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Breaking the endosymbiont-organelle border: the case of *Paulinella chromatophora*

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Primary endosymbiosis gave rise to photosynthetic eukaryotes (glaucophytes, red algae and green plants) about 1.5 billion years ago, when a phagotrophic eukaryote enslaved a cyanobacterium, transforming it into a true cellular organelle called primary plastid. Such cyanobacterium-to-organelle transformation had been considered to be a singular event in the history of life on our planet until *Paulinella chromatophora* was discovered. This testate amoeba carries two photosynthetic endosymbionts (chromatophores) of cyanobacterial origin, which were acquired relatively recently, about 60-140 million years ago. The chromatophores have already experienced a significant genome reduction, endosymbiotic gene transfer of more than 30 genes, including those involved in photosynthesis, and evolved import machinery for nuclear-encoded proteins. These features make the chromatophores *bona fide* organelles but, in contrast to primary plastids, they import only proteins characterised by low molecular weight and nearly neutral charge. These restrictions in the protein import seem to result from the presence of the peptidoglycan wall and the lack of a channel-like translocon in the outer chromatophore membrane, which could perforate this wall together with a channel in the inner membrane.

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Primary plastids and their photosynthetic legacy

For as long as two billion years photosynthesis was restricted to cyanobacteria (Des Marais 2000). About 1.5 billion years ago, the know-how was co-opted by a unicellular phagotrophic protozoan (e.g., Yoon et al. 2004; Archibald 2015). This eukaryote instead of digesting engulfed cyanobacteria learned to benefit from their prolonged upkeep and subsequently transformed them into two-membrane photosynthetic organelles called primary plastids (reviewed by Ku et al. 2014; McFadden 2014). The cyanobacterium-to-organelle transformation, termed primary endosymbiosis, involved: (i) endosymbiotic gene transfer (EGT), (ii) loss of many genes, and (iii) evolution of a protein import system in endosymbiont membranes (the Toc-Tic apparatus; Figure 1A) for proteins encoded by nuclear genes including those derived from the EGT (Cavalier-Smith and Lee 1985; Bodył et al. 2009a; Kleine et al. 2009).

The primary endosymbiosis triggered evolutionary radiation of the supergroup Archaeplastida, composed of glaucophytes, red algae and green plants (Mackiewicz and Gagat 2014), and subsequent eukaryote-eukaryote endosymbioses initiated by the enslavement of red and green algae. The latter endosymbioses resulted in three- or four-membrane (complex) plastids of chlorarachniophytes, euglenids, cryptophytes, haptophytes, stramenopiles, dinoflagellates, and even parasitic apicomplexans, spreading the photosynthesis throughout the eukaryotic tree of life (Figure 2; reviewed by Archibald 2009; Keeling 2010).

How frequent are origins of primary photosynthetic organelles?

As morphological, molecular and phylogenetic data strongly support a single endosymbiotic origin of all primary plastids, all extant plastids, including complex ones, must stem from a single cyanobacterial endosymbiosis (Archibald 2009; Keeling 2010). One of the arguments in support of a single endosymbiotic event is the Toc-Tic apparatus in primary plastid membranes (Paila et al. 2015) and in their evolutionary derivatives, i.e. two innermost membranes of complex plastids (Sheiner and Striepen 2013). The presumed difficultly in establishment

of an efficient protein import system in endosymbiont membranes was behind formulation of a paradigm postulating minimization of the number of endosymbioses to explain the observed plastid diversity (Cavalier-Smith and Lee 1985). This paradigm of cellular evolution seems to be, especially, of paramount importance for organelles of prokaryotic origin. In contrast to complex plastids, which inherited the efficient Toc-Tic apparatus and use the existing endomembrane pathway to traverse outer, eukaryote-derived plastid membranes (Sheiner and Striepen 2013), prokaryotic organelles have to evolve protein import machinery. This, however, does not have to be as difficult as it is assumed because it can occur by a kind of molecular tinkering from pre-existing components of both the cyanobacterial endosymbiont and the eukaryotic host (Shi and Theg 2013). Further, it is possible to reconstruct gradual evolution of the Toc and Tic translocons, which suggests that their origin may not represent a unique evolutionary process (discussed in Bodył et al. 2007; Bodył et al. 2009a).

Interestingly, the possibility of multiple origins of primary plastids is still considered and some phylogenies indeed break the monophyly of Archaeplastida, questioning the paradigm that endosymbiont-to-organelle transformation is indeed difficult, and therefore, exceptionally rare (Stiller 2007; Howe et al. 2008; Kim and Graham 2008; Burki et al. 2016). Such claims are especially tempting in light of the discovery that primary photosynthetic endosymbiosis is not a unique event in the history of life on our planet, but has happened at least twice, as the example of *P. chromatophora* shows (Figure 2).

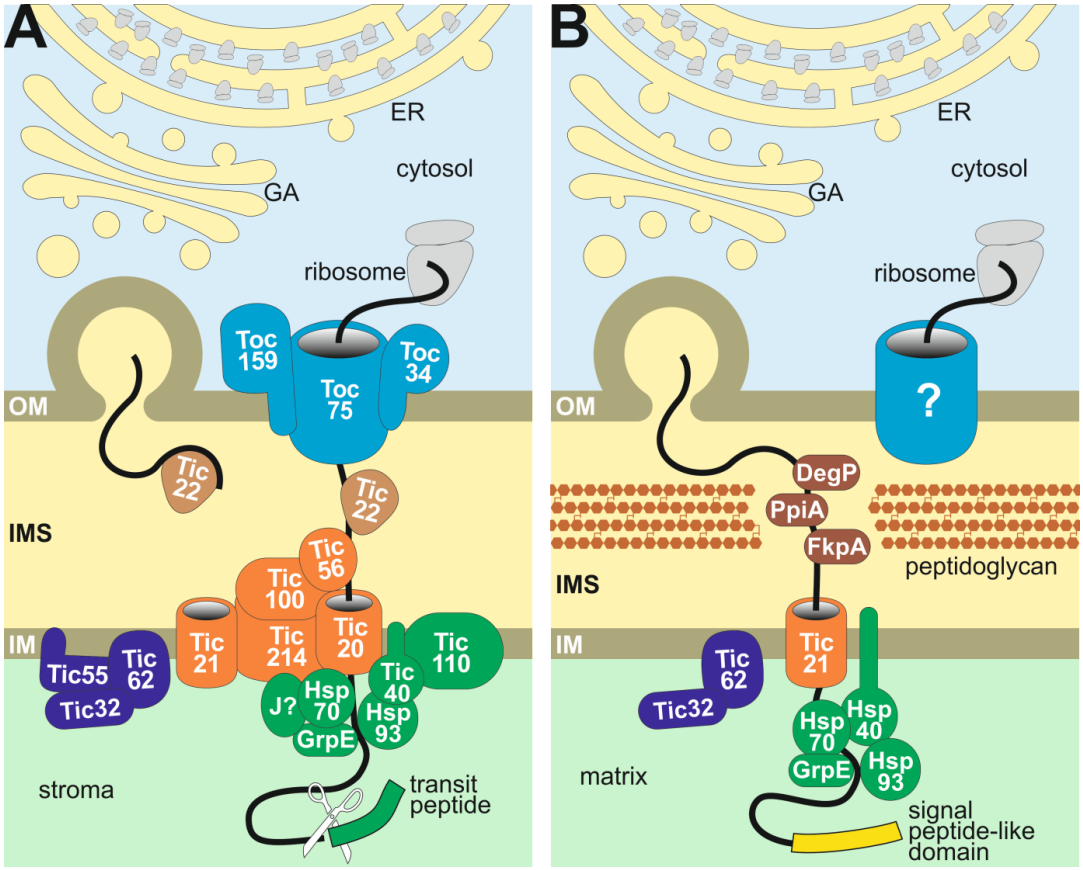

Figure 1: Model for protein import into primary plastids (A) and the photosynthetic bodies of *P. chromatophora* (B). (A) Most proteins imported into primary plastids carry transit peptides at their N-termini and are post-translationally translocated across the plastid envelope with the help of Toc-Tic apparatus, a translocon at the outer (OM) and inner (IM) chloroplast membranes. Only a handful of proteins are imported into primary plastids in vesicles via the endomembrane system, involving the endoplasmic reticulum (ER) and the Golgi apparatus (GA) (Gagat et al. 2013). The Toc translocon contains a protein-conducting channel Toc75, and two transit peptide receptors Toc159 and Toc34. The structure of the Tic translocon is still unclear but it seems to be composed of several complexes: (i) the main translocation complex (Tic20, Tic214, Tic100, Tic56 and Tic21), (ii) the main molecular motor (Tic110, Tic40 and Hsp93), (iii) an additional import motor (J?, Hsp70, GrpE) and (iv) a regulation complex (Tic62, Tic55, Tic32). Tic22 is responsible for protein movement through the intermembrane space (IMS) of primary plastids. (B) Nuclear-encoded, chromatophore-targeted proteins are usually equipped with a signal peptide-like domain, and therefore likely enter Paulinella chromatophores in vesicles of the endomembrane system. After their release into the intermembrane space (IMS), they have to cross the peptidoglycan wall, which could be facilitated by molecular chaperones, such as DegP, FkpA, PpiA, and Hsp70. The final import step, i.e. translocation across the inner chromatophore membrane, may proceed via a simplified Tic-like apparatus, composed of Tic21 (protein-conducting channel), Tic32 and Tic62 (calcium and redox-sensing regulatory proteins) as well as a molecular motor responsible for pulling imported proteins into the organelle matrix. The latter could consist of Hsp93, Hsp70, Hsp40 and GrpE.

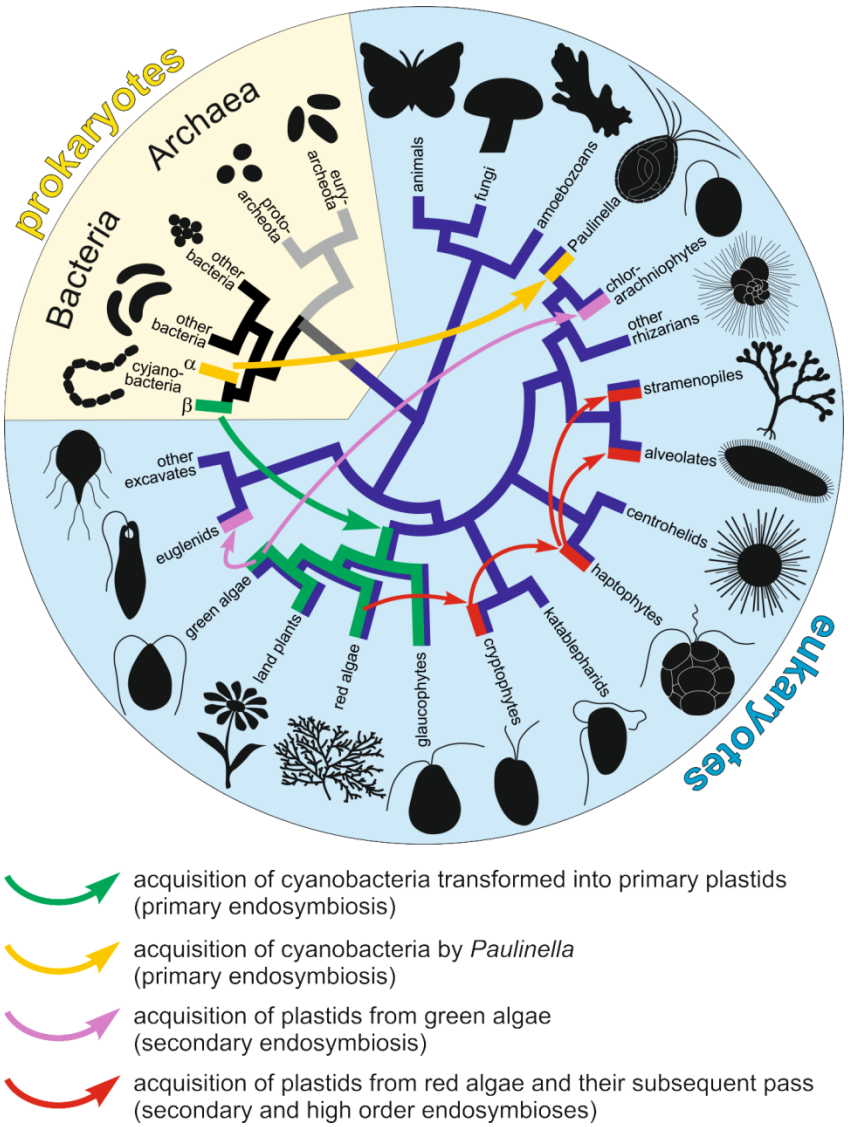

Figure 2: Evolutionary history of primary and complex plastids. Primary plastids of glaucophytes, red algae and green plants are photosynthetic organelles surrounded by two membranes that directly descend from a β-cyanobacterial endosymbiont engulfed by the phagotrophic eukaryote in the process of primary endosymbiosis. Independently and much later, *P. chromatophora* acquired an α-cyanobacterial endosymbiont. The primary plastids of Archaeplastida were horizontally passed to other eukaryotic lineages in subsequent endosymbiotic events. Green algae gave rise to three and four membrane plastids of chlorarachniophytes and euglenids, respectively, whereas red algae to three membrane plastids of peridinin dinoflagellates and four membrane plastids of cryptophytes, haptophytes, stramenopiles and apicomplexans. According to the Chromalveolata hypothesis (Cavalier-Smith 1999), there was only one secondary red algae endosymbiosis; consequently, all protists with red alga-derived plastids evolved from a single, red alga-carrying ancestor. However, more and more data support alternative evolutionary scenarios which postulate serial and multiple endosymbioses and independent horizontal plastid transfers (Bodył 2005; Bodył et al. 2009b; Burki et al. 2012, 2016). The "serial and multiple endosymbioses" model is also supported by the fact that some dinoflagellates swapped their peridinin plastid for haptophyte (fucoxanthin dinoflagellates) and stramenopile (dinotom dinoflagellates) endosymbionts, whereas Dinophysis regularly acquires cryptophyte plastids from the ciliate Myrionecta (Gagat et al. 2014). The presented relationships between eukaryotic lineages should be considered tentative because the tree of life is still not completely resolved. They are mainly based on phylogenies obtained by Burki et al. (2016) but we retained the monophyly of Archaeplastida. The depicted directions of secondary and higher order endosymbioses involving red algal plastid are also hypothetical and are mainly based on plastid gene phylogenies.

P. chromatophora and its photosynthetic bodies

P. chromatophora is a testate amoeba, enclosed within a shell composed of silica plates, that moves through sediments of freshwater and brackish bodies, and even marine sand flats, by means of thread-like (filose) pseudopodia (reviewed by Bodyl et al. 2016, see also Kim and Park 2016). It belongs to the supergroup Rhizaria, a lineage that shows much closer affiliation to stramenopiles and alveolates than archaeplastidians (Figure 2; Burki 2014; Burki et al. 2016). Paulinella was discovered by

Robert Lauterborn (1895), a German biologist, in a material collected from an old riverbed of the river Rhine on Christmas Eve of 1894, a true Christmas present for the endosymbiosis researches (see also Melkonian and Mollenhauer 2005).

What is interesting about Paulinella is that it harbours two double membrane-bound photosynthetic bodies (called chromatophores), acquired independently of primary plastids from a cyanobacterium ~60-140 million years ago (Marin et al. 2005; Nowack et al. 2008; Delaye et al. 2016). Although Paulinella chromatophores are of cyanobacterial origin, they are derived from a distinct cyanobacterial group than the primary plastids of Archaeplastida (Figure 3; Marin et al. 2005). In accordance with their prokaryotic origin, Paulinella chromatophores have preserved several cyanobacterial features such as: (i) a bacterial cell wall composed of peptidoglycan layers, (ii) phycobilisomes on the outer surface of thylakoid membranes and (iii) carboxysomes which are responsible for concentrating CO_2 (Kies 1974). The relatively recent acquisition of the chromatophores renders Paulinella an immensely interesting subject from the point of view of endosymbiont-to-organelle transformation (discussed in Bodył et al. 2012; Nakayama and Archibald 2012).

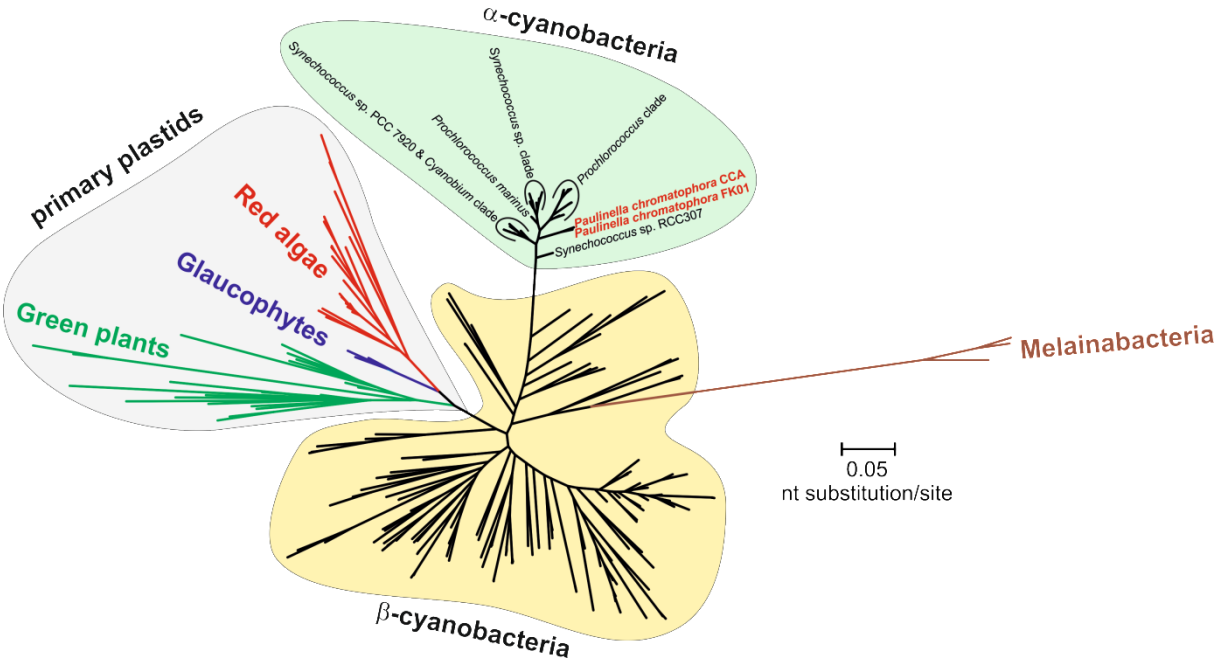

Figure 3: A Bayesian phylogenetic tree obtained in MrBayes (Ronquist et al. 2012) based on the concatenated alignment of 16S and 23S rRNA showing independent cyanobacterial origin of primary plastids and Paulinella photosynthetic organelles. The chromatophores show close affiliation to Synechococcus/Prochlorococcus group. In the analyses, we assumed separate mixed+I+Γ(5) models for these two partitions and two independent runs, each using 8 Markov chains. Trees were sampled every 100 generations for 10,000,000 generations. After obtaining the convergence, the last 2,912,000 trees from each chain were collected to compute the posterior consensus.

Paulinella chromatophores are very deeply integrated with the host cell permitting it fully autotrophic existence. They divide synchronously with the host and exchange metabolites with its cytosol (Kies 1974; Kies and Kremer 1979; Marin et al. 2005). Further, the chromatophore genomes of two different Paulinella strains CCAC 0185 (Nowack et al. 2008) and FK01 (Reyes-Prieto et al. 2010) have been sequenced and they indicate significant genome reduction in terms of its size and gene number. Compared to their closest free-living relative, the cyanobacterium *Cyanobium gracile* PCC 6307, the chromatophore genomes have been reduced from ~3 Mb to ~1 Mb and their coding capacity form ~3.300 genes to ~900 genes (Nowack et al. 2008; Reyes-Prieto et al. 2010).

Endosymbiotic gene transfer and protein import into Paulinella chromatophores

Such substantial genome reduction raises a question about possible endosymbiotic gene transfers, and indeed more than 30 EGT-derived genes have been found in the Paulinella nuclear genome (Nakayama and Ishida 2009; Reyes-Prieto et al. 2010; Nowack et al. 2011). Among these genes, there are also those involved in photosynthesis or photosynthesis-related processes, indicating evolution of protein import route, or routes through chromatophore membranes. The putative protein import into Paulinella chromatophores has been mainly investigated *in silico* and as a result a model for protein import into Paulinella

chromatophores has been proposed (Bodył et al. 2010; Mackiewicz et al. 2012a, 2012b; Gagat and Mackiewicz 2014). The model involves: (i) vesicular trafficking to the outer chromatophore membrane, (ii) a simplified Tic-like complex at the inner chromatophore membrane and (iii) a molecular motor responsible for pulling imported proteins into the chromatophore matrix (Figure 1B). Interestingly, the model was partly proved experimentally by Nowack and Grossman (2012), who demonstrated that some proteins are imported in vesicles of the endomembrane system to the outer chromatophore membrane.

The presence of vesicular trafficking to the outer membrane of Paulinella chromatophores requires this membrane be compatible with the host system for vesicle formation and fusion. Thus, it could be suggested that this membrane is derived from the phagosomal membrane of the host, as hypothesized by Kies (1974). Such compatibility might have also been ensured by membrane chimerization, which means acquisition of some host lipids and proteins, either during endosymbionts' escape from the phagosome and/or by establishing vesicular connection based on the initial bacterial vesicular pathway (discussed in Bodył et al. 2016). The former process was also suggested to have contributed to the chimerization of primary plastid outer membrane (Bodył et al. 2009a). The inner chromatophore membrane, similarly to primary plastids, is derived from the cyanobacterial plasmalemma (Bodył et al. 2016).

Paulinella chromatophores are true cellular organelles

The fact that Paulinella endosymbionts are so deeply integrated with their host has been the subject of a hot debate, for many years, whether they really represent true cell organelles or barely endosymbionts (see Bhattacharya and Archibald 2006; Theissen and Martin 2006; Bodył et al. 2007, 2012). The presence of protein import machinery indicates that the chromatophores are true organelles, but they differ in some aspects from primary plastids.

The presence of protein import machinery is the essence of the commonly accepted discrimination between endosymbionts and organelles provided by Cavalier-Smith and Lee (1985). According to them endosymbionts should preserves all genes important for their functioning and, as a result, they do not require to import proteins from the host cell. In contrast, organelles retain only a small fraction of their original gene pool, and depend on nuclear-encoded proteins imported via established import mechanisms using proper targeting signals.

In light of the definition of Cavalier-Smith and Lee (1985), Paulinella chromatophores are *bona fide* organelles. They have experienced significant genome reduction and EGT. Furthermore, some of the transferred genes, including genes involved in photosynthesis, are imported into the chromatophores in vesicles of the host's endomembrane system (see previous sections). Mackiewicz et al. (2012a) also found that some of the EGT-derived proteins carry signal peptides, that could be used by the classical co-translational transport pathway. These signal peptides could have evolved either from host or cyanobacterial/endosymbiont sequences, by exon shuffling or modifications of the N-terminal hydrophobic parts of the proteins, respectively.

Why Paulinella chromatophores import only a special subset of nuclear-encoded proteins?

The eight EGT-derived proteins presumably imported into Paulinella chromatophores and the two for which import was confirmed experimentally are characterised by low molecular weight and nearly neutral charge (Nowack et al. 2011; Mackiewicz et al. 2012a). Although these features constitute good adaptations to their passage through the peptidoglycan wall, still preserved between the outer and inner chromatophore membrane (Figure 1B), they also gives us a very important clue about the protein import machinery itself. It suggests that the system is best suited for small proteins and perhaps cannot serve larger polypeptides (Nowack et al. 2011; Mackiewicz et al. 2012a).

Undoubtedly, Paulinella photosynthetic bodies are true cellular organelles, but they import much less nuclear-encoded proteins than primary plastids (Kleine et al. 2009). This phenomenon seems to result from both the presence of the peptidoglycan wall and the import of nuclear-encoded proteins via the endomembrane system. Evolution of a channel-based translocon in the outer chromatophore membrane and its connection with the Tic-like translocon in the inner membrane, as it is observed in galucophyte plastids with the peptidoglycan wall (Wunder et al. 2007), could bypass these obstacles in future.

Further candidates for cyanobacterium-derived organelles

There are many other interesting endosymbiotic associations between eukaryotes and cyanobacteria, in addition to *P. chromatophora* (Rai et al. 2003). Some of them have reached a relatively advanced level of integration with their host cells. Especially notable is a diatom *Rhopalodia gibba*, which harbours coccoid cyanobacteria responsible for nitrogen fixation (Prechtl et al. 2004). Interestingly, these endosymbionts are vertically transmitted and their genome have been subjected to reductive evolution characteristic of other endosymbionts. The genome have become rich in AT and decreased its size, whereas many genes were lost, fused, truncated or became pseudogenes (Prechtl et al. 2004; Kneip et al. 2008). Such endosymbionts seem to be on a good way to become true cellular organelles.

References

Archibald JM. (2009) The puzzle of plastid evolution. *Curr Biol.* 19:R81-R88.

Archibald JM. (2015) Endosymbiosis and eukaryotic cell evolution. *Curr Biol.* 25:R911-R921.

Bhattacharya D, Archibald J. (2006) The difference between organelles and endosymbionts. Response to Theissen and Martin. *Curr Biol.* 16:R1017-R1018.

Bodył A, Cieśla J, Mackiewicz P, Gagat P. (2016) How frequent are transformations of bacterial endosymbionts into cell organelles? The case of the thecate amoeba *Paulinella chromatophora*. In: Grube M, Seckbach J, Muggia L (eds) Algal and Cyanobacteria Symbioses. World Scientific Publishing and Imperial College Press. *In Press*.

Bodył A. (2005) Do plastid-related characters support the chromalveolate hypothesis? *J Phycol.* 41:712-719.

Bodył A, Mackiewicz P, Stiller JW. (2007) The intracellular cyanobacteria of *Paulinella chromatophora*: endosymbionts or organelles? *Trends Microbiol.* 15:295-296.

Bodył A, Mackiewicz P, Stiller JW. (2009a) Early steps in plastid evolution: current ideas and controversies. *BioEssays.* 31:1219-1232.

Bodył A, Stiller JW, Mackiewicz P. (2009b) Chromalveolate plastids: direct descent or multiple endosymbioses? *Trends Ecol Evol.* 24:119-121.

Bodył A, Mackiewicz P, Stiller JW. (2010) Comparative genomic studies suggest that the cyanobacterial endosymbionts of the amoeba *Paulinella chromatophora* possess an import apparatus for nuclear-encoded proteins. *Plant Biol.* (Stuttg) 12:639-649.

Bodył A, Mackiewicz P, Gagat P. (2012) Organelle evolution: Paulinella breaks a paradigm. *Curr Biol.* 22:R304-R306.

Burki F, Okamoto N, Pombert J-F, Keeling PJ. (2012) The evolutionary history of haptophytes and cryptophytes: phylogenomic evidence for separate origins. *Proc R Soc Lond*. [Biol] 279:2246-2254.

Burki F. (2014) The eukaryotic tree of life from a global phylogenomic perspective. *Cold Spring Harb Perspect Biol.* 6:a016147.

Burki F, Kaplan M, Tikhonenkov DV, Zlatogursky V, Minh BQ, Radaykina LV, Smirnov A, Mylnikov AP, Keeling PJ. (2016) Untangling the early diversification of eukaryotes: a phylogenomic study of the evolutionary origins of Centrohelida, Haptophyta and Cryptista. *Proc R Soc Lond.* [Biol] 283:20152802.

Cavalier-Smith T, Lee JJ. (1985) Protozoa as hosts for endosymbioses and the conversion of symbionts into organelles. *J Eukaryot Microbiol.* 32:376-379.

Cavalier-Smith T. (1999) Principles of protein and lipid targeting in secondary symbiogenesis: euglenoid, dinoflagellate, and sporozoan plastid origins and the eukaryote family tree. *J Eukaryot Microbiol.* 46:347-366.

Delaye L, Valadez-Cano C, Pérez-Zamorano B. (2016) How really ancient is *Paulinella chromatophora*? *PLOS Currents: Tree of Life.* doi:10.1371/currents.tol.e68a099364bb1a1e129a17b4e06b0c6b.

Des Marais DJ (2000) When did photosynthesis emerge on Earth? *Science.* 289:1703-1705.

Gagat P, Bodyl A, Mackiewicz P. (2013) How protein targeting to primary plastids via the endomembrane system could have evolved? A new hypothesis based on phylogenetic studies. *Biol Direct.* 8:18.

Gagat P, Bodył A, Mackiewicz P, Stiller J. (2014) Tertiary plastid endosymbioses in Dinoflagellates. In: Löffelhardt W (ed) Endosymbiosis. Springer Vienna, pp 233-290.

Gagat P, Mackiewicz P. (2014) Protein translocons in photosynthetic organelles of *Paulinella chromatophora*. *Acta Soc Bot Pol.* 83:399-407.

Howe CJ, Barbrook AC, Nisbet RE, Lockhart PJ, Larkum AW. (2008) The origin of plastids. *Philos Trans R Soc Lond B Biol Sci.* 363:2675-2685.

Keeling PJ. (2010) The endosymbiotic origin, diversification and fate of plastids. *Philos Trans R Soc Lond B Biol Sci.* 365:729-748.

Kies L. (1974) Electron microscopical investigations on *Paulinella chromatophora* Lauterborn, a thecamoeba containing blue-green endosymbionts (Cyanelles). *Protoplasma.* 80:69-89.

Kies L, Kremer BP. (1979) Function of cyanelles in the tecamoeba *Paulinella chromatophora*. *Naturewissenschaften.* 66:578-579.

Kim E, Graham LE. (2008) EEF2 analysis challenges the monophyly of Archaeplastida and Chromalveolata. *PLoS One.* 3:e2621.

Kim S, Park MG. (2016) *Paulinella longichromatophora* sp. nov., a new marine photosynthetic testate amoeba containing a chromatophore. *Protist.* 167:1-12.

Kleine T, Maier UG, Leister D. (2009) DNA transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis. *Annu Rev Plant Biol.* 60:115-138.

Kneip C, Voss C, Lockhart PJ, Maier UG. (2008) The cyanobacterial endosymbiont of the unicellular algae Rhopalodia gibba shows reductive genome evolution. *BMC Evol Biol* 8:30

Ku C, Roettger M, Zimorski V, Nelson-Sathi S, Sousa FL, Martin WF. (2014) Plastid origin: who, when and why? *Acta Soc Bot Pol.* 83:281-289.

Lauterborn R. (1895) Protozoenstudien II. *Paulinella chromatophora* nov. gen., nov. spec., ein beschalter Rhizopode des Süsswassers mit blaugrünen chromatophorenartigen Einschlüssen. *Z Wiss Zool.* 59:537-544.

Mackiewicz P, Bodyl A, Gagat P. (2012a) Possible import routes of proteins into the cyanobacterial endosymbionts/plastids of *Paulinella chromatophora*. *Theory Biosci.* 131:1-18.

Mackiewicz P, Bodyl A, Gagat P. (2012b) Protein import into the photosynthetic organelles of and its implications for primary plastid endosymbiosis. *Symbiosis.* 58:99-107.

Mackiewicz P, Gagat P. (2014) Monophyly of Archaeplastida supergroup and relationships among its lineages in the light of phylogenetic and phylogenomic studies. Are we close to a consensus? *Acta Soc Bot Pol.* 83:263-280.

Marin B, Nowack EC, Melkonian M. (2005) A plastid in the making: evidence for a second primary endosymbiosis. *Protist.* 156:425-432.

McFadden GI. (2014) Origin and evolution of plastids and photosynthesis in eukaryotes. *Cold Spring Harb Perspect Biol.* 6:a016105.

Melkonian M, Mollenhauer D. (2005) Robert Lauterborn (1869-1952) and his *Paulinella chromatophora*. *Protist.* 156:253-262.

Nakayama T, Ishida K-I. (2009) Another acquisition of a primary photosynthetic organelle is underway in *Paulinella chromatophora*. *Curr Biol.* 19:R284-R285.

Nakayama T, Archibald JM. (2012) Evolving a photosynthetic organelle. *BMC Biol.* 10:35.

Nowack EC, Melkonian M, Glockner G. (2008) Chromatophore genome sequence of Paulinella sheds light on acquisition of photosynthesis by eukaryotes. *Curr Biol.* 18:410-418.

Nowack EC, Grossman AR. (2012) Trafficking of protein into the recently established photosynthetic organelles of *Paulinella chromatophora*. *Proc Natl Acad Sci USA.* 109:5340-5345.

Nowack ECM, Vogel H, Groth M, Grossman AR, Melkonian M, Glöckner G. (2011) Endosymbiotic gene transfer and transcriptional regulation of transferred genes in *Paulinella chromatophora*. *Mol Biol Evol.* 28:407-422.

Paila YD, Richardson LG, Schnell DJ. (2015) New insights into the mechanism of chloroplast protein import and its integration with protein quality control, organelle biogenesis and development. *J Mol Biol.* 427:1038-1060.

Prechtl J, Kneip C, Lockhart P, Wenderoth K, Maier UG. (2004) Intracellular spheroid bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin. *Mol Biol Evol.* 21:1477–1481.

Rai AN, Bergman B, Rasmussen U. (eds) (2003) *Cyanobacteria in symbiosis*. Kluwer Academic Publishers, Dordrecht.

Reyes-Prieto A, Yoon HS, Moustafa A, Yang EC, Andersen RA, Boo SM, Nakayama T, Ishida K, Bhattacharya D. (2010) Differential gene retention in plastids of common recent origin. *Mol Biol Evol.* 27:1530-1537.

Ronquist F, Teslenko M, van der Mark P et al. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 61:539–542.

Sheiner L, Striepen B. (2013) Protein sorting in complex plastids. *Biochim Biophys Acta.* 1833:352-359.

Shi LX, Theg SM. (2013) The chloroplast protein import system: from algae to trees. *Biochim Biophys Acta.* 1833:314-331.

Stiller JW. (2007) Plastid endosymbiosis, genome evolution and the origin of green plants. *Trends Plant Sci.* 12:391-396.

Theissen U, Martin W. (2006) The difference between organelles and endosymbionts. *Curr Biol.* 16:R1016-R1017.

Wunder T, Martin R, Löffelhardt W, Schleiff E, Steiner JM. (2007) The invariant phenylalanine of precursor proteins discloses the importance of Omp85 for protein translocation into cyanelles. *BMC Evol Biol.* 7:236-236.

Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. (2004) A molecular timeline for the origin of photosynthetic eukaryotes. *Mol Biol Evol.* 21:809-818.