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## **CHAPTER 1: How did thylakoids emerge in cyanobacteria and how were the primary chloroplast and chromatophore acquired?**

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**Running Head:** At the origin of plastids

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## **Abstract**

The emergence of thylakoid membranes in cyanobacteria is a key event in the evolution of all oxygenic photosynthetic cells, from prokaryotes to eukaryotes. Recent analyses show that they could originate from a unique lipid phase transition rather than from a supposed vesicular budding mechanism. Emergence of thylakoids coincided with the great oxygenation event, more than two billion years ago. The acquisition of semi-autonomous organelles, such as the mitochondrion, the chloroplast, and more recently, the chromatophore, are critical steps in the evolution of eukaryotes. They resulted from primary endosymbiotic events that seem to share general features, i.e. an acquisition of a bacterium/cyanobacteria likely via a phagocytic membrane, a genome reduction coinciding with an escape of genes from the organelle to the nucleus, and finally the appearance of an active system translocating nuclear-encoded proteins back to the organelles. An intense mobilization of foreign genes of bacterial origin, via horizontal gene transfers, plays a critical role. Some third partners, like Chlamydia, might have facilitated the transition from cyanobacteria to the early chloroplast. This chapter further details our current understanding of primary endosymbiosis, focusing on primary chloroplasts, thought to have appeared over a billion years ago, and the chromatophore, which appeared around a hundred million years ago.

## **Keywords**

Primary endosymbiosis, chloroplast, mitochondria, chromatophore, Archaeplastida.

## **1. Introduction - Photosynthetic membranes in prokaryotic and eukaryotic cells**

Membrane structures specialized in oxygenic photosynthesis (called thylakoids) are present in both prokaryotic and eukaryotic cells. In the Bacteria domain of life, thylakoid lamellae are present in the cytoplasm of cyanobacteria cells, with the notable exception of a group considered as archaic, comprising *Gloeobacter* species, in which photosystems are anchored in the cytoplasmic membrane [1]. Thylakoids adopt a much broader variety of structures in cyanobacteria compared to chloroplasts in eukaryotic cells, from multiple parietal membrane layers, to sacs piled in stacks [2]. The emergence and biogenesis of thylakoids in cyanobacteria have remained unresolved questions, until recent hypotheses based on non-vesicular elaboration of thylakoids [3].

Contrasting with prokaryotes, eukaryotic cells are characterized by a complex system of intracellular membranes. These compartments have a variety of shapes, from spherical vesicles to tubular or flattened cisternae, reticulated membrane networks, large size multilayer sub-spherical systems etc. An immense task has consisted in the description and definition of these organelles, mainly based on microscopy imaging techniques, but also cell fractionation and biochemistry, and this task is still incomplete. In eukaryotic cells, large size organelles can be observed with a basic light microscope. This is the case for the nuclei, as well as vacuoles in plant cells. The chloroplasts are the easiest organelle to observe simply because of their pigmentation: they 'look' like cyanobacteria inside the cytoplasm. Smaller organelles or intricate membrane systems need higher resolution imaging methods, such as electron microscopy, and more recently three-dimensional electron microscopy or tomography. The organization of eukaryotic cells and its dynamics have been initially described in simple cellular models. The organizational principles were then extrapolated to more elaborate models, such as multicellular animals and plants and all kinds of eukaryotic cells that have

arisen from simple endosymbiotic events, described in this chapter. Higher levels of cellular complexity have emerged from secondary endosymbiosis events treated in the next chapter.

When focusing on photosynthetic membranes, shared by cyanobacteria and chloroplasts, thylakoids form membrane lamellae, with nearly identical lipid compositions [4] and conserved function. Although some proteins are conserved, a large number of chloroplast proteins have diverged or are very different from their cyanobacteria counterparts. This is particularly evident in lipid biosynthesis pathways [4].

Two questions about emergence are therefore puzzling in relation to this remarkable structural conservation: how did thylakoids emerge in cyanobacteria and was a cyanobacteria at the origin of primary chloroplasts?

## **2. How did thylakoids emerge in cyanobacteria?**

The origin of a membrane compartment may look simple, when it results from the budding or protrusion of a parent membrane, fusing with other vesicles or growing by membrane lateral expansion. It becomes a difficult question when we lack evidence of any vesicle budding. The biophysical rearrangement of membrane components, most notably lipids, during a fusion process, has usually a positive enthalpy and requires an external energy source, usually provided by ATP or GTP. Such reorganization is therefore not spontaneous and involves also specific machineries.

Thylakoid formation in cyanobacteria is unlikely vesicle-based, firstly because of the lack of ultrastructural observation of vesicular structures or protrusions generated from the plasma membrane toward the cell interior, but also because primitive thylakoids are concentric, uninterrupted, and it is not clear where their biogenesis could be initiated. A simple hypothesis was recently proposed to resolve the mechanism of this non-vesicular process [3]. It is based on the gradual production of lipids forming a non-bilayer phase, called Hexagonal

II (or *HexII*), at the inner periphery of the cytosolic membrane of a primitive cyanobacterium, transformed in a few nanoseconds and without any external energy source into multilayer prothylakoids (Fig. 1). This model is derived from the opposite mechanism described by Marrink and Mark [5], in which lipids arranged in a lamellar phase (*Lm*) can be converted into a *HexII* phase when provided with energy (Fig. 1A). As a prerequisite for this model, cyanobacteria cells contain both types: *HexII*-forming lipids, most importantly monogalactosyldiacylglycerol (MGDG) and *Lm*-forming lipids, one being neutral, digalactosyldiacylglycerol (DGDG) and two being anionic, phosphatidylglycerol (PG) and sulfoquinovosyldiacylglycerol (SQDG). Most ancient cyanobacteria of *Gloeobacter* clade, which were unable to generate any thylakoid membrane, had a poor content in anionic *Lm* lipids, represented only by PG, whose proportion is controlled by the level of phosphorus in the environment. They contain their photosystems in foci at their cell periphery (Fig. 2A) [6]. The acquisition of the SQDG biosynthetic pathway contributed to a more stable supply of anionic lipids, allowing the *HexII* → *Lm* phase transition (Fig. 1A) to occur, enabling the formation of parietal thylakoids (Fig. 2B). The emergence of thylakoid coincides with the oxygenation of the atmosphere, during the great oxygenation event (GOE), which have occurred ~2.4 billion years ago. Thus the oxygenation of our atmosphere was not driven directly by the appearance of oxygenic photosynthesis, but on a latter metabolic innovation in thylakoid lipid biosynthesis [3].

This model for thylakoid emergence was elaborated before the description of the spatiotemporal biogenesis process of thylakoid membranes in the rod-shaped cyanobacterium *Synechococcus elongatus* PCC 7942 [7]. In this cyanobacterium, the plasma membrane and concentric layers of thylakoids have no physical connections. Newly synthesized thylakoid membranes emerge between the plasma membrane and pre-existing thylakoids (Huokko et al., 2021). It should be kept in mind that this non-vesicular lipid phase transition does not exclude

the involvement of proteins. Nevertheless the role of companion proteins, such as Vipp1, whose characterization in a hypothetical vesicle-based biogenetic process remained unsatisfactory both in cyanobacteria and in chloroplasts, is probably not that of a biogenesis driver, and needs to be reevaluated.

### **3. Was a cyanobacteria at the origin of primary chloroplasts?**

#### **3.1. Eukaryotic cell membrane compartmentalization.**

In a simple eukaryotic cell, two major categories of membrane compartments can be considered: the endomembrane system and the semi-autonomous organelles, *i.e.* the mitochondrion and the chloroplast.

##### **3.1.1. The endomembrane system**

The ‘endomembrane system’ consists of the endoplasmic reticulum (ER), nuclear envelope, Golgi apparatus, trans-Golgi network, endosomes, vacuolar network, the plasma membrane, etc. All the membranes of the endomembrane system are dynamically connected either by vesicular shuttles or tubules. It is well-known that the ‘eukaryotic’ cell is named after the presence of the nucleus, however, the nuclear envelope ‘disappears’ in some eukaryotes during mitosis (in so-called ‘open’ mitosis). The ER is the only membrane sack that is always present and it can be considered as the core of the endomembrane system. Expansions of the ER make the nuclear envelope; vesicles budding from the ER are at the origin of other compartments, such as the Golgi network, vacuoles, etc., and eventually the plasma membrane. Membrane biogenesis, combining lipid biosynthesis with protein co-translational membrane insertion, is therefore very intense at the level of the ER, and then membrane lipids and proteins are sorted, remodeled, modified, etc., fitting with the desired composition in the final (or transitory) functional compartments, in which they sit.

##### **3.1.2. The mitochondrion, the primary chloroplast and the chromatophore.**

Some membrane structures are not connected to this endomembrane system and are called ‘semi-autonomous organelles’. They contain DNA and need to divide to be transmitted to daughter cells. If they are not transmitted following mitosis or if they are lost or degraded, they cannot be reconstituted from another membrane system. Best-known semi-autonomous organelles are the mitochondrion and the primary chloroplast, both limited by an envelope made of two membranes. A less common semi-autonomous organelle, containing DNA and bounded by two membranes, is the chromatophore, currently restricted to a group of amiboids called the Paulinellidae.

Secondary plastids are limited by 3 to 4 membranes and have a hybrid membrane organization, having their outermost membrane connected to the endomembrane system. The architecture of some secondary plastids can even include direct connections with the nuclear envelope and also contain DNA inherited from a nuclear material, called the nucleomorph (see Chapter 2). In the case of secondary plastids, the architecture of the photosynthetic ‘organelle’ is hybrid, and a cooperation between the endomembrane system and the organelle is required for maintenance and transmission; for such subcellular architecture, the definition as ‘semi-autonomous organelles’ makes little sense. Therefore, the term ‘semi-autonomous organelle’ needs to be restricted to the mitochondrion, the primary chloroplast and the chromatophore.

### **3.1.3. The origin of eukaryotes and the question of the acquisition of the semi-autonomous organelles**

Understanding the evolution of eukaryotes and the acquisition of semi-autonomous organelles is a difficult task, probably unattainable [8] and so is the reconstruction of a putative universal tree of life [9]. Reasonable hypotheses are based on molecular features found in genetic sequences, protein structures, conserved metabolites, function, cell architecture etc. and a few experimental data.



Since the 1980's, it is considered that ribosome encoding organisms, excluding viruses, comprise three major groups or domains, called 'Archaea', 'Bacteria' and 'Eukarya' [9]. Life is supposed to have emerged from self-replicating systems in an RNA-rich environment. Maybe as early as 4.5 to 3.8 billion year ago, this initial RNA-based system has transitioned to the modern DNA/RNA/protein system [10,11]. Whereas limiting membranes predated the origin of life is still a matter of debate; nevertheless, the Last unicellular common ancestor (LUCA) is proposed to have emerged more than 3.5 billion years ago, followed by the divergence between Bacteria and Archaea [10-12]. Based on the most recent phylogenetic studies, Eukarya are now considered as a sister group of a superphylum of Archaea, called the 'Asgard Archaea' [13]. The transition from Asgard Archea to Eukarya is supposed to have occurred via an intense remodeling of genetic material, including, on the hand, transmission of Archaea genes, tending to be involved in information processes, and on the other hand, horizontal gene transfers (HGT) from Bacteria, linked to metabolic processes [13]. This results in the current questioning of the definition of the Eukarya as an actual 'domain of life' or more likely a 'hybrid' domain originating from Bacteria and Archaea [14].

The acquisition of the mitochondrion and the primary chloroplast are probably one of the most important events in the evolution of eukaryotes. Mitochondria provided a unique gain in bioenergetics efficiency influencing the formation of the eukaryotic cell, as we know it today [15]. Both the mitochondrion and the primary chloroplast are limited by two membranes, both organelles are central in the bioenergetic metabolism, both contain circular DNA, with (nearly) all the necessary machineries for DNA replication and gene translation. They are like guests ensuring the energetic supply of their host cells, and this function is therefore likely central in their successful integration.

The reconstruction of the acquisition of these organelles has fascinated biologists for more than a century. In 1905, the Russian biologist C. Mereschkowsky [16] developed a theory

postulating that plastids were the evolutionary descendants of endosymbiotic-like organisms, calling them the “little green slaves”. From this first visionary insight, the endosymbiotic theory was not considered until the 1970’s [17]. Cytological and molecular evidence accumulated and confirmed that plastids have probably originated from the engulfment of an unknown cyanobacterial ancestor inside a eukaryotic cell, predated by the enslavement of a mitochondrion inherited from an  $\alpha$ -proteobacterium-like ancestor. Evidence for an endosymbiotic origin includes (1) the presence of “naked” (without histones) organelle-specific DNA, (2) high degrees of sequence homology between DNA of mitochondria and plastids and of bacteria and cyanobacteria, respectively, (3) organelle ribosomes similar to those of prokaryotes and sensitive to chloramphenicol, (4) initiation of messenger RNA translation by means similar to those in prokaryotes, (5) lack of actin/tubulin system, (6) fatty acid biosynthesis occurring via a prokaryote-like fatty acid synthase and coupled to an acyl carrier protein (ACP), and (7) occurrence of  $\beta$ -barrel-membrane proteins in the outer envelope membrane like in gram-negative bacteria and cyanobacteria, etc.

This being said, how and when did these endosymbiotic events occur? How many times?

How did this association become irreversible, with such a high level of dependence with the host cell?

### **3.2. The mechanism of entry and the question of the outermost membranes of the mitochondrion and the chloroplast**

The First eukaryotic common ancestor (FECA) is inferred to be identical to the last common ancestor of Archaea and Eukarya [13]. Since we do not know how the endomembrane system was acquired, FECA is shown either containing an ER and a nucleus in Fig. 3 or without any membrane compartments in Fig. 2. About 1 to 1.9 billion years ago, features of modern eukaryotes are evidenced [13], and this is therefore during this period that the Last eukaryotic common ancestor (LECA) is positioned. The mitochondrion and the primary chloroplast are

bounded by two membranes. Alternative models are possible regarding the origin of these membranes (Fig. 3).

The ancestral prokaryotes are supposed to have entered the cell via either syntrophy (when two organisms interact without endosymbiosis), specific bacteriotrophy or unspecific phagocytosis (these two latter mechanisms requiring a phagocytosis) [15,18]. No contemporary example of a bacterial endosymbiont resident in a archaeal host has been described to date, which would support such hypothesis for the acquisition of the mitochondrion [19]. The mechanism of acquisition of the mitochondrion is difficult to reconstruct: it was possibly captured in a net of membrane protrusions acting as a phagocytic membrane (or phagosome) ending with an invagination of the plasma membrane of the host cell (Fig. 1, (2) and (4)). The acquisition of the chloroplast seems more simply due to a more classical phagocytic process. Some textbooks propose that the outermost membrane of the mitochondria and the primary chloroplast envelope derive from this phagosome. However, the protein and lipid compositions of mitochondria and chloroplast outer envelope membranes do not support this hypothesis and are rather reminiscent of the outermost membrane of an ancestral  $\alpha$ -proteobacterium and cyanobacteria, respectively. One can therefore consider that a reasonable scenario is either an entry within the cell, without any phagocytic membrane, or a loss of the phagosome over evolution.

An alternative option is that a hybrid membrane system might have appeared, combining features from the ancestral prokaryotic endosymbionts, the eukaryotic host, and possibly other prokaryotic partners (see below).

Phylogenetic and molecular analyses support that the mitochondria derive from a unique endosymbiotic event, with a debate whether the  $\alpha$ -proteobacterium might be related to the free-living *Pelagibacter* group or the parasitic *Rickettsiales* [20]. Different models for

eukaryotic origins were proposed, which were compatible with both mitochondrial-early and mitochondria-late scenarios [19], positioning therefore this event in a very broad period from 1.9 to 1 billion years ago. The primary chloroplast derives from a single endosymbiotic event occurring later, from 1.5 to 1 billion years ago [21]. It is believed to originate from, at least, a close relative of the deep-branching *Gloeomargarita* cyanobacterium, in combination with gene transfers from a variety of other sources [22-24].

### **3.3. The genetic and molecular integration of the mitochondrion and the chloroplast**

Mitochondrial and chloroplast chromosomes exist in both circular and linear forms [25,26]. They contain conserved genes coding for ribosomes components, tRNA and organellar proteins. They vary in size from a few kilobase pairs to thousands of kilobase pairs. A common feature is that none of these chromosomes is sufficient to ensure the complete biogenesis and maintenance of these organelles. A considerable reduction of the ancestral eubacterial genomes has occurred, following the massive loss and/or transfer of genes to the nucleus of the post-endosymbiotic cell [27,28]. This loss of autonomy is probably the most achieved in mitochondrial evolution, since DNA-free organelles, bounded by two membranes, are evidenced to derive from mitochondria, i.e. the hydrogenosome, which continues to generate energy for the host cell, and the mitosome, which does not [11]. Presence of cardiolipin, the mitochondrial specific phospholipid, has been demonstrated in hydrogenosomes [29], whereas at least some of the mitosomes are cardiolipin-free [30]. No such DNA-free organelle deriving from chloroplasts has been observed to date. The most reduced form of a plastid is a secondary plastid, found in Apicomplexa parasites, missing galactolipids that are specific to all plastids studied to that date [4, 31-33]. The function of genes transferred to the nucleus was preserved in many cases, schematically by introducing a short additional sequence at the N-terminus of nuclear-encoded proteins, allowing protein precursors to be actively translocated back from the cytosol to the endosymbiotic organelles

[34]. Mechanisms allowing the transfer of genetic material from the mitochondrion or the chloroplast to the nucleus are still active. This is actually one of the most robust aspects in our understanding of endosymbiosis, since this transition from free living organisms to full integration, based on a gene escape from the organelles to the nucleus, is still ongoing and can be evidenced by experiments. The principle is simply to incorporate a gene in the mitochondria of a yeast [35] or in the chloroplast of tobacco [36], carrying a nuclear selectable marker gene that allows the efficient selection of yeasts or plants, with a nuclear genome that carries pieces transferred from the mitochondria or the chloroplast genome, respectively. The rate of a gene escape from the mitochondrion to the nucleus is in the range of  $2 \cdot 10^{-5}$  per cell per generation in yeast [35]. The rate at which DNA migrates from the nucleus to mitochondria seems at least 100,000 times less [35]. The frequency of a gene escape from the chloroplast to the nucleus is at least of 1 per 5 million cells in tobacco [36]. A more puzzling question is how organellar sequences could be remodeled following their integration in the nuclear chromosomes, so as to contain domains allowing their addressing back to the mitochondrion, the chloroplast, and sometimes both. Specific N-terminal sequences, called the mitochondrial and chloroplast transit peptides (Mtp and Ctp) thus allow the entry in the mitochondrion *via* a large translocon complex, at the outer and inner envelope membrane (called TOM/TIM) and in the chloroplast *via* another translocon complex (called TOC/TIC). Following entry inside the organelles, the Mtp and Ctp are cleaved by specific peptidases in the mitochondrial matrix and chloroplast stroma, respectively. A convincing model has been introduced recently, proposing that these addressing N-terminal sequences derived from a specific property of the ancestors of the organelles, i.e. a resistance strategy to antimicrobial peptides that consisted in their rapid internalization and proteolytic disposal by microbial peptidases [27].

### **3.4. Inheritance of non $\alpha$ -proteobacterial and non-cyanobacterial genes by horizontal gene transfers**

The description we have given on the current reconstruction of the evolution of early eukaryotes seems simple. This picture might be satisfying if only genes tracing back to FECA (initial pool of Archaea, Bacteria and unique Eukarya genes),  $\alpha$ -proteobacterial and cyanobacterial genes were detected. The reduction of mitochondrial and chloroplast genomes should therefore coincide with an increase of nuclear-encoded genes with  $\alpha$ -proteobacterial and cyanobacterial signatures. However, this simple scheme is far from being satisfactory. The major issue is that a large number of genes have other signatures, i.e. non- $\alpha$ -proteobacterial genes and proteins in mitochondria [20] and non-cyanobacterial genes and proteins in chloroplasts [4,24,37]. This presence of mostly bacterial genes, and sometimes genes with no prokaryotic signatures highlight the importance of horizontal gene transfers (HGT), from other Eukarya and Bacteria [13]. In Fig. 4, HGT is considered at all stages of Eukarya evolution, and is still ongoing. Some bacterial genes could be incorporated via Bacteria-to-Archaea HGT before and after FECA emergence. The acquisition of the mitochondrion could involve Bacteria-to- $\alpha$ -proteobacterium HGT, followed by the escape of some of the mitochondrial genes to the nucleus. Likewise, the acquisition of the chloroplast could involve Bacteria-to-cyanobacteria HGT, followed by the escape of some genes to the nucleus. Some lateral gene transfers between both organelles could then have occurred.

### **3.5. Could an ancestral *Chlamydia* have helped primary chloroplast emergence? The Ménage-à-trois hypothesis (MATH).**

A novel hypothesis has been recently proposed based on the intriguing detection of genes having Chlamydial signatures in photosynthetic eukaryotes [21,38], often harboring a plastid-targeting signal [39]. Up to 55 genes are proposed to be transferred from Chlamydiae to

primary photosynthetic eukaryotes [40-43]. *Chlamydia* are obligate intracellular bacteria, including pathogens of animals and found in unicellular organisms such as amoebae [21]. The primary endosymbiosis at the origin of chloroplasts might have therefore involved an ancestral cyanobacterium and an ancestral *Chlamydia*, and be conserved thanks to Chlamydia-to-eukaryote LGT [40-41, 44]. The presence of the bacterial pathogen would have been critical in transmitting genes allowing the long-term residence of the cyanobacterial symbiont. Two models are currently considered (Fig. 5). In the first model, cyanobacteria and *Chlamydia* entered the host cell simultaneously but in distinct phagosomal vacuoles (Fig. 5, left). In the second model, both cyanobacteria and *Chlamydia* occupied the same phagocytic vacuole (Fig. 5, right). The co-occurrence of a cyanobacteria and *Chlamydia* is then supposed to have led to the development of a proto-chloroplast, combining genes obtained via intense LGT, making the basis for modern primary plastids.

### **3.6. Three lineages deriving from chloroplast primary endosymbiosis:**

#### **Glaucocystophytes, Chlorophytes and Rhodophytes.**

Following the primary endosymbiosis, which has led to the emergence of the chloroplast, three major lineages have been defined, based on pigments in photosynthetic machineries [45] (Fig. 6, A).

- The *green lineage of primary endosymbionts* (Viridiplantae), in which chlorophyll *a* is associated to chlorophyll *b*, contains the “green algae” (Chlorophyta), such as *Chlamydomonas reinhardtii*, and the so-called “plants” (Streptophyta), such as *Arabidopsis thaliana* ;
- The *red lineage of primary endosymbionts*, in which chlorophyll *a* is associated to phycobiline, contains the “red algae” (Rhodophyta) such as *Cyanidioschyzon merolae*.
- A non-green, non-red (sometimes called “blue”) *lineage of primary endosymbionts*, in which chlorophyll *a* is associated to phycocyanin and allophycocyanin, is a small group of

unicellular organisms (Glaucocystophytes), such as *Cyanophora paradoxa*, in which the chloroplast still contains a peptidoglycan cell wall.

#### **4. A second and independent cyanobacterial endosymbiosis in Rhizaria at the origin of the chromatophore**

The study of *Paulinella chromatophora*, a unicellular photosynthetic eukaryote, has led to the discovery of a second independent primary endosymbiosis leading to the integration of a cyanobacterium into a Rhizarian amoeba, 100 to 60 million years ago [46-48] (Fig. 4, B). The organelle is not called a chloroplast, but a chromatophore. Phylogenetic analyses have shown that a quarter of protein-encoding genes could be found in modern free-living *Synechocystis* species [36]. Just like in chloroplasts, a genome reduction of the chromatophore is ongoing and genes have escaped to the nucleus and encode proteins imported back into the organelle (almost 450 nuclear-encoded proteins targeted to the chromatophore) [48-52]. Like mitochondria and chloroplasts, the chromatophore function relies on a large number of bacterial genes originating from HGT [53]. This discovery is therefore fascinating. Firstly, this endosymbiotic event shows that, although it is rare, the emergence of a semi-autonomous organelle can still occur. Secondly, features observed in the  $\alpha$ -proteobacterium-to-mitochondrion and cyanobacteria-to-chloroplast transition are also observed. Thirdly, we are witnessing the emergence of a novel eukaryotic organization and the evolution of chromatophore-containing species might lead to a very rich and complex biodiversity in terms of molecular, structural and functional diversity that we cannot extrapolate today.

#### **5. Conclusion**

In this chapter, the current understanding of thylakoid emergence in cyanobacteria and the acquisition of chloroplasts by primary endosymbiosis has been summarized, leaving some frustrating unresolved questions, which might actually stay unresolved unless novel data are



made available. The presented model for the emergence of thylakoid membranes points to the actual function of companion proteins, such as Vipp1, and to the overlooked role of *HexII* ↔ *Lm* phase transitions promoted by such lipids as SQDG, in the biogenesis of membrane compartments. The emergence of semi-autonomous organelles seems to share general features, i.e. an acquisition of a bacterium/cyanobacteria likely via a phagocytic membrane, a genome reduction coinciding with an escape of genes from the organelle to the nucleus, and finally the appearance of an active system translocating nuclear-encoded proteins back to the organelles. An intense mobilization of foreign genes of bacterial origin, via HGT, plays a critical role. Some third partners, like *Chlamydia*, might even have facilitated the transition from cyanobacteria to chloroplasts. Interestingly, although the evolution of mitochondria can lead to the complete loss of DNA, occurring independently in multiple lineages, forming mitochondria-derived hydrogenosomes and mitosomes, no such phenomenon is currently demonstrated for chloroplasts. Altogether, this chapter illustrates also the diversity of organelle forms and functions that can derive from primary endosymbiosis. An increase in complexity has arisen from multiple events of secondary endosymbioses, between non-photosynthetic and photosynthetic eukaryotes and has raised superbly complicate cell structures. The understanding of the evolution, structure and function of these secondary or complex plastids are far less advanced compared to primary plastids, representing therefore a major goal and challenge for future research.

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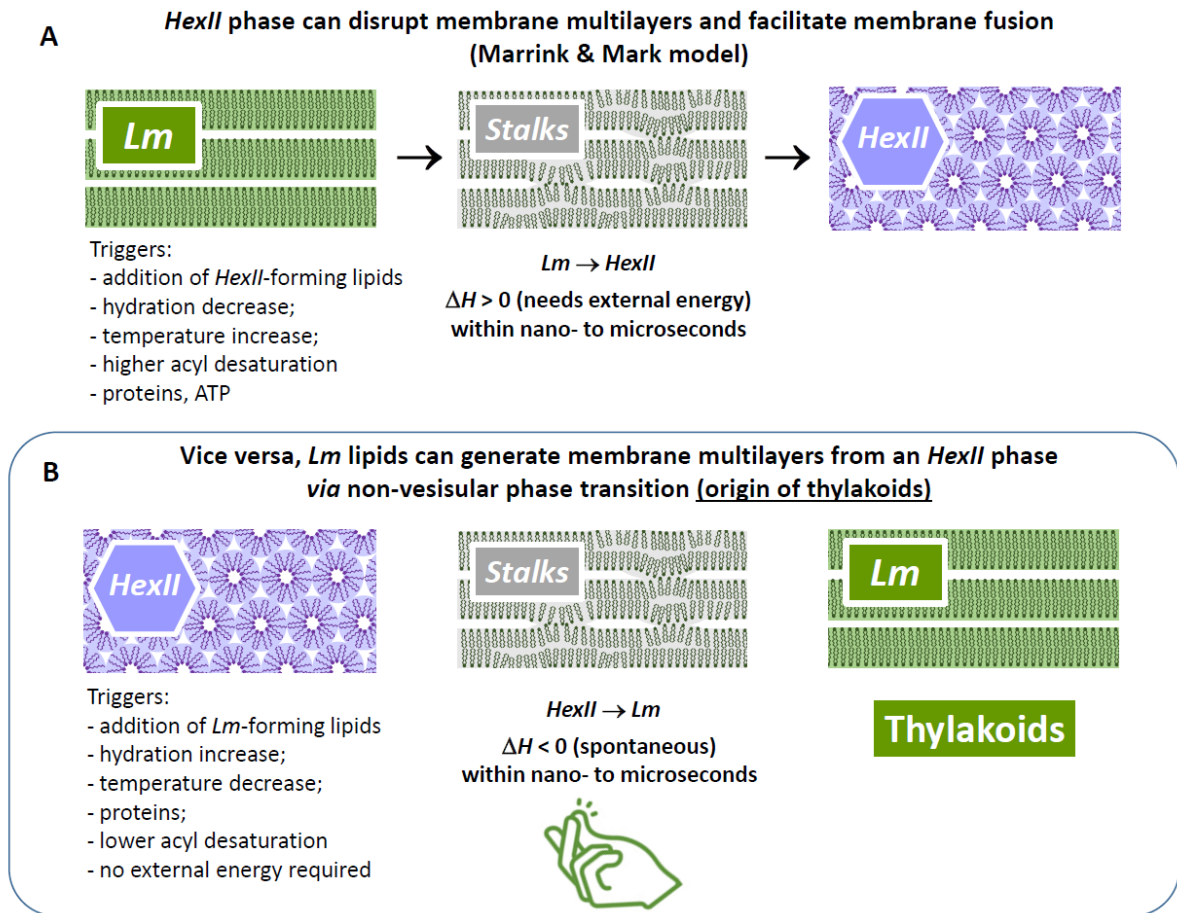
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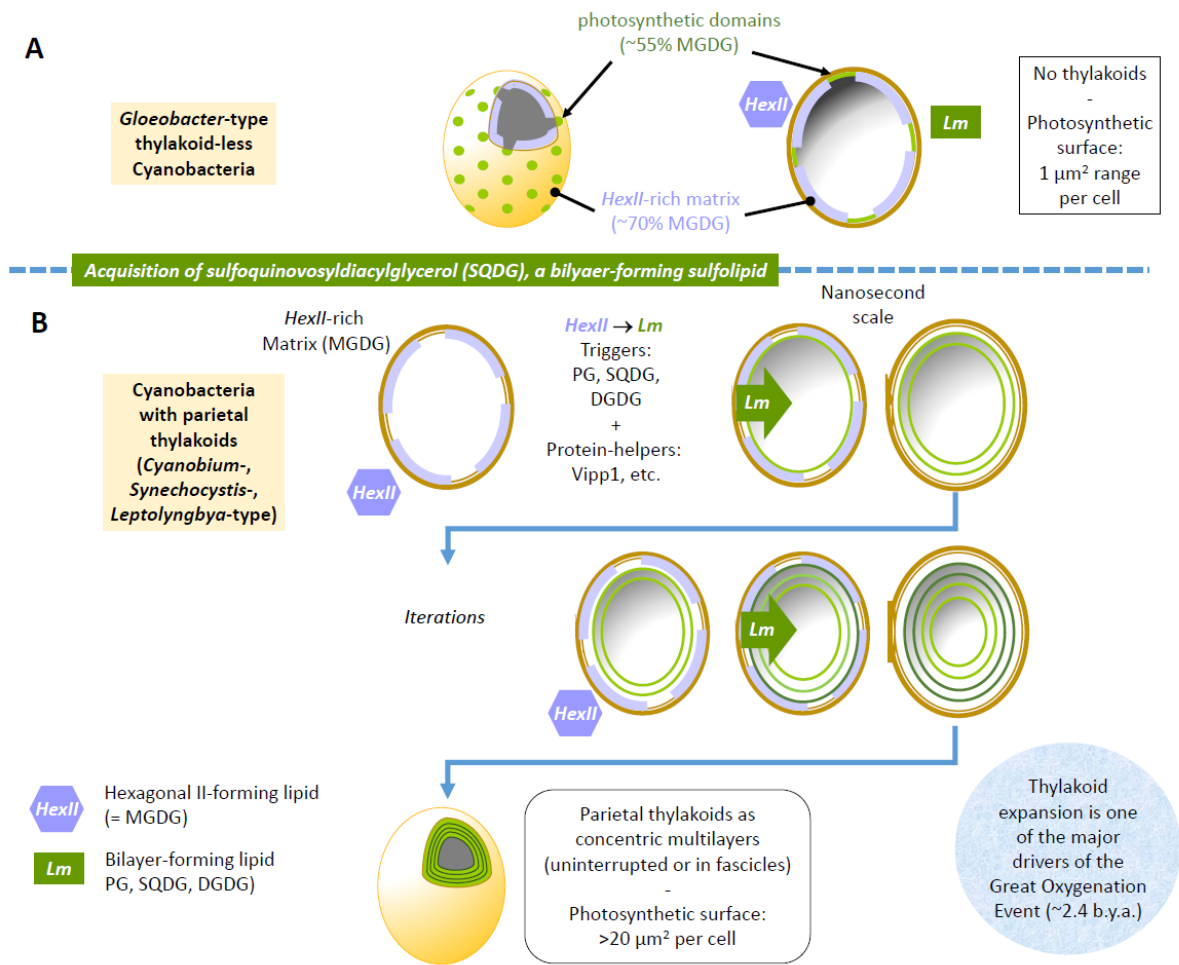
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## Figure Legends

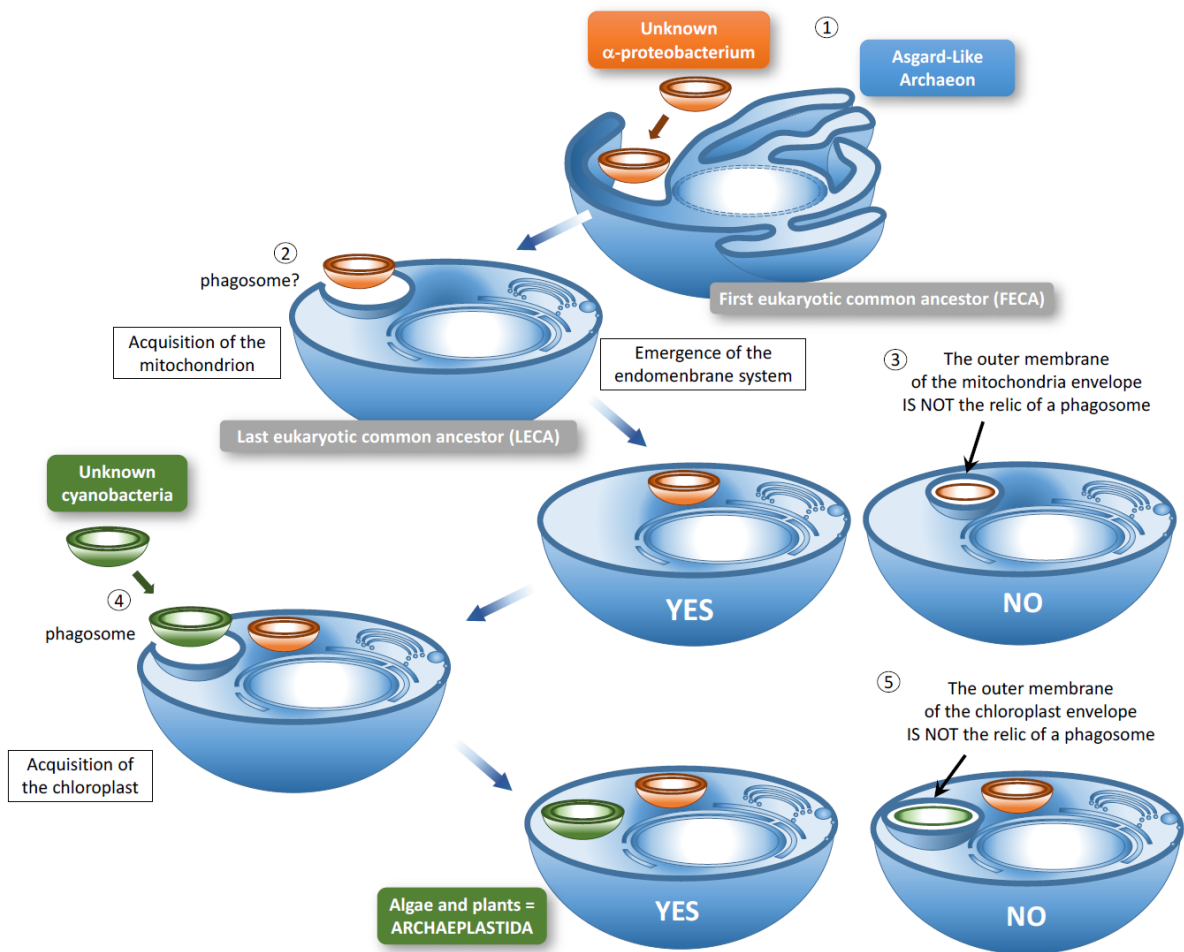


**Figure 1. Non lamellar-to-lamellar lipid phase transition at the origin of thylakoids.** **A.** The *Lm*-to-*HexII* phase transition has been described as a driving process of membrane fusion, conditioned by a supply of an external energy source. The intermediate phase, forming “stalks” according to the model of Marrink and Mark is shown. **B.** *HexII* to *Lm* phase transition as a driving process for thylakoid emergence. The major thylakoid lipid, monogalactosyldiacylglycerol, is a *HexII* lipid forming such structures. Based on the principle of reversibility, the phase transition is spontaneous, occurring within nanoseconds. It can be facilitated by molecular triggers, including *Lm*-forming lipids such as sulfoquinovosyldiacylglycerol.

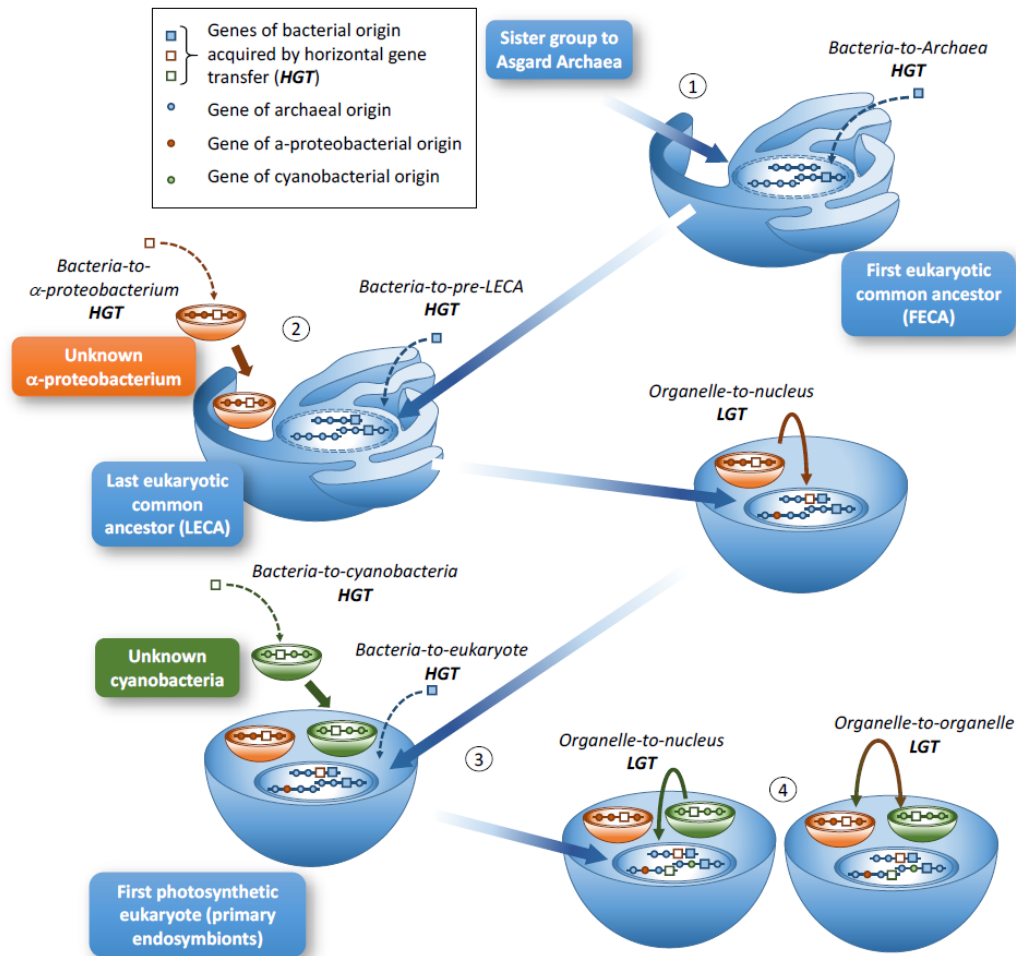




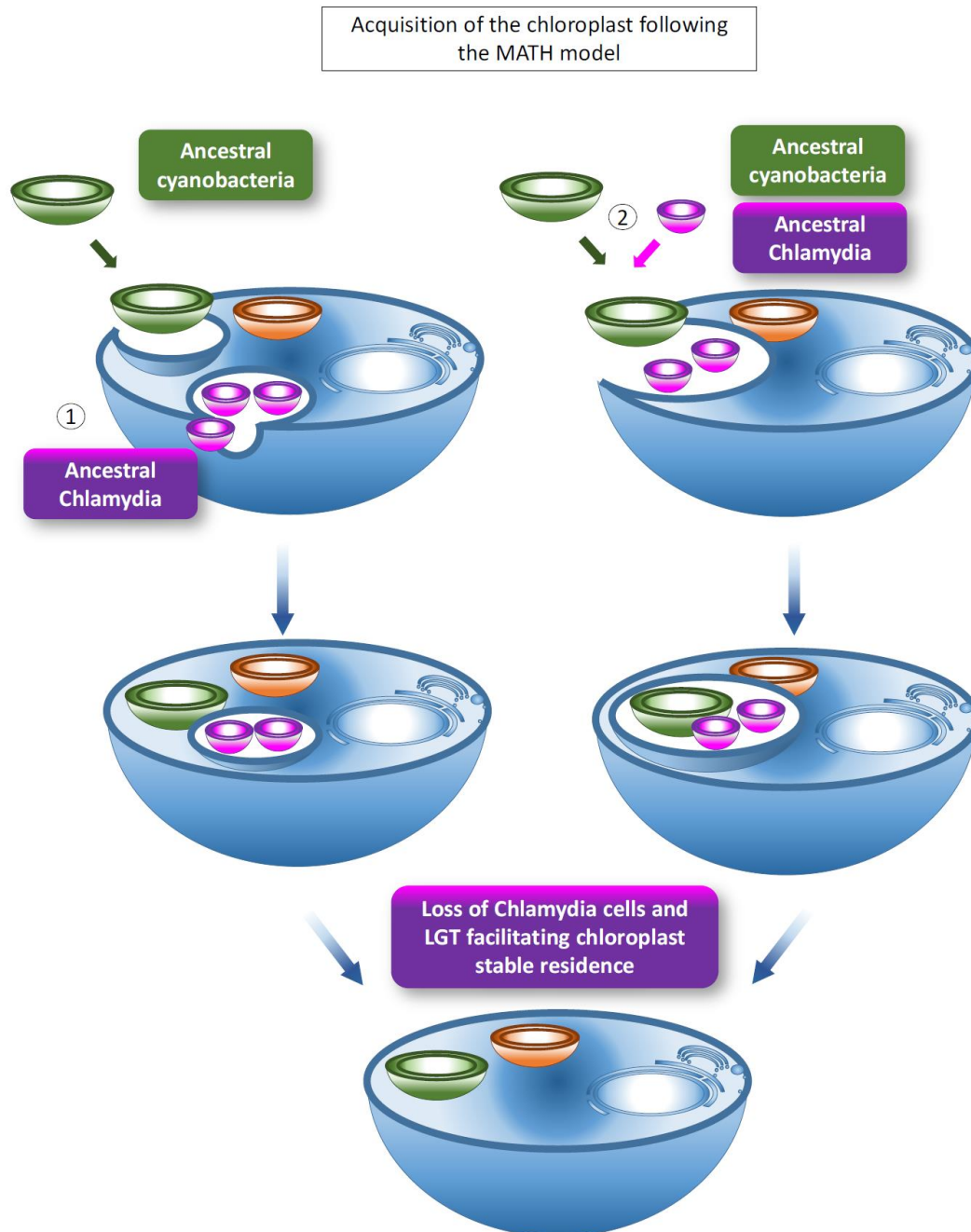
**Figure 2. Origin of thylakoids in early cyanobacteria.** **A.** In the ancient type of cyanobacteria like *Gloeobacter*, cells have domains in their cytosolic limiting membrane, in which photosystems are concentrated, with a balanced composition of *HexII* and *Lm*-forming lipids. A MGDG-rich matrix can therefore create local *HexII*-rich domains. This matrix can possibly form an *HexII* phase protruding inward. In *Gloeobacter*, the level of *Lm* lipids is not sufficient to trigger a transition from *HexII* to *Lm* phase. **B.** Later in the evolution, in the first SQDG-containing cyanobacteria, *Lm*-forming lipids are not limiting and can trigger serial *HexII*-to-*Lm* phase transitions, giving rise to concentric parietal thylakoids. Parietal thylakoids can be uninterrupted in *Cyanobium* cells (as illustrated) or forming fascicles in *Synechocystis* or *Leptolyngbya*. Given the increase of photosynthetic membrane surface thus introduced, a multiplier effect is compatible with a decisive role in the accumulation of  $\text{O}_2$  during the great oxygenation event (GOE).



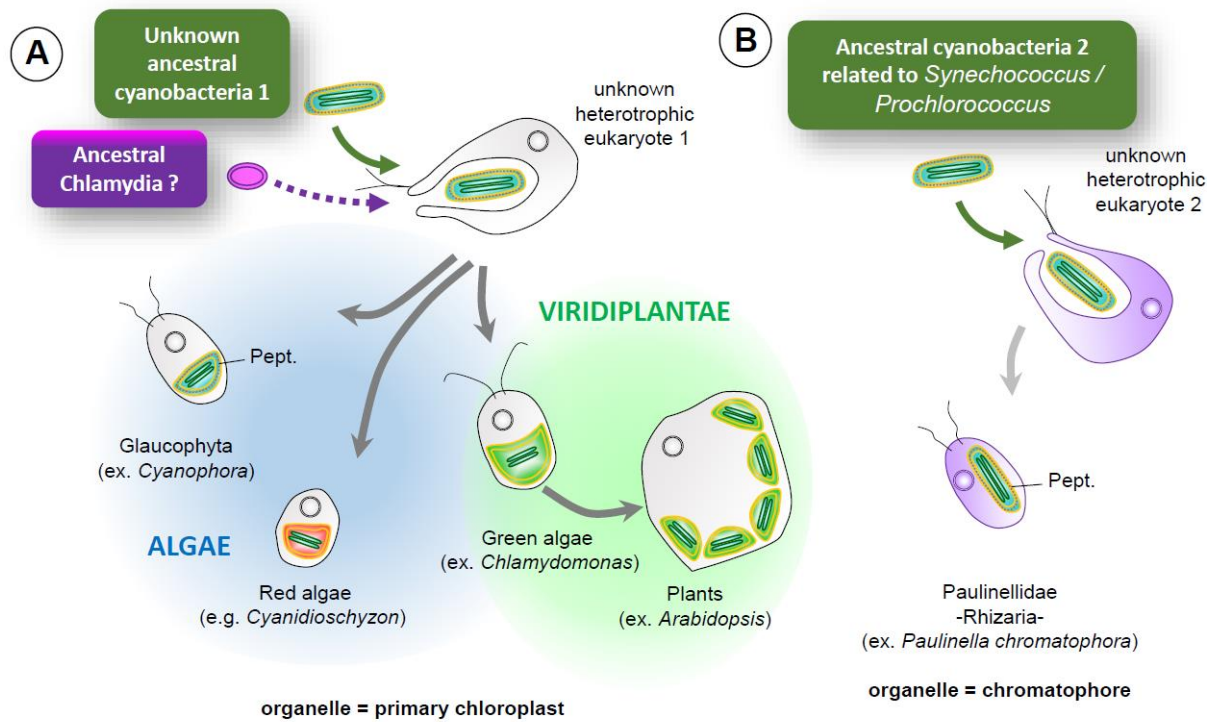
**Figure 3. From syntrophy and phagocytosis to endosymbiosis.** In this scheme, the First eukaryotic common ancestor (1) is shown containing an endomembrane system (in blue). The Last eukaryotic common ancestor (2) appears when an unknown  $\alpha$ -proteobacterium is engulfed within the cell, giving rise to the mitochondrion. Should it be via the formation of a network of cellular ‘tentacles’ or of actual phagosome, the phagosome-like structure is not conserved (3). In this simple scheme, the primary chloroplast derives from the engulfment of an unknown cyanobacterium (4). Again, the phagosome is not conserved (5). The two membranes limiting the mitochondrion and the chloroplast are therefore supposed to derive, mainly, from the outermost membranes of the  $\alpha$ -proteobacterium and the cyanobacterium, respectively.



**Figure 4. Importance of horizontal gene transfers in Eukarya evolution and in primary endosymbioses.** In this scheme, the First eukaryotic common ancestor (1) contains genes originating from Archaea and unique Eukarya origin (blue circles). Some bacterial genes (blue square) could be incorporated via Bacteria-to-Archaea horizontal gene transfer (HGT). The acquisition of the mitochondrion could involve Bacteria-to- $\alpha$ -proteobacterium HGT (2), explaining the presence of genes who do not carry an  $\alpha$ -proteobacterial signature in mitochondria (brown square). This endosymbiotic event was followed by the escape of some of the mitochondrial genes to the nucleus by a specific HGT, called here lateral gene transfer (LGT). Likewise, the acquisition of the chloroplast could involve Bacteria-to-cyanobacteria HGT (3), explaining the presence of genes who do not carry a cyanobacterial signature in chloroplasts (green square). Primary endosymbiosis of the chloroplast was followed by the escape of some genes to the nucleus by LGT. Some LGT between both organelles could then have occurred (4).



**Figure 5. Acquisition of the primary chloroplast following the M $\acute{e}$ nage-à-Trois Hypothesis (MATH).** In this scheme, the acquisition of the ancestral cyanobacteria coincides with the presence of parasitic Chlamydia, either in distinct (1) or identical (2) phagocytic vacuoles. The presence of Chlamydia cells provides a genetic environment adapted to the residence of a bacterium within a eukaryote. Following HGT, Chlamydia genes are proposed to have facilitated the cyanobacteria-to-chloroplast transition.



**Figure 6. Chloroplast-containing and chromatophore-containing eukaryotes.** (A) The organisms containing a primary chloroplasts belong to the Archaeplastida kingdom. The primary endosymbiosis has occurred more than 1 billion years ago. Three lineages have been defined: the green lineage comprising Green Algae and Plants (also called Viridiplantae), the red lineage comprising Red algae, and the non-green, non-red lineage comprising Glaucophyta, such as *Cyanophora* species. Glaucophyta contain a chloroplast with peptidoglycans, which origin might be distinct from those of the ancestral cyanobacteria. (B) An independent primary endosymbiosis has occurred 100-60 million years ago leading to the emergence of another type of photosynthetic organelle, the chromatophore, in Paulinellidae. The organelle contains peptidoglycans, like in cyanobacteria. Pept, peptidoglycans.