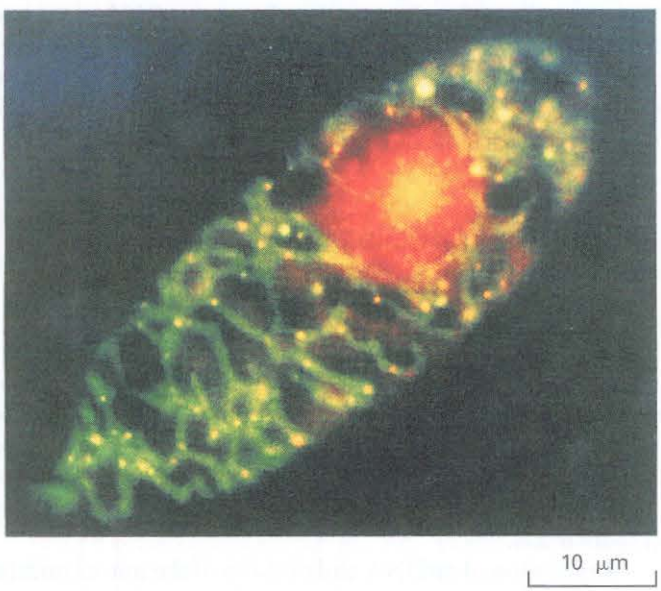


FIGURE 6-20 Endosymbiont hypothesis model of mitochondria and chloroplast evolution. Endocytosis of a bacterium by an ancestral eukaryotic cell would generate an organelle with two membranes, the outer membrane derived from the eukaryotic plasma membrane and the inner one from the bacterial membrane. Proteins localized to the ancestral bacterial membrane retain their orientation, such that the portion of the protein once facing the extracellular space now faces the intermembrane space. Budding of vesicles from the inner chloroplast membrane, such as occurs during development of chloroplasts in contemporary plants, would generate the thylakoid membranes of chloroplasts. The organellar DNAs are indicated.

multiple mtDNA molecules. Thus the total amount of mtDNA in a cell depends on the number of mitochondria, the size of the mtDNA, and the number of mtDNA molecules per mitochondrion. Each of these parameters varies greatly between different cell types.



EXPERIMENTAL FIGURE 6-21 Dual staining reveals the multiple mitochondrial DNA molecules in a growing *Euglena gracilis* cell. Cells were treated with a mixture of two dyes: ethidium bromide, which binds to DNA and emits a red fluorescence, and DiOC6, which is incorporated specifically into mitochondria and emits a green fluorescence. Thus the nucleus emits a red fluorescence, and areas rich in mitochondrial DNA fluoresce yellow—a combination of red DNA and green mitochondrial fluorescence. [From Y. Hayashi and K. Ueda, 1989, *J. Cell Sci.* **93**:565.]

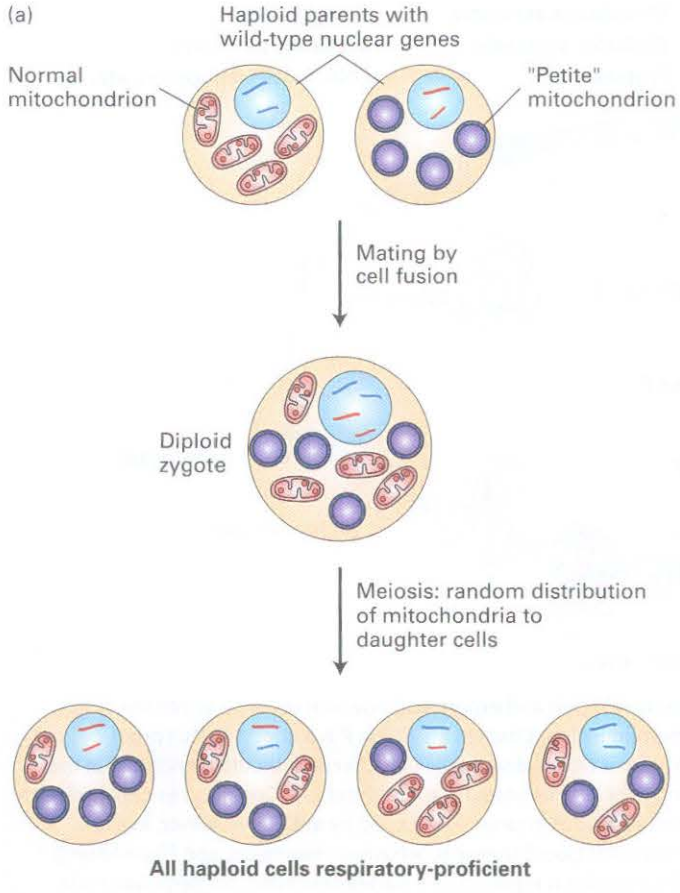
mtDNA Is Inherited Cytoplasmically

Studies of mutants in yeasts and other single-celled organisms first indicated that mitochondria exhibit *cytoplasmic inheritance* and thus must contain their own genetic system (Figure 6-22). For instance, *petite* yeast mutants exhibit structurally abnormal mitochondria and are incapable of oxidative phosphorylation. As a result, petite cells grow more slowly than wild-type yeasts and form smaller colonies. Genetic crosses between different (haploid) yeast strains showed that the *petite* mutation does not segregate with any known nuclear gene or chromosome. In later studies, most petite mutants were found to contain deletions of mtDNA.

In the mating by fusion of haploid yeast cells, both parents contribute equally to the cytoplasm of the resulting diploid; thus inheritance of mitochondria is biparental (see Figure 6-22a). In mammals and most other multicellular organisms, however, the sperm contributes little (if any) cytoplasm to the zygote, and virtually all the mitochondria in the embryo are derived from those in the egg, not the sperm. Studies in mice have shown that 99.99 percent of mtDNA is maternally inherited, but a small part (0.01 percent) is inherited from the male parent. In higher plants, mtDNA is inherited exclusively in a uniparental fashion through the female parent (egg), not the male (pollen).

The Size, Structure, and Coding Capacity of mtDNA Vary Considerably Between Organisms

Surprisingly, the size of the mtDNA, the number and nature of the proteins it encodes, and even the mitochondrial genetic code itself vary greatly between different organisms. The mtDNAs of most multicellular animals are ≈16-kb circular molecules that encode intron-less genes compactly arranged on both DNA strands. Vertebrate mtDNAs encode



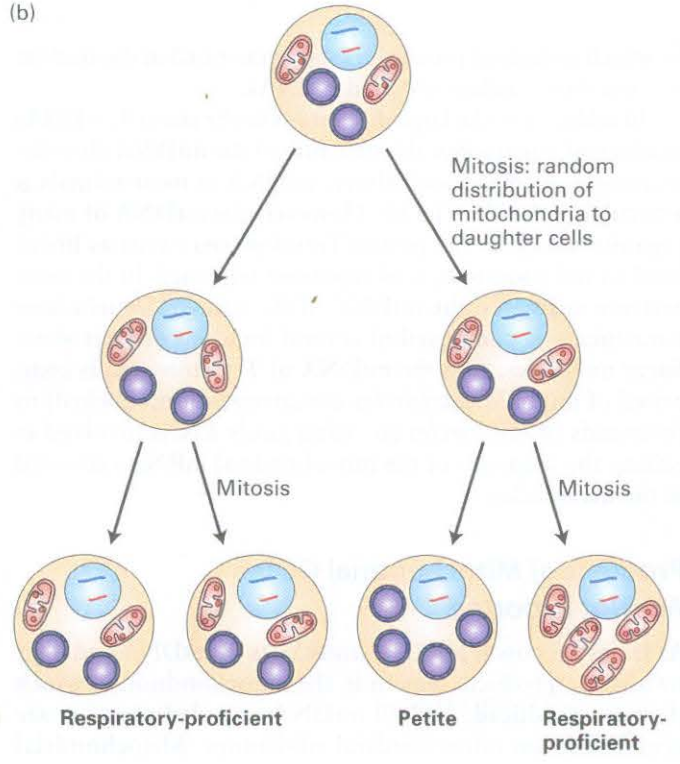


FIGURE 6-22 Cytoplasmic inheritance of an mtDNA *petite* mutation in yeast. Petite-strain mitochondria are defective in oxidative phosphorylation owing to a deletion in mtDNA. (a) Haploid cells fuse to produce a diploid cell that undergoes meiosis, during which random segregation of parental chromosomes and mitochondria containing mtDNA occurs. Note that alleles for genes in nuclear DNA (represented by large and small nuclear chromosomes colored red and blue) segregate 2:2 during meiosis (see Figure 5-5). In contrast, since yeast normally contain ≈50 mtDNA molecules per cell, all products of meiosis usually contain both normal and petite mtDNAs and are capable of respiration. (b) As these haploid cells grow and divide mitotically, the cytoplasm (including the mitochondria) is randomly distributed to the daughter cells. Occasionally, a cell is generated that contains only defective petite mtDNA and yields a petite colony. Thus formation of such petite cells is independent of any nuclear genetic marker.

the two rRNAs found in mitochondrial ribosomes, the 22 tRNAs used to translate mitochondrial mRNAs, and 13 proteins involved in electron transport and ATP synthesis (Chapter 12). The smallest mitochondrial genomes known are in *Plasmodium*, single-celled obligate intracellular parasites that cause malaria in humans. *Plasmodium* mtDNAs are only ≈6 kb, encoding five proteins and the mitochondrial rRNAs.

The mitochondrial genomes from a number of different metazoan organisms (i.e., multicellular animals) have now been cloned and sequenced, and mtDNAs from all these sources encode essential mitochondrial proteins (Figure 6-23). All proteins encoded by mtDNA are synthesized on mitochondrial ribosomes. Most mitochondrially synthesized polypeptides identified thus far are subunits of multimeric complexes used in electron transport, ATP synthesis, or insertion of proteins into the inner mitochondrial membrane or intermembrane space. However, most of the proteins localized in mitochondria, such as those involved in the processes listed at the top of Figure 6-23, are encoded by nuclear genes, synthesized on cytosolic ribosomes, and imported into the organelle by processes discussed in Chapter 13.

In contrast to metazoan mtDNAs, plant mtDNAs are many times larger, and most of the DNA does not encode protein. For instance, the mtDNA in the important model plant *Arabidopsis thaliana* is 366,924 base pairs, and the largest known mtDNA is ≈2 Mb, found in cucurbit plants (e.g., melon and cucumber). Most plant mtDNA consists of long introns, pseudogenes, mobile DNA elements restricted to the mitochondrial compartment, and pieces of foreign (chloroplast, nuclear, and viral) DNA that were probably inserted into plant mitochondrial genomes during their evolution. Duplicated sequences also contribute to the greater length of plant mtDNAs.

Differences in the number of genes encoded by the mtDNA from various organisms most likely reflect the movement of DNA between mitochondria and the nucleus during evolution. Direct evidence for this movement comes from the observation that several proteins encoded by mtDNA

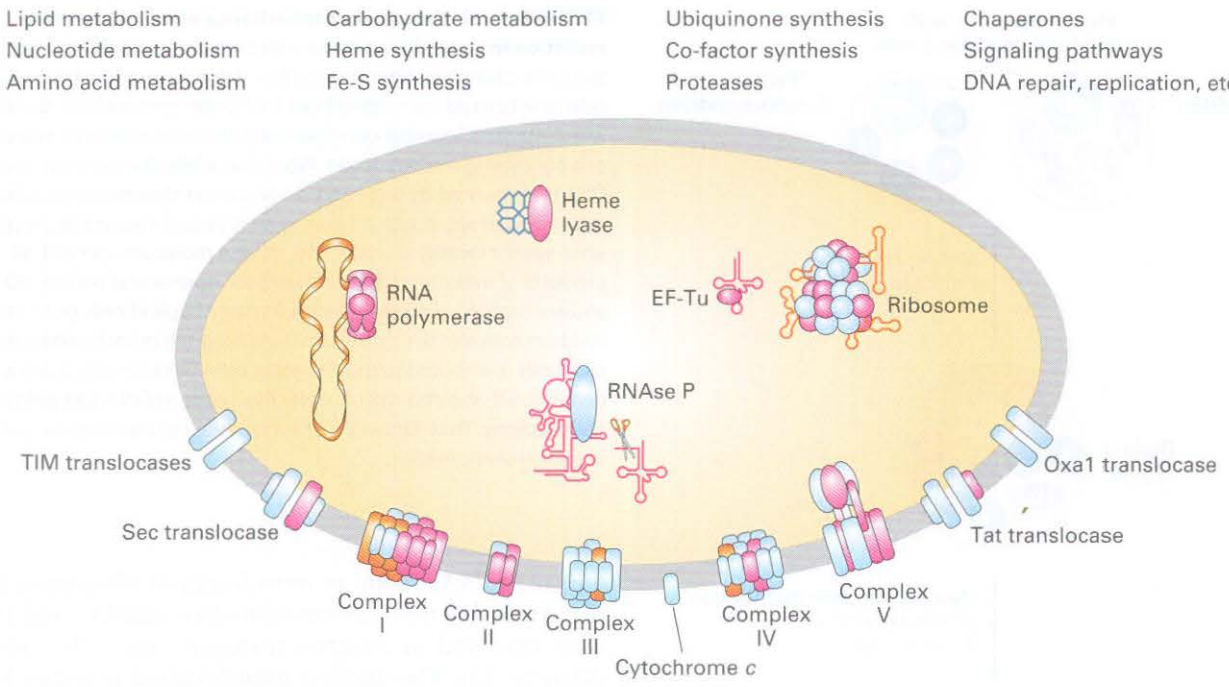


FIGURE 6-23 Proteins encoded in mitochondrial DNA and their involvement in mitochondrial processes. Only the mitochondrial matrix and inner membrane are depicted. Most mitochondrial components are encoded by the nucleus (blue); those highlighted in pink are encoded by mtDNA in some eukaryotes but by the nuclear genome in other eukaryotes, whereas a small portion are invariably specified by mtDNA (orange). Mitochondrial processes that have exclusively nucleus-encoded components are listed at the top. Complexes I–V are involved in electron transport and oxidative phosphorylation. TIM, Sec, Tat, and Oxa1 translocases are involved in protein import and export, and insertion of proteins into the inner membrane (see Chapter 13). RNase P is a ribozyme that processes the 5′ end of tRNAs (discussed in Chapter 8). It should be noted that the majority of eukaryotes have a multisubunit Complex I as depicted, with three subunits invariantly encoded by mtDNA. However, in a few organisms (*Saccharomyces, Schizosaccharomyces,* and *Plasmodium*), this complex is replaced by a nucleus-encoded, single-polypeptide enzyme. For more details on mitochondrial metabolism and transport, see Chapters 12 and 13. [Adapted from G. Burger et al., 2003, *Trends Genet.* **19:**709.]

in some species are encoded by nuclear DNA in other, closely related species. The most striking example of this phenomenon involves the *cox II* gene, which encodes subunit 2 of cytochrome *c* oxidase, which constitutes complex IV in the mitochondrial electron-transport chain (see Figure 12-16). This gene is found in mtDNA in all multicellular plants studied except for certain related species of legumes, including the mung bean and the soybean, in which the *cox II* gene is nuclear. The *cox II* gene is completely missing from mung bean mtDNA, but a defective *cox II* pseudogene that has accumulated many mutations can still be recognized in soybean mtDNA.

Many RNA transcripts of plant mitochondrial genes are edited, mainly by the enzyme-catalyzed conversion of selected C residues to U, and occasionally U to C. (RNA editing is discussed in Chapter 8.) The nuclear *cox II* gene of mung bean corresponds more closely to the edited *cox II* RNA transcripts than to the mitochondrial *cox II* genes found in other legumes. These observations are strong evidence that the *cox II* gene moved from the mitochondrion to the nucleus during mung bean evolution by a process that involved an RNA intermediate. Presumably this movement involved a reverse-transcription mechanism similar to that by which processed pseudogenes are generated in the nuclear genome from nucleus-encoded mRNAs.

In addition to the large differences in the sizes of mtDNAs in different eukaryotes, the structure of the mtDNA also varies greatly. As mentioned above, mtDNA in most animals is a circular molecule ≈16 kb. However, the mtDNA of many organisms such as the protist *Tetrahymena* exists as linear head-to-tail concatemers of repeating sequence. In the most extreme examples, the mtDNA of the protist *Amoebidium parasiticum* is composed of several hundred distinct short linear molecules. And the mtDNA of *Trypanosoma* is comprised of multiple *maxicircles* concatenated (interlocked) to thousands of *minicircles* encoding *guide RNAs* involved in editing the sequence of the mitochondrial mRNAs encoded in the maxicircles.

Products of Mitochondrial Genes Are Not Exported

As far as is known, all RNA transcripts of mtDNA and their translation products remain in the mitochondrion in which they are produced, and all mtDNA-encoded proteins are synthesized on mitochondrial ribosomes. Mitochondrial

DNA encodes the rRNAs that form mitochondrial ribosomes, although most of the ribosomal proteins are imported from the cytosol. In animals and fungi, all the tRNAs used for protein synthesis in mitochondria also are encoded by mtDNAs. However, in plants and many protozoans, most mitochondrial tRNAs are encoded by the nuclear DNA and imported into the mitochondrion.

Reflecting the bacterial ancestry of mitochondria, mitochondrial ribosomes resemble bacterial ribosomes and differ from eukaryotic cytosolic ribosomes in their RNA and protein compositions, their size, and their sensitivity to certain antibiotics (see Figure 4-22). For instance, chloramphenicol blocks protein synthesis by bacterial and mitochondrial ribosomes from most organisms, but cycloheximide, which inhibits protein synthesis on eukaryotic cytosolic ribosomes, does not affect mitochondrial ribosomes. This sensitivity of mitochondrial ribosomes to the important aminoglycoside class of antibiotics that includes chloramphenicol is the main cause of the toxicity that these antibiotics can cause. ■

Mitochondria Evolved from a Single Endosymbiotic Event Involving a *Rickettsia*-like Bacterium

Analysis of the mtDNA sequences from various eukaryotes, including single-celled protists that diverged from other eukaryotes early in evolution, provides strong support for the idea that the mitochondrion had a single origin. Mitochondria most likely arose from a bacterial symbiote whose closest contemporary relatives are in the *Rickettsiaceae* group. Bacteria in this group are obligate intracellular parasites. Thus, the ancestor of the mitochondrion probably also had an intracellular lifestyle, putting it in a good location for evolving into an intracellular symbiote. The mtDNA with the largest number of encoded genes so far found is in the protist species *Reclinomonas americana*. All other mtDNAs have a subset of the *R. americana* genes, strongly implying that they evolved from a common ancestor with *R. americana*, losing different groups of mitochondrial genes by deletion and/or transfer to the nucleus over time.

In organisms whose mtDNA includes only a limited number of genes, the same set of mitochondrial genes is retained, independent of the phyla that include these organisms (see Figure 6-23, orange proteins). One hypothesis for why these genes were never successfully transferred to the nuclear genome is that their encoded polypeptides are too hydrophobic to cross the outer mitochondrial membrane, and therefore would not be imported back into the mitochondria if they were synthesized in the cytosol. Similarly, the large size of rRNAs may interfere with their transport from the nucleus through the cytosol into mitochondria. Alternatively, these genes may not have been transferred to the nucleus during evolution because regulation of their expression in response to conditions within individual mitochondria may be advantageous. If these genes were located in the nucleus, conditions within each mitochondrion could not influence the expression of proteins found in that mitochondrion.

Mitochondrial Genetic Codes Differ from the Standard Nuclear Code

The genetic code used in animal and fungal mitochondria is different from the standard code used in all prokaryotic and eukaryotic nuclear genes; remarkably, the code even differs in mitochondria from different species (Table 6-3). Why and how these differences arose during evolution is mysterious. UGA, for example, is normally a stop codon, but is read as tryptophan by human and fungal mitochondrial translation

TABLE 6-3 Alterations in the Standard Genetic Code in Mitochondria

		Mitochondria				
Codon	Standard Code *	Mammals	*Drosophila*	*Neurospora*	Yeasts	Plants
UGA	Stop	Trp	Trp	Trp	Trp	Stop
AGA, AGG	Arg	Stop	Ser	Arg	Arg	Arg
AUA	Ile	Met	Met	Ile	Met	Ile
AUU	Ile	Met	Met	Met	Met	Ile
CUU, CUC, CUA, CUG	Leu	Leu	Leu	Leu	Thr	Leu

*For nuclear-encoded proteins.

SOURCES: S. Anderson et al., 1981, *Nature* **290**:457; P. Borst, in *International Cell Biology 1980–1981*, H. G. Schweiger, ed., Springer-Verlag, p. 239; C. Breitenberger and U. L. Raj Bhandary, 1985, *Trends Biochem. Sci.* **10**:478–483; V. K. Eckenrode and C. S. Levings, 1986, *In Vitro Cell Dev. Biol.* **22**:169–176; J. M. Gualber et al., 1989, *Nature* **341**:660–662; and P. S. Covello and M. W. Gray, 1989, *Nature* **341**:662–666.

systems; however, in plant mitochondria, UGA is still recognized as a stop codon. AGA and AGG, the standard nuclear codons for arginine, also code for arginine in fungal and plant mtDNA, but they are stop codons in mammalian mtDNA and serine codons in *Drosophila* mtDNA.

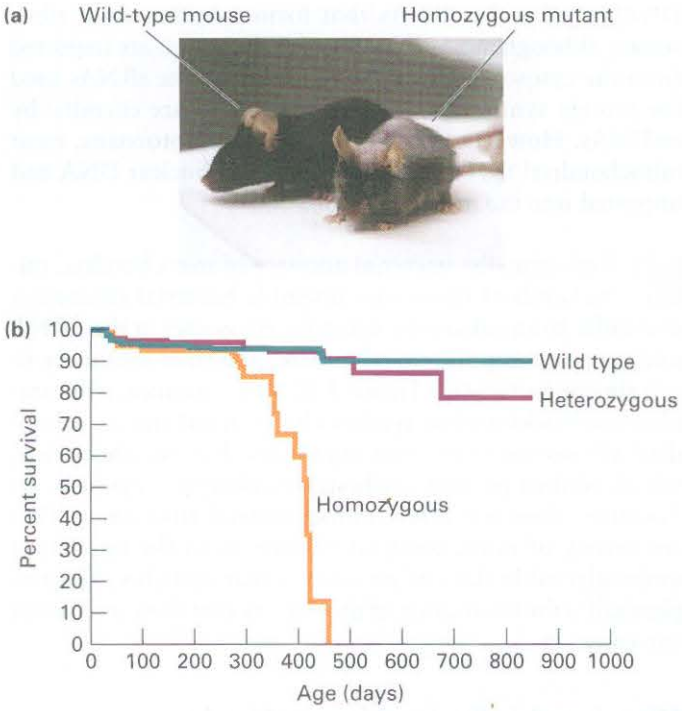
As shown in Table 6-3, plant mitochondria appear to utilize the standard genetic code. However, comparisons of the amino acid sequences of plant mitochondrial proteins with the nucleotide sequences of plant mtDNAs suggested that CGG could code for *either* arginine (the "standard" amino acid) or tryptophan. This apparent nonspecificity of the plant mitochondrial code is explained by editing of mitochondrial RNA transcripts, which can convert cytosine residues to uracil residues. If a CGG sequence is edited to UGG, the codon specifies tryptophan, the standard amino acid for UGG, whereas unedited CGG codons encode the standard arginine. Thus the translation system in plant mitochondria does utilize the standard genetic code. ■

Mutations in Mitochondrial DNA Cause Several Genetic Diseases in Humans

The severity of disease caused by a mutation in mtDNA depends on the nature of the mutation and on the proportion of mutant and wild-type mtDNAs present in a particular cell type. Generally, when mutations in mtDNA are found, cells contain mixtures of wild-type and mutant mtDNAs—a condition known as *heteroplasmy*. Each time a mammalian somatic or germ-line cell divides, the mutant and wild-type mtDNAs segregate randomly into the daughter cells, as occurs in yeast cells (see Figure 6-22b). Thus, the mtDNA genotype, which fluctuates from one generation and from one cell division to the next, can drift toward predominantly wild-type or predominantly mutant mtDNAs. Since all enzymes required for the replication and growth of mammalian mitochondria, such as the mitochondrial DNA and RNA polymerases, are encoded in the nucleus and imported from the cytosol, a mutant mtDNA should not be at a "replication disadvantage"; mutants that involve large deletions of mtDNA might even be at a selective advantage in replication, because they can replicate faster.

Recent research suggests that the accumulation of mutations in mtDNA is an important component of aging in mammals. Mutations in mtDNA have been observed to accumulate with aging, probably because mammalian mtDNA is not repaired in response to DNA damage. To study this hypothesis, researchers used gene "knock-in" techniques to replace the nuclear gene encoding mitochondrial DNA polymerase with normal proofreading activity (see Figure 4-34) with a mutant gene encoding a polymerase defective in proofreading. Mutations in mtDNA accumulated much more rapidly in homozygous mutant mice than in wild-type mice, and the mutant mice aged at a highly accelerated rate (Figure 6-24).

With few exceptions, all human cells have mitochondria, yet mutations in mtDNA affect only some tissues. Those most commonly affected are tissues that have a high requirement for ATP produced by oxidative phosphorylation and tissues that require most or all of the mtDNA in the cell to synthesize sufficient amounts of functional mitochondrial proteins. For instance, *Leber's hereditary optic neuropathy* (degeneration of the optic nerve) is caused by a missense mutation in the mtDNA gene encoding subunit 4 of the NADH-CoQ reductase (complex I), a protein required for ATP production by mitochondria (see Figure 12-16). Any of several large deletions in mtDNA causes another set of diseases, including *chronic progressive external ophthalmoplegia*, characterized by eye defects, and *Kearns-Sayre syndrome*, characterized by eye defects, abnormal heartbeat, and central nervous system degeneration. A third condition, causing "ragged" muscle fibers (with improperly assembled mitochondria) and associated uncontrolled jerky movements, is due to a single mutation in the TΨCG loop of the mitochondrial lysine tRNA. As a result of this mutation, the translation of several mitochondrial proteins apparently is inhibited. ■



EXPERIMENTAL FIGURE 6-24 Mice with a mitochondrial DNA polymerase defective for proofreading exhibit premature aging. A line of "knock-in" mice were prepared by methods discussed in Chapter 5 with an aspartic acid-to-alanine mutation in the gene encoding mitochondrial DNA polymerase (D257A), inactivating the polymerase's proofreading function. (a) Wild-type and homozygous mutant mice at 390 days old (13 months). The mutant mouse displays many of the features of an aged mouse (>720 days, or 24 months of age). (b) Plot of survival versus time of wild-type (+/+), heterozygous (D257A/+) and homozygous (D257A/D257A) mice. [From G. C. Kujoth et al., 2005, *Science* **309:**481. Part (a) courtesy of Jeff Miller/University of Wisconsin-Madison and Gregory Kujoth, Ph.D.]

Chloroplasts Contain Large DNAs Often Encoding More Than a Hundred Proteins

Like mitochondria, chloroplasts are thought to have evolved from an ancestral endosymbiotic photosynthetic bacterium (see Figure 6-20). However, the endosymbiotic event giving rise to chloroplasts occurred more recently (1.2–1.5 billion years ago) than the event leading to the evolution of mitochondria (1.5–2.2 billion years ago). Consequently, contemporary chloroplast DNAs show less structural diversity than do mtDNAs. Also similar to mitochondria, chloroplasts contain multiple copies of the organellar DNA and ribosomes, which synthesize some chloroplast-encoded proteins using the standard genetic code. Like plant mtDNA, chloroplast DNA is inherited exclusively in a uniparental fashion through the female parent (egg). Other chloroplast proteins are encoded by nuclear genes, synthesized on cytosolic ribosomes, and then incorporated into the organelle (Chapter 13). ■

In higher plants, chloroplast DNAs are 120–160 kb long, depending on the species. They initially were thought to be circular DNA molecules because in genetically tractable organisms such as the model plant protozoan *Chlamydomonas reinhardtii*, the genetic map is circular. However, recent studies have revealed that plant chloroplast DNAs are actually long head-to-tail linear concatemers plus recombination intermediates between these long linear molecules. In these studies, researchers have used techniques that minimize mechanical breakage of long DNA molecules during isolation and gel electrophoresis, permitting analysis of megabase-size DNA.

The complete sequences of several chloroplast DNAs from higher plants have been determined. They contain 120–135 genes, 130 in the important model plant *Arabidopsis thaliana*. *A. thaliana* chloroplast DNA encodes 76 protein-coding genes and 54 genes with RNA products such as rRNAs and tRNAs. Chloroplast DNAs encode the subunits of a bacterial-like RNA polymerase and express many of their genes from polycistronic operons as in bacteria (see Figure 4-13a). Some chloroplast genes contain introns, but these are similar to the specialized introns found in some bacterial genes and in mitochondrial genes from fungi and protozoans, rather than the introns of nuclear genes. As in the evolution of mitochondrial genomes, many genes in the ancestral chloroplast endosymbiote that were redundant with nuclear genes have been lost from chloroplast DNA. Also, many genes essential for chloroplast function have been transferred to the nuclear genome of plants over evolutionary time. Recent estimates from sequence analysis of the *A. thaliana* and cyanobacterial genomes indicate that ≈4500 genes have been transferred from the original endosymbiote to the nuclear genome.

Methods similar to those used for the transformation of yeast cells (Chapter 5) have been developed for stably introducing foreign DNA into the chloroplasts of higher plants. The large number of chloroplast DNA molecules per cell permits the introduction of thousands of copies of an engineered gene into each cell, resulting in extraordinarily high levels of foreign protein production. Chloroplast transformation has recently led to the engineering of plants that are resistant to bacterial and fungal infections, drought, and herbicides. The level of production of foreign proteins is comparable with that achieved with engineered bacteria, making it likely that chloroplast transformation will be used for the production of human pharmaceuticals and possibly for the engineering of food crops containing high levels of all the amino acids essential to humans. ■

KEY CONCEPTS of Section 6.4

Organelle DNAs

- Mitochondria and chloroplasts most likely evolved from bacteria that formed a symbiotic relationship with ancestral cells containing a eukaryotic nucleus (see Figure 6-20).
- Most of the genes originally within mitochondria and chloroplasts were either lost because their functions were redundant with nuclear genes or moved to the nuclear genome over evolutionary time, leaving different gene sets in the organellar DNAs of different organisms (see Figure 6-23).
- Animal mtDNAs are circular molecules, reflecting their probable bacterial origin. Plant mtDNAs and chloroplast DNAs generally are longer than mtDNAs from other eukaryotes, largely because they contain more noncoding regions and repetitive sequences.
- All mtDNAs and chloroplast DNAs encode rRNAs and some of the proteins involved in mitochondrial or photosynthetic electron transport and ATP synthesis. Most animal mtDNAs and chloroplast DNAs also encode the tRNAs necessary to translate the organellar mRNAs.
- Because most mtDNA is inherited from egg cells rather than sperm, mutations in mtDNA exhibit a maternal cytoplasmic pattern of inheritance. Similarly, chloroplast DNA is exclusively inherited from the maternal parent.
- Mitochondrial ribosomes resemble bacterial ribosomes in their structure, sensitivity to chloramphenicol, and resistance to cycloheximide.
- The genetic code of animal and fungal mtDNAs differs slightly from that of bacteria and the nuclear genome and varies among different animals and fungi (see Table 6-3). In contrast, plant mtDNAs and chloroplast DNAs appear to conform to the standard genetic code.
- Several human neuromuscular disorders result from mutations in mtDNA. Patients generally have a mixture of wild-type and mutant mtDNA in their cells (heteroplasmy): the higher the fraction of mutant mtDNA, the more severe is the mutant phenotype.