

## Origin and evolution of plastids and mitochondria : the phylogenetic diversity of algae

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**Abstract:** This review presents an account of the current knowledge concerning the endosymbiotic origin of plastids and mitochondria. The importance of algae as providing a large reservoir of diversified evolutionary models is emphasized. Several reviews describing the plastidial and mitochondrial genome organization and gene content have been published recently. Therefore we provide a survey of the different approaches that are used to investigate the evolution of organellar genomes since the endosymbiotic events. The importance of integrating population genetics concepts to understand better the global evolution of the cytoplasmically inherited organelles is especially emphasized.

**Résumé :** Cette revue fait le point des connaissances actuelles concernant l'origine endosymbiotique des plastes et des mitochondries en insistant plus particulièrement sur les données portant sur les algues. Ces organismes représentent en effet des lignées eucaryotiques indépendantes très diverses, et constituent ainsi un abondant réservoir de modèles évolutifs. L'organisation et le contenu en gènes des génomes plastidiaux et mitochondriaux chez les eucaryotes ont été détaillés exhaustivement dans plusieurs revues récentes. Nous présentons donc une synthèse des différentes approches utilisées pour comprendre l'évolution de ces génomes organitiques depuis l'événement endosymbiotique. En particulier nous soulignons l'importance des concepts de la génétique des populations pour mieux comprendre l'évolution des génomes à transmission cytoplasmique dans la cellule eucaryote.

**Keywords :** algae, endosymbiosis, evolution, genetic conflicts, mitochondria, plastids

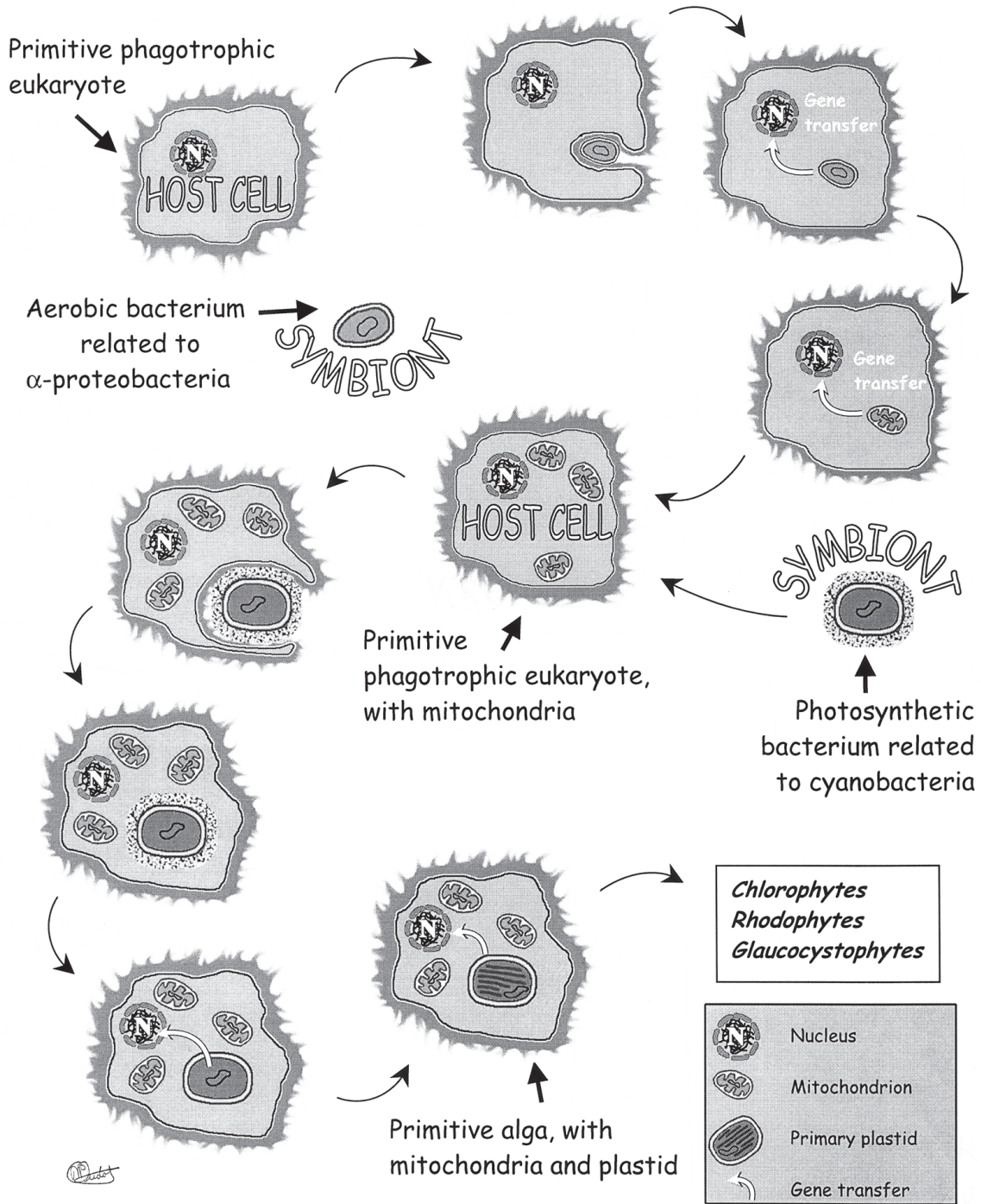
### Introduction

According to the theory of endosymbiosis, unicellular phagotrophic eukaryotes, or protists, engulfed prokaryotic organisms, giving rise to some eukaryotic organelles (Figs. 1, 2). The establishment of these symbiotic relationships conferred new biochemical activities to the host cell, such as aerobic respiration and photosynthesis. The endosymbiotic origin of plastids and mitochondria is now widely accepted (Fig. 1), especially since phylogenetic analyses have clearly

demonstrated that plastids and mitochondria are derived from eubacterial lineages, related respectively to cyanobacteria and  $\alpha$ -proteobacteria (Gray & 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 organelles (mitochondria or plastids), two protagonists are clearly involved: the organelle, which is the present representative of the symbiont, and the nucleus, corresponding to the host.

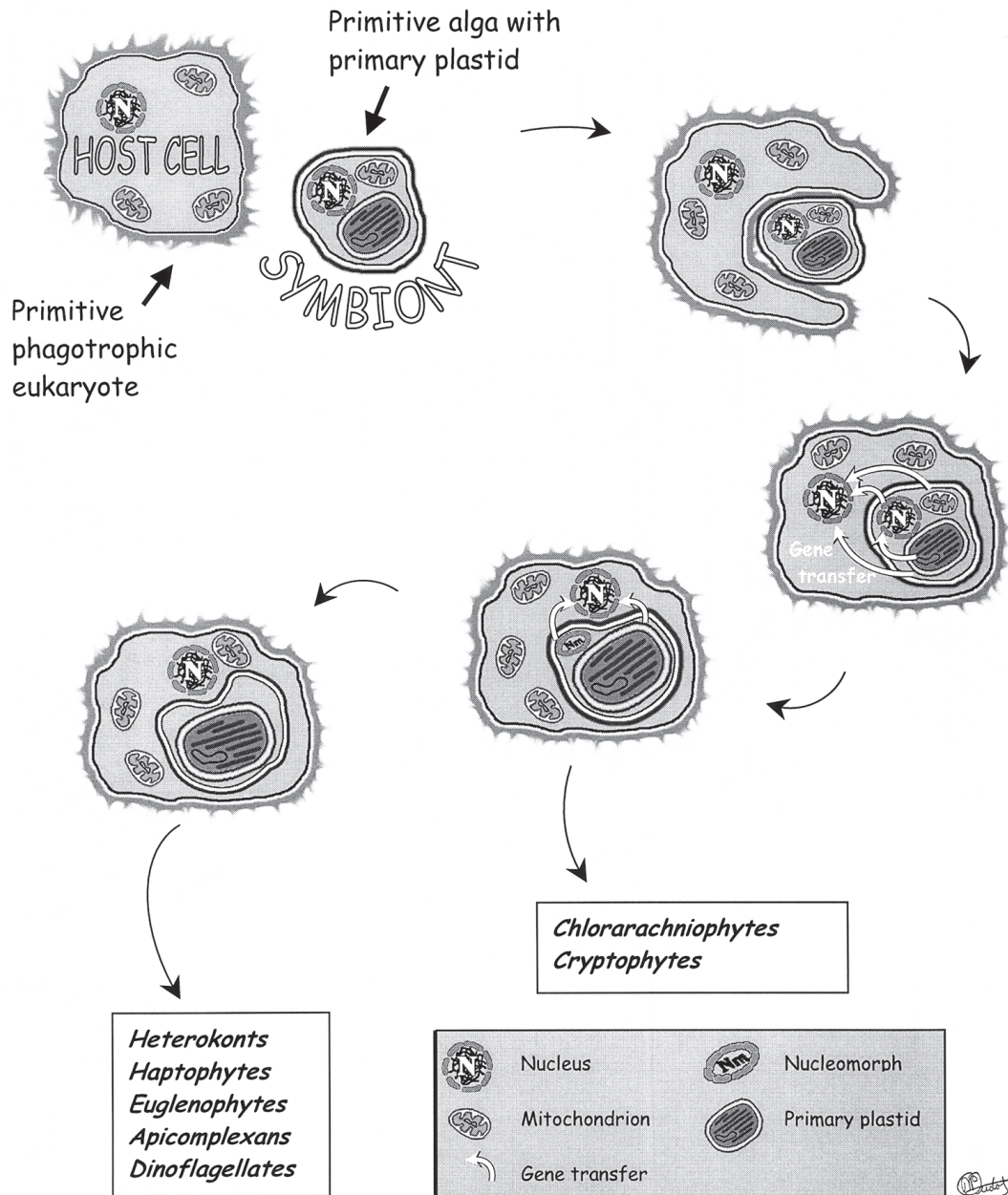
# ORIGIN OF MITOCHONDRIA AND PLASTIDS BY PRIMARY ENDOSYMBIOSIS



**Figure 1.** Origin of mitochondria and primary plastids by primary endosymbiosis. A phagotrophic heterotrophic eukaryote (the host cell) engulfs a prokaryotic cell which becomes the endosymbiont.

**Figure 1.** Représentation schématique des endosymbioses primaires à l'origine des mitochondries et des plastes primaires. Au cours de cette endosymbiose, une cellule eucaryote hétérotrophe ou cellule hôte, phagocyte un organisme procaryote qui deviendra l'endosymbionte.

## ORIGIN OF PLASTIDS BY SECONDARY ENDOSYMBIOSIS



**Figure 2.** Origin of secondary plastids by secondary endosymbiosis involving a heterotrophic eukaryotic cell and a photosynthetic eukaryotic cell, itself arising from primary endosymbiosis. In the course of evolution several modifications occurred such as gene transfer from the endosymbiont to the host cell, as well as progressive loss of cellular structures (cell wall, mitochondria, cytoskeleton) which led to the extant plastids. Certain algae such as Cryptophytes and Chlorarachniophytes have retained a vestigial nucleus, the nucleomorph.

**Figure 2.** Représentation schématique de l'endosymbiose secondaire. Une cellule eucaryote hétérotrophe capture une autre cellule eucaryote photosynthétique provenant elle-même d'une endosymbiose primaire. Au cours de l'évolution, de nombreuses modifications telles que le transfert de gènes de l'endosymbionte vers le noyau de la cellule hôte et la disparition progressive de structures cellulaires du symbionte (paroi, mitochondries, cytosquelette) conduisent à la structure des plastes actuels. Chez certaines algues telles que les Cryptophytes et les Chlorarachniophytes, il existe un noyau résiduel, le nucléomorphe.

In order to understand the evolution of the mosaic cell, three main questions can therefore be raised: (1) did the organelle arise from a single endosymbiotic event (monophyly) or were several symbionts and/or several hosts (polyphyly) involved in the evolution of organelles? (2) Is the uptake of organelles 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?

Several complementary approaches can be considered to answer these questions. Using gene/protein sequence comparisons, it is possible to look at the host history through nuclear phylogenies and at the symbiont history through mitochondrial or plastid phylogenies. Comparison of mitochondrial and plastidial genome organization, as well as their gene expression can also help to elucidate the evolution of organelles. Tracking the ancestral genome through the analysis of primitive organelles aims to gain insight into the early evolutionary steps. In that context the term "primitive" characterizes a mitochondrial or plastidial genome that has retained in the course of evolution ancestral prokaryotic features, irrespective of the real phylogenetic emergence of the referred organism, or, a truly primitive and early eukaryote that can be considered as a relic of the ancestral stage. Recent advances resulting from large scale sequencing programs have provided sequence data for total genomes from various prokaryotes. Among these we can find organisms believed to be descendants of the original symbionts ( $\alpha$ -proteobacteria and cyanobacteria). Comparing their sequence and gene content with those of extant organelles is of great interest and helps in understanding the evolution of these genomes.

The origin and evolution of mitochondria and plastids, as well as their gene content and genome structures, have been reviewed recently (Gray, 1992; Gray, 1995; Bhattacharya, 1997; Leblanc et al., 1997). This paper will therefore provide a survey of the different approaches used to tackle questions of the endosymbiotic origin of plastids and mitochondria. Moreover, we emphasize the great value of algae as research organisms to gain insight into the origin and evolution of organelles as they provide a large reservoir of diversified models.

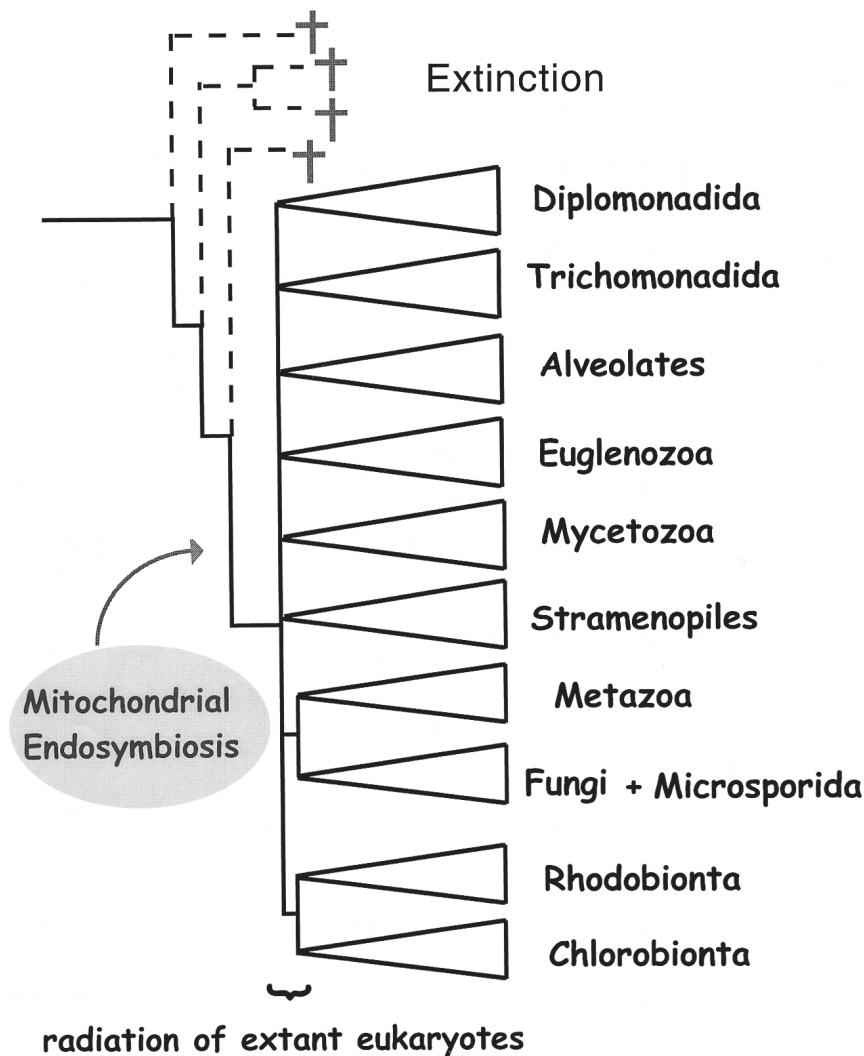
## Mitochondria

### *Origin of the host: nuclear phylogenies*

Over the past few years considerable effort has been focused on resolving the branching order of the so called "early eukaryotes" that usually emerge at the base of the nuclear phylogenies (Leipe et al., 1993). Among these taxa that lack mitochondria are the Microsporidians (*Vairimorpha necatrix*), Diplomonads (*Giardia lamblia*, *Hexamita*

*inflata*), and Trichomonads, which have been collectively called "archeozoans" by Cavalier-Smith (1993). Owing to their primitive organization, and their deep branching in phylogenetic trees, archeozoans were considered as relics of the evolutionary stage before mitochondrial endosymbiosis. They could, therefore, be related to the original host involved in progressive acquisition of mitochondria and plastids. This view, which has prevailed for a relatively long time, is now disputed due to the accumulation of new molecular data and results obtained by new analytical methods. There is now good evidence indicating that mitochondrial endosymbiosis occurred early in eukaryote evolution and was followed in several phyla by the loss of these organelles. Moreover, several data indicate that mitochondria and hydrogenosomes share a common ancestor (Dyall et al., 2000; Bradley et al., 1997). Philippe and Adoutte (1998) proposed a new hypothesis concerning the evolution of eukaryotes. They argued that all amitochondriate early eukaryotic lineages have gone extinct and that the so-called "primitive" eukaryotes actually belong to a relatively recent broad radiation of extant eukaryotes. Their grouping at the base of phylogenetic trees was considered to be due to a long-branch attracting phenomenon. According to Philippe and Adoutte's "big bang" hypothesis "Extant eukaryotes are assumed to have arisen during a period of rapid diversification, perhaps correlated with the establishment of mitochondrial endosymbiosis" (Fig. 3). Philippe and Adoutte suggest that the mitochondrial endosymbiotic event took place 700-1000 millions years ago. However, this dating is in contradiction with fossil records suggesting that multicellular eukaryotes are 950-1250 millions years old (Knoll, 1992). This hypothesis not only challenges the established view of the protist evolution, but also emphasizes the driving force of endosymbiosis in the diversification of eukaryotes. Recently several models have been proposed that question the prevailing view of the origin of eukaryotic cell, implying a symbiosis between an aerobic bacterium and a primitive eukaryotic host. Comprehensive debate of this hypothesis is beyond the scope of this review, however we briefly mention them.

Two of these models argue that the driving force for symbiosis was a requirement for hydrogen by an autotrophic methanogenic **archaeal** host cell. Both hypotheses suggest an anaerobic eubacteria for the origin of protomitochondrial symbiosis but differ in the bacterial affiliation. In the Hydrogen hypothesis (Martin & Müller, 1998) the bacterial partner is a  $\delta$ -proteobacterium whereas the Syntrophy theory (Moreira & Lopez-Garcia, 1998; Lopez-Garcia & Moreira, 1999) proposes two successive bacterial associations, a first one implying a  $\delta$ -proteobacterium and a second one implying a methanogenic  $\alpha$ -proteobacterium. Moreover these hypotheses entail that



**Figure 3.** The “big bang hypothesis” redrawn, with permission from Philippe & Adoutte (1998). Extant eukaryotes are assumed to have arisen during a period of rapid diversification, perhaps correlated with the establishment of mitochondrial endosymbiosis. No extant representative of the earliest eukaryote lineages are left.

**Figure 3.** Représentation schématique de l’hypothèse du “big bang”, avec la permission de Philippe & Adoutte (1998). Selon cette hypothèse les eucaryotes actuels sont apparus au cours d’une période d’intense diversification, peut être corrélée à la mise en place de l’endosymbiose mitochondriale. Il n’existe plus de représentants des eucaryotes primitifs. Ceux-ci auraient disparu précocement au cours de l’évolution.

the origin of mitochondria is strongly connected to the emergence of a eukaryotic cell. The “ox-tox” hypothesis (Andersson & Kurland, 1999; Vellai et al., 1998) states that the driving force for symbiosis was the scavenging of oxygen correlated to the occurrence of the “oxygen-spike” 2000 million years ago. These scenarios also involve the contribution of  $\alpha$ -proteobacteria at the origin of mitochondria. An alternative theory based on functional

comparisons of the biogenesis of hydrogenosomes and mitochondria suggests that mitochondria may have arisen from trichomonad hydrogenosomes (Dyall & Johnson, 2000). This two-step endosymbiotic scenario involves a methanogenic Archaeon that engulfed first a hydrogen-producing bacterium, leading to a proto-hydrogenosome, then an aerobic  $\alpha$ -proteobacterium acting as a detoxifying partner when the atmospheric oxygen increased sharply. Both proto-organelles would then have fused to give rise to a “hydrogenosome/mitochondrion conglomerate”. Although all these theories have to be tested and validated, they might soon make obsolete the classical scheme presented in Figure 1 concerning the origin of mitochondria.

#### *The symbiont origin: mitochondrial phylogenies*

In SSU rRNA global phylogenies, mitochondria cluster with eubacteria, and more precisely with a subgroup of  $\alpha$ -proteobacteria (Yang et al., 1985). In particular, studies based upon the 16S rRNA gene, as well as on the heat-shock protein HSP 70, show a close relationship with the  $\alpha$ -proteobacterial symbiont *Rickettsia* (Olsen et al., 1994; Gupta, 1995).

As mentioned earlier (Fig. 3), the acquisition of mitochondria is thought to have been an early event in eukaryote evolution and the symbiont probably became an integral part of the eukaryote cell before the radiation of all extant eukaryotes. Therefore, one may expect the evolution of mitochondrial genes to follow the host cell history and to observe congruence between mitochondrial and nuclear phylogenetic trees.

In this respect, the emergence of land plants at the base of the SSU ribosomal RNA mitochondrial tree, inconsistent with nuclear phylogenies (Yang et al., 1985; Gray et al., 1989; Leblanc et al., 1995a), was first thought to reveal the occurrence of at least two endosymbiotic events for the origin of mitochondria. The topology was subsequently recognized as an artefact resulting from the “long-branch attracting” effect (Felsenstein, 1978). Ribosomal

phylogenetic trees, therefore, do not exclusively reflect mitochondrial evolution but, rather, can be negatively affected by differences in evolutionary rates among different organisms. In protein-based mitochondrial phylogenies, the use of amino acids as molecular markers significantly decreases the differences in evolutionary rates, resulting in more homogenous branch lengths (Boyen et al., 1994; Wolff et al., 1994; Paquin et al., 1995). In mitochondrial protein trees, the topologies are indeed similar to that of nuclear phylogenies and are in favour of a monophyletic origin for mitochondria. Phylogenetic analyses that include concatenated amino acid sequences from the *cob*, *cox1*, and *cox2* mitochondrial genes from the red algae *Chondrus crispus* and *Cyanidium caldarium* also support the hypothesis of a single origin for mitochondria (Leblanc et al., 1997).

#### *The connection to the prokaryotic ancestor*

The genome sequence of *Rickettsia prowasekii*, an obligate intracellular parasite belonging to the  $\alpha$ -proteobacteria and which is, up to now, the closest known relative to mitochondria, has been recently determined (Andersson et al., 1998). The genome of *R. prowasekii* is small (ca. 1.1 Mbp) compared to other bacterial genomes and contains 25% noncoding sequence, a significantly high proportion for a prokaryotic genome. According to these authors, these features result from a reductive evolution typical of adaptation to the parasitic lifestyle and thus, independent from the mitochondrial history. Nevertheless, the genome of *R. prowasekii* contains 834 protein-encoding genes among which are those exhibiting similarities to typical mitochondrial genes. Moreover, about 300 predicted *R. prowasekii* proteins share significant sequence homologies with yeast nucleus-encoded mitochondrial proteins. Access to the total genome of *R. prowasekii* gives us clues to the ancestral genome of the mitochondrial symbiont.

#### *Diversity of the mitochondrial genomes*

The ancestral prokaryotic mitochondrial genome has undergone substantial changes that gave rise to present-day mitochondrial genomes. These changes include massive gene transfer from the primary symbiont to the host nucleus (Fig. 1), which now controls the majority of mitochondrial biogenesis and functions (Gray, 1993), as well as the probable loss of genes that were redundant in the newly established eukaryotic cell. Several factors can account for the evolution of mitochondrial genome size and organization, such as changes in the proportion of intergenic regions, the presence or absence of introns and the occurrence of intra- and inter-molecular recombinations. Overall, mitochondrial genomes are highly diverse in their

size and physical organization, both among eukaryotic phyla and within lineages (Leblanc et al., 1997). At one extreme, animal mitochondria have small (16 kb), circular genomes characterized by compactness of the genetic information, a high substitution rate and a modified genetic code (Wolstenholme, 1992). At the other extreme, land plants exhibit the largest mitochondrial genomes (200-2400 kb), have more genes, display extended molecular recombination, have RNA editing, and use the universal genetic code (Gray, 1992).

In terms of recent data, studies have particularly focused on unexplored eukaryotic lineages e.g., algae and other heterotrophic protists, that could throw new light on the evolution of mitochondria. The mitochondrial genome of the first red alga was characterized by Leblanc et al. (1995b, 1997). More recently, the complete mitochondrial sequences of two other red alga were released, one from the very primitive unicellular *Cyanidioschyzon merolae* (Ohta et al., 1998) and the other from *Porphyra purpurea*, a member of the Bangiales (Burger et al., 1999). A 10-kb fragment of the mitochondrial genome from *Cyanidium caldarium*, a member of the Porphyridiales is also available (Viehman, 1995). In short, these red algal genomes are rather small (26-38 kb), exhibit a high coding capacity and contain the genes for three subunits of succinate dehydrogenase (SDHB, SDHC, SDHD) that have been transferred to the nuclear genomes of most other eukaryotes. The primitive unicellular rhodophytes *Cyanidium caldarium* and *C. merolae* use the universal code, whereas in the multicellular rhodophytes, *Chondrus crispus* and *Porphyra purpurea*, the tryptophane codon is specified by UGA. Globally, red algal mitochondrial genomes have features similar to the animal type of mitochondria, although phylogenetic analyses suggest that the red and green algal lineages share a common ancestor (Leblanc et al., 1997).

The mitochondrial genome of the brown alga (Heterokonta) *Pylaiella littoralis* (Oudot-Le Secq, 2000) has retained several ancestral features, such as the presence of many genes coding for ribosomal proteins, and rRNA genes closely related to those of  $\alpha$ -proteobacteria (Fontaine et al., 1995a,b). It also contains typical proteobacterial  $\sigma$ -70-like promoter regions not reported in the other known mitochondrial genomes (Delaroque et al., 1996) and a T7-phage-type RNA polymerase (Rousvoal et al., 1998). The genome of this brown alga could represent an early-intermediate stage in the general evolution of mitochondria.

The mitochondrial genome of the early diverging protist *Reclinomonas americana* (Lang et al., 1997) contains more genes (97) than other mitochondrial genomes. It displays many typical eubacterial features found in the genome of *Rickettsia prowasekii*, such as conserved operons and four genes specifying a multisubunit eubacterial-type  $\sigma$ -70 RNA

polymerase. The mtDNA of *R. americana* appears as the most ancestral mitochondrial genome identified to date. Moreover, these data provide evidence that ancestral mitochondrial genomes functioned with eubacterial machinery and that the phage-type RNA polymerase (absent in the *Rickettsia* genome) has probably been recruited after, although early, in the evolution of mitochondria (Cermakian et al., 1997; Rousvoal et al., 1998). The diagram presented in Figure 4 summarizes the state of knowledge concerning the origin and evolution of mitochondria.

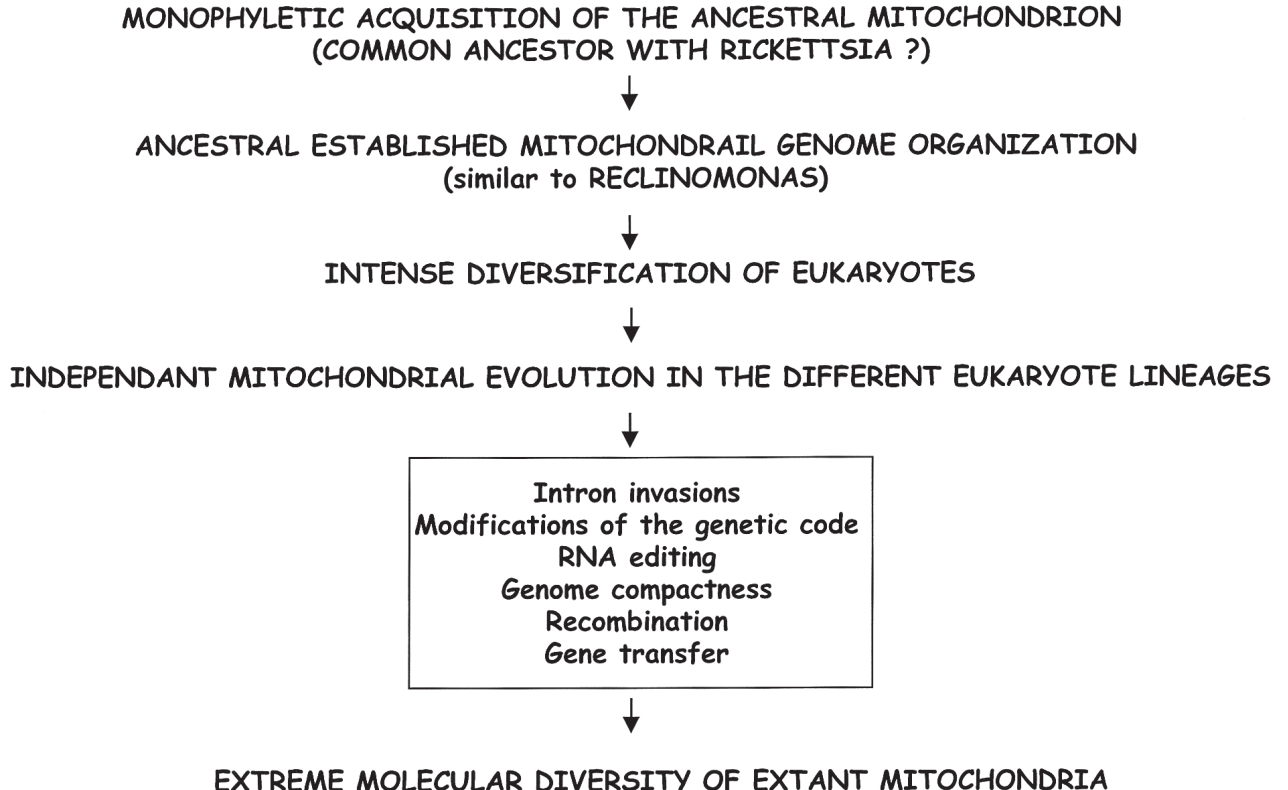
### Plastids

Based on their accessory light-harvesting pigments, algae were traditionally grouped into the Chlorophyta and Euglenophyta (chl *a+b*), "Chromophyta" (chl *a+c*), and Rhodophyta (chl *a* + phycobiliproteins). It is now largely recognized, however, that plastids have arisen through two different types of endosymbiotic events: i) one (or more than one) primary endosymbiosis (Fig. 1), where a photosynthetic prokaryote, related to extant cyanobacteria, entered a phagotrophic eukaryote, leading to plastids

surrounded by two membranes, such as those of Glaucocystophyta, green and red algae, and land plants; and ii) various secondary endosymbioses (Fig. 2) involving several phagotrophic eukaryotes and plastid-containing eukaryotic cells, giving rise to eukaryotes that contain plastids surrounded by three or four-membranes such as those of Heterokontophyta, Cryptophyta, Euglenophyta, Chlorarachniophyta, Haptophyta and Dinophyta (Loiseaux-de Goër, 1994; Bhattacharya & Medlin, 1995). It follows that an algal classification based only on pigment composition does not reflect the complex phylogeny of plastids. Furthermore, such a classification does not reflect the endosymbiotic theory, as it does not take into account the host phylogeny.

#### *The host history: nuclear phylogenies*

Based on phylogenetic analysis of the 18S rRNA gene (Fig. 5), and of other nuclear genes from the main lineages, algae fall into several diverse lineages, irrespective of common traits in their plastidial ultrastructure and pigment composition. Chlorophyta associate with land plants in a group referred to as the green lineage (or Chlorobionta), the



**Figure 4.** Hypothetical diagram summarizing our current knowledge of the origin and evolution of mitochondria.

**Figure 4.** Diagramme hypothétique de l'origine et de l'évolution des mitochondries dans l'état actuel des connaissances.

monophyly of which is confirmed in all protein and ribosomal phylogenies available to date (Sogin, 1991; Hasegawa et al., 1993; Cavalier-Smith, 1993; Bhattacharya & Stickel, 1994; McFadden et al., 1994a; Nordnes et al., 1994; Ragan & Gutell, 1995). Chlorarachniophyta on the other hand belong to an independent lineage associated with the filose amoebae *Euglyphyna* (Bhattacharya et al., 1995a; McFadden et al., 1994b). Chlorarachniophytes exhibit complex chloroplasts probably derived from the secondary endosymbiosis of a green algal-like organism (McFadden et al., 1995; McFadden et al., 1997a). Euglenophyta include heterotrophic and photosynthetic euglenoids. These unicellular organisms resolve with the heterotrophic kinetoplastids to form a monophyletic group named Euglenozoa (Cavalier-Smith, 1981). As for Chlorarachniophyta, they probably resulted from a secondary endosymbiosis with a green algal-like ancestor.

The so-called Chromophyta or chlorophyll *a+c* - containing algae are phylogenetically separated into divergent groups: 1) dinoflagellates, which have all sorts of different plastids, belong to the Alveolata lineage including heterotrophs such as the Apicomplexa (*Plasmodium falciparum*) and ciliates (*Paramecium aurelia*) (Wolters, 1991; Sogin, 1991; Gajadhar et al., 1991; Cavalier-Smith, 1993; Medlin et al., 1994; Bhattacharya & Stickel, 1994; Leipe et al., 1994). Recently, a reduced non-photosynthetic plastid containing a small 35 kb circular genome has been identified in two members (*Plasmodium* and *Toxoplasma*) of the phylum Apicomplexa and sequenced (McFadden et al., 1997b; Blanchard & Hicks, 1999); 2) Heterokontophyta, which include the Bolidophyceae, Diatomophyceae, Phaeophyceae, Pelagophyceae, Xanthophyceae, Eustigmatophyceae, Chrysophyceae, Rhaphidophyceae and Synurophyceae. Based on ribosomal (Sogin, 1991; Ariztia et al., 1991; Cavalier-Smith, 1993; Andersen et al., 1993; Leipe et al., 1994; Bhattacharya et al., 1995b; Guillou et al., 1998) and protein (Bhattacharya & Stickel, 1994) gene phylogenies, they are grouped with heterotrophic protists such as the Oomyceta previously classified as lower fungi. The Heterokonta occasionally resolve as a sister group to the Alveolata in 18S rRNA nuclear phylogenetic trees (Medlin et al., 1994); 3) The Prymnesiophyta or Haptophyta form a group distinct from the Heterokonta and Alveolata, with no close relationship to either phyla (Ariztia et al., 1991; Cavalier-Smith, 1993; McFadden et al., 1994a; Leipe et al., 1994; Medlin et al., 1994; Bhattacharya et al., 1995b).

Rhodophyta, once considered as primitive organisms because of their morphology, the nature of their plastids and from 5S rRNA phylogenies (Hori & Osawa, 1987), 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 &

Medlin, 1995) and protein-encoding gene trees (Bouget et al., 1995; Ragan & Gutell, 1995). This phylum is not associated with any heterotrophic lineage and its position among the eukaryotic "crown taxa" is not well identified. However, increasing molecular and biochemical evidence suggests that rhodophytes and green plants are sister groups (Ragan & Gutell, 1995; Cerff, 1995; Leblanc et al., 1995b; Burger et al., 1999). This idea is also supported by the claim of a monophyletic origin of green and red plastids (Reith, 1995).

Cryptophyta also emerge as an independent branch, convincingly related to the heterotrophic phagotrophic flagellate *Goniomonas truncata* (McFadden et al., 1994a). Nucleomorph genes show that the endosymbiotic eukaryote that gave rise to cryptophyte plastids is related to Rhodophyta (Douglas et al., 1991; McFadden et al., 1994a). Glaucocystophyta (Chl *a* + phycobiliproteins) form a small distinct group of cyanelle-containing photosynthetic protists that share a common ancestry with Cryptophyta in the phylogenetic tree presented by Bhattacharya et al. (1995b).

#### *The symbiont history: plastid phylogenies*

When looking at the endosymbiont history through plastid gene phylogenies, it is important to consider primary plastids apart from secondary plastids. Phylogenetic analyses of plastid genes usually confirm that plastids share a common ancestor with the modern oxygenic photosynthetic bacteria (i.e., cyanobacteria). In particular, studies based on the 16S rRNA gene (Nelissen et al., 1995) and the *tufA* gene (Delwiche et al., 1995), which include a comprehensive set of cyanobacterial genes as well as a representative sampling among the three primary lineages of plastids are in favour of a monophyletic origin of plastids. The only obvious contradiction comes from analyses based on the *rbc* genes for large and small subunits of ribulose-1,5- bisphosphate carboxylase oxygenase, which indicates that rhodophyte *rbc* plastid genes are of proteobacterial origin (Delwiche & Palmer, 1996). This result has been attributed to a horizontal gene transfer or to an early duplication of the *rbc* operon followed by differential loss in the three primary plastid lineages (for review, see Martin et al., 1992; Delwiche & Palmer, 1997). Yet, whether all plastids arose from one unique cyanobacterium-like ancestor or from closely related cyanobacterial ancestors, and by one or several endosymbiotic event(s), is difficult to prove and will not be debated further in this paper. Moreover phylogenetic analysis of extant cyanobacteria and plastid genes (cited in Bhattacharya, 1997) indicates no strong candidate among the ten identified monophyletic groups of photosynthetic bacteria as sister taxon to the plastids.

#### *The connection to the ancestral genomes*

The only entirely sequenced cyanobacterial genome analysed so far is that of *Synechocystis* sp. PCC6803. The



total length of the circular genome is 3.5 Mb and contains a total of 3168 potential protein coding genes (Kaneko & Tabata, 1997). Even though we admit that *Synechocystis* is probably not the closest evolutionary relative to extant plastids, we can venture a few comparisons. For example *Synechocystis* contains twelve times as many genes as the largest and most primitive plastid genome described so far, that of the rhodophyte *Porphyra purpurea* (Reith & Munholland, 1995). As mentioned earlier for mitochondria, the genome of the prokaryotic progenitor of modern plastids has been tremendously reduced and restructured through loss and transfer of genes to the host nucleus. Some operons (*rpoB/C1/C2*, *psbD/C*, *psaA/B*, *psbE/F/L/J*) have kept the same arrangement in cyanobacteria and in the plastids of green plants and *P. purpurea*, confirming a common ancestor (Reith & Munholland, 1995). On the other hand, some gene clusters (*psbB/N/H* and *atp/rps/rpo*) are maintained among the different plastid lineages, but appear to be different in characterized cyanobacterial genomes. This trait is considered by some authors as a derived shared character supporting a monophyletic origin of plastids, although it might result equally from convergent evolution.

#### *Plastid genome organization*

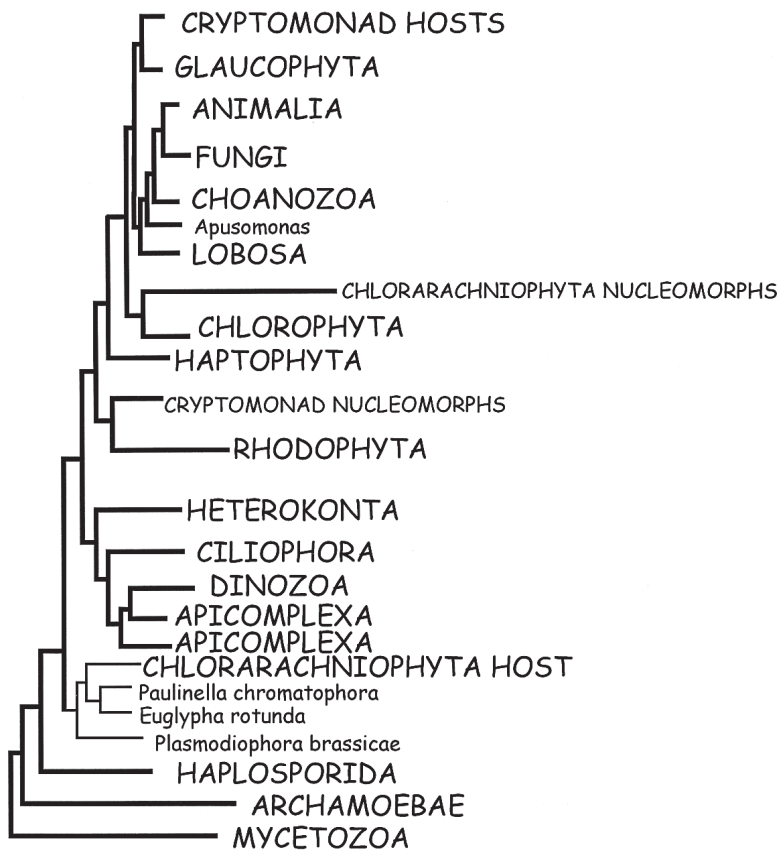
Compared to mitochondrial genome diversity, plastid genomes are much more homogenous. Their size usually ranges between 120 and 200 kb. Plastid genomes are highly specialized in the sense that they have retained mainly genes involved in photosynthesis and expression. Plastidial genomes belonging to parasitic organisms such as the higher plant *Epifagus virginiana* (70 kb), the colourless euglenophyte *Astasia longa* (70 kb) and the apicomplexan *Plasmodium falciparum* and *Toxoplasma* (35 kb) are devoid of photosynthetic genes and are, therefore, highly reduced. A comprehensive review of the size, gene content and genome organization of plastid DNA may be found in Delwiche & Palmer (1997). Presently, complete plastid sequences for at least one representative of six different algal lineages are known, namely: *Cyanidium caldareum* (AF022186, Gloeckner, Rosenthal and Valentin, unpublished) and *Porphyra purpurea* (Reith & Munholland, 1995) for Rhodophyta; *Odontella sinensis* (Kowallik et al., 1995) for Heterokonta; *Cyanophora paradoxa* (Stirewalt et al., 1995) for Glaucocystophyta; *Euglena gracilis* (Hallick et al., 1993) for Euglenophyta; *Mesostigma viride* (Lemieux et al., 2000), *Nephroselmis olivacea* (Turmel et al., 1999) and *Chlorella vulgaris* (Wakasugi et al., 1997) for Chlorophyta; *Guillardia theta* (Douglas & Penny, 1999) for Cryptophyta. The red algal plastid genome appears as the most primitively organized and, as such, the most cyanobacterium-like. It contains no introns, a nearly complete set of tRNA genes, a high gene-coding capacity,

and has retained many typical cyanobacterial operons (Reith & Munholland, 1993, 1995). Sequencing of additional secondary plastid genomes of different Heterokontophyta, Chlorarachniophyta, Dinophyta and Haptophyta are still required to provide a better view of plastid evolution. Secondary plastid endosymbiosis implies serial functional relocations of genes among various nuclear compartments, a process that is far from being deciphered. Recent studies concerning the plastid DNA of dinoflagellates with secondary plastids show that these have evolved in a very particular way, leading to minicircular DNA molecules encoding just one plastidial gene each (Barbrook & Howe, 2000; Zang et al., 2000). Partial data concerning gene content of the nucleomorph chromosomes in the Cryptophyta and the Chlorarachniophyta is now available allowing important insights into the process of eukaryote/eukaryote cell endosymbiosis (Gilson et al., 1997). To add to the confusion, it seems that some dinoflagellates have reacquired plastids by different secondary or even tertiary endosymbioses, involving haptophytic, heterokont or cryptophytic algae (Chesnick et al., 1996; Horiguchi & Pienaar, 1994; Taylor et al., 1969). Such organisms can contain up to six coordinated genomes of different origins. The flow diagram in Figure 5 is an attempt to synthesize our current understanding of the diversity of plastid origins.

#### *Genetic conflicts*

The endosymbiotic relationship is usually considered only from a functional point of view, where both partners live together for mutual benefit. In this perspective, endosymbiosis confers a new significant biochemical function (aerobic respiration or photosynthesis) to the host while the "dominated" symbiont makes a profit from the intracellular lifestyle. Then, the host's nucleus aims to completely control the various compartments of the cell to the point where the organelles might be totally deprived of their genome. This oversimplified way of looking at endosymbiosis might explain certain aspects of the evolution of organelles, such as sustained gene transfer from the mitochondria and plastid to the nucleus. However, it does not account for other aspects, such as the extreme diversity of mitochondrial genomes all descending from the same ancestor.

On the other hand, the concept of genetic conflict throws new light on the rules that might govern intracellular relationships. From the conflicting interest perspective, genetic interactions between organelles and the nucleus are considered as an "arms race" where each competing genome is acting for its own reproductive interest. For example, according to population genetic reasoning, it is argued that the uniparental inheritance of organelle DNA is a way to



**Figure 5.** Maximum likelihood tree inferred from the comparison of nuclear small subunit (18S) rRNA sequences, modified from McFadden et al. (1997). This tree displays the phylogenetic diversity of algae, belonging to various independent eukaryotic lineages.

**Figure 5.** Arbre phylogénétique construit à partir de la comparaison des séquences du gène de la petite sous-unité de l'ARN ribosomique (18S) rRNA, utilisant la méthode du maximum de vraisemblance. Cet arbre a été modifié à partir de l'arbre publié par McFadden et al., (1997). Il met en évidence la diversité phylogénétique des algues, qui appartiennent à de nombreuses lignées évolutives indépendantes.

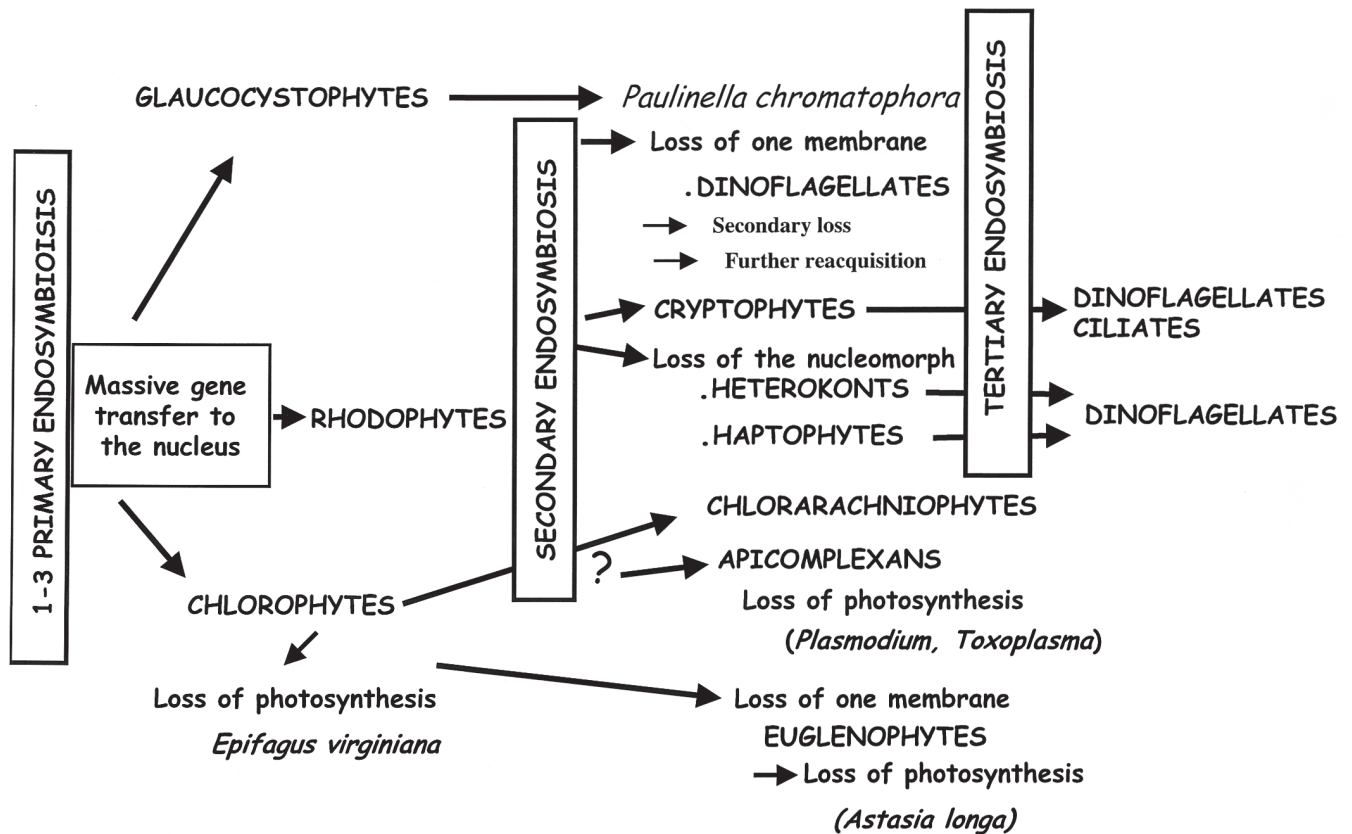
prevent the spread of self-promoting deleterious cytoplasmic genes (Hurts et al., 1996). Uniparental inheritance means that organelle genes are inherited primarily or exclusively from one parent and thus, that organelle genes obey non-Mendelian laws. This clonal type of reproduction results in a competition within the micro-population of organelles that favours the smaller and faster replicating genomes. Such a phenomenon described as the "genomic economisation process" (Kurland, 1992), leads to the reduction of genome size by deletion or transfer of mitochondrial genes to the nuclear genome. According to Kurland (1992), it is the capacity of the nuclear genome to

integrate functionally the transferred mitochondrial genes, which 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 (super wobble). In plants and in some fungi this deletion process has been balanced by recombination mechanisms within mitochondria that allowed the recovery of deleted sequences (Kurland, 1992).

In addition, Atlan (1991) proposed that there is a relation between the mt genome sizes, mtDNA copy number and mutation rate in these uniparentally inherited genomes. According to this model, two opposite strategies could have been selected to prevent the accumulation of deleterious mutations, leading to the two major trends in the organization of mitochondrial genomes. 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 (Atlan & Couvet, 1993). 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., 1995b; Leblanc et al., 1997). With regards to the plastidial

genomes, the presence of inverted repeats, also existing in some fungal and oomycete mitochondrial genomes, involving intramolecular recombination through isomeric molecules might as well result from evolutionary constraints to prevent the accumulation of deleterious mutations (Atlan, 1991).

In the present state of knowledge these hypotheses should be considered as working grounds, which need more experimental data, especially concerning organelle transmission in organisms other than higher plants or animals. Nevertheless our understanding of the origin and evolution of organellar genomes must integrate the



**Figure 6.** Summary diagram showing the evolution and diversity of plastids derived from primary, secondary or tertiary endosymbiosis.

**Figure 6.** Schéma récapitulatif de l'évolution et de la diversité des plastes provenant d'endosymbioses primaires, secondaires ou tertiaires.

population genetics concepts together with the comparison of total organellar genomes and their expression mechanisms.

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