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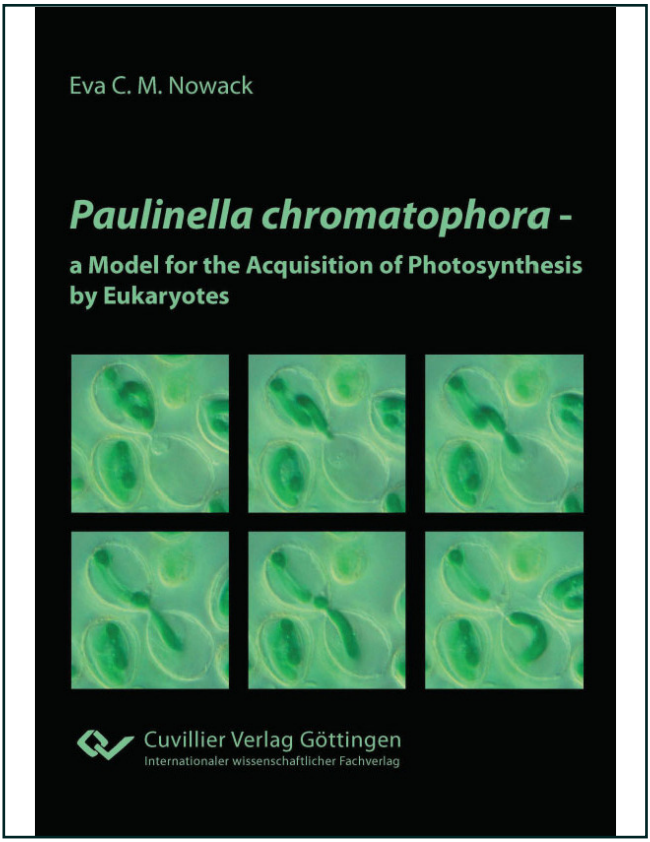
# Paulinella chromatophora – a Model for the Acquisition of Photosynthesis by Eukaryotes

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# 1 Introduction

## 1.1 On the Origin and Diversity of Plastids

Cyanobacteria arose 3.5 - 2.5 billion years ago (Ga), when the atmosphere of the Earth was still anoxic (Brocks et al., 1999; Knoll, 2008; Schopf and Packer, 1987). Using $CO_2$ as virtually infinite carbon source, light as virtually infinite energy source, and water as virtually infinite electron donator for the production of energy-rich sugars, the cyanobacteria established an extraordinarily successful bioenergetic concept: oxygenic photosynthesis. Probably as a result of the massive expansion of cyanobacteria, the Earth experienced the first dramatic rise of atmospheric $O_2$ around 2.3 Ga (Bekker et al., 2004; Raven, 1997). Interestingly, despite their fundamentally different type of cell organization, phototrophic eukaryotes that emerged at least 1.2 Ga (Butterfield, 2000; Butterfield et al., 1990), display the same type of photosynthesis as cyanobacteria, using $H_2O$ as electron donator for $CO_2$ fixation and producing $O_2$ by the action of two photosystems.

Influenced by the work of Richard Altmann, Julius Sachs, and Andreas Schimper (Altmann, 1890; Sachs, 1882; Schimper, 1883), in 1905 the Russian botanist Constantin Mereschkovsky postulated that plastids, the photosynthetic organelles of phototrophic eukaryotes, arose from once free-living cyanobacteria by an intracellular symbiotic association with a heterotrophic eukaryote (Mereschkowsky, 1905). Though initially very popular, the endosymbiont hypothesis for the origin of plastids in eukaryotes was not seriously considered until the 1970s (Kutschera and Niklas, 2005), when it experienced a revival and an expansion mainly driven by the American biologist Lynn Sagan, better known under her later name Lynn Margulis (Margulis, 1970; Sagan, 1967). Although some aspects of the symbiotic theory of cell evolution still provoke controversy, the endosymbiont hypothesis for the origin of plastids (and mitochondria) is commonly accepted today for there exist plenty of data supporting this view, particularly from the comparison of cyanobacterial and plastid genome sequences (Archibald, 2006; Delwiche, 1999; Douglas, 1998; Melkonian, 2001).

Moreover, the comparison of genomes revealed that the most distinguishing feature between cyanobacteria and plastids is the tremendous reduction in size and coding capacity of plastid genomes: Typically, cyanobacteria encode several thousand genes (e.g. 5,368 protein-coding genes in *Anabaena* sp. PCC 7120 (Kaneko et al., 2001), 3,168 protein-coding genes in *Synechocystis* sp. PCC 6803 (Kaneko et al., 1996b)), whereas the plastid genome with the largest coding capacity reported to date, that of the rhodophyte *Porphyra purpurea*, encodes a meager 251 genes (Reith and Munholland, 1995). Following endosymbiosis most cyanobacterial genes were either lost completely due to dispensability of their encoded function in an intracellular environment, or were transferred

to the nucleus of the eukaryotic host by endosymbiotic gene transfer (EGT). Their products are either targeted and reimported into the plastid or obtained new functions in other compartments of the eukaryotic cell (Martin et al., 2002; Reyes-Prieto et al., 2006). At least in species with several plastids per cell, where lysis of single plastids may casually occur, transfer of genetic material from the plastid to the nucleus is an ongoing process, observed at surprisingly high rates (Huang et al., 2003; Stegemann et al., 2003). Contrary, in organisms with a single plastid per cell, frequent EGT could not be detected, suggesting that gene transfer is driven by genetic material escaping from lysing plastids (Lister et al., 2003). When a plastidial gene enters the nucleus, its plastidial copies are initially retained in other plastids of the cell. To permit complete loss of the plastidial gene, expression and targeting of the newly acquired nuclear copy have to be achieved. This means that the gene needs to fall under the control of an appropriate promotor and acquire a plastid targeting signal (Martin and Herrmann, 1998). Additionally complicating is the fact that the gene moves from a prokaryote-derived genomic environment (i.e. compact, operon-containing, and intron-poor) to a eukaryotic one (i.e. inflated, operon-dividing, and intron-rich). Despite all these obstacles the majority of plastid-localized proteins are nuclear-encoded.

Relocation of genes from the plastid to the host nucleus appears to be favored for several reasons: First, plastid DNA is subjected to mutagenesis by free radicals that form during photosynthesis (Allen and Raven, 1996). Second, gene transfer to the host is supposed to facilitate integration and regulation of the endosymbiont. And finally, the haploid asexual lifestyle of an organelle may lead to fixation of deleterious mutations, so moving genes to the sexual milieu in the nucleus seems to be advantageous (Blanchard and Lynch, 2000). Thus, EGT combined with protein import is often regarded as hallmark for integrated organelles that discriminates them from mere endosymbionts (Cavalier-Smith and Lee, 1985).

Analysis of dozens of extant plastid genomes revealed that plastids had a monophyletic origin within cyanobacteria and most likely were acquired via a single cyanobacterial endosymbiosis (McFadden and van Dooren, 2004; Moreira et al., 2000; Palmer, 2003; Rodríguez-Ezpeleta et al., 2005). However, owing to the ancient divergence, the issue of what kind of cyanobacterium participated in the origin of plastids is difficult to resolve by phylogenetic methods. Only recently an extensive comparative study of complete genomes of photosynthetic and non-photosynthetic pro- and eukaryotes suggested that the ancestor of plastids might have been an organism similar to filamentous, heterocyst-forming cyanobacteria (Deusch et al., 2008).

In the course of evolution, the ancestor of photosynthetic eukaryotes that emanated from the initial plastid-forming endosymbiosis (=primary endosymbiosis) diverged into the three extant lineages

carrying primary plastids (kingdom Plantae, Figure 1): the Viridiplantae, comprising chlorophytes and streptophytes, the rhodophytes, and the glaucophytes (Rodríguez-Ezpeleta et al., 2005).

The plastids of the glaucophytes, which are referred to as cyanelles, are morphologically still most similar to their cyanobacterial ancestor in that they retained a residual peptidoglycan wall and

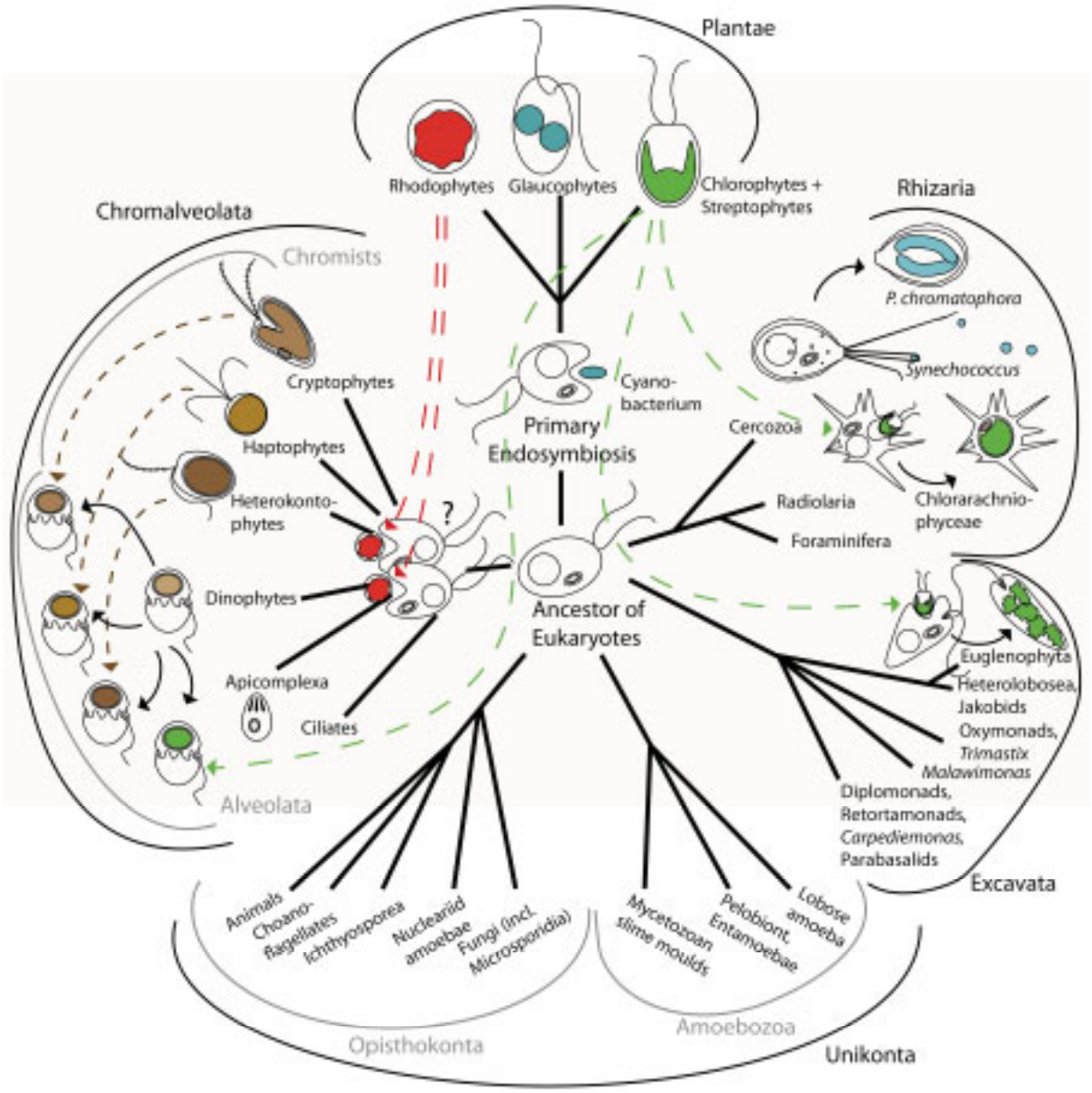


**Figure 1: Evolutionary history of plastid acquisition by primary, secondary, and tertiary endosymbioses.** In the center of the figure, the nucleate, mitochondrion-bearing ancestor of eukaryotes is depicted, giving rise to 5 to 7 superphyla of eukaryotes. Primary plastids evolved only once by the engulfment of a cyanobacterium by a heterotrophic host cell leading to the kingdom of Plantae. Chlorophytes and rhodophytes gave rise to the secondary plastids of the chlorarachniophytes, eugleophytes, Apicomplexa, dinophytes, heterokontophytes, haptophytes, and cryptophytes (highlighted by long-dashed lines, color-coded by the origin of plastid). Plastid-bearing members of the Chromists gave rise to tertiary plastids in the dinophytes that secondarily lost their former plastid (highlighted by short-dashed lines). The phylogenenetic tree underlying the figure is adopted from the current view of eukaryotic superphylogeny (compare Simpson and Roger, 2004). Branch lengths are chosen arbitrarily and do not reflect evolutionary distance. Nucleus and mitochondria are left out in plastid-bearing cells for reasons of clarity.

carboxysomes (Kies and Kremer, 1990). Nonetheless, genomically cyanelles are unmistakably fully integrated plastids (Stirewalt et al., 1995). All primary plastids are surrounded by two envelope membranes, derived from the inner and outer cyanobacterial membrane (Duy et al., 2007), and are endowed with a complicated protein import machinery partly derived from channel proteins of the former endosymbiont (Bölter et al., 1998; Reumann and Keegstra, 1999).

Later, by the sequential engulfment of photosynthetic unicellular eukaryotes by larger heterotrophic unicellular eukaryotes (=secondary and tertiary endosymbioses) a still wider diversity of plastid-containing eukaryotes evolved (Figure 1), namely the Euglenophyta, Chlorarachniophyta, Cryptophyta, Heterokontophyta, Haptophyta, dinoflagellates, and Apicomplexa (e.g. Delwiche and Palmer, 1997; Keeling, 2004). These organisms are characterized by the possession of 3 - 4 envelope membranes surrounding their plastids, more complex protein import mechanisms, and sometimes remnants of the nucleus of the eukaryotic endosymbiont, known as the nucleomorph (van Dooren et al., 2001). The majority of nuclear genes of the endosymbiont in secondary and tertiary plastids, however, has also been transferred to the new host nucleus (Douglas et al., 2001; Gilson et al., 2006).

The uptake of cyanobacteria did not only provide eukaryotes with a photosynthetic machinery but also with a new compartment for biosynthetic pathways such as fatty acid, isoprenoid, and amino acid biosynthesis (Gould et al., 2008). Today photosynthetic eukaryotes exhibit a bewildering biodiversity of primary producers that provides the Earth with most of its biomass.

## 1.2 The Exceptional Evolutionary Position of *Paulinella chromatophora*

*Paulinella chromatophora* (Figure 2) is a photosynthetic thecate amoeba of cercozoan affiliation (Bhattacharya et al., 1995; Cavalier-Smith and Chao, 2003) that is globally found in the sediments of freshwater ponds and lakes (Melkonian and Mollenhauer, 2005) and was first described in 1895 by Robert Lauterborn (Lauterborn, 1895). The Cercozoa encompass numerous flagellates and amoebae that mainly feed by means of pseudopodia and, together with the Foraminifera and Radiolaria, form the kingdom Rhizaria (Cavalier-Smith and Chao, 2003; Moreira et al., 2007). Within the Cercozoa *Paulinella* is member of the Euglyphida, an order of uninucleate cells with a surface that is covered with imbricated silica scales except for a terminal or lateral aperture for the naked filopodia (Cavalier-Smith and Chao, 2003). Besides *P. chromatophora*, there is a single clade among the Cercozoa that exhibits phototrophy: the chlorarachniophytes (see Figure 1). These net-forming amoebae that are only distantly related to the Euglyphida obtained a plastid by secondary endosymbiosis of a green alga (McFadden et al., 1994).

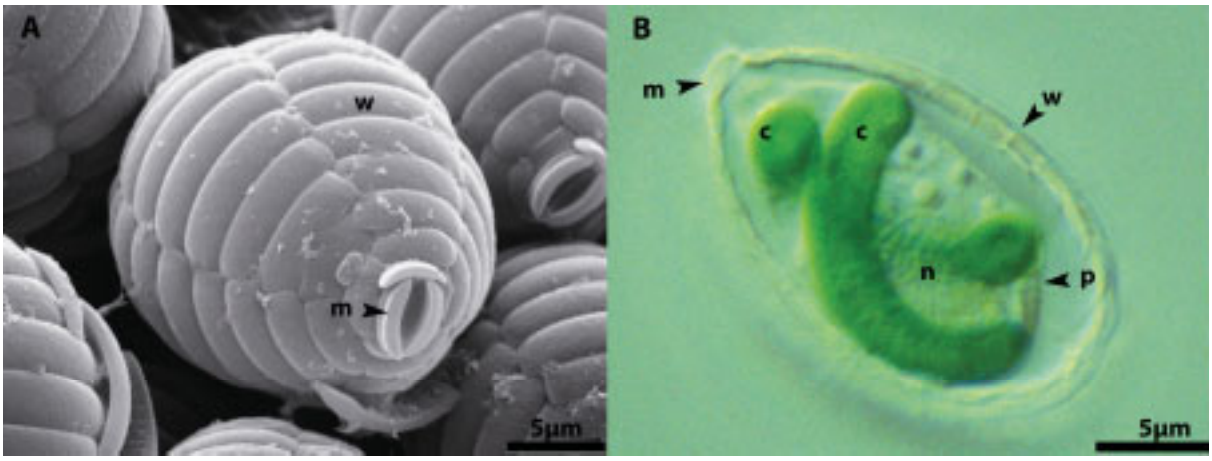


**Figure 2: *Paulinella chromatophora* cells.** (A) Scanning electron microscopic image. (B) Light microscopic image (differential interference contrast). Abbreviations: c, chromatophore; m, mouth opening; n, nucleus; p, plasma membrane; w, cell wall composed of silica scales.

In comparison, the two large, sausage-shaped intracellular photosynthetic entities present in *P. chromatophora,* termed chromatophores, clearly differ from green plastids but resemble in color and ultrastructure (possession of carboxysomes, unstacked thylakoids, and a peptidoglycan wall (Kies, 1974)) cyanobacteria of the *Synechococcus* type and cyanelles. Lauterborn clearly recognized the importance of his discovery for symbiosis research and biology in general (Melkonian and Mollenhauer, 2005): He postulated that the chromatophores 'might represent either autonomous organisms of the Cyanophytes, living in tight symbiosis with the rhizopod, or integrated parts, i.e. real organs, of the rhizopod body' (translated from Lauterborn, 1895).

In nature, *P. chromatophora* has never been observed without its chromatophores (Lauterborn, 1895; Penard, 1905), and in experimental culture the association between host and chromatophore has been stable for more than 15 years (personal communication, Melkonian, 2008). Furthermore, chromatophore and host cell cycle are strictly synchronized (Hoogenraad, 1927) and attempts to cultivate the symbiont separately from the host have failed thus far. These observations imply that the symbiosis is obligatory for both partners and that a high level of integration has been achieved. In contrast to their closest marine relatives that feed on cyanobacteria (Hannah et al., 1996; Johnson et al., 1988), food particles have never been observed in the hyaline cytoplasm of *P. chromatophora* (Kies, 1974; Lauterborn, 1895; Penard, 1905), suggesting that *P. chromatophora* has dispensed with phagotrophic nutrition. Instead Kies and Kremer detected photosynthetic radiocarbon assimilation by *P. chromatophora* following incubation of the cells in $H^{14}CO_3^-$ medium in the light and $^{14}C$ accumulation mainly in polysaccharides and proteins as well as low molecular weight organic compounds such as glucose, organic phosphates and amino acids (Kies and Kremer, 1979). Hence, providing energy in form of reduced carbon compounds to the host by using its photosynthetic machinery seems to be the main function of the chromatophore.