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Evolution of Chloroplast Genome

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The discovery that chloroplasts contain DNA and possess several characteristics similar to those of prokaryotes, has led to many speculations regarding their origin. Attempt is made here to evaluate two main hypotheses (symbiotic and internal origin). Taking into account most of the facts known about the chloroplast and its genome, a new model has been suggested for their possible origin.

Key Words: **Model, Chloroplast genome, Origin, Endosymbiosis,** *Cyanocystis*, **Prokaryotic genome,** *Cyanella*, **Ribosomal cistrons, Nuclear DNA**

Introduction

Acquisition of the photosynthetic ability by primitive organisms at certain time in the course of organic evolution was a very important event. It was significant not only for the subsequent evolution of photosynthetic organisms but also for changing the course of nonphotosynthetic ones. The organisms which acquired the photosynthetic capacity became autotrophic and a large amount of their photosynthetic products was also useful to the heterotrophic organisms as nutrients. The other important outcome of the acquisition of the photosynthetic ability by the organisms was the supply of oxygen to the primitive atmosphere as its by-product. The accumulation of oxygen in the atmosphere brought in dramatic event in the origin of aerobic organisms (figure 1).

The basic photosynthetic apparatus of the eukaryotes such as higher plants and lower algae is the chloroplast. This organelle is independent of other cell organelles morphologically as well as functionally. The photosynthetic materials of prokaryotes, photosynthetic bacteria and blue-green algae, are simply developed membrane systems (figure 2). These are not independent organelles and are generally connected to the protoplasmic membrane. On the other hand a well-developed chloroplast of higher plants is quite a complex structure of membrane systems possessing its own DNA and protein-synthesising machinery (figure 3).

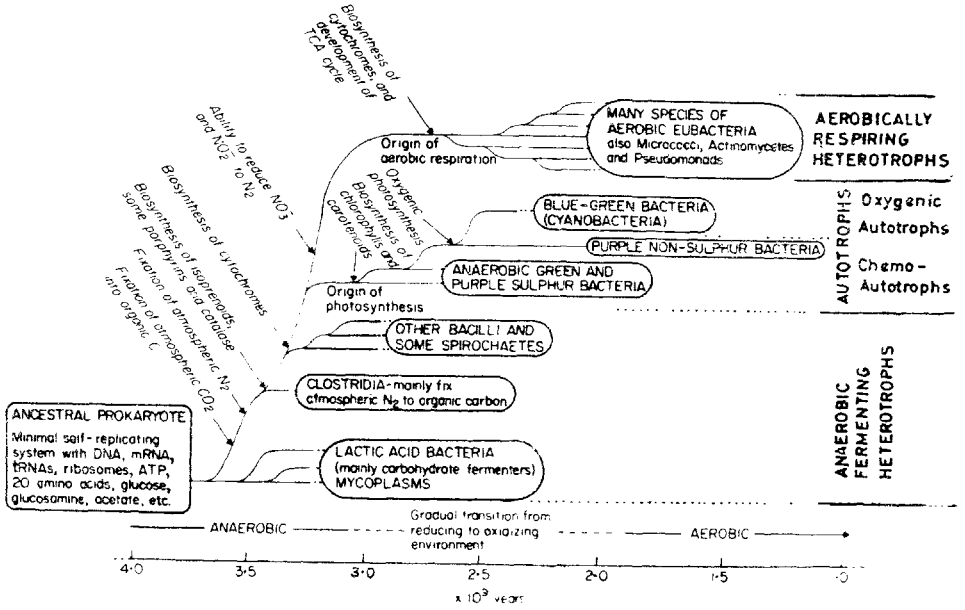


Figure 1 Postulated evolutionary pathways taken by prokaryotic cells (simplified and modified after Margulis)

These facts suggest that there is a morphological and a functional (biochemical) gap between the photosynthetic organelles of eukaryotic and prokaryotic cells.

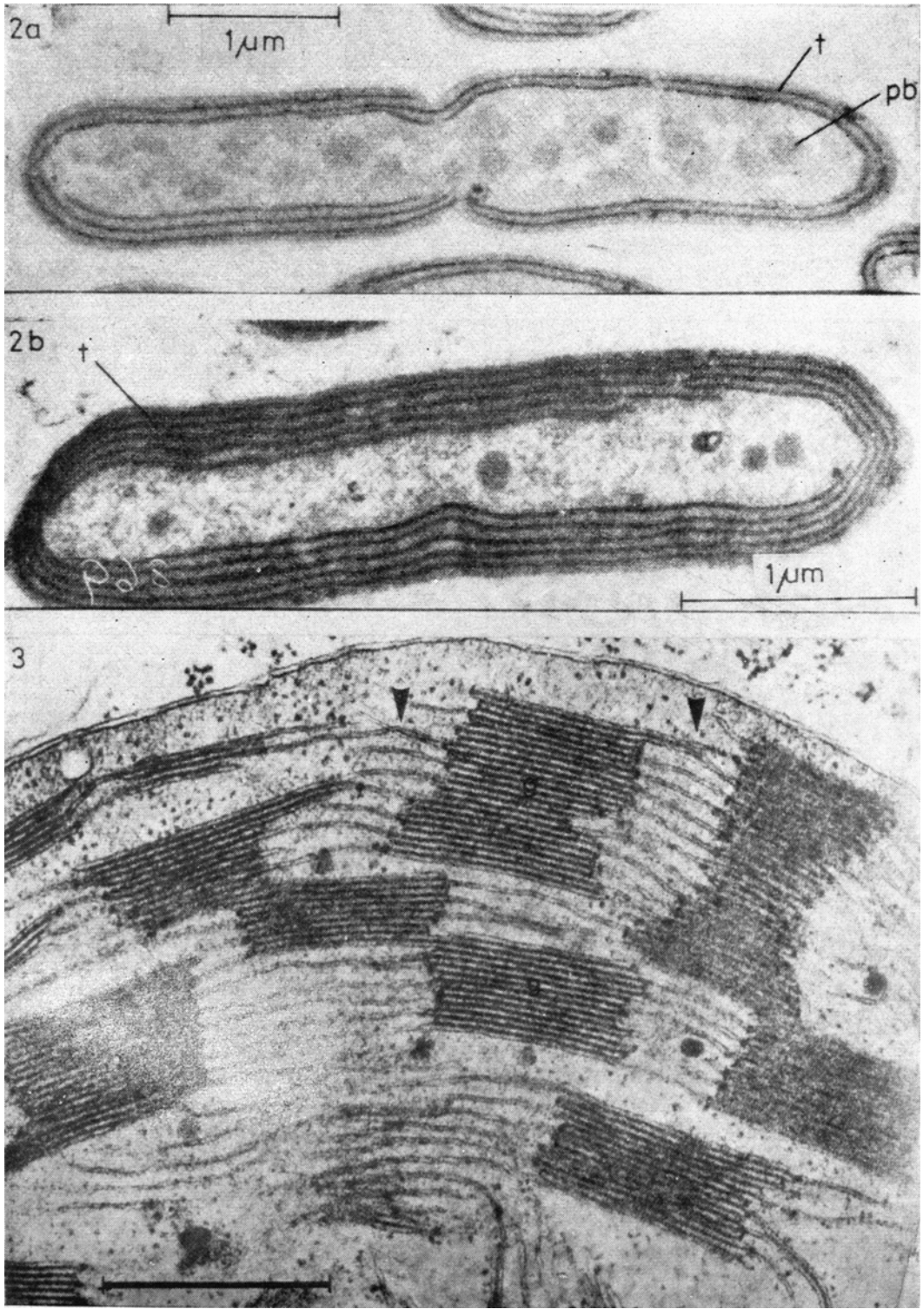
It is important to know when and how the photosynthetic organelle chloroplast developed in the course of biological evolution. Several views have been advanced. However, they are grouped under two main hypotheses.

Hypotheses on Evolution of Chloroplasts

Endosymbiotic hypothesis

One view is that chloroplasts are derived from the endosymbiotic blue-green algae living in the host cells (Schimper 1885, Ris & Plaut 1962, Margulis 1970 and Margulis et al. 1978). It was believed that after the photosynthetic prokaryotes such as unicellular blue-green algae immigrated into the colourless eukaryotic cells as symbionts they gradually became adapted to the environment of the host cells. Finally the symbionts became dependent on the nuclear genome of the host cells. Such a symbiotic blue-green algae became the present day eukaryotic chloroplast.

Recently, the symbiotic hypothesis on the origin of cell organelles, not only chloroplasts but also mitochondria and flagella have been proposed. According to this hypothesis, chloroplasts and mitochondria have evolved from symbiotic blue-green algae and aerobic bacteria, respectively. It also suggests that flagella have evolved from spirochete-like microbes. Thus all these organelles have evolved through serial endosymbiosis (figure 4). However, proponents of the endosymbiotic theory are not agreed on the actual sequence of events. The universal presence of mitochondria in eukaryotes would tend to suggest that the acquisition of mitochondria preceded that of chloroplasts.



Figures 2a, b & 3; 2, Prokaryotic cells of (a) *Anacystis nidulans* (2 or 3 thylakoid membranes) and (b) *Anabaena cylindrica*; 4 or 5-thylakoid membranes. All restricted to the periphery only; **3**, Part of a thin-sectioned chloroplast from barley seedling leaf. Thylakoid membranes are differentiated into sacked regions call grana (g) and unsacked regions (arrows). Also visible are ribosomes (r) in stroma

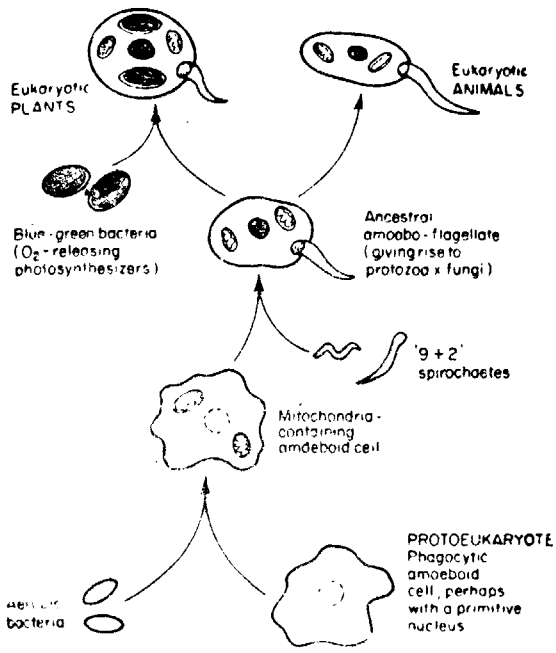


Figure 4 The postulated evolution of eukaryotic cells by serial endosymbiosis

Internal Origin Hypothesis

In contrast to the endosymbiotic hypothesis, some investigators (Cavalier 1975 and Nakamura 1975) have proposed the theory of internal origin for the organelles. According to their view, the nuclei, chloroplasts, mitochondria, cillia, flagella etc., have appeared in normal course of evolution as part of favourable, accumulated mutations and the process of natural selection. The process of differentiation and division of labour as acts of specialization can explain the formation of these organelles. It is assumed that the original genome may have fragmented, redistributed as per functional specialisations followed by compartmentalisation into organelles. This can explain the partial dependence of these organelles on nuclear genome for their development and functions.

Prokaryotic Nature of Chloroplast

A great deal of information is now available concerning the similarities between chloroplasts, mitochondria and contemporary prokaryotes. Table 1 summarises some important characteristics of several constituents of eukaryotic cell organelles and prokaryotic cells. The chloroplasts possess circular DNA molecules but lack nucleosomes as seen in eukaryotic nucleus. They do not contain the basic proteins—histones. Moreover, in the chloroplasts, chromosomes characteristic of the eukaryotic cell nucleus are not formed at any stage of their life cycle.

The redundancy of rRNA cistrons in chloroplast DNA molecules is very low, generally only two copies, similar to bacterial genomes which also have a few copies. On the other hand, very high redundancy (as much as 10^{-3}–10^{-6}) of this gene is found in the nucleus of higher plants and animals. Chloroplasts possess bacterial type 70S ribosomes, different from the cytoplasmic 80S type ones. The S-values of the rRNA subunits from chloroplasts are 16S and 23S which are also different in molecular species from the 18S and 25-29S rRNA of cytoplasm. Sensitivity to inhibition modes of RNA and protein synthesis in chlroplasts is similar to that observed in bacteria but not to the systems of eukaryotic cells. Another notable similarity is in the translation systems of prokaryotes and eukaryotic organelles such as chloroplasts. In bacteria, mitochondria and chloroplasts, a formylation step follows charging of the tRNA with methionine. By contrast in the eukaryota the formylation step is omitted. These findings suggest that the chloroplasts have many features in common with the prokaryotes and that the chloroplasts might have evolved through endosymbiosis.

Table 1 *Cytological nature of cell constituents in eukaryotes and prokaryotes*

Subjects	Organells in Eukaryotic Cells			Prokaryotic Cells	
	Nucleu/cytol.	Mitochondria	Chloroplasts	Blue-green algae	Bacteria
Molecular shape of DNA	Linear (?)	Circle	Circle	(?)	Circle
DNA organization	High organized (Chromosome)	Low (Bacteria type)	Low (Bacteria type)	Low (Bacteria type)	Low (Double strand)
5-methyl cytosine*	+	—	—	—	—
Redundancy of rRNA cistrons	Very high 10^{-5}–$10)^{-5}$	Very Low	Very Low	(?)	Very Low
DNA content (g)***	10^{-9}~10^{-14}	10^{-16}~10^{-17}	10^{-14}~10^{-16}	10^{-14}~10^{-15}	10^{-15}(*E. col*)
Mitotic apparatus	+	—	—	—	—
Histone	+	—	—	—	—
S-values of ribosome	80 S type	70 S type	70 S type	70 S type	70 S type
S-values of rRNA	1. S, 25—29 S W	15 S,21 S	16 S,23 S	16 S,23 S	16 S,23 S
Protein synthesis chloramphenicol	No inhibition	Inhibition	Inhibition	Inhibition	Inhibition
cycloheximide	Inhibition	No inhibition	No inhibition	No inhibition	No inhibition
Initiation of protein synthesis (tM—tRNA)**	—	—	+	+	+
DNA deep RNA polymerase Rifampicine	No inhibition	Inhibition	Inhibition	Inhibition	Inhibition

(*) Presence; (+) or absence (—) in DNA Molecules; (**) Initiated by N-formylmethionine tRNA (+) or not (—); (***) Per nucleus per organelle or per bacterial cell

Further Evidences from Molecular Biology

Recent information suggests molecular homology between chloroplasts and some prokaryotes like blue-green algae (Bonnen & Doolittle 1975, 1976 and Zablen et al. 1975). Very high homology was observed *in vitro*, between rRNA from blue-green algae and chloroplast DNA of *Euglena*. For instance, the homology of *Euglena* DNA to *Gloeocapsa aplicota* rRNA is 47%. It is 32% to *Nostoc mucorum*, 32% *Chlorogloea fritschii* and 13% to *Anacystis nidulans*. It is important to note that the homology between *Euglena* chloroplast DNA and rRNA from photosynthetic bacteria such as *Rhodomicrobium vannielli* and *Rhodospirillum rubrum* is low (5.9 and 3.9% values respectively). The values between *Euglena* and non-photosynthetic bacteria are also lower than those of photosynthetic bacteria. No hybridisation was observed between chloroplast DNA and cytoplasmic rRNA encoded by nuclear DNA in *Euglena*. These observations indicate that from the evolutionary point of view chloroplasts are closer to blue-green algae than to the photosynthetic bacteria or any other groups (Ishida 1979) (table 2).

Another evidence has been obtained from oligonucleotide analysis. When 16S rRNA of *Euglena* chloroplasts is digested by T_1 RNase, and when the small oligonucleotide fragments are subjected to the

Table 2 *Hybridization Ch-DNA of Euglena with rRNA*

	Source of rRNA	% Hybridization
Blue	Gloeocapsa	47
Green	Nostoc	32
Algae	Anacystis	13
Photosynthetic	Rhodomicrobium	5.9
Bacteria	Rhodosprillum	3.6
Algae	Euglena cytoplasmic (Nuclear coded)	0

sequence study, the extent of similarities of sequences can be related to their phylogenic closeness. The 16S rRNA from *Euglena* has a large number of oligonucleotide sequences that are also present in prokaryote 16S RNA but appear to be less common in 18S rRNA species. Thus chloroplast 16S rRNAs show extensive homology with prokaryote 16S RNAs but very little homology with cytosolic 18S rRNA molecules (Pigott & Carr 1972).

Furthermore the genome size of DNA of some endosymbiotic blue-green algae, e.g. *Cyanocystis korschikoffiana* is 1.17×10^8 daltons which is very close to the value 9.2×10^7 daltons of chloroplast DNA. The GC (guanine plus cytosine) content of the above blue-green algae is also of the same order of the GC content of chloroplast i.e. 37 mol per cent. The copy number of circular DNA molecules contained in *Cyanocystis* is about 60, i.e. it shows high polyploidy like the chloroplast DNA which has usually 20–80 copies per organelle.

Instances of Endosymbiosis in the Present Day Organisms

There are many instances of endosymbiotic relations between several species.

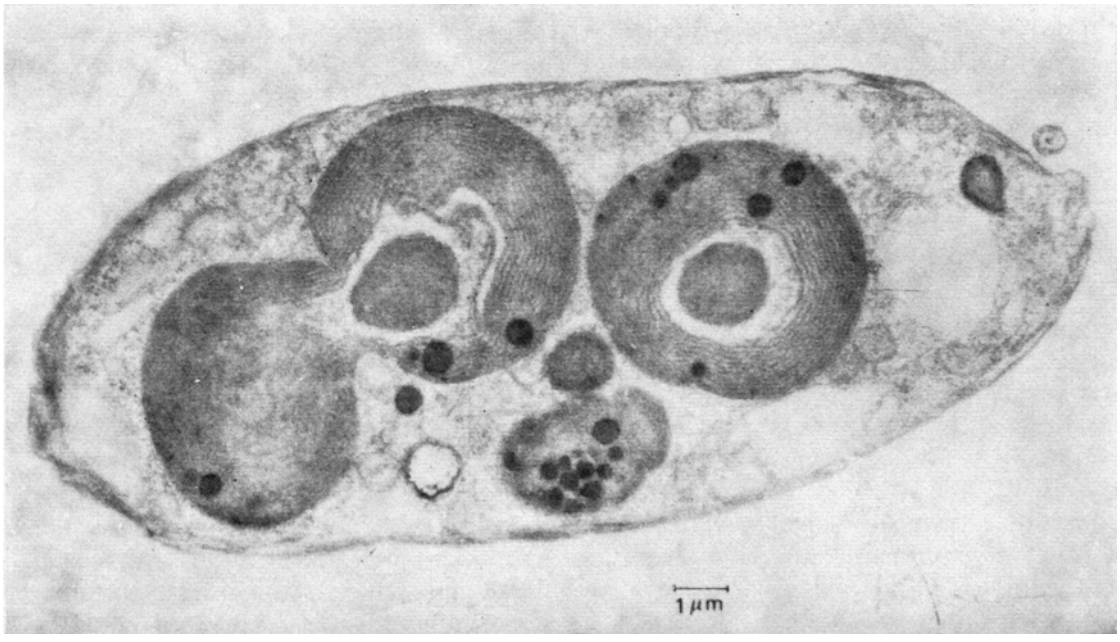


Figure 5 Electron micrograph of *Cyanophora paradoxa*. The photosynthetic structures present within the cell are known as cyanelles

However, relevant to the theme of chloroplast evolution, a few examples are cited here. One of the most studied cases of endosymbiosis is that of *Paramecium* which often harbours either endosymbiotic green algae or bacteria. In one well investigated example, involving *Paramecium aurelia*, some of the endosymbiotic bacteria produce bright *Kappa* particles. The association of *Kappa* within *Paramecium* is governed by nuclear genes. Another striking example is the 'green' algae *Glaucocystis hostochinearum*, which has blue-green bacterial endosymbionts. One more example of particular interest is the anomalous eukaryotic alga *Cyanophora paradoxa* which harbours blue green alga *Cyanocystis korschikoffiana* (Broda 1975) (figure 5). Such blue-green algae living in eukaryotic host cells are called *Cyanelle*. These cyanelles have properties intermediate between blue-green bacteria and red-algal chloroplasts. Their photosynthetic pigments and thylakoids appear to be very similar to red-algal chloroplasts but they possess a cell wall similar to blue-green bacteria. The existence of these "*protochloroplasts*" argues strongly in favour of the endosymbiotic theory.

Evidences Supporting the Internal Origin Hypothesis

None of the prokaryotes possess all the characteristic pigments found in the chloroplasts. For example, although some prokaryotes possess chlorophyll *a* none contains chlorophyll *b* (Whatley et al. 1979). The chloroplasts of all higher plants contain chlorophyll *a*+*b*. This fact is difficult to explain from the view of endosymbiotic origin of chloroplasts from prokaryotes. Recently a prokaryotic alga *Prochloron* has been found to contain both chlorophyll *a* and *b*. It still remains to be seen if it can be a good precursor of chloroplast.

It is now increasingly becoming clear that by far many characters and functions of chloroplasts are determined and controlled by nuclear genes (Ishida 1980). Figure 6 shows these genetic systems. The dotted lines in this figure show the routes of the genetic information from the nucleus to the chloroplasts. The cistrons of the enzymes which catalize all steps of chlorophyll synthesis are located in the nuclear genome and not in chloroplasts. Several kinds of photosynthetic membrane proteins and chloroplast 70S ribosomal proteins are also directed by nuclear genes (Machold & Aurich 1972). The enzymes such as aldolase and phosphoribulokinase for carbon dioxide fixation cycle in chloroplasts are also directed by nuclear genes.

Ribulose diphosphate carboxylase which catalizes the first step of carbondioxide fixation is composed of large and small subunits of peptides. Of these the cistrons for the large subunit are found in the chloroplast genome, whereas those for the small one are in the nucleus (Kawashima & Wildman 1972).

A number of such important characters of chloroplast photosynthetic apparatus are nuclear controlled. In other words, the chloroplast genome does not contribute entirely to the photosynthesis process. These facts are hard to explain from the endosymbiotic hypothesis.

Protagonists of endosymbiotic hypothesis often fail to comment on the nonprokaryotic features of mitochondria and chloroplasts. For example many eukaryotic genomes-including those of mitochondria and chloroplasts-have 'split genes', whilst those of bacteria

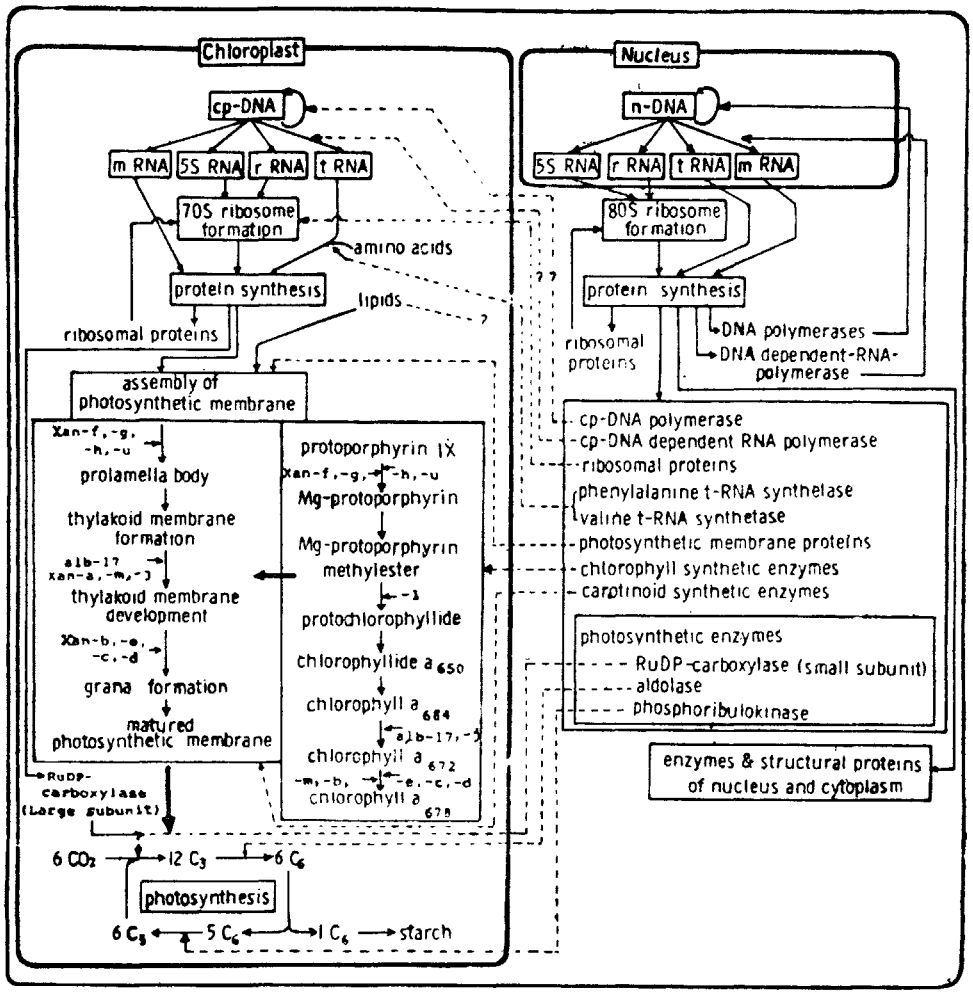


Figure 6 Interaction between the chloroplast and nucleus. The dotted lines indicate the functions of the chloroplasts controlled by nuclear genes

do not (Tribe et al. 1981).

It has been recently discovered that the genetic code employed by mitochondria and chloroplasts deviates from that of both prokaryote genes and eukaryotic nuclear genes (Tribe et al. 1981). Another distinct feature of mitochondria and chloroplasts is that of their DNA dependent RNA polymerases. According to a number of investigators the DNA dependent RNA polymerases from mitochondria as well as chloroplasts are composed of single subunits, their molecular weights being of the order of 50,000 to 65,000. Eukaryotic nuclear RNA polymerases and bacterial RNA polymerases are different from this, but are similar to each other in being composed of several subunits with total molecular weights in the range of 400,000 to 500,000.

All these facts go against the assumption that some prokaryotes evolved into the present day chloroplasts through

endosymbiosis. However, a large number of prokaryotic features of chloroplasts cannot be explained by any other way or by internal origin hypothesis.

Chloroplast Genome

Chloroplast DNA molecules are generally circular in form and their contour lengths are almost constant—about 40 mμ throughout the plant kingdom with few exceptions. The GC contents of DNA from chloroplasts show values around 37 mol per cent from eukaryotic alga and higher plants. As mentioned earlier GC values of *Cyanelle* and some blue-green algae, fall into the same level as that of chloroplast DNA. In the rRNA-DNA molecular hybridization experiments, a high homology between rRNA from blue-green algae and *Euglena* chloroplast DNA has been observed (Ishida 1978).

The gene maps of the genomes of chloroplasts show one symmetrical region containing two inverted symmetrical regions and one asymmetrical region (Ishida 1978). The symmetrical region contains sequential arrangements of 16S, 23S, 4.5S RNA polycistrons, two cistrons of leucine t-RNA and two cistrons of isoleucine t-RNA, all placed in two inverted symmetrical regions. Such organisation may indicate the two symmetrical regions containing bacterial type r-RNA polycistrons were originally one molecule but it replicated once followed by dimerization. The asymmetrical region may have also been duplicated to produce a number of genes. Among these genes, some have mutated followed by the appearance of new genes.

A New Model

Considering all the above information a new model is proposed on the process of evolutionary origin of the chloroplast genome. It is likely that a prokaryote similar to *Cyanella*—(*Cyanocystis*) or *Prochloron* might have associated as a symbiont of some colourless unicellular or multicellular eukaryote host. During the course of evolution the genome of the symbiont must have broken off at positions *a* and *b* as shown in figure 7.

The segment containing the group of genes for photosynthesis must have recombined with the nuclear genome of the host cell. These break off positions must have been random since it became separated into two cistrons of RUDP carboxylase subunits as well as some photosynthetic membrane proteins. After break off the other prokaryotic genome segment which was left behind, must have closed its ends to become a circular DNA. Again the circular genome must have replicated to form or dimer molecule in the symbiont. At this time, the symmetrical inverted regions containing cistrons of rRNA and leucine tRNA genes must have come into existence. The asymmetrical region might have been made by the addition of the new sequences produced as a result of repeated replications of the small part, near the replication point after the formation of the symmetrical regions. The other possible way is that the asymmetrical region might have been derived from a broken off segment of the nuclear genome and recombined with the dimer molecule of the symbiont DNA.

Thus in this model, an attempt is made to explain the organisation of chloroplast genome and nuclear genome containing genes interacting with chloroplast genome by assuming the possibility of recombination after random breaks

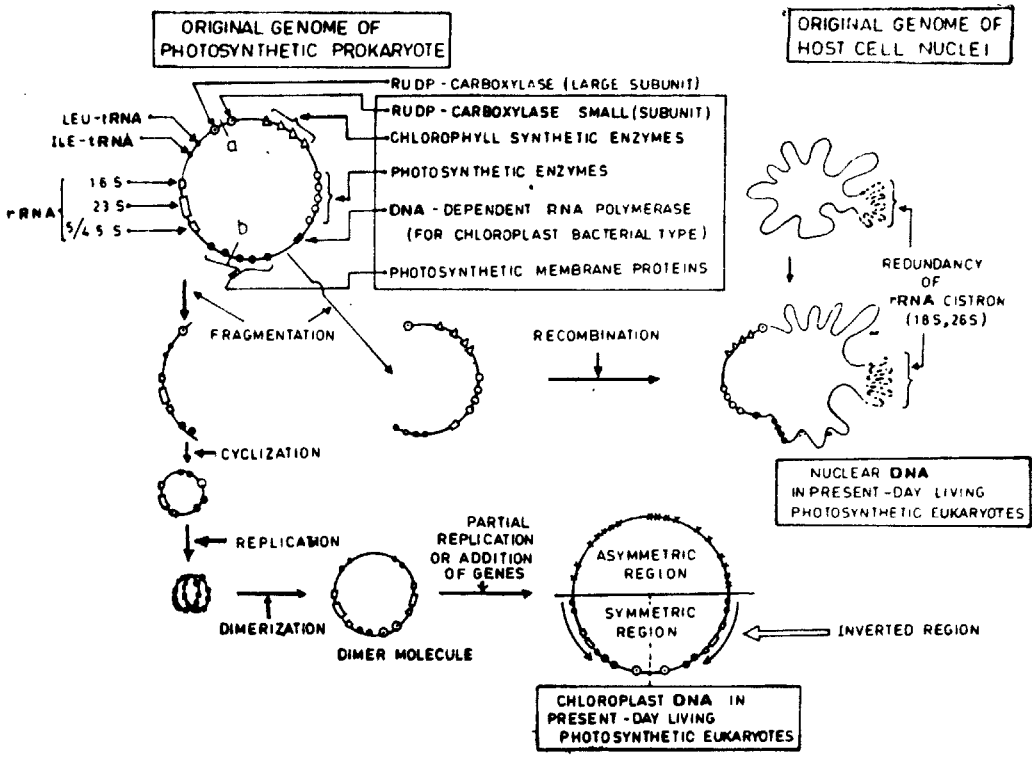


Figure 7 Model showing recombinant processes of the evolutionary formation of chloroplast. Points *a* and *b* are shown as possible break off points for the separated treatment to recombine with nuclear genome. The chloroplast genome circularised, dimerised and some segmental genes mutated. Inverted repeated regions are shown in symmetrical region

both in symbiont genome and the host nuclear genome. Such visualisation of recombination can explain the evolution of chloroplast on the basis of the information available until now.

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