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REVIEW

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Origin and evolution of mitochondria: what have we learnt from red algae?

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Abstract The purpose of this review is to present an account of our current understanding of the structure, organization and evolution of mitochondrial genomes, and to discuss the origin and evolution of mitochondria from the perspective recently provided by the extensive sequencing of various mitochondrial genomes. Mitochondrial-encoded protein phylogenies are congruent with nuclear phylogenies and strongly support a monophyletic origin of mitochondria. The newly available data from red-algal mitochondrial genomes, in particular, show that the structural and functional diversity of mitochondrial genomes can be accounted for by paralogous evolution. We also discuss the influence of other constraints, such as uniparental inheritance, on the evolution of genome organization in mitochondria.

Key words Mitochondrial genome · Red algae · Evolution

Introduction

According to the theory of endosymbiosis, unicellular phagotrophic eukaryotes, or protists, engulfed prokaryotic organisms, giving rise to eukaryotes with organelles. The establishment of this symbiotic relationship conferred new biochemical activities on the host cell, such as aerobic respiration and/or photosynthesis. The endosymbiotic origin of plastids and mitochondria is now widely accepted, especially since phylogenetic analyses have clearly demonstrated that plastids and mitochondria are derived from eubacterial lineages, related respectively to cyanobacteria and α-proteobacteria (Gray and Doolittle 1982; see Loiseaux-de Goër 1994). The eukaryotic cell should thus be considered as a genetic chimera, descended from the association of different organisms (Sitte 1993).

With respect to the origin and evolution of mitochondria, two protagonists are clearly involved: the mitochondrion, which is the present representative of the symbiont, and the nucleus, corresponding to the host. In order to understand the evolution of such a mosaic cell, three main questions can be raised: (1) did the mitochondrion arise from a single endosymbiotic event (monophyly) or were several symbionts and/or several hosts (polyphyly) involved in the evolution of mitochondria ?, (2) is the uptake of mitochondria an ancient or a recent event in the evolution of eukaryotes ?, (3) can we unravel the nature of the unicellular phagotrophic eukaryote(s) or, at least, its (their) connections with extant protists ? An answer to these questions can perhaps be found by looking at the host history through nuclear phylogenies and at the symbiont history through mitochondrial phylogenies. The comparison of various mitochondrial genome organizations should also help to elucidate the evolution of the mitochondrion.

Before 1991 our knowledge of mitochondrial genomes was restricted to a few clades, i.e. animals, ciliates, land plants and fungi. Given the extreme diversity in size and organization of these genomes, a common ancestor was hardly distinguishable. Over recent years, however, a variety of mitochondrial genomes have been characterized from other eukaryote lineages, including green and red algae, heterokonts, and the rhizopod *Acanthamoeba castellanii*. We review here these recent results and present an account of our present understanding of the structure, organization and evolution of mitochondrial genomes. In particular, as we were involved in the description of the mitochondrial genomes of *Chondrus crispus* (Leblanc et al. 1995a, b) and *Cyanidium caldarium* (Viehmann et al. 1996), an evolved and an ancestral rhodophyte, respectively, emphasis is placed on the interest of this phylum for an understanding of the origin and evolution of mitochondria.

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The origin of the host: nuclear phylogenies

The early eukaryotes and the acquisition of mitochondria

Present-day protists may be thought of both as remnants of the primary host cell and as evolutionary hinges involved in the origin of multicellular eukaryotic lineages. In this respect, the recent accumulation of molecular data concerning unicellular eukaryotes throws a new light on the early history of eukaryotes, as shown in Fig. 1. At the base of ribosomal RNA phylogenies, taxa that lack mitochondria emerge first, in a branching order still somewhat unresolved. They include Microsporidians (*Vairimorpha necatrix*), Diplomonads (*Giardia lamblia*, *Hexamitata inflata*), and Trichomonads, which have also collectively been called "archezoans" (Cavalier-Smith 1993), as well as the primitive parasite *Entamoeba histolytica* (Sogin 1991; Leipe et al. 1993; Ragan and Gutell 1995). Phylogenies based on the elongation factor EF-1α (Hasegawa et al. 1993; Hashimoto et al. 1994; Nordnes et al. 1994), as well as on RNA polymerase (Hashimoto et al. 1992), confirm these early branchings. Most of these organisms are large-sized unicellular parasites which have survived in a poorly oxygenated habitat. Endowed with primitive systems for uptake and digestion, these eukaryotes are devoid of mitochondria. They could be related to the ancestral phagocyte and therefore considered as relics of the evolutionary stage before mitochondrial endosymbiosis (Schlegel 1994). It has been suggested from ultrastructural data (Cavalier-Smith 1993), however, that the hydrogenosomes of Trichomonads have been derived from mitochondria. In addition, genes encoding typically mitochondrial proteins, the *cpn-60* chaperonin genes, have been recently identified in the nuclear genomes of the amitochondriate protists *Trichomonas vaginalis* and *Naegleria fowleri* (Horner et al. 1996) as well as of *E. histolytica* (Clark and Roger 1995). From these findings Horner et al. (1996) hypothesize that mitochondrial endosymbiosis occurred before the divergence of *Trichomonas*. On the other hand, Henze et al. (1995) argue that the amitochondriate protist *G. lamblia* has evolved through "cryptic" (possibly ephemeral) endosymbioses, "resulting in abortive organelle genesis". Based on the available data one cannot yet discriminate between the above-mentioned hypotheses on the origin of mitochondria. In one scenario (Horner et al. 1996), the mitochondrial endosymbiosis occurred very early in evolution, and was followed in several phyla by the loss of the original organelle or by its modification into hydrogenosomes. If this is true, only the microsporidians, which were shown recently to emerge first in the eukaryotic lineage (Galtier and Gouy 1995), would be genuine amitochondriates. The acquisition of mitochondria should therefore be dated around the emergence of archezoans (Fig. 1, arrow a). In the other scenario (Henze et al. 1995) various cryptic symbiotic events occurred in amitochondriate protists, independent from the one that led to the extant mitochondrion. In this case, the mitochondrial endosymbiosis should be placed at the point of divergence of the first mitochondria-containing species (Fig. 1, arrow b).

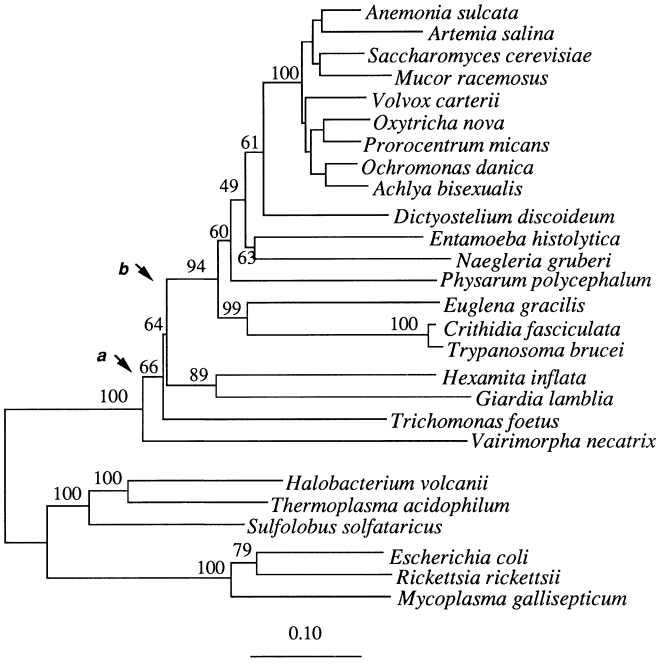


Fig. 1 The multi-kingdom unrooted tree inferred from structural similarities of small-subunit rRNA sequences. Bootstrap percentage values based on 200 re-samplings of the data are shown at the internal nodes. The bar scale corresponds to 10 changes per 100 positions. Reprinted from Leipe et al. (1993), with permission. *Arrow b* indicates the position in the tree after which all taxa possess mitochondria (or have lost them secondarily in the case of *Entamoeba*). *Arrow a* shows another possible location for the acquisition of mitochondria, which postulates a secondary loss or modification of mitochondria in various taxa (see text for more details)

The radiation of higher eukaryotes and algal positions

Based on both ribosomal RNA and protein-coding gene phylogenies, the radiation of the "crown" group of higher eukaryotes is still not well resolved as it corresponds to a period of intensive diversification (Leipe et al. 1993; McFadden et al. 1994a). However, in spite of variations in the global topologies, some groups or clades are clearly monophyletic (Schlegel 1994). Animals represent a robust monophyletic clade, even though the detailed evolutionary relationships within this group are not completely clarified (Christen et al. 1991; Wainright et al. 1993). Eumycetic fungi, i.e. Basidiomycetes and Ascomycetes, also constitute a monophyletic lineage. Animal and Eumycete lineages are closely related in several phylogenetic analyses, whether based on the 18*s* rRNA genes (Sogin 1991; Sitte 1993; Wainright et al. 1993; McFadden et al. 1994a; Bhattacharya et al. 1995a; Ragan and Gutell 1995), on the EF-1α gene (Hasegawa et al. 1993; Nordnes et al. 1994), or on the actin gene (Bhattacharya and Stickel 1994). Metaphyta (Bryophytes, Pteridophytes, and Spermatophytes) also belong to a monophyletic group, which again seems to be closely related to the animal and fungal lineages (Sogin 1991; Wainright et al. 1993; Ragan and Gutell 1995).

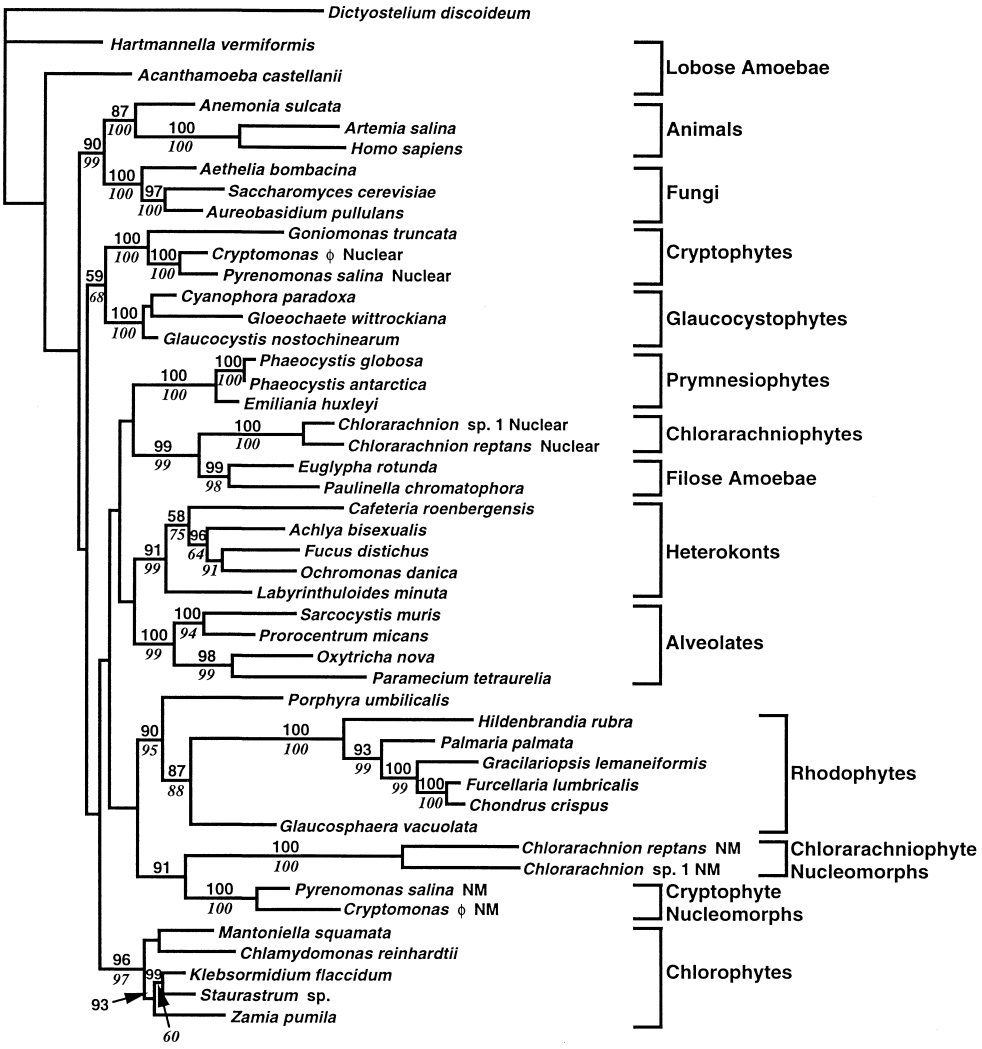


Fig. 2 Phylogeny of eukaryotes based on small-subunit rRNA sequence comparisons inferred with the maximum-likehood method. The phylogeny is rooted within the branch leading to *Dictyostelium discoideum*. Bootstrap analysis (100 replications) was done with the distance and maximum parsimony methods. See Bhattacharya et al. (1995 a) for more details. Reprinted from Bhattacharya et al. (1995a), with permission

Based on their accessory light-harvesting pigments algae were classically divided into four main groups, namely Chlorophytes and Euglenophytes (chl *a*+*b*), "Chromophytes" and Dinophytes (chl *a*+*c*), Cryptophytes (chl *a*+*c*, phycobiliproteins), as well as Rhodophytes and Glaucocystophytes (chl *a* + phycobiliproteins). It is now largely recognized, however, that plastids have arisen through two types of endosymbiotic events: (1) one (or more than one) primary endosymbiosis, in which a photosynthetic prokaryote, the ancestor of extant cyanobacteria, entered a phagotrophic eukaryote, leading to plastids surrounded by two membranes, such as those of Cyanophora, of green and red algae, and of land plants; (2) various secondary endosymbioses, involving several phagotrophic eukaryotes and plastid-containing eukaryotic cells, giving rise to eukaryotes that contain plastids surrounded by three- or four-membraned plastids such as those of "Chromophytes", Cryptophytes, Chlorarachniophytes, Euglenophytes and Dinophytes (Loiseaux-de Goër 1994; Bhattacharya and Medlin 1995). It follows that an algal classification based on pigment composition does not reflect the complex phylogeny of plastids. Furthermore, such a classsification does not reflect the endosymbiotic theory, as it does not take into account the host phylogeny.

Based on the phylogenetic analysis of the 18*s* rRNA gene (Fig. 2) and of other nuclear genes from the main lineages, algae fall into several paraphyletic lineages, i.e. irrespective of common traits in their plastidial ultrastructure and pigment composition. Chlorophytes associate with land plants in a group referred to as the green lineage, the monophyly of which is confirmed in all of the protein and ribosomal phylogenies available so far (Sogin 1991; Hasegawa et al. 1993; Cavalier-Smith 1993; Bhattacharya and Stickel 1994; McFadden et al. 1994a; Nordnes et al. 1994; Ragan and Gutell 1995). Chlorarachniophytes belong to an independent new lineage, associated with the filose amoebae Euglyphyna (McFadden et al. 1994b; Bhattacharya et al. 1995b). They exhibit complex chloroplasts probably derived from the secondary endosymbiosis of a green algal-like organism (McFadden et al. 1995).

The so-called Chromophytes or chlorophyll *a*+*c*-containing algae are phylogenetically separated into different groups: Dinoflagellates which have all sorts of different plastids belong to the Alveolate lineage, which also includes such heterotrophic organisms as Apicomplexa (*Plasmodium falciparum*) and Ciliates (*Paramecium aurelia*) (Gajadhar et al. 1991; Sogin 1991; Wolters 1991;

Cavalier-Smith 1993; Bhattacharya and Stickel 1994; Leipe et al. 1994; Medlin et al. 1994). Heterokonts encompass the Diatomophyceae, Phaeophyceae, Xanthophyceae, Eustigmatophyceae, Chrysophyceae, Rhaphydophyceae and Synurophyceae. Based on the phylogeny of ribosomal (Sogin 1991; Ariztia et al. 1991; Cavalier-Smith 1993; Leipe et al. 1994; Bhattacharya et al. 1995 a) and protein (Bhattacharya and Stickel 1994) genes, they are grouped with the so-called lower fungi, the Oomycetes. Heterokonts radiate sometimes as a sister group of the Alveolates in the 18*s* rRNA nuclear phylogenetic tree (Medlin et al. 1994). Prymnesiophytes or Haptophytes form a group distinct from Heterokonts and Alveolates, with no close relationship to the other phyla (Ariztia et al. 1991; Cavalier-Smith 1993; Leipe et al. 1994; McFadden et al. 1994a; Medlin et al. 1994; Bhattacharya et al. 1995 b).

Once considered as primitive organisms, because of their morphology, the nature of their plastids and from 5*s* rRNA phylogenies (Hori and Osawa 1987), Rhodophytes are now recognized as a lineage that emerged contemporaneously with the other higher eukaryote lineages, based on evidence from both ribosomal RNA trees (Hendriks et al. 1991; Cavalier-Smith 1993; Ragan et al. 1994; Bhattacharya and Medlin 1995) and protein-encoding gene trees (Bouget et al. 1995; Ragan and Gutell 1995). This phylum is not associated with any other heterotrophic lineage and its emerging position within the eukaryotic "crown taxa" is not well identified. However, increasing molecular and biochemical evidence suggests that Rhodophytes and green plants are sister groups (Ragan and Gutell 1995; Cerff 1995). This idea is supported by the claim for a monophyletic origin of green and red plastids (Reith 1995). Cryptophytes also emerge as an independent branch, convincingly related to the flagellate, phagotrophic heterotrophic, *Goniomonas truncata* (McFadden et al. 1994a). At the origin of their plastids the endosymbiotic eukaryotes are related to Rhodophytes (Douglas et al. 1991; McFadden et al. 1994a; Fig. 2). Glaucocystophytes (chl *a* + phycobiliprotein) form a small distinct group of cyanelle-containing photosynthetic protists that share a common ancestry with Cryptophytes in the phylogenetic tree presented by Bhattacharya et al. (1995a).

Structure and organization of mitochondrial genomes

The ancestral, prokaryotic mitochondrial genome has undergone important re-structuring which gave rise to the present-day mitochondrial genome. This includes massive gene transfer from the primary symbiont to the nucleus, which now controls the major part of mitochondrial biogenesis and functions (Gray 1993), as well as a probable loss of those genes that were redundant in the newly established eukaryotic cell. This evolutionary process is still in progress, as gene transfer to the nucleus has been shown to have occurred recently in leguminous plants (Brennicke et al. 1993). Besides the transfer or the deletion of mitochondrial genes, several factors can account for the evolution of mitochondrial genome size and organization. These include a change in the proportion of intergenic regions, the presence or absence of introns, and the occurrence of intra- and inter-molecular recombination. Overall, mitochondrial genomes are highly diverse in their size and physical organization (Table 1), both between various eukaryotic phyla as well as within several lineages. As the mitochondrial genome organization of the main eukaryotic taxa was recently extensively reviewed (Gray 1992), only a brief survey of mtDNA architecture is provided below, with more emphasis on the newly characterized genomes.

Genome sizes

Mitochondrial genome organization ranges between two extremes, which are epitomized by the animal and plant mitochondrial genomes, respectively (Table 1). Animal mitochondria display small, circular genomes with a typical, uniform architecture (Wolstenholme 1992). Their size ranges from 14 kb (*Caenorhabditis elegans*) to 42 kb (*Placopecten magellanicus*). Such a small size is associated with a large compactness of the genetic information. Genes are either separated by a few nucleotides, or are contiguous or overlap and, with the exception of the sea anemone *Metridium senile* mtDNA (Wolstenhome 1992), they lack introns. Animal mitochondrial DNAs do not normally exhibit recombination and their gene arrangement is well conserved, especially within vertebrates. Animal mitochondrial genes, however, are characterized by a high substitution rate.

In contrast, land plants exhibit the largest-sized mitochondrial DNAs having the most complex genome architecture. They display extended molecular recombination that involves large, direct or inverse repeated sequences leading to an equilibrium of various subgenomic molecules. The only exceptions known so far are those of *Marchantia polymorpha* (184 kb) and *Brassica hirta* (208 kb), in which the mitochondrial genomes are unicircular. Other plant mitochondrial genomes are described as a master circle that encompasses all of the subgenomic information. Altogether, plant mitochondrial genomes are large, yet quite heterogenous in size, ranging from 184 kb in the bryophyte *M. polymorpha* to 2400 kb in the watermelon *Cucumis melo* (for reviews see Gray 1992; Lonsdale and Grienenberger 1992; Schuster and Brennicke 1994). The mitochondrial DNA of the bryophyte *M. polymorpha* has been completely sequenced. The size difference between this genome and the small, compact animal mtDNAs is mainly due to a large proportion (70%) of extended intergenic regions, to the presence of duplicated sequences and, to a smaller extent, to the presence of introns and additional genes. In angiosperms the even larger mtDNA size is accounted for by the frequency of recombination that duplicates both the coding and non-coding regions and also by the integration of non-mitochondrial sequences such as plastidial sequences (for a review see Schuster and Brennicke 1994). Recombination in plant mitochondrial genomes also results in rapid gene rearrangement. In contrast

Table 1 Physical caracteristics of mitochondrial genomes. For references see: animals (Wolstenholme 1992); fungi (Clark-Walker 1992; Paquin and Lang 1996; Internet site: FMGP at http://megasun.bch.unmontreal.ca); green plants (Lonsdale and Grienenberger 1992; Schuster and Brennicke 1994; Oda et al. 1992a); green algae (Wolff et al. 1994; Gray 1992; Denovan-Wright and Lee 1992; Kessler and Zetsche 1995); *C. crispus* (Boyen et al. 1994b; Leblanc et al. 1995a); *P. purpurea* (Burger, Lang and Gray, personal communication); *C. cadarium* (Viehmann and Zetsche, unpublished data); other red algae and *E. gracilis* (Coleman and Goff 1991); Heterokonts (Lang and Forget 1993; Internet site: OGMP at http://megasun.bch.unmontreal.ca); *P. littoralis* (Fontaine et al. 1995a, b); *A. castellanii* (Burger et al. 1995); other protists (Gott et al. 1993; Cole and Williams 1994; Angata et al. 1995); ciliates (Cummings 1992); *P. falciparum* (Feagin et al. 1992); kinetoplasts (Stuart and Feagin 1992). Symbols: +, feature present; –, feature absent; ?, partial or insufficient data

Organism	Size (kpb)	Configuration	AT%	Non-coding sequence	Recombination	Intron (groups I, II)
Animals						
Vertebrates	16	Circular	54–64%	<<10%	–	–
Others	14–42	Circular (cnidarian excepted)	45–80%	<<10%	–	– (except anemone)
Fungi						
Filamentous ascomycetes	20–115	Circular + subgenomes	70–82%	Variable	+	+
Ascomycetous yeasts	17–85	Circular + subgenomes	70–82%	15–63%	+	+
Basidiomycetes	33–180	Circular	78%[a]	Variable	–?	+
Zygomycetes	25; 54[b]	Circular	73.9%[c]	?	?	+
Chytridiomycetes	17–95	Circular	60%[d]	?	?	+
Green plants						
Angiosperms	200–2400	Multicircular	52%	>90%	+	+
Marchantia polymorpha	184	Circular	57%	70%[e]	–	+
Green algae						
Protheca wickerhamii	55	Circular	74%	35%	–	+
Chlamydomonas reinhardtii	15.8	Linear	54.8%	17%	–	–
Chlamydomonas eugametos	24	Circular	?	?	–	+
Chlamydomonas moewusii	22	Circular	?	?	–	+
Tetraselmis subcordiformis	48.2	Circular	65%	?	?	–
Red algae						
Gracilaropsis lemaneiformis	40	Circular	?	?	?	?
Griffithsia pacifica	25–28	Circular	?	?	?	?
Chondrus crispus	26	Circular	72.1%	4.8%	–	1
Porphyra purpurea	38	Circular	66.5%	?	+	2
Cyanidium caldarium	33	Circular	73%	?	–	?
Heterokonts						
Oomycetes	36–73	Circular	76%[f]	?	–	–
Pylaiella littoralis	58	Circular	?	?	–	+
Cafeteria roenbergensis	43.2	Circular	?	?	?	–
Ochromonas danica	41.1	Linear	?	?	?	–
Chrysodidymus synuroideus	33	Circular	?	?	?	–
Protists						
Acathamoeba castellanii	41	Circular	70%	6.8%	–	+
Dictyostelium discoideum	54	Circular	70%	Low	?	–?
Physarum polycephalum	60	Circular	?	?	?	?
Ciliates						
Paramecium aurelia	41	Linear	59%	44%	–	–
Tetrahymena pyriformis	55	Linear	75%	?	–	–
Apicomplexa						
Plasmodium falciparum	6	Linear	68%	?	–	–
Euglenozoa						
Kinetoplasts	20–35 0.4–2.5	“Maxi-Circles” “Mini-Circles”	?	?	?	–
Euglena gracilis	60	?	?	?	?	?

[a] *Schizophyllum commune* mtDNA has an AT% of 78
[b] The size of *Phycomyces blakesleanus* mtDNA is 25 kbp and of *Rhizopus stolonifer* is 54 kbp
[c] *Rhizopus stolonifer* mtDNA has a AT% of 73.9
[d] *Allomyces macrogenus* mtDNA has an AT% content of 60
[e] The % of non-coding sequences has been calculated including all ORFs identified by the authors
[f] *Phytophthora infestans* mtDNA has an AT% of 76

with other eukaryotes, however, plant mitochondrial gene sequences are characterized by a very low mutation rate.

Mitochondrial genomes of fungi fall in between the two extreme trends described above. More heterogeneous in their size and organization than animal genomes, they consist of circular molecules ranging from 17 to 180 kb (Table 1; for a review see Clark-Walker 1992). Compared to animal mtDNAs the presence of introns and of non-coding sequences accounts for the size difference. In filamentous Ascomycetes the presence or lack of introns is the main factor that explains mtDNA inter- and intra-species heterogeneity. In budding Ascomycetes (yeasts), introns also contribute to the increase of the genome size but the main variations are due to the proportion of non-coding regions. In these AT-rich intergenic regions, small, mobile GC-rich repeated elements are targets for recombination, which generates subgenomic molecules and gene rearrangements (Clark-Walker 1992).

Little information is available on the organization of the mitochondrial genomes of other eukaryotes. Within the green lineage, the unicellular alga *Chlamydomonas reinhardtii* (Volvocales) has a linear genome (15.8 kb), while the mtDNAs of *C. eugametos* and *C. moewusii* (24 and 22 kb, respectively) are circular. Compared to animal mtDNAs, these three genomes encode a reduced number of genes (Denovan-Wright and Lee 1992; Gray 1992). In contrast, the primitive green alga *Tetraselmis* (*Platymonas*) *subcordiformis* (Prasinophyceae) (Kessler and Zetsche 1995) and the colourless "green" alga *Prototheca wickerhamii* (Chlorococcales) (Wolff et al. 1994) exhibit larger circular mtDNAs – 48.2 kb and 55 kb respectively – with an approximately five-fold increased gene content compared with *Chlamydomonas*. While no introns have been detected so far in the mtDNA of *T. subcordiformis*, several introns and intergenic repeated sequences are present in the mtDNA of *P. wickerhamii*. The mt genome of *T. subcordiformis* contains an inverted repeat of about 1.5 kb. The mitochondrial DNA of the rhizopod *A. castellanii* (41 kb) contains approximately the same genetic information as that of *P. wickerhamii* mtDNA, yet in a more compact form, including the presence of overlapping genes and reduced intergenic regions (Burger et al. 1995). The oomycete *Phytophthora infestans* (Lang and Forget 1993) and the brown alga *Pylaiella littoralis* (Loiseaux-De Goër, personal communication) feature mitochondrial genomes globally similar to those of *A. castellanii* and *P. wickerhamii* in both their size (from 33 to 58 kb) and their gene content. A conspicuous difference is the lack of introns in *P. infestans* whereas several group-IIB introns have been described in *P. littoralis* and group-I introns in *A. castellanii* mitochondrial LSU rDNA (Burger et al. 1995; Fontaine et al. 1995 a).

The partial data available concerning the mitochondrial genome of the slime mold *Dictyostelium discoideum* suggest a high information density (Cole and Williams 1994; Angata et al. 1995). Its relatively large size (54 kb) may therefore be due to the presence of additional genes that have been transfered to the nucleus in other more-evolved organisms (Cole and Williams 1994). In Ciliates, the mtDNA is linear, with a size of 55 kb for *Tetrahymena pyriformis* and of 45 kb for *P. aurelia* (Cummings 1992). In kinetoplastid trypanosomes the mitochondrial genome is composed of several circular molecules with variable sizes, referred to as mini-circles (0.6–2.5 kb) and maxi-circles (20–35 kb), which coexist in a dynamic equilibrium (Stuart and Feagin 1992). The smallest mitochondrial genome known so far (6 kb), is that of the parasite *P. falciparum* (Apicomplexa) which includes only five genes (Feagin et al. 1992).

The mitochondrial genome of the red alga *C. crispus* (Leblanc et al. 1995 a) resembles the animal mt genomes in its small size (26 kb) and its high coding density. It does not contain recombinating repeated sequences, harbors only one intron, and the non-coding regions total only 4.8% of the genome. The mt genome of the red alga *Porphyra purpurea* (Burger, Lang and Gray, personal communication) is also a small molecule, 38 kbp in length. This mtDNA is characterized by the presence of inverted repeats and contains two group-II introns in the *rnl* gene. At 33 kb the mtDNA of the primitive unicellular red alga *C. caldarium* is within the same range as that of the multicellular red algae. No introns or repeated sequences were detected in the mtDNA of this alga (Viehmann and Zetsche, unpublished).

Gene content

In most eukaryotes mitochondrial genomes have conserved the genes that encode the hydrophobic subunits of the respiratory complexes. In particular, the genes of cytochrome b (*cob*, complex III) and subunits 1–3 of cytochrome oxidase (*cox1–3*, complex IV) have been identified in nearly all mitochondrial genomes studied so far (Table 2). Similarily, in all of the eukaryotes investigated to-date the genes encoding the small and large subunits of mt-rRNA have remained localized in the mitochondrial genome (Table 3). We will only discuss genes that are endowed with evolutionary significance.

Respiratory chain proteins and genes with unidentified function

Most mitochondrial genomes possess at least seven genes encoding subunits of NADH dehydrogenase (respiratory complex I). In addition subunits NAD7 and NAD9 are encoded in mitochondria of plants, of *P. wickerhamii* and of a few protists. Moreover in these protists, as well as in heterokonts, NAD11 is mitochondria-encoded (Table 2). Several mitochondrial genomes also encode subunits α, 6, 8 and 9 of ATP synthase (complex V, *atp1*, *atp6*, *atp8* and *atp9* genes, respectively). The *atp8* gene, however, has been identified only in mtDNAs from animals (except nematodes), from fungi, and from two chrysophytes (Table 2). The *atp1* gene has been identified in the mitochondrial genomes of land plants and in those of *P. wickerhamii*, *A. castellanii*, *P. infestans* and *Cafeteria roenbergensis* (Table 2).

Table 2 Respiratory chain genes encoded by mitochondrial genomes. This table is modified from Gray (1992) and completed with data from newly-characterized mitochondrial genomes: fungi (Brown 1993; Collins 1993; Grivell 1993; Lang 1993; Sekito et al. 1995; Paquin and Lang 1996 and Internet site: FMGP at http://megasun.bch.unmontreal.ca), higher plants (Schuster and Brennicke 1994), *T. subcordiformis* (Kessler and Zetsche 1995), *C. crispus* (Leblanc et al. 1995a), *C. caldarium* (Viehmann and Zetsche, unpublished data), *A. castellanii* (Burger et al. 1995), *D. discoideum* (Cole and Williams 1994; Angata et al. 1995), *P. infestans* (Lang and Forget 1993), *C. roenbergensis* and *O. danica* (Internet site: OGMP at http://megasun.bch.umontreal.ca) *T. brucei* (Accession number M94286). Gene designations: *nad*, subunit of NADH dehydrogenase (Complex I); *cob*, apocytochrome *b* component of ubiquinol-cytochrome *c* oxydoreductase (Complex III); *cox*, subunit of cytochrome *c* oxidase (Complex IV); *atp*, subunit of ATP synthase (Complex V); *sdh*, subunit of succino dehydrogenase (Complex II). The unassigned ORFs are named according to publications. Symbols: +, gene present; –, gene absent; (+), pseudogene; [+], additional unassigned ORFs present; ?, partial or insufficient data; FA, Filamentous Ascomycetes; AY, Ascomycetous Yeasts; B, Basidiomycetes; Z, Zygomycetes; C, Chytridiomycetes. a) According to the Commission on Plant Gene Nomenclature (Plant Mol Biol Rep vol 12, Number 2, Suppl, June 1994) the gene encoding the iron-sulfur protein subunit of the NADH-UBQ oxidoreductase is named *nad8* and the gene encoding the 20-kDa protein subunit of the NADH-UBQ oxidoreductase is named *nad10*

Organism	*nad*												*cob*	*cox*			*atp*				*sdh*			Unassigned ORFs			
	1	2	3	4	4L	5	6	7	8[a]	9	10[a]	11		1	2	3	1	6	8	9	2	3	4	*orfB*	*orf25*	*orfx*	Others
Animals																											
Animals except nematodes	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–
Nematodes	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–
Fungi																											
Aspergillus nidulans FA	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	+	(+)	–	–	–	–	–	–	*urfA3*
Schizosaccharomyces pombe AY	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	+	+	+	–	–	–	–	–	–	*urfa*
Hansenula wingei AY	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	+	+	–	–	–	–	–	–	[+]
Schizophyllum commune B	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	+	+	–	–	–	–	–	–	[+]
Rhizopus stolonifer Z	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	+	+	–	–	–	–	–	–	?
Allomyces macrogynus C	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	+	+	–	–	–	–	–	–	[+]
Green plants																											
Angiosperms	+	+	+	+	+	+	+	+	–	+	–	?	+	+	+	+	+	+	?	+	–	–	?	+	+	+	[+]
Marchantia polymorpha	+	+	+	+	+	+	+	(+)	–	+	–	–	+	+	+	+	+	+	–	+	–	*orf137*	+	*orf172*	*orf183*	*orf244*	[+]
Green algae																											
Prototheca wickerhamii	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	+	+	+	–	+	–	–	–	*orf234.2*	*orf183*	*orf234.1*	[+]
Chlamydomonas reinhardtii	+	+	–	+	–	+	+	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	*rtl*
Tetraselmis subcordiformis	?	+	+	+	?	+	+	+	?	?	?	?	+	+	+	+	?	+	?	?	+	+	?	?	?	?	?
Red algae																											
Chondrus crispus	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	–	+	+	+	+	*orf142*	*orf183*	*orf262*	[+]
Cyanidium caldarium	+	+	+	?	+	+	+	?	?	?	?	?	+	+	+	+	?	+	?	?	+	+	+	?	?	?	?
Protists																											
Acanthamoeba castellanii	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	–	–	–	*orf137*	*orf124*	–	[+]
Dictyostelium discoideum	+	?	+	?	?	+	+	?	?	+	?	=80-kDa subunit	+	+	?	?	?	+	?	+?	?	?	?	?	?	?	*orf104*
Heterokonts																											
Phytophthora infestans	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	–	–	–	–	*orf248*	–	[+]
Cafeteria roenbergensis	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	–	–	–	?	–	–	[+]
Ochromonas danica	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	+	+	–	–	–	?	–	–	[+]
Cilliate																											
Paramecium aurelia	+	+	+	+	–	+	–	+	–	+	*psbG*	?	+	+	+	–	–	–	–	+	–	–	–	–	–	–	[+]
Kinetoplast																											
Trypanosoma brucei	+	?	?	+	?	+	?	+	+	–	–	?	+	+	+	+	?	+	?	?	?	?	?	?	?	?	MURF1–6
Apicomplexa																											
Plasmodium falciparum	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–

Table 3 Translation-apparatus genes encoded by mitochondrial genomes. This table is modified from Gray (1992) and completed with data from newly-characterized mitochondrial genomes (see Table 2 for references). Gene designations: *rrn*, ribosomal RNA (SSU, small subunit; LSU, large subunit); *trn*, transfer RNA; *rps*, SSU ribosomal protein; *rpl*, LSU ribosomal protein; *dpo*, DNA polymerase gene. Symbols: +, gene present; –, gene absent; ?, partial or insufficient data. (a): recent data (Lang et al. 1996) would seem to rule out the presence of a 5*s* rRNA gene in red algal mtDNA generally

Organism	*rrn*			*trn*	*rps*												*rpl*								Others
	SSU	LSU	5*s*		1	2	3	4	7	8	10	11	12	13	14	19	2	5	6	11	12	14	16	20	
Animals	+	+	–	22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fungi																									
Aspergillus nidulans	+	+	–	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Schizosaccharomyces pombe	+	+	–	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hansenula wingei	+	+	–	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*var1*
Schizophyllum commune	+	+	–	24	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Rhizopus stolonifer	+	+	–	24	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Allomyces macrogynus	+	+	–	25	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Green plants																									
Angiosperms	+	+	+	<20	+	?	+	?	+	?	?	?	+	+	+	+	+	+	?	?	?	?	+	?	?
Marchantia polymorpha	+	+	+	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–
Green algae																									
Prototheca wickerhamii	+	+	+	26	–	+	+	+	+	–	+	+	+	+	+	+	–	+	+	–	–	–	+	–	–
Chlamydomonas reinhardtii	+	+	–	3	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–
Tetraselmis subcordiformis	+	+	?	<7	?	?	?	?	?	?	?	?	?	?	?	+	?	?	?	?	?	?	+	?	?
Red algae																									
Chondrus crispus	+	+	? (a)	23	–	–	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–
Cyanidium caldarium	+	+	?	<8	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	+	?	+	?
Protist																									
Acanthamoeba castellanii	+	+	–	16	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	–	–
Heterokonts																									
Phytophthera infestans	+	+	–	25	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–
Cafeteria roenbergensis	+	+	–	20	–	+	–	+	–	+	–	–	+	+	+	+	+	–	+	–	–	+	+	–	–
Ochromonas danica	+	+	–	23	–	–	+	–	+	+	+	–	+	+	+	+	+	–	+	–	–	+	+	–	*dpo*
Ciliate																									
Paramecium aurelia	+	+	–	4	–	–	–	–	–	–	–	–	+	–	+	–	+	–	–	+	–	–	–	–	–
Kinetoplast																									
Trypanosoma brucei	+	+	–	0	?	?	?	?	?	?	?	?	+	?	?	?	?	?	?	?	?	?	?	?	?

The systematic sequencing of various mitochondrial genomes has led to the identification of several, apparently functional open reading frames (orf), the products of which are not yet characterized. Among them are orf25, orfB and orfx, first identified in land-plant mtDNAs and which were thought to be specific to this lineage. Homologous mitochondrial orfs, however, have since been characterized in the green alga *P. wickerhamii*, the amoeba *A. castellanii*, the oomycete *P. infestans* and in the red alga *C. crispus* (Table 2). In addition, the genes for two subunits of succinate dehydrogenase, SDH2 and SDH3 (complex II), which are usually encoded by the nucleus, were located on *C. crispus* mitochondrial DNA (Leblanc et al. 1995a). This finding has been confirmed for two other rhodophytic algae, *C. caldarium* (Viehmann et al. 1996) and *P. purpurea* (Burger et al. 1996). Moreover, ORF 137 encoded on the *M. polymorpha* mitochondrial genome presents sequence homology with yeast SDH3 (Daignan-Fornier et al. 1994). The *orf84* gene from *C. crispus*, as well as *orf86a* from *M. polymorpha*, exhibit sequence homology with a putative *sdh4* gene from *Rickettsia prowazekii* (Burger et al. 1996).

Genes involved in translation

Compared to eubacteria, the organization of the mitochondrial ribosomal genes is poorly conserved in mitochondrial genomes (Table 3). With the exception of land plants, the genes of small and large subunits of ribosomal RNA, *rns* and *rnl* respectively, are no longer organized in an operon in mitochondrial genomes. They may even be discontinuous, as in the green alga *C. reinhardtii* (for a review see Gray 1992). There are also important differences in the size and secondary structure of the products of these genes. Animal and fungal mitochondria exhibit rRNAs with reduced sizes (12*s* and 16*s*, and 15*s* and 21*s*, respectively), resulting from the deletion of peripheral loops in their secondary structure. In contrast, green plants harbor mitochondrial *rns* and *rnl* rRNA genes that contain large additional loops compared to eubacteria. Moreover, land-plant mitochondrial DNA encodes a 5*s* rRNA, whose gene is always linked to the small subunit rRNA gene. This gene has been considered as an evolutionary trait specific to mitochondria from the green lineage (Wolff et al. 1994). Based on sequence comparisons among several species, Lang et al. (1996) have identified a 5*s* rRNA gene in the mitochondrial genome of the primitive protist *Reclinomonas americana* but did not find traces of 5*s* rRNA genes in the two red algae *P. purpurea* and *Gracilariopsis lemaneiformis*. They conclude that there is no 5*s* rRNA gene or pseudogene in red-algal mtDNA. These data question our proposed identification of a sequence in the *cox2-cox3* intergenic region of *C. crispus* mtDNA (Leblanc et al. 1995a) as a 5*s* rRNA pseudogene.

In animals, *Chlamydomonas*, and most fungi, all genes encoding ribosomal proteins are nuclear (Table 3). In contrast, the mitochondrial genomes of *M. polymorpha* (Takemura et al. 1992) (and probably that of angiosperms), of *P. wickerhamii*, of *A. castellanii* and of *P. infestans* display a large number of ribosomal protein-encoding genes (10–12 *rps* and 3–6 *rpl* genes, encoding SSU and LSU ribosomal proteins, respectively). Most of these genes are organized in operons that are fairly conserved relative to those of *Escherichia coli* (Takemura et al. 1992; Wolff et al. 1994; Burger et al. 1995). In this respect the mitochondrial genome of the rhodophyte *C. crispus*, which contain three *rps* and one *rpl* gene only, shows a trend similar to that of animals and fungi.

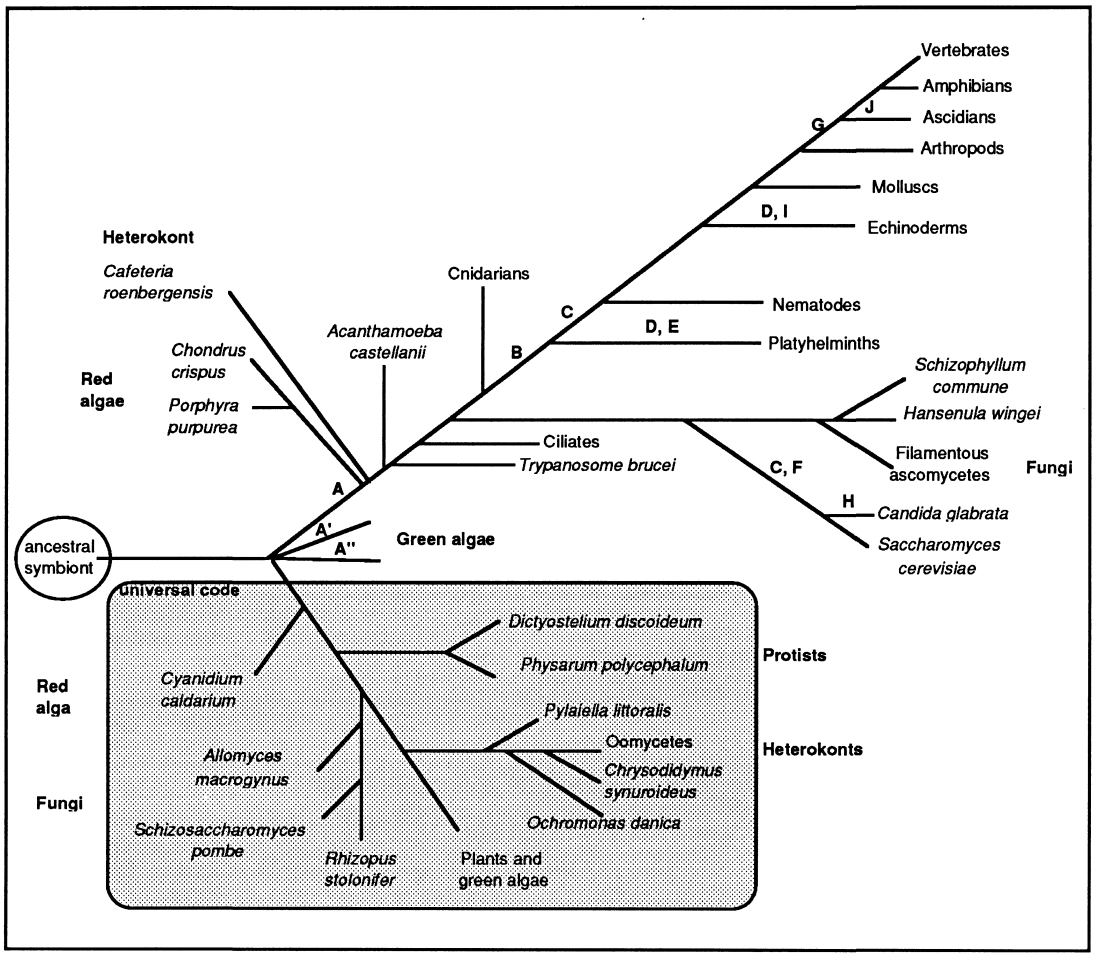
As demonstrated in Table 3, mitochondrial genomes also differ in their number of tRNAs. Animal and fungal mtDNAs encode a variety of tRNAs (22–28) that are sufficient for the translation of all of the protein-encoding genes. In contrast, the mitochondrial set of tRNAs is incomplete in *P. aurelia*, *A. castellanii*, *C. reinhardtii*, and *M. polymorpha* (Gray 1992; Oda et al. 1992b; Burger et al. 1995), as well as in angiosperms where some of the mitochondrial tRNA genes are of plastidal origin (Maréchal-Drouard et al. 1993). In *C. crispus* also, the set of mitochondrial tRNAs does not comprise the minimum necessary for complete translation in mitochondria, indicating a requirement for the importation of two tRNAs from the cytoplasm (Leblanc et al. 1995a).

Peculiarities in the expression of mitochondrial genomes

A modified genetic code

The universal genetic code is used in the mitochondrial genomes of land plants and some green algae (Osawa et al. 1992; Wolff et al. 1994), in those of the oomycete *P. infestans* (Karlovsky and Fartmann 1992), of the brown algae *P. littoralis* (Fontaine et al. 1995b) *O. danica* and *C. synuroideus* (OGMP at http://megasun.bch.umontreal.ca), of the slime molds *D. discoideum* (Cole and Williams 1994; Angata et al. 1995) and *Physarum polycephalum* (Gott et al. 1993), and of various fungi such as the Chytridiomycete *Allomyces macrogynus* (Paquin and Lang 1996), the Ascomycete *Schizosaccharomyces pombe* (Lang 1993) and the Zygomycete *Rhizopus stolonifer* (Paquin et al. 1995b). Deviations from the universal code, however, are widespread in mitochondrial genomes, ranging from one modification (the UGA termination codon to tryptophan) to as many as eight re-assignments in ascidians (Fig. 3). Very recently it has been shown that in several green-algal mitochondria, UGA is a sense codon (Hayashi-Ishimaru et al. 1996). In the same way, the mitochondrial genome of the Basidiomycete *Schizophyllum commune*, which was first supposed to possess the universal code (Specht et al. 1992), actually uses a modified (UGA = tryptophan) code (Paquin et al. 1995 b; FMGP at http://megasun.bch.umontreal.ca).

The evolution of the genetic code does not correspond to the phylogeny of eukaryotes, as closely related groups may use different codes whereas identical modifications appear in distinct nuclear lineages. The modification of the genetic code of mitochondrial genomes is therefore a typ-



	A	A'	A''	B	C	D	E	F	G	H	I	J
codon	UGA	UGA	UGA	AGR	AUA	AAA	UAA	CUN	AGR	CGN	AUA	AGR
univ. code	stop	stop	stop	Arg	Ile	Lys	stop	Leu	Arg	Arg	Ile reass. Met (C)	Arg
mod. code	Trp	Arg	Leu	Ser	Met	Asn	Tyr?	Thr	stop or non coding	non coding	Ile	Gly

Fig. 3 Evolution of the mitochondrial genetic code, modified from Jukes and Osawa (1993). *Letters A–J* refer to codon changes from the universal code, as explained in the table above. Please note that this is not a phylogenetic tree as branches integrate the number and nature of codon reassignments, irrespective of the phylum position. All the organisms in the *shaded box* use the universal genetic code

ical convergence phenomenon, perhaps influenced by similar mutation pressures in mitochondria. According to Osawa et al. (1992), the genetic code has evolved under the influence of mutational bias generated during DNA replication. According to the codon-capture hypothesis proposed by these authors, codon reassignments are selectively neutral events driven by variations of genomic A+T content. On the other hand, Kurland (1992) has argued that mutational bias alone cannot account for the evolution of the genetic code in mitochondria. He suggests that, in response to the forces tending to reduce the size of mitochondrial genomes, codon re-assignments are functionally selected events, leading to an overall decrease in the number of genes coding for tRNA species.

In any case, with the exception of green-algal mtDNA (Hayashi-Ishimaru et al. 1996), re-assignment of the UGA codon from Stop to Trp appears as the earliest modifica-

tion in mitochondria, as it is present in all of the mitochondrial genomes that use a modified genetic code (Fig. 3). In this respect, it is interesting to note that in the multicellular red algae *C. crispus* (Boyen et al. 1994a) and *P. purpurea* (Burger, Lang and Gray, personal communication) Trp is specified by UGA, whereas the primitive unicellular Rhodophyte *C. caldarium* uses the universal code (Zetsche et al., unpublished). However there is no difference in AT content between *C. crispus* (72%) and *C. caldarium* (73%).

RNA editing

In mitochondria of several eukaryotic groups, RNA editing, a post-transcriptional maturation process, modifies the genetic information at the RNA level. Two types of RNA editing have been described, the insertional and the substitutional types. With the exception of the bryophyte *M. polymorpha* (Hiesel et al. 1994), the latter prevails in the mRNAs of land plants (for reviews see Bonnard et al. 1992; Pring et al. 1993). No RNA editing was detected in the *cox3* transcripts of the red alga *C. crispus* and editing is not required in any other of its genes in order to maintain highly conserved amino-acid sequences (Boyen et al. 1994a; Leblanc et al. 1995a).

The symbiont origin: mitochondrial phylogenies

Mitochondrial ribosomal RNA phylogenies

In SSU rRNA global phylogenies, mitochondria cluster with eubacteria, suggesting that they have arisen from an ancestor of a subgroup of α-proteobacteria (Yang et al. 1985). In particular, studies based on the 16*s* rRNA gene, as well as on the heat-shock protein HSP 70, have shown a close relationship with the eubacterial symbiont *Rickettsia* (Olsen et al. 1994; Gupta 1995).

As mentioned earlier (Fig. 1), the acquisition of mitochondria is thought to have been an early event in the course of eukaryote evolution and the symbiont probably became an integral part of the eukaryote cell before the radiation of higher eukaryotes. One may therefore expect the evolution of mitochondrial genes globally to follow the host-cell history and observe congruence between mitochondrial and nuclear phylogenetic trees. In this respect, the emergence of land plants at the base of the ribosomal mitochondrial tree was considered as inconsistent with nuclear phylogenies (Yang et al. 1985; Gray et al. 1989; Leblanc et al. 1995b). This discrepancy led Gray et al. (1989) originally to propose that the angiosperm ribosomal mitochondrial operon (and maybe their mitochondria) may have been gained recently, by a secondary endosymbiotic event. Plant mitochondrial and eubacterial rRNA coding genes, however, exhibit a low mutation rate whereas those of other eukaryotes have evolved more rapidly. Marked differences in evolutionary rates may introduce bias in the construction of phylogenetic trees, such as the "long-branch attracting" effect (Felsenstein 1978), and give rise to artefactual topologies (Cavalier-Smith 1992). Ribosomal phylogenetic trees would thus not reflect mitochondrial evolution but, rather, differences in evolutionary rates among genes from different organisms. Comparison of the secondary structures of both the large and small subunits of mitochondrial rRNA indeed indicates that these genes from red and brown algae and from the green lineage have a close common ancestor (Fontaine et al. 1995a; Leblanc et al. 1995b, c). A separate origin of plant mitochondrial rRNA genes is thus now discounted, even by the original authors (see Gray 1995).

Mitochondrial protein phylogenies

In mitochondrial protein-based phylogenies, the use of amino acids as molecular markers significantly decreases the differences in evolutionary rates, resulting in more homogenous branch sizes (Wolff et al. 1993; Boyen et al. 1994a). Using COX1 and COX3 sequences, a similar approach was applied recently to construct a robust fungal mitochondrial phylogeny (Paquin et al. 1995a). In mitochondrial protein trees, the topology is indeed similar to that of nuclear phylogenies. In particular, the higher-plant lineage radiates contemporaneously with fungal and animal groups in trees based on the *atp9* gene (Recipon et al. 1992), as well as on phylogenies based on the heat-shock protein HSP70 (Gupta 1995). COX1 phylogenies also support the existence of a close common ancestor between the mitochondria of land plants and of the green alga *P. wickerhamii* (Wolff et al. 1993). Moreover, phylogenetic analyses of COX1 and COX2 amino-acid sequences (Sachay et al. 1993), as well as of COB (Angata et al. 1995), position the Oomycete *P. megasperma* and the Myxomycete *D. discoideum* as close relatives of higher plants. Such phylogenies, which bridge land-plant mitochondrial sequences with those of other eukaryotes, favor the monophyly of mitochondria.

Phylogenetic analyses that include mitochondrial proteins from the red alga *C. crispus* also support the hypothesis of a single origin for mitochondria. In the COX3 phylogenetic tree, red algae indeed appear localized at the base of the green lineage, showing that *C. crispus* mitochondria share a relatively close common ancestor with those of green plants (Boyen et al. 1994a). This finding is confirmed when using concatenated amino-acid sequences from the *cob*, *cox1*, and *cox2* genes, including those from the ancestral rhodophyte *C. caldarium* (Fig. 4 and similar analyses presented elsewhere by Lang, personal communication). As mentioned before, in nuclear phylogenies Rhodophytes are considered as an independent phylum, in particular unrelated to the green lineage. If mitochondrial protein phylogenies are considered as congruent with nuclear evolution, and therefore suitable to assess eukaryotic relationships (Paquin et al. 1995a), the above results are in favor of a direct nuclear parenthood between green plants and red algae, as already proposed by Ragan and Gutell (1995).

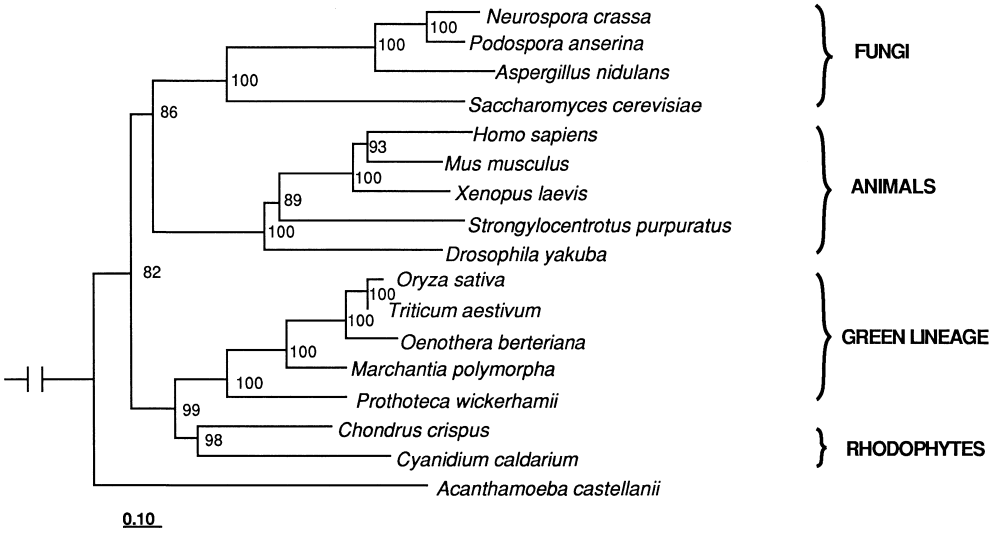


Fig. 4 Phylogenetic analysis of concatenated COX1-COX2-COB amino-acid sequences. The tree was built with the neighbour-joining method applied to a "categories distance matrix" (PHYLIP package) using 1260 informative amino-acid positions. The robustness was tested by bootstrap analysis (PHYLIP 3.5c, Protdist program, Felsenstein 1993, unpublished). *Paramecium aurelia* was chosen as the outgroup. The *horizontal bar* represents 0.1 substitution per nucleotide and branch lengths are drawn to scale. The global topology of the tree is congruent with nuclear trees. The branching of the rhodophytes as a sister group of the green lineage can be inferred with high confidence in this tree but more data are needed to assess this grouping. In particular a more comprehensive phylogeny would include sequence data from other photosynthetic eukaryotes such as Cryptophytes, Heterokonts, Prymnesiophytes, Chlorarachniophytes, Glaucocystophytes as well as from lower eukaryotes. Source of the sequences: *N. crassa* (P03945; P00411; P00162), *P. anserina* (M61734), *A. nidulans* (P00402; P13588; P00162), *S. cerevisiae* (P00401; P00410; P00175), *H. sapiens* (J01415), *M. musculus* (J01420), *X. laevis* (M10217), *S. purpuratus* (X12631), *D. yakuba* (X03240), *O. sativa* (P14578; P04373; P14833), *T. aestivum* (P08741; P00413; P07747), *O. berteriana* (P08743; P05490; P09843), *M. polymorpha* (M68929), *P. wickerhamii* (U02970), *C. crispus* (Z47547) *C. caldarium* (Z48930) *A. castellanii* (U12386). Essentially similar conclusions have been reached by others, using similar methods (Lang, personal communication)

Conclusion

During more than approximately 1-billion years, i.e. from the acquisition of the ancestor of mitochondria to the intense diversification of eukaryotes, the original symbiont evolved in the host cell, leading to an ancestral mitochondrial organization. However, this primary genome that once was shared by all eukaryotes has undergone independent evolution in the different eukaryote lineages, so that the primary mitochondrial genome can hardly be recognized among the extreme molecular diversity of extant mitochondria. In particular, higher-plant mitochondrial genomes exhibit a large size and a marked tendency to recombination, whereas mitochondria of ciliates, animal and fungi are characterized by a reduction of their genome size. Until as late as 1991 no clear evolutionary pattern could be drawn to account for the distant structural and phylogenetical relationships between the land-plant mitchondrial genomes and those of animals and fungi. Over the last 4 years, however, the accumulation of data on the mitochondrial genomes of eukaryotes other than animals, land plants and fungi has unravelled a part of the *terra incognita* between the green-plant mitochondria and those of other eukaryotes.

In particular, Rhodophytes have provided additional links in delineating the evolution of mitochondria between the green-plant lineage and the "non-plant" lineages. In global small subunit (SSU) rRNA trees, green plants branch very close to the root of the mitochondrial subtree, a topology which markedly differs from the branching position of higher plants in nuclear phylogenies. We (Leblanc et al. 1995 b) and others (Paquin et al. 1995 a), however, have stressed the limits of ribosomal phylogenies for the reconstruction of the evolution of mitochondria. In contrast, mitochondrial protein-encoding genes have proven better adapted to address the phylogenetic relationships of mitochondrial genomes. Since they point to a monophyletic origin of mitochondria (e.g. Fig. 4), these results led to the rejection of the hypothesis that a secondary endosymbosis was required to account for the strongly eubacterial character of the ribosomal operons from plant mitochondria (Gray 1993; Leblanc et al. 1995 b; Paquin et al. 1995 a). In addition, since the monophyly of mitochondria implies congruence between their evolution and that of the nucleus, mitochondrial protein genes might provide useful markers to resolve doubtful nuclear phylogenies. For example, nuclear phylogenies are not consistent in inferring the emerging position of red algae. Yet mitochondrial protein phylogenies comprising genes from *C. crispus* and *C. caldarium* (Fig. 4) suggest that red algae are a sister group to green plants. It is obvious, however, that a larger number and variety of mitochondrial protein sequences from other eukaryotic lineages are required to assess the robustness of this putative relationship.

If mitochondria are monophyletic, one may wonder how, after the radiation of higher eukaryotes, they have evolved such a diversity in their genome sizes and organizations, with two major evolutionary trends, i.e. large ge-

nomes with long non-coding regions and which have retained eubacterial features versus reduced, compact, fast-evolving genomes using modified codes. The evidence provided by newly available, complete mitochondrial sequences (Tables 1–3), from *M. polymorpha* (Bryophytes), *P. wickerhamii* (Chlorophytes), *P. anserina* and *A. macrogynus* (Chytridiomycetes), *P. infestans* (Oomycetes), and *A. castellanii* (rhizopods), shows that the apparent inconsistencies between genomic size and the genetic code, on the one hand, and between gene content and phylogenies, on the other, can be accounted for by paralogous evolution resulting from similar evolutionary constraints and strategies.

In this respect, *C. crispus* mtDNA is an interesting example of the convergence phenomena that affect the organization of mitochondrial genomes. As stated above, phylogenetic analyses suggest that red-algal and higher-plant mitochondria share an immediate common ancestor and both have retained primitive features of the ancestral mitochondrial genome in their genes. Yet the mitochondrial genome of *C. crispus* features "non-plant" characteristics, such as a small size, a high coding density, the use of a modified code and the absence of RNA editing. This raises the question of the biological basis of the diversity of mitochondrial genome sizes. A major force driving mitochondrial genome evolution is the genomic economization process, i.e. reduction of genome size by deletion or transfer of mitochondrial genes to the nuclear genome. This process results from competition taking place within a micro-population of organelles, which favors the smaller, faster-replicating mt genomes. According to Kurland (1992), it is the capacity of the nuclear genome to functionally integrate the transferred mitochondrial genes which has allowed the establishment of a successful endosymbiosis. In animal mitochondria, a reduction in the number of tRNA genes has been made possible by the development of a rearranged genetic code via expanded codon recognition (superwobble). In plants and some fungi, this deletion process has been balanced by recombination mechanisms within mitchondria that allowed the recovery of deleted sequences (Kurland 1992).

In addition, Atlan and Couvet (1993) have proposed that there is a relation between genome size, mtDNA copy number, and mutation rate in these uniparentally inherited genomes. According to their model, two opposite strategies could have been selected to prevent the accumulation of deleterious mutations, leading to two major trends in the organization of mitochondrial genomes (Atlan and Couvet 1993). In angiosperms, frequent mitochondrial DNA recombination events would have contributed to the elimination of deleterious mutations, resulting in an overall conservation of gene sequence but leading to the progressive incorporation of non-coding and foreign sequences. In contrast, maintenance of the functional integrity of mitochondrial genomes in animals would result from the compactness of their mtDNAs, which allows for a high copy number but leads to a higher level of non-deleterious mutations correlated with the higher frequency of replication. Within this hypothesis, red-algal mitochondria might have evolved a genome organization similar to that of animals and yet have retained genes that remain phylogenetically close to those of green plants (Leblanc et al. 1995a).

In conclusion, it is obvious that we still do not have either a comprehensive overview of the diversity of mitochondrial genomes or a clear understanding of the evolution of mitochondria. This will require sequencing of mitochondrial DNAs from additional representatives of a variety of higher and lower eukaryote lineages. Algal taxa that belong to lineages other than the Rhodophytes, such as Phaeophytes, Cryptophytes, Dinophytes and Euglenoids, should be useful models in providing insights into the evolution of mitochondria from various, different perspectives.

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