Glaucophyta

Dana C. Price, Jürgen M. Steiner, Hwan Su Yoon,
Debashish Bhattacharya, and Wolfgang Löffelhardt

Abstract

The Glaucophyta is by far the least species-rich phylum of the Archaeplastida comprising only four described genera, *Glaucocystis*, *Cyanophora*, *Gloeochaete*, and *Cyanoptyche*, and 15 species. However, recent molecular and morphological analyses reveal that glaucophytes are not as species poor as hitherto assumed with many novel lineages existing in natural environments. Glaucophytes are freshwater phototrophs of moderate to low abundance and retain many ancestral plastid traits derived from the cyanobacterial donor of this organelle, including the remnant peptidoglycan wall in their envelope. These plastids were originally named "cyanelles," which was later changed to "muroplasts" when their shared ancestry with other Archaeplastida was recognized. The model glaucophyte, *Cyanophora paradoxa*, is well studied with respect to biochemistry, proteomics,

D.C. Price
Department of Plant Biology and Pathology, The State University of New Jersey, New Brunswick, NJ, USA
e-mail: d.price@rutgers.edu

J.M. Steiner
Institute of Biology, Plant Physiology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany
e-mail: juergen_steiner@gmx.at

H.S. Yoon
Department of Biological Sciences, Sungkyunkwan University, Suwon, Republic of Korea
e-mail: hsyoon2011@skku.edu; hwansu@gmail.com

D. Bhattacharya
Department of Ecology, Evolution and Natural Resources, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA
e-mail: bhattacharya@rutgers.edu

W. Löffelhardt (✉)
Department of Biochemistry and Cell Biology, University of Vienna, Vienna, Austria
e-mail: wolfgang.loeffelhardt@univie.ac.at

J.M. Archibald et al. (eds.), *Handbook of the Protists*,
DOI 10.1007/978-3-319-32669-6_42-1

and the gene content of the nuclear and organelle genomes. Investigation of the biosynthesis of cytosolic starch led to a model for the transition from glycogen to starch storage during plastid endosymbiosis. The photosynthetic apparatus, including phycobilisome antennae, resembles that of cyanobacteria. However, the carbon-concentrating mechanism is algal in nature and based on pyrenoids. Studies on protein import into muroplasts revealed a primordial Toc/Tic translocon. The peptidoglycan wall was elucidated with respect to composition, biosynthesis, and involvement of nuclear genes. The muroplast genome is distinct, not due to the number of encoded genes but, rather, because of the presence of unique genes not present on other plastid genomes. The mosaic nature of the gene-rich (27,000) nuclear genome came as a surprise, considering the relatively small genomes of unicellular red algae.

Keywords

Archaeplastida • *Cyanophora paradoxa* • Muroplasts • Single primary endosymbiotic event • Phylogenomics • Carbon-concentrating mechanism • Eukaryotic peptidoglycan • Phycobilisomes

Contents

Summary Classification

● **Glaucophyta** (Skuja 1954) Glaucocystophyta (Kies and Kremer 1986)
●● **Glaucophyceae** Bohlin
●●● **Glaucocystales** Bessey
●●●● **Glaucocystaceae** G.S. West (*Gloeochaete*, *Cyanoptyche*, *Glaucocystis*)
●●● **Cyanophorales** Kies and Kremer
●●●● **Cyanophoraceae** Kies and Kremer (*Cyanophora*)

Introduction

General Characteristics

The phylum Glaucophyta Kies and Kremer 1986 (synonym: Glaucophyta Skuja 1954) contains a single class, the Glaucocystophyceae Schaffner 1922. It comprises a small group of unicellular mastigotes (monadoid members), unicellular and colonial organisms devoid of flagella with persistent contractile vacuoles (capsalean members), and unicellular and colonial organisms lacking any characters of mastigotes in the vegetative stage (coccoid members). Currently, four genera are known with at least 15 species. Glaucophytes live photoautotrophically with the aid of their unique plastids that are surrounded by a remnant peptidoglycan wall. These organelles were named cyanelles by Pascher (1929), a denomination which was later proven to be incorrect and thus was replaced by the more appropriate term "muroplast" coined by Schenk (1994) (Fig. 1). Muroplasts owe their origin to cyanobacteria, providing direct proof for the endosymbiotic theory of plastid evolution. The glaucophytes are thought to be the most ancient phylum of phototrophic eukaryotes although molecular data provide inconclusive data regarding this hypothesis (Martin et al. 1998; Reyes-Prieto and Bhattacharya 2007a; Price et al. 2012). Together with rhodophytes and chlorophytes/streptophytes, they constitute the Archaeplastida (Adl et al. 2005) that contain "primary" plastids surrounded by two envelope membranes. The major reason that we understand the evolutionary importance of glaucophytes is the excellent and meticulous ultrastructural studies conducted by Ludwig Kies as summarized in Kies (1992). The unifying characters of this phylum are the presence of muroplasts with peptidoglycan layers in their envelopes (Fig. 1) and a number of shared morphological features (see below). This grouping was later corroborated by phylogenetic analyses based on 16S (Helmchen et al. 1995) and 18S rRNA (Bhattacharya et al. 1995a; Marin et al. 1998) and concatenated protein sequences (Rodríguez-Ezpeleta et al. 2005).

Occurrence

Glaucophytes are relatively rare in nature, occupying niches. All members inhabit freshwater environments in the plankton or benthos of lakes, ponds, or ditches. Only

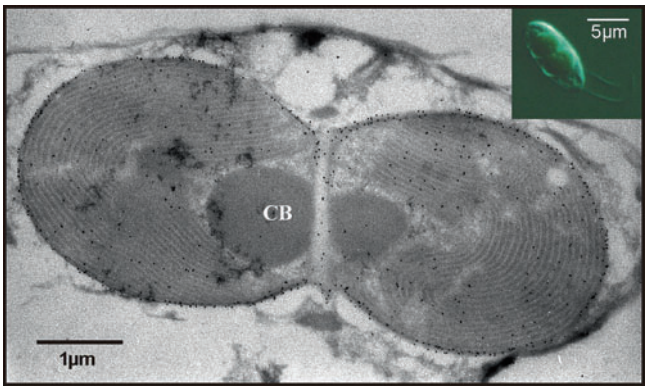

Fig. 1 *Cyanophora paradoxa* SAG 29.80. Immuno-EM of a dividing muroplast. Primary antibodies directed against peptidoglycan from *E. coli*. Gold particles mainly decorate the envelope and the newly formed septum. The division furrow neatly cleaves the RuBisCo-containing central body (CB), the genetic material surrounding it, and the concentric thylakoids into two halves destined for the daughter muroplasts. *Insert*: Interference contrast micrograph showing the ovoid cell, the flagella, and two muroplasts

four genera are maintained in culture collections, i.e., *Cyanophora*, *Gloeochaete*, *Cyanoptyche*, and *Glaucocystis* (Table 1), and thus are available for research. Almost all biochemical and molecular data acquired during the past 25 years (after the review by Kies and Kremer 1990) were obtained from *Cyanophora paradoxa*, which is the model organism for this phylum. A relatively fast growth rate, ease of cell lysis, and stable muroplasts account for its wide usage in research. Species that once were grouped together with the glaucophytes but were not deposited in an algal culture collection are not further dealt with here.

Literature and History of Knowledge

Kies and Kremer (1990) review the early literature, until the end of the 1980s, and explore the morphological criteria characteristic of glaucophytes. The excellent EM work of Ludwig Kies is presented in this chapter whenever possible. Bhattacharya and Schmidt (1997) review the phylogenetic analyses supporting the phylum Glaucophyta. Löffelhardt et al. (1997a) and Löffelhardt and Bohnert (2001) include the forthcoming molecular (muroplast genome sequence) and biochemical (fine structure of muroplast peptidoglycan) data until the end of the 1990s. The important issue of protein targeting to the muroplasts of *C. paradoxa* is dealt with in two reviews (Steiner and Löffelhardt 2002, 2005). Genomic data from *C. paradoxa* and *G. nostochinearum* and microarray data revealing CO_2-responsive genes and their involvement in the inorganic carbon-concentrating mechanism (CCM) are presented in Rodríguez-Ezpeleta et al. (2005) and Burey et al. (2007), respectively. The landmark paper describing the nuclear genome sequence of *C. paradoxa* (Price

Table 1 Strains of Glaucophyta available from culture collections of algae

Taxon	Culture collection and number	Isolator and year of isolation	Origin	Remarks
Cyanophora biloba	UTEX LB 2766	P. Kugrens 1997	USA	Ephemeral alpine pond
Cyanophora cuspidata T. Takahashi and Nozaki	NIES-3645	T.Takahashi and Nozaki	Japan	
	SAG 45.84	L. Kies 1967	Germany	1555 (Kies strain), axenic
	=CCAC 0091			
Cyanophora paradoxa Korsh	CCAP 981/1	G. Pringsheim 1943	England	Pringsheim strain, ovoid, axenic
	= UTEX LB 555			
	= SAG 29.80			
	= CCMP329			
	= NIES-547			
	= CCAC 0074			
Cyanophora kugrensii T. Takahashi and Nozaki	NIES-763	S. Suda 1991	Japan	Axenic
Cyanophora sudae T. Takahashi and Nozaki	NIES-764	S. Suda 1991	Japan	Broad bean shape, generally four plastids (2–8), axenic
Cyanophora tetracyanea[a]	Not available (NA)			Similar to *C. sudae*
Cyanoptyche gloeocystis	SAG 34.90	L. Kies 1984	Austria	2643 (Kies strain)
	SAG 4.97	O. Lourenco 1989	Portugal	ACOI 387 (Santos strain)
Cyanoptyche sp.	CCAC 2322 B	E. Kusel 1994	Austria	ASW 10005
Glaucocystis geitleri	SAG B 229–3 (= UTEX 1929?)	R. A. Lewin 1963		Designated *G.* cf. *nostochinearum* by Schnepf et al. (1966), G1 clade in Chong et al. (2014)
Glaucocystis geitleri	UTEX B 1929 (NA)	R. A. Lewin		Designated Lewin CY-11, G1 clade in Chong et al. (2014)

(*continued*)

Table 1 (continued)

Taxon	Culture collection and number	Isolator and year of isolation	Origin	Remarks
Glaucocystis geitleri	SAG 28.80	R.M. Brown 1975		G1 clade in Chong et al. (2014)
Glaucocystis nostochinearum Itzigs.	SAG 16.98	U. G. Schlösser 1997	Germany	G2 clade in Chong et al. (2014)
Glaucocystis nostochinearum Itzigs.	SAG 45.88	D. Mollenhauer 1983	Germany	Axenic, G2 clade in Chong et al. (2014)
Glaucocystis miyajii	NIES-1961	A. Kai 2003	Japan	G3 clade in Chong et al. (2014)
Glaucocystis oocystiformis	NIES-966	F. Kasai 1987	Japan	G4 clade in Chong et al. (2014)
Glaucocystis oocystiformis	NIES-1369	A. Kai 2003	Japan	G4 clade in Chong et al. (2014)
Glaucocystis bhattacharyae	SAG 27.80	Tassigny 1966	France	G5 clade in Chong et al. (2014)
Glaucocystis bhattacharyae	HS30 (NA)	H.S. Yoon 2010	USA	G5 clade in Chong et al. (2014)
Glaucocystis bhattacharyae	BBH (NA)	H.S. Yoon 2009	USA	G5 clade in Chong et al. (2014)
Glaucocystis incrassata	SAG 229–2	G. Pringsheim 1955	Denmark	Designated *G. incrassata* Lemmermann by Schnepf et al. (1966), G6 clade in Chong et al. (2014)
Glaucocystis incrassata	SAG B 229–1	E. A. George 1952	England	Designated *G. geitleri* nom. Prov. Pringsheim by Schnepf et al. (1966), G6 clade in Chong et al. (2014)
	= UTEX 64 (NA)			
	= NIES-2141			
	= CCAP 229/1			

(*continued*)

Table 1 (continued)

Taxon	Culture collection and number	Isolator and year of isolation	Origin	Remarks
Glaucocystis sp. (no molecular data)	CCAC 0088 B	B. Marin 1993	Germany	Axenic
	CCAC 2233 B	L. Kies 1980	Germany	2523 (Kies strain)
	CCAC 2234 B	L. Kies 1975	Germany	2343 (Kies strain)
	CCAC 2235 B	L. Kies 1977	Germany	2395 (Kies strain)
	CCAC 2323 B	E. Kusel 1993	Austria	ASW 10006
	CCAC 2877 B	M. Melkonian 2006	Russia	Collector A. Gontcharov
	CCAC 2994 B	M. Melkonian 2007	Germany	
	CCAC 3352 B	M. Melkonian 2007	Switzerland	
	CCAC 3353 B	M. Melkonian 2010	Switzerland	
Gloeochaete wittrockiana Lagerheim	SAG 46.84	L. Kies 1973	Germany	2323 (Kies strain)

UTEX The Culture Collection of Algae at the University of Texas at Austin, Texas 78,712, USA, *SAG* Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität, D-3400 Göttingen, Germany, *CCAC* Culture Collection of Algae at the University of Cologne, Cologne, Germany, *CCAP* Culture Collection of Algae and Protozoa, Scottish Marine Institute, Oban, UK, *NIES* Microbial Culture Collection at the National Institute for Environmental Studies, Tsukuba, Japan, *NA* currently not available

Data on strains of Glaucocystophyceae have been compiled from website of culture collections below: http://www.uni-goettingen.de/; http://www.ccap.ac.uk; http://www.ccac.uni-koeln.de/, http://www.utex.org; http://mcc.nies.go.jp

[a]Might correspond to *C. paradoxa* (Kugrens 2001)

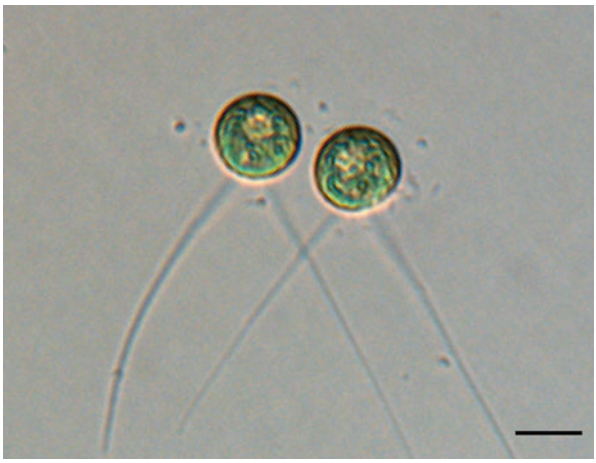

Fig. 2 Two cells of *Gloeochaete wittrockiana*, strain SAG 46.84 (Kies strain IAB 2323). Each cell contains two long pseudocilia. Interference contrast light micrograph. Scale line=10μm

et al. 2012) provided distinct support for a single plastid primary endosymbiotic event and gave rise to a number of related reviews (Bhattacharya et al. 2014; Löffelhardt 2014; Facchinelli and Weber 2015; Jackson et al. 2015).

The recognition of Glaucophyta is intimately connected to the concept of endosymbiosis between protists and cyanobacteria and the theory of the evolution of eukaryotic cells (Mereschkowsky 1905; Margulis 1981; Margulis and Sagan 2003). After thorough investigations, both Geitler (1959a) and Pascher (1929) concluded that *Cyanophora* species (Fig. 1), *Gloeochaete wittrockiana* (Figs. 2, 3, and 5) and *Glaucocystis nostochinearum* (Figs. 4a, b, 6, and 7), were cases of symbioses between heterotrophic host cells and modified autotrophic cyanobacterial endosymbionts functioning like plastids. Such endosymbionts were named "cyanelles" by Pascher (1929) who created the terms "endocyanome" for the whole consortium and "endocyanosis" for this particular type of endosymbiosis. Nowadays, the kingdom "Archaeplastida," also known as "Plantae," is thought to have resulted from a single successful primary endosymbiotic event between a cyanobacterium and a heterotrophic protist. Once this immensely complicated and lengthy process was successfully completed, the "protoplastid" became the ancestor of all plastids known to date, regardless of differences in traits such as pigmentation and morphology. This does not rule out much more recent instances of endosymbiotic organellogenesis as in *Paulinella* (Nowack et al. 2008) or *Rhopalodia* (Kneip et al. 2008).

In contrast to all other plastids, with the potential exception of the moss *Physcomitrella patens* (Hirano et al. 2016), the muroplasts of *Cyanophora paradoxa* (Fig. 1), *Gloeochaete wittrockiana* (Fig. 5), *Glaucocystis nostochinearum* (Fig. 6), and *Cyanoptyche gloeocystis* (Fig. 4c, d) have thin lysozyme-sensitive cell walls clearly recognizable with electron microscopy (EM) between the two envelope membranes (Kies 1992), which in *C. paradoxa* (Schenk 1970; Aitken and Stanier 1979; Pfanzagl et al. 1996a), *G. nostochinearum* (Scott et al. 1984; Pfanzagl et al. 1996b), and *C. gloeocystis* (Pfanzagl et al. 1996b) have been identified as peptidoglycan layers. Skuja's taxonomic treatment of the phylum (Skuja 1954) was adopted:

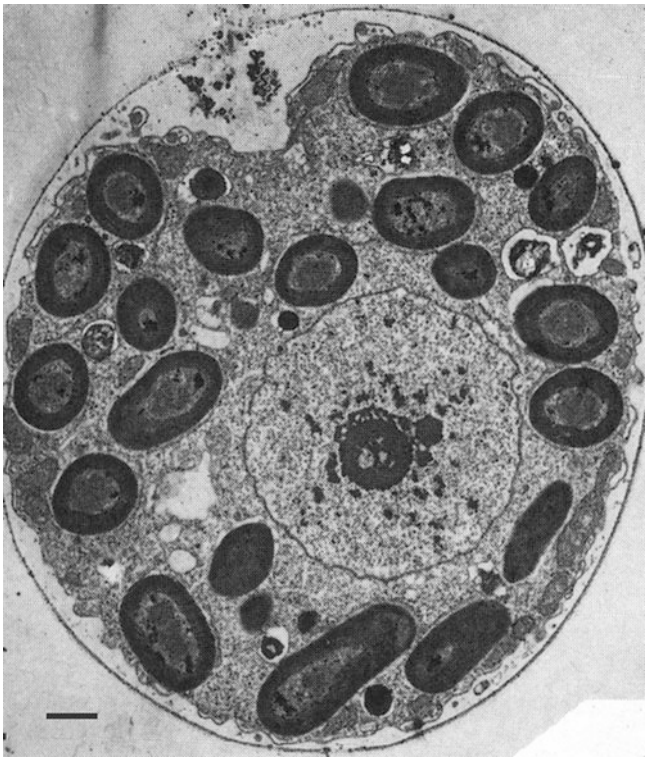

Fig. 3 *Gloeochaete wittrockiana*, strain SAG 46.84 (Kies strain IABH 2323), in longitudinal section, with apical depression, numerous muroplasts, and in the center of the cell a conspicuous nucleus with a nucleolus. Transmission electron micrograph. *Scale line* = 1 μm (Taken from Kies and Kremer (1990))

Skuja included in his phylum Glaucophyta *Gloeochaete*, *Glaucocystis*, and all endocyanomes described by Korshikov, Pascher, Geitler, and Skuja. Motile endocyanomes such as *Cyanophora* were not included in this framework. Kies (1979) suggested reviving the class Glaucocystophyceae (Skuja 1954) to accommodate the genera *Cyanophora*, *Gloeochaete*, *Glaucocystis*, and *Glaucosphaera*, which share ultrastructural characters not encountered together in any other algae (see Table 2). An emendation including a typification of several taxa of the Glaucophyta is given by Kies and Kremer (1986). *Cyanoptyche* was confirmed as a new member in 1989 (Kies 1989), whereas *Glaucosphaera* was removed in 1995 (Bhattacharya et al. 1995a).

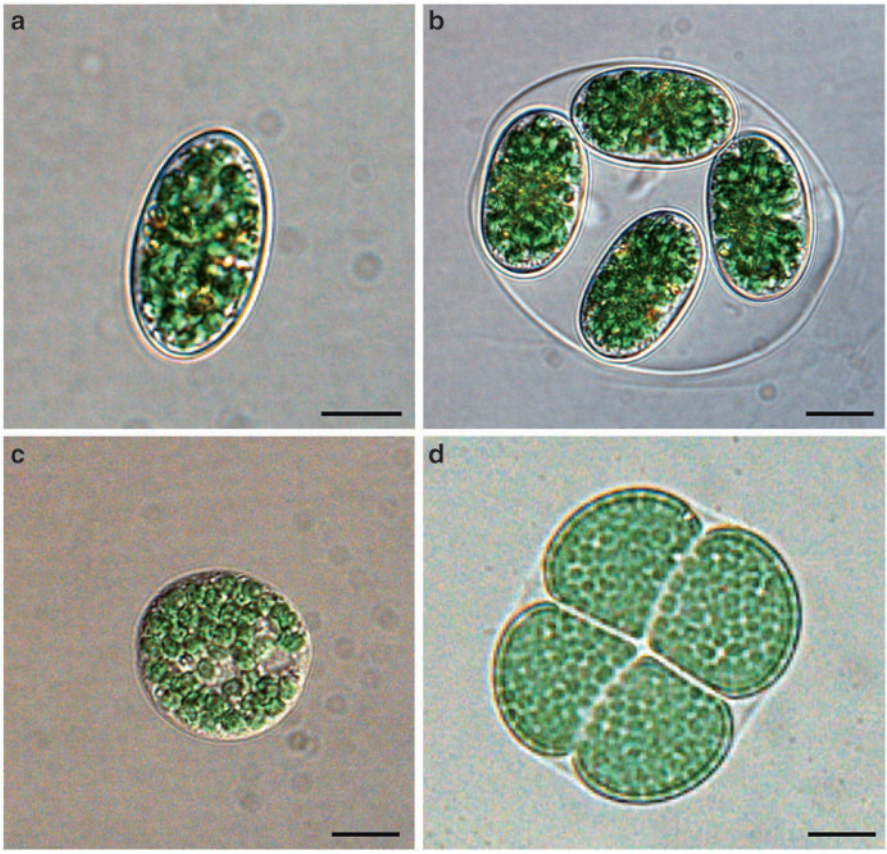

Fig. 4 **a**, **b** *Glaucocystis nostochinearum* SAG 45.88; C-D: *Cyanoptyche gloeocystis* SAG 4.97. A vegetative cell and autospores are shown for each species. *Scale bar* = 10 μm

Practical Importance

The Glaucophyta have not been exploited for economic or medical applications. A potential use of the eukaryotic peptidoglycan is as a model for the impact of beta-lactam antibiotics on eukaryotes, because the doses effective on *C. paradoxa* are similar to those for *E. coli* (Berenguer et al. 1987). In addition, a pigment extract of *C. paradoxa* containing pheophorbide *a*, beta-cryptoxanthin, and zeaxanthin as the main components has been shown to have strong antiproliferative activity against three cancer cell lines (Baudelet et al. 2013).

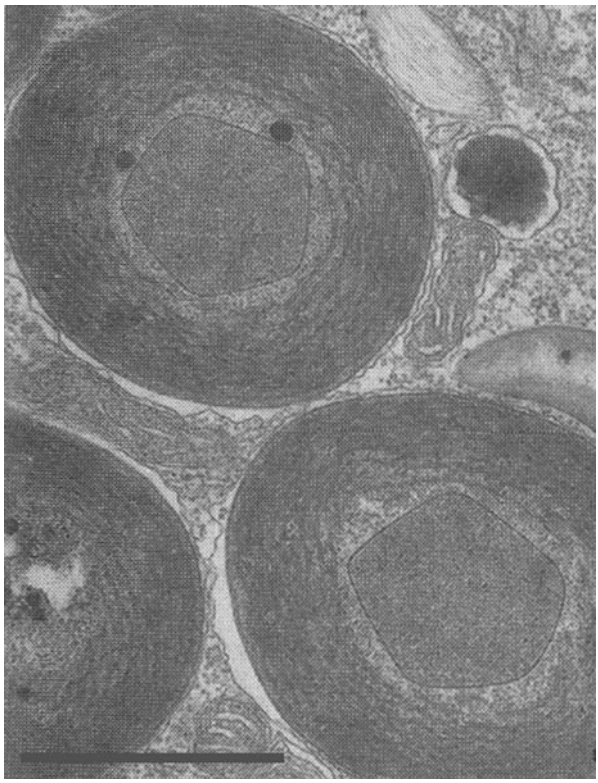

Fig. 5 Muroplasts of *Gloeochaete wittrockiana*, strain SAG 46.84 (Kies strain IABH 2323) with concentric thylakoid membranes. The central part contains a large polyhedral body confined by an electron-dense layer. Transmission electron micrograph. *Scale line* = 1 μm (Taken from Kies and Kremer (1990))

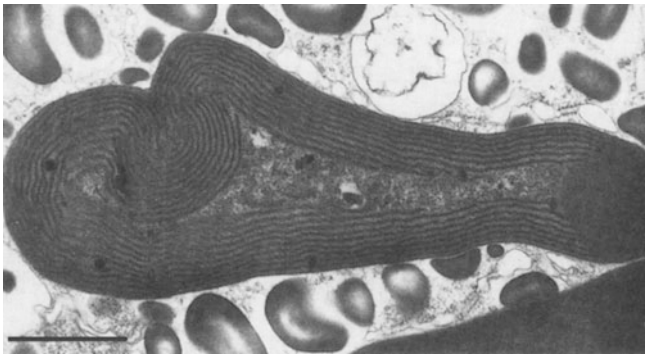

Fig. 6 A muroplast of *Glaucocystis nostochinearum*, strain IABH 2344 (Kies strain), in longitudinal section. Note the irregular, rodlike shape (spherical in all other glaucophytes) and the polar position (central in all other glaucophytes) of the RuBisCo microcompartment (Transmission electron micrograph. Scale line = 1 μm (Taken from Kies and Kremer (1990))

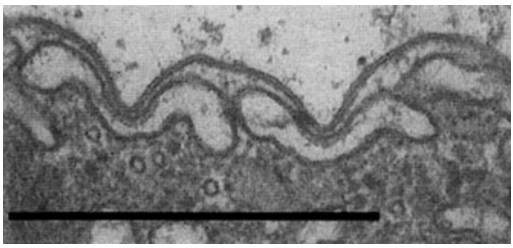

Fig. 7 Pellicle of *Glaucocystis nostochinearum*, strain IABH 2344 (Kies strain). Flat vesicles (lacunae) associated with microtubules form a layer beneath the plasma membrane. Transmission electron micrograph. *Scale line* = 0.5 μm (Taken from Kies and Kremer (1990))

Habitats and Ecology

In terms of being reported in the literature, the cosmopolitan *G. nostochinearum* is most frequent, followed by *C. paradoxa* and *G. wittrockiana* (see also Table 1). Because the knowledge of their distribution pattern and ecological niches is incomplete, and there is only a limited and dispersed literature on their ecology, glaucophytes are not easy to collect.

Cyanophora paradoxa, originally found in small eutrophic ditches near Kharkov, Ukraine (Korshikov 1924), was isolated by Pringsheim in England from alkaline water and from a soil sample taken from a fishpond near Erlangen, Germany, by Kies (Kies 1979; Pringsheim 1958). *Cyanophora tetracyanea* has been collected from river plankton in the Gorki district of Belarus and from the littoral zone of Lake Fibysjon, Sweden (Skuja 1956). *Cyanoptyche gloeocystis* and its subspecific taxa have been found in *Sphagnum* bogs (Pascher 1929) and in ponds rich in submerged cormophytes and diatoms (Geitler 1959b). It has been found on the underside of floating leaves of *Potamogeton natans*, a monocotyledonous angiosperm (Pascher 1929). In some instances it occurred together with other glaucophytes such as *Gloeochaete* and *Glaucocystis*. *Gloeochaete wittrockiana* is epibiotic on filamentous chlorophytes such as *Oedogonium*, *Rhizoclonium*, *Chara*, and *Nitella*, the xanthophyte *Vaucheria*, and the leaves of aquatic mosses and submerged angiosperms. It has been found both in acidic *Sphagnum* bogs, soft water lakes poor in plant nutrients (Skuja 1956), and ditches with medium levels of inorganic nutrients (Kies 1979). Skuja frequently found its zoospores in the plankton of some Swedish lakes. It often occurred together with *Glaucocystis nostochinearum*. *Glaucocystis nostochinearum* has been found in acid and alkaline waters (Geitler 1959a); it was reported from the plankton of Swedish lakes and ponds where it occurred together with *Gloeochaete wittrockiana* (Skuja 1956) and from swamps and bogs. It was collected from a drainage ditch near Hamburg, Germany, rich in submerged land plants and also containing *Gloeochaete wittrockiana* (Kies 1979). The pH was 6.5–8.2.

Table 2 Morphological characters of Glaucophyta

Character	*Cyanophora*	Cyanoptyche	*Gloeochaete*		*Glaucocystis*
			Zoospore	Vegetative cell	Vegetative cell
Organization	Monadoid	Palmelloid	Monadoid?	Capsalean	Coccoid
Reproduction	Bipartition	Binary fission	*	Successive bipartition	Progressive cleavage autospores
Cell wall	No wall	Mucopoly-saccharidic	No wall	Non-cellulosic	Cellulosic [a, b]
Layer of flat vesicles underneath the plasmalemma	+	+	+	+	+
Apical depression	+	–	+	+	+
pulsating vacuoles	+	+	+	+	+
Golgi bodies	Parabasal	Parabasal perinuclear?	Parabasal	Parabasal	Parabasal
Symmetry of monadoid stages	Dorsoventral	Dorsoventral *	Dorsoventral	*	*
Flagella	2, with mastigoneme	*	2, with mastigonemes	2 pseudocilia	2 reduced flagella
Cross section of flagella	(9 + 9) + 2	(9 + 9) + 2	(9 + 9) + 2	(9 + 9) + 0	(9 + 9) + 0
Kinetid	Cruciate, 2 MLS[c, d]	*	Cruciate 4 MLS	Cruciate 4 MLS	Cruciate[c] 4 MLS
Nuclear membrane fragments during mitosis, open spindle	+	?	*	+	+
Centrioles	–	?	*	–	–

(*continued*)

Table 2 (continued)

Character	*Cyanophora*	Cyanoptyche	*Gloeochaete*		*Glaucocystis*
Phycoplast	–	?	*	–	–
Persistent telophase spindle	+	?	*	+	+
Division by infurrowing	+[e]	?	*	+	+
Starch grains free in cytoplasm	+	+	+	+	+
Mitochondria with flattened cristae	+	+	+	+	+
Muroplasts with peptidoglycan wall	+	+	+	+	+

+ = character present, – = character absent, * = not applicable, ? = not investigated; MLS = multilayered structure
References: Kies 1992
Additional references in Kies (1979) and Trench (1982)
[a]Schnepf (1965)
[b]Robinson and Preston (1971)
[c]Rogers et al. (1981)
[d]Melkonian (1983)
[e]Pickett-Heaps (1972)

Characterization and Recognition

Glaucophytes are distinguished by ultrastructural and biochemical characters. The phylum Glaucophyta can be defined as follows (for references see Tables 1 and 2): Glaucophyta (glaucophytes) are mastigote (Fig. 1) or coccoid algae (Fig. 4a), single or in colonies (Fig. 2). Typical carotenoids of cyanobacteria such as echinenone and myxoxanthophyll are absent. They display characters of oxygenic prokaryotic photosynthesizers (photosystems I and II). The thylakoids are concentrically arranged (Figs. 1, 5, and 6), and the muroplast pigments are chlorophyll *a*, β-carotene, zeaxanthin, β-cryptoxanthin, allophycocyanin, and C-phycocyanin.

Flagellated vegetative cells and asexual reproductive cells (mastigotes), if present, have a dorsoventral construction. In motile forms, two flagella (Heimann et al. 1989; Fig. 1) both with mastigonemes arise in an apical groove. One is directed toward the direction of swimming, the other laterally. In vegetative cells of *Gloeochaete*, stiff, hairlike extensions called pseudocilia arise (Fig. 2) in an apical depression (Fig. 3). In *Glaucocystis*, reduced flagella are present. The cruciate kinetid contains four multilayered structures (MLS) (Table 2) in *Gloeochaete* and *Glaucocystis* and two in *Cyanophora.*

Glaucophytes contain mitochondria with flattened cristae. An open spindle appears during mitosis, but centrioles and phycoplasts are absent. Cytokinesis occurs by infurrowing of the plasma membrane. Reproduction is by longitudinal binary fission in the mastigotes, by multiple mastigotes or immotile reproductive cells ("autospores," Fig. 4b, d) in capsalean and coccoid members. Sexuality has not yet been reported; a lacuna pellicular system is present (Heimann et al. 1997; Fig. 7). The polysaccharide reserve product (starch) accumulates in the cytoplasm of the host cell in the form of minute granules.

Classification

A classification scheme was first proposed by Skuja (1954). A more recent treatment of the Glaucophyta (Kies and Kremer 1986; Kies 1992) differs from Skuja's scheme in that:

1. The diagnosis of the phylum and class is emended to include ultrastructural and biochemical characters. Typified names instead of descriptive names are used for all taxa.
2. Mastigotes, which comply with the emended diagnosis, are included.
3. Separate orders are established for monadoid (Fig. 1), capsalean (Fig. 3), and coccoid (Fig. 4a) genera.
4. Taxa of uncertain affiliation with the Glaucophyta due to incomplete description and/or lacking ultrastructural and biochemical evidence are treated here as genera and species inquirendae.

Maintenance and Cultivation

Gloeochaete and *Glaucocystis*, the only common glaucophytes, are isolated by use of a capillary pipette (Hoshaw and Rosowski 1973). From fresh natural collections, single cells or colonies are removed and transferred with a sterile capillary pipette in a Petri dish through at least ten drops of sterile culture medium to dilute out undesired organisms. Between each step the capillary pipette is newly pulled through a flame. *Gloeochaete* living epibiotically on filamentous freshwater algae should be isolated together with parts of the filaments. In fresh culture medium multiple mastigotes are formed readily and may be isolated as described.

Axenic cultures have been established from *Cyanophora* and *Glaucocystis* species (see Table 1). Fluorescence-activated cell sorting (FACS) proved to be the method of choice. The criteria adopted were maximum chlorophyll autofluorescence and maximum forward scatter. A total of 20–30% of the sorted single cell cultures grew successfully, and among these more than 20% were axenic (Sensen et al. 1993). Isolates from four genera were deposited in culture collections (Table 1), among them at least five species of *Cyanophora*, whereas the other genera appeared to be monospecific: *Cyanoptyche gloeocystis*, *Glaucocystis nostochinearum*, and *Gloeochaete wittrockiana*. However, recently a more thorough investigation of the genus *Glaucocystis* (Chong et al. 2014; Takahashi et al. 2016) led to a splitting into several species as has also happened for *Cyanophora* (Takahashi et al. 2014). A compilation including the latest results is presented in Fig. 8. The best sources are the SAG (Göttingen, Germany), the NIES (Tsukuba, Japan), and the CCAC (Cologne, Germany) that keep several Kies strains (Table 1).

Culture media recipes can be found in the SAG catalogue and web site (http://www.uni-goettingen.de/). *Cyanophora paradoxa* cultures show a requirement for vitamin B_{12} as an essential cofactor for methionine biosynthesis, which in natural habitats likely is provided by environmental bacteria (Croft et al. 2005).

Biochemistry, Molecular Biology, and Cell Biology

Here, research done during the past 25 years will be reviewed. Almost all data were obtained with *C. paradoxa*, and most of them deal with various aspects of muroplast biology. The clear outcome is that muroplasts are primary plastids sensu stricto.

The Muroplasts of *Cyanophora paradoxa*: Protein Import, Biochemical Pathways, and Plastome Organization

Emphasis is given to processes and structures for which biochemical and cell biological experiments corroborate and extend the information obtained from plastome and genome sequencing.

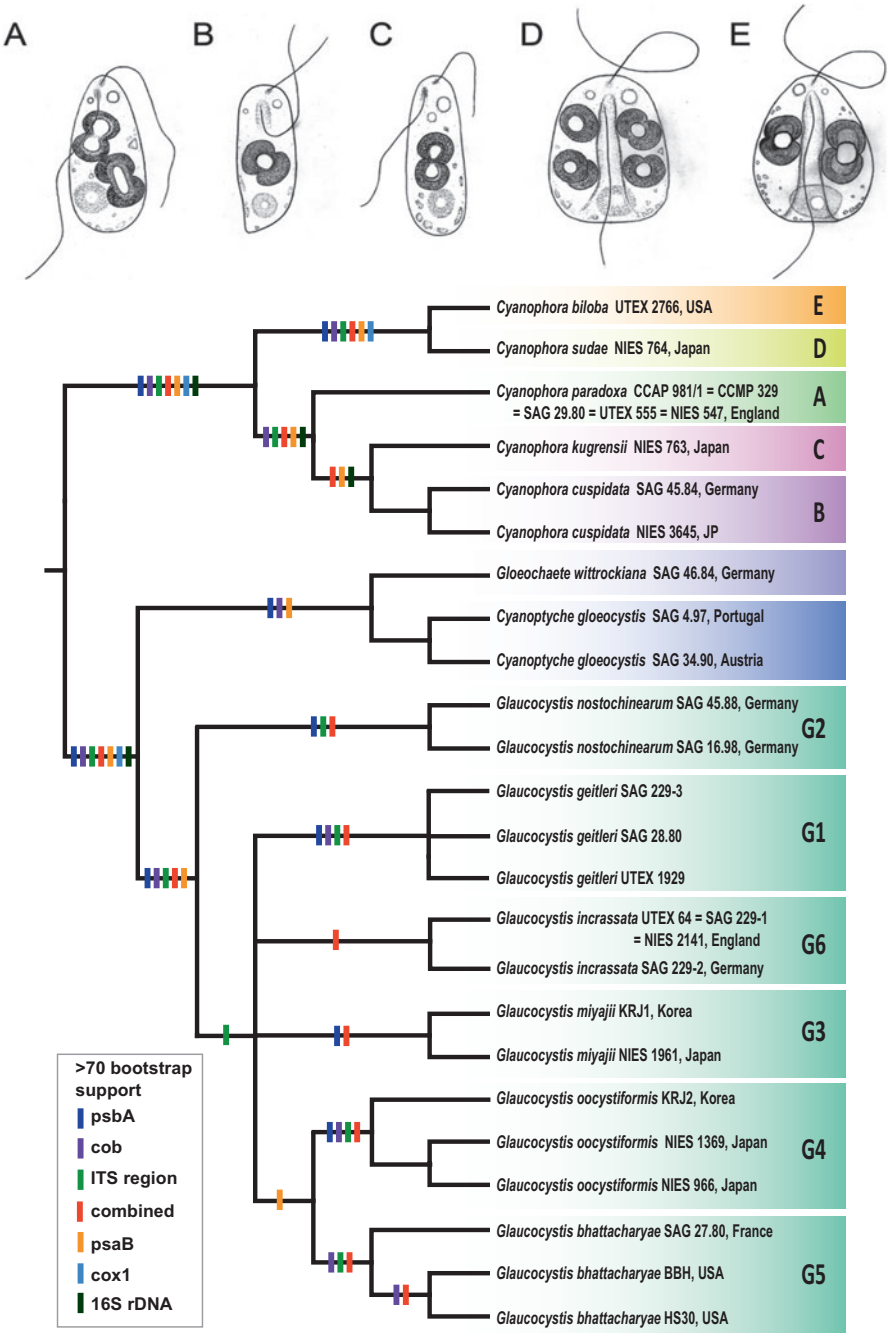

Fig. 8 Phylogenetic relationship of the Glaucophyta based on plastidal psbA, psaB, 16S rRNA, mitochondrial cox1, cob, and nuclear ITS region including ITS 1 and 2, 5.8S, partial SSU, and LSU

Protein Import into Muroplasts

Considerable progress has been made during the past 20 years with respect to components and mechanism of the import apparatus of land plant chloroplasts. It consists of two independent but cooperating translocons, Toc and Tic (Paila et al. 2015), at the outer envelope membrane (OEM) and the inner envelope membrane (IEM), respectively. Important translocon components are Toc75 (channel), Toc34 and Toc159 (receptors), Tic110 (putative channel), Tic20 (putative channel), Tic21, Tic22, and Tic40. GTP is the energy source for OEM translocation, and ATP energizes further translocation across the IEM via chaperone action. There is agreement that the import apparatus constitutes a eukaryotic "invention" which does not preclude the recruitment of suitable cyanobacterial membrane proteins (Reumann et al. 2005; Kalanon and McFadden 2008).

Nucleus-encoded muroplast polypeptides are synthesized in the cytosol as precursors containing cleavable N-terminal transit sequences that are 35–90 aa in length (Steiner and Löffelhardt 2002). These resemble chloroplast stroma-targeting peptides (Bruce 2000) in domain structure, amino acid c006Fmposition (especially at the processing site; Köhler et al. 2015), and positive net charge. However, the N-terminal motif MA(A)FVxxVP is found with slight variation in nearly all muroplast transit sequences (Steiner and Löffelhardt 2002, 2005) but not in those for land plant or green algal chloroplasts. Pre-FNR and pre-transketolase from *C. paradoxa* were efficiently imported into isolated muroplasts (Ma et al. 2009; Jakowisch et al. 1996). Other precursors as pre-cytochrome c_6 and pre-RuBisCO activase performed even better during in vitro import and were completely internalized after 3–7 min incubation (Burey et al. 2005; Steiner et al. 2000). The energy requirements (ATP, temperature) corresponded to those for chloroplast import. The observed stability of muroplasts due to their peptidoglycan armor is misleading: even a slight osmotic shock causes damage of the OEM and loss of import competence (Steiner and Löffelhardt 2002), CO_2 fixation (Trench 1982), and in organello protein synthesis (Löffelhardt and Bohnert 2001).

In addition to pre-FNR (Jakowitsch et al. 1996), all other *Cyanophora* precursors tested are readily imported into isolated chloroplasts from spinach or pea (Ma et al. 2009; Steiner and Löffelhardt 2002, 2005). However, the inverse heterologous import, i.e., of precursors from land plants into isolated muroplasts, did not occur. Therefore, the N-terminal consensus sequences appear to be the sole recognizable difference between muroplast and chloroplast stroma- targeting peptides. The phenylalanine residue, usually at position three or four, is conserved and might be

Fig. 8 (continued) rDNA (Modified from Chong et al. 2014; Takahashi et al. 2014). Strain number and its origin were indicated beside the species name. Six clades of *Glaucocystis* species complex were marked as G1–G6 (Chong et al. 2014), while three new *Cyanophora* species from Takahashi et al. (2014) have been adopted in this phylogeny. Color bars indicate >70% bootstrap support values for each node from each individual gene. Ink drawings for A–E (*Cyanophora* species) were taken from Takahashi et al. 2014

crucial for successful translocation across the muroplast envelope. Its prevalence was recently confirmed through proteomic studies on isolated muroplasts (Köhler et al. 2015). Indeed, deletion or exchange of this amino acid from *C. paradoxa* pre-FNR led to impeded or even completely abolished import into muroplasts (Steiner et al. 2005a). The obvious next step was to engineer a chloroplast precursor, pre-FNR from *Mesembryanthemum crystallinum*, with the missing phenylalanine in the N-terminal region of the transit sequence. This enabled heterologous import with an efficiency comparable to homologous import (Steiner et al. 2005a).

The high gene content of their plastomes, the PBS light-harvesting antennae, and results of phylogenetic analyses make it possible to categorize muroplasts and rhodoplasts as "plastids with ancestral characteristics." An inspection of putative transit sequences of nucleus-encoded rhodoplast proteins from various red algae revealed N-terminal consensus sequences very similar to those for muroplast stroma-targeting peptides. The crucial phenylalanine residue is always present, even in precursors targeted to secondary plastids derived from endosymbiotic red algae where a phenylalanine residue is created as the first amino acid of the transit sequence after cleavage of the preceding signal sequence (Patron and Waller 2007; Gould et al. 2006; Kilian and Kroth 2005). On the other hand, this is not found in precursors to chloroplasts or secondary plastids from the "green lineage." When this feature is considered as typical for primordial plastids, it might as well have been taken over or adapted from the prokaryotic ancestor: phenylalanine has been reported to occupy a prominent position in the sequence of bacterial proteins targeted to the outer membrane (Struyvé et al. 1991) as porins (C-terminus) or type IV pilins (N-terminus, created by prepilin peptidase cleavage). An outer membrane protein, Omp85, acting as receptor/chaperone for such proteins recognizes their exposed phenylalanine residue and assists in their correct membrane assembly (Voulhoux and Tommassen 2004). When such a preexisting cyanobacterial protein was recruited (after transfer of its gene to the nucleus) for the development of a protein import apparatus in the endosymbiont envelope, it could have been oriented inversely so that precursors with a phenylalanine signature coming from outside, i.e., from the eukaryotic cytosol, would be recognized. Indeed, this reorientation could recently be demonstrated (Sommer and Schleiff 2014). Thus, an Omp85-like protein (due to its sequence similarity, chloroplast Toc75 is included in the Omp85 family) could have been adapted to fulfil dual functions, that of the "Phe-receptor" and that of the protein import channel (Steiner and Löffelhardt 2005; Steiner et al. 2005a). Blue-native gels of isolated muroplasts yielded a distinct signal (αToc75) for the Toc complex at about 550 kDa. (Yusa et al. 2008). The muroplast import apparatus is considered as a prototype that has not undergone many changes relative to that of the ancestral protoplastid which might also apply to rhodoplasts and, likely, to secondary plastids derived from red algal endosymbionts (with respect to the two innermost membranes). Omp85 proteins are suitable for the proposed dual role because: (i) these are the only members of the Omp85 family that can form pores of sufficient diameter to allow protein translocation and (ii) they display a presequence (Phe)-binding domain. Phe in the transit sequence of pre-FNR from *C. paradoxa* was shown to reduce unspecific binding to liposomes but to enhance binding to

proteoliposomes containing Omp85 from *Anabaena variabilis* (Wunder et al. 2007). There is now evidence for the minimal set of components of the Toc/Tic complexes (see section on Genome Analysis of Glaucophytes; Bhattacharya et al. 2014; Löffelhardt 2014). The interaction of both complexes might be more pronounced than in chloroplasts, resulting in fixed positions of import sites coinciding with localized lesions in the organelle wall. The latter are necessary to allow translocation of large proteins that would have problems with the narrow mesh size of the peptidoglycan network and could be generated through the action of lytic transglycosylases bound to the import complex (Steiner and Löffelhardt 2005).

Conservative Sorting

The "conservative sorting" hypothesis posits that organelles (mitochondria, plastids) that are derived from prokaryotic endosymbionts not only had to develop a selective protein import apparatus at their envelope but also retained prokaryotic preprotein translocases at their inner envelope (mitochondria) and thylakoid membranes (chloroplasts). Conservative sorting in land plant chloroplasts is widely accepted (Smeekens et al. 1990). Bipartite presequences, i.e., a transit sequence followed by a signal sequence, are indicative of intraplastidic sorting to the thylakoid lumen or thylakoid integration. According to the translocons involved and the respective energy requirements, the Sec pathway transporting unfolded passenger proteins and the ΔpH-dependent or Tat pathway for folded proteins are defined (Cline and Dabney-Smith 2008), both being paradigms for conservative sorting of (largely) lumenal proteins. Cyanobacteria, the ancestors of plastids, are capable of "exporting" cytosolically synthesized preproteins either to the periplasmic space or into the thylakoid lumen (Mackle and Zilinskas 1994). Muroplasts also possess a periplasmic space between IEM and OEM containing the peptidoglycan wall, seven penicillin-binding proteins, enzymes of peptidoglycan degradation and modification, cytochrome c_6, etc. (Steiner et al. 2000; Löffelhardt and Bohnert 2001). Therefore it is justified to postulate conservative sorting for both the thylakoid and inner envelope membranes (Fig. 9) as was first shown for cyanobacteria with respect to the Sec translocase (Nakai et al. 1993).

Sec pathway: Here, muroplasts have played a leading role for some time because *secY* is a muroplast gene and was shown to complement the thermosensitive *secY24* mutation in *E. coli* (Flachmann et al. 1993). In subsequent work, an expressed sequence tag (EST) for nuclear-encoded SecA was found in *C. paradoxa*. Muroplast SecA appeared to be quite susceptible to inhibition by sodium azide during import experiments with homologous precursors: the amount of mature cytochrome c_6 was reduced and intermediate accumulated in the stroma, whereas thylakoid translocation of the larger intermediate form of PsbO was completely abolished (Steiner et al. 2005b). Cyanobacterial thylakoids do not form tight vesicles upon isolation and thus are not suitable to demonstrate protease protection of internalized, processed lumenal proteins. With improved muroplast fractionation methods, it was possible, at least for PsbO, to show Sec-dependent translocation in organello and, after

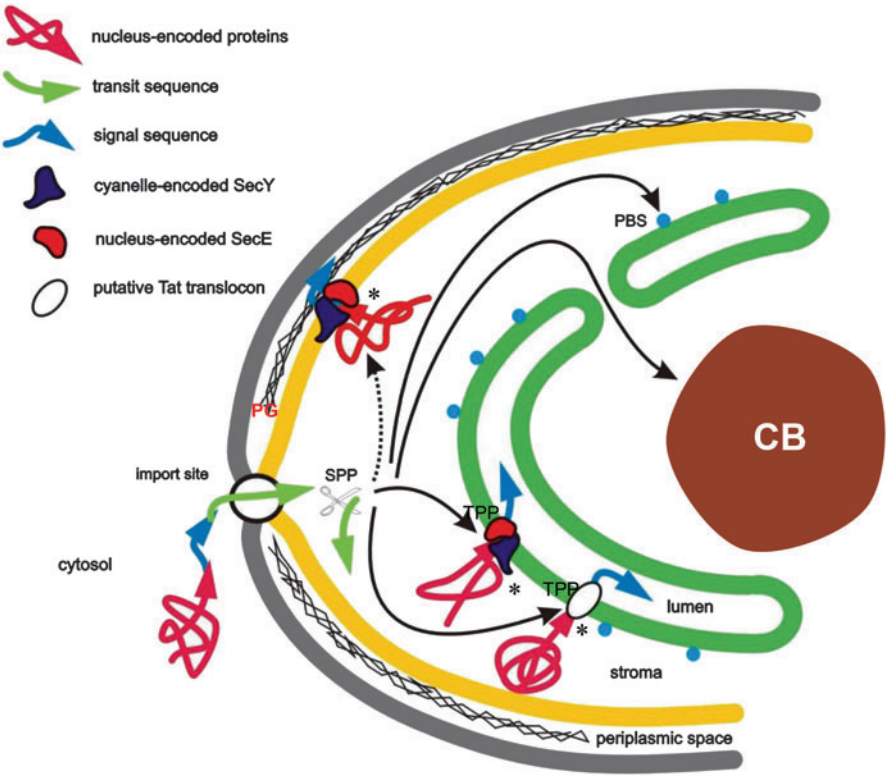

Fig. 9 Conservative sorting within the muroplasts of *Cyanophora paradoxa*. Proteins directed by a specific transit sequence (muroplast stroma-targeting peptide) across the muroplast envelope into the stroma can either stay there, or can be integrated into a microcompartment, or can be sorted by a signal sequence (in case of a bipartite presequence) to the thylakoid lumen or the periplasmic space, respectively. *CB* central body, *PBS* phycobilisome, *PG* peptidoglycan, *Spp* stroma processing peptidase, *TPP* thylakoid processing peptidase, * conservative sorting

muroplast lysis and thylakoid isolation, for the first time protease protection of the mature protein inside of phycobilisome-bearing thylakoids. Nigericin did not interfere; addition of azide to the import assay abolished protease protection of PsbO by inhibiting thylakoid translocation (Steiner et al. 2005b). However, import experiments into isolated thylakoids are only possible in land plant systems. The *Cyanophora* Genome Project revealed contigs for secA and thylakoid processing proteases (TPP) but no additional, nucleus-encoded SecY (Table 3; Steiner et al. 2012): there is but one *secY* gene and one *secA* gene, as in cyanobacteria. The generation of specific antisera directed against muroplast SecY allowed the demonstration of dual localization (Fig. 9) of the Sec translocon in muroplasts (Yusa et al. 2008). SecY-containing bands of distinct size were immuno-decorated on blue-native gels of thylakoid membranes and IEM, respectively (Koike et al. 2007). In land plant chloroplasts, a second Sec translocase was recently shown at the IEM, but

Table 3 Genes for components of protein sorting pathways within the muroplasts of *Cyanophora paradoxa* and for candidate passengers undergoing spontaneous membrane insertion (Steiner et al. 2012)

Protein	Function	Comments
SecY	Sec translocase	One copy on the muroplast genome
SecE (n.d.)	Sec translocase	Should be present. Low sequence conservation
SecA	Sec translocase	N-terminal fragment with STP[a]
TatC	Tat translocase	5 TM domains, negatively charged N-terminus[a]
TatA	Tat translocase	STP, 1 TM domain, highly polar C-terminus[b]
TPP	Signal peptide cleavage	LepB1 homolog[a], 1 TM domain
TPP	Signal peptide cleavage	Fragment, putative LepB2 homolog[a]
mpSRP54	Signal recognition particle	STP, GTP-binding domain[a]
mpFtsY	SRP receptor	GTP-binding domain[b]
Albino3	D1 insertase	STP, 5 TM domains[b]
Vipp1	Thylakoid stabilization	STP, amphipathic α-helix at C-terminus[a]
PsbW	Spontaneous insertion?	STP, SP, 1 TM domain[b]
PsaK	Spontaneous insertion?	2 TM domains[a]

n.d. not detectable, *mp* muroplast, *STP* stroma-targeting peptide, *SP* signal peptide, *TM* transmembrane

[a]Best hits among cyanobacteria

[b]Best hits among green algae and plants

SecY and SecA are derived from nuclear genes different from those giving rise to the thylakoid Sec translocon (Skalitzky et al. 2011).

Tat pathway: In *C. paradoxa* EST databases, nucleus-encoded candidate passengers were found, as pre-PsbU and pre-PsbQ', with bipartite presequences containing the typical "twin-arginine" motif in the signal sequence (Cline and Dabney-Smith 2008) that did not respond to azide. The problem is that the effect of nigericin on muroplasts obviously is weaker than on land plant chloroplasts. In the non-cleavable signal-anchor sequence immediately after the transit sequence, a KR motif is found in both cases (RR only in cyanobacterial pre-PetC). In the presence of azide, but not of nigericin, it was possible to detect low amounts of protease-protected (i.e., internalized) mature protein trimmed by removal of five N-terminal amino acids (preceding the single transmembrane domain) protruding into the stroma. This was interpreted as evidence for operation of the Tat pathway in the muroplast thylakoid membrane (Steiner et al. 2005b). With the availability of the genomic sequence, genes for TatA and TatC could be identified (Table 3; Steiner et al. 2012). There is now also evidence for dual localization of the Tat translocase in cyanobacteria (Aldridge et al. 2008). In the absence of any experimental data, this is also a likely scenario for muroplasts resulting in fully conservative sorting, whereas for chloroplasts an IEM-resident Tat translocase is rather not envisaged (Skalitzky et al. 2011). The muroplast signal recognition particle (SRP) protein and the corresponding receptor (distinct from the cytosolic counterparts) were also identified and included in the compilation of Table 3. Since LHCP is missing from glaucophytes and rhodophytes, a posttranslational SRP pathway should not be operative in the plastids

from both phyla. However, the cotranslational SRP pathway with the important function of thylakoid integration of PSII and PSI reaction centers (Ossenbühl et al. 2006) can be considered as another example of conservative sorting and is expected to be active in muroplasts and rhodoplasts. Consequently, the genes for Albino3/Oxa1/YidC and Vipp1 were identified, whereas the SRP-RNA which is encoded on all rhodoplast genomes could not be found on muroplast DNA (M. Rosenblad, personal communication). Obviously, mpSRP54 alone can fulfill its function without an RNA component. Table 3 is completed by two candidates for spontaneous (i.e., unassisted) thylakoid insertion (Tissier et al. 2002) which seems to be a special feature of galactolipid-rich plastid membranes.

Structure and Biosynthesis of the Unique Eukaryotic Peptidoglycan

In contrast to chloroplasts, isolated muroplasts of *C. paradoxa* are stable in hypotonic medium. This is due to the presence of a lysozyme-sensitive murein sacculus in the muroplast envelope (Schenk 1970). This "organelle wall" with an estimated thickness of 7 nm has hitherto only been found in the eubacterial kingdom. Such a peculiar prokaryotic wall around a eukaryotic organelle, perhaps the most striking biochemical evidence for the cyanobacterial origin of plastids, was assumed to mimic early stages of primary endosymbiosis and justified (for some time) consideration of *C. paradoxa* and glaucophytes in general as "living fossils." The basic components of muroplast peptidoglycan were identified as those known for the $A_1\gamma$-type found in Gram-negative bacteria: *N*-acetylmuramic acid, *N*-acetylglucosamine, L-alanine, D-glutamic acid, *m*-diaminopimelic acid, and D-alanine (Aitken and Stanier 1979). Analogous results were reported for the muroplast wall from *G. nostochinearum* (Scott et al. 1984). Cleavage of purified muroplast peptidoglycan from *C. paradoxa* with *Chalaropsis* muramidase and separation by HPLC yielded a muropeptide pattern different from that of *E. coli*: Only 7 of the 29 major muropeptides investigated by a combination of amino acid analysis and mass spectrometry were identical to bacterial counterparts. The remaining 22 appeared to be derived from known muropeptides of *E. coli* by a substitution leading to an increment in MW of 112 or multiples thereof (Pfanzagl et al. 1996a). The modification was localized to the C-1 carboxylic group of the D-isoglutamoyl moiety, and *N*-acetylputrescine was identified as the substituent (Pittenauer et al. 1993). The structures of all 29 major muropeptides (4 monomers, 8 dimers, 11 trimers, and 6 tetramers) have been elucidated (Pfanzagl et al. 1996a). In fact, the muroplast wall is thicker and more cross-linked than the cell wall of *E. coli*. The substitution (not detected in cyanobacteria) and the reduced thickness (as compared to the cyanobacterial wall) could thus both serve the purpose of increasing the permeability of the peptidoglycan network. This might be especially important for a cell organelle which requires extensive protein import from the cytoplasm. Indeed, *N*-acetylputrescine was also found in the muroplast walls from two other glaucophytes, *G. nostochinearum* and *C. gloeocystis* (Pfanzagl et al. 1996b), indicating that it really constitutes a signature for muroplasts in general, i.e., for the "eukaryotic"

peptidoglycan of an armored organelle. An alternative, less likely function might be in connecting the PG layer to the OM in the absence of murein lipoprotein (Pfanzagl et al. 1996a) as was reported for some rare cases of anaerobic Gram-negative bacteria that show cadaverine or putrescine linked to C-1 of the isoglutamoyl moiety (Kojima et al. 2010).

The biosynthetic pathway of *C. paradoxa* murein appears to be analogous to that of *E. coli* with respect to intermediates, the participating enzymes, and their compartmentation. Penicillin-binding proteins (PBPs) possess transglycosylase and/or transpeptidase activity and perform the last steps of bacterial peptidoglycan biosynthesis by introducing new monomeric building blocks into the growing carbohydrate chain and cross-linking the peptide side chains (Sauvage et al. 2008). Seven PBPs in the size range from 110 to 35 kDa were identified in the muroplast envelope by labelling with a radioactive derivative of ampicillin (Berenguer et al. 1987). Accordingly, β-lactam antibiotics are lethal for *C. paradoxa* in much the same concentrations as for eubacteria. Also, differential sensitivity of individual PBPs toward different penicillin derivatives was demonstrated (Berenguer et al. 1987). Muroplast division is arrested whereas cell division continues, finally leading to colorless, nonviable cells. Dumbbell-shaped muroplasts were also observed upon benzyl penicillin and vancomycin treatment of *C. paradoxa* (Iino and Hashimoto 2003). Indirect evidence was obtained for a periplasmic localization in the muroplasts of *C. paradoxa* of DD- and LD-carboxypeptidases and DD-endopeptidase, enzymes hydrolyzing defined bonds in peptidoglycan (Plaimauer et al. 1991). As in the cytosol of *E. coli* (Barreteau et al. 2008), the biosynthesis of the soluble precursor of peptidoglycan, UDP-*N*-acetylmuramoyl pentapeptide, was shown to occur in the muroplast stroma (Plaimauer et al. 1991). The membrane-bound steps, i.e., the transfer of UDP-*N*-acetylmuramoyl pentapeptide to undecaprenylphosphate (yielding Lipid I) and disaccharide formation with *N*-acetyl glucosamine (yielding lipid II), occur in analogy to *E. coli* (Bouhss et al. 2008) at the inner envelope membrane of muroplasts followed by putrescinylation at C-1 of the D-isoglutamyl moiety and then *N*-acetylation (Pfanzagl and Löffelhardt 1999). Amidation of *Staphylococcus aureus* PG at the same position was recently reported to also occur at the stage of lipid II (Münch et al. 2012). Surprisingly, the muroplast genome encodes only a single protein potentially involved in peptidoglycan biosynthesis during septum formation, FtsW (Löffelhardt et al. 1997). One proven function of *E. coli* FtsW is the recruitment of PBP3 (FtsI) to the divisome. More than 30 eukaryotic genes specifying enzymes responsible for building up the prokaryotic organelle wall must therefore reside in the nuclear genome of *C. paradoxa*. Recently, a homolog to the cyanobacterial division protein SepE, which has a role in assembly and stability of the FtsZ ring (Hamoen et al. 2006), was also identified on the muroplast genome. The expression of *ftsW* and *sepE* appear to be cell cycle independent (Miyagishima et al. 2012).

Muroplast division in *C. paradoxa* shows intermediate features between cyanobacterial and plastid division (Iino and Hashimoto 2003; Sato et al. 2009). This was to be expected since it is strictly dependent upon the formation of a peptidoglycan septum in contrast to all other plastid types which nevertheless rely

on a number of cell division genes of bacterial origin as *ftsZ*, *ftn2* (*arc6*), *minD*, *minE*, etc. (Yang et al. 2008). Interestingly, there is but one gene for (muroplast-targeted) FtsZ on the *C. paradoxa* genome (as in cyanobacteria) and no mitochondrial counterpart, whereas algae and plants possess at least two genes for the chloroplast proteins and, more recently (e.g., in case of stramenopiles), additional genes for the mitochondrial FtsZ were described (Leger et al. 2015). Muroplasts and chloroplasts show in the stroma a distinct inner plastid division (PD) ring, corresponding to the FtsZ ring superimposed by a thicker, electron-dense ring. However, muroplasts lack the outer chloroplast division ring and the adjacent ring formed by the dynamin-related protein ARC5 (DRP5B). These components of the chloroplast division machinery are considered as host cell contributions after the endosymbiotic event. Nuclear genes for MinD and MinE, proteins determining the site of the division septum, and for ARC6 (assumed to tether the FtsZ ring to the IEM) were identified in *C. paradoxa*, and their expression was shown to be regulated by the cell cycle (Miyagishima et al. 2012). In contrast, FtsZ was found to be constitutively expressed. In (cyano)bacteria, various hydrolases function in PG splitting during septum formation. Recently, a homolog of the gene for DipM was detected on the nuclear genome of *C. paradoxa*, and the protein was shown to localize to the intermembrane space of dividing muroplasts at the site of septum formation (Miyagishima et al. 2014a). Again, the expression of DipM followed the cell cycle with a peak in the S phase.

In the *Cyanophora* Genome Project, three different approaches were used for PBP gene identification: (1) domain searches, (2) BLAST searches against the eight PBP genes of *Synechocystis* sp. PCC6803 (Marbouty et al. 2009) and the *Anabaena* sp. PCC7120 homologs, and (3) BLAST searches against *Physcomitrella patens* PBP-like genes. In most cases, the results converged leading to the identification of at least 11 genes or gene fragments (Bhattacharya et al. 2014); examples of which are shown in Table 4. In general, sequence similarity was higher to homologs in cyanobacteria than to those in *P. patens*. In some cases of periplasmic proteins, bipartite presequences consisting of a transit peptide and a signal peptide could be found. This suggests import to the muroplast stroma, followed by export to the periplasmic space. This special variant of "conservative sorting" would necessitate a dual location of Sec (already documented) and Tat (seems possible as another parallel to cyanobacteria) translocases on thylakoid and inner envelope membranes of muroplasts. In a Gram-negative background, the low molecular weight (MW) peptidases VanX and VanY are not linked to vancomycin resistance but rather to D-alanine recycling and to an additional endolysin, respectively. Peptidoglycan biosynthesis requires cleavage of existing glycan chains to allow for insertion of new material. This is performed by soluble and membrane-bound lytic transglycosylases: one gene of this kind could also be identified in *C. paradoxa*. A lysozyme family protein with significant similarity to protist lysozymes displays a signal peptide indicating a vacuolar (lysosomal) location that is likely involved in the autophagosomal digestion of damaged muroplasts. Genes for stromal proteins that are involved in the synthesis of the soluble precursor are also listed in Table 4. The N-terminal transit peptide identifies one such gene in *C. paradoxa* (*glmS*, specifying

Table 4 Nuclear genes involved in biosynthesis and degradation of muroplast peptidoglycan in *Cyanophora paradoxa*

Gene/protein	Function[a]	Localization
PBP1, PBP2	PG transglycosylase/transpeptidase	PS, IEM
PBP1, PBP2	PG transglycosylase/transpeptidase	PS, IEM
ftsI/PBP3	PG transglycosylase/transpeptidase	PS, IEM (septal ring)
PBP4	PG transglycosylase/transpeptidase	PS
dacB/PBP 5	D-Ala-D-Ala-carboxypeptidase,	PS
	D-Ala-D-Ala-endopeptidase	
PBP 8	D-Ala-D-Ala-carboxypeptidase C	PS
vanX	D-Ala-D-Ala-dipeptidase	PS
vanY/endolysin	D-Ala-D-Ala-carboxypeptidase	PS
Lysozyme-like	Muramidase	PS
mlt	Lytic transglycosylase	PS
dipM	PG splitting enzyme	PS (septum site)
glmS	Glucosamine-6-P synthase	Stroma
murA	UDP-*N*-acetylglucosamine-	Stroma
	1-carboxyvinyl transferase	
murB	UDP-*N*-acetylenolpyruvoyl-	Stroma
	glucosamine reductase	
murC	UDP-*N*-acetylmuramate:	Stroma
	L-Ala ligase	
murI	Glutamate racemase	Stroma
murD	D-Glu-adding enzyme	Stroma
murE	DAP-adding enzyme	Stroma
alr	Alanine racemase	Stroma
ddl	D-Ala:D-Ala ligase	Stroma
murF	UDP-*N*-acetylmuramoyl	Stroma
	tripeptide/D-Ala-D-Ala ligase	
mraY	Lipid I synthesis	IEM
murG	Lipid II synthesis	IEM

PS periplasmic space, *IEM* inner envelope membrane
[a]The high MW (1–4) and the medium MW (5–8) PBPs are redundant in *Synechocystis* sp. PCC6803

D-glucosamine-1-phosphate synthase) as a member of the muroplast-resident PG biosynthesis pathway, whereas the cytosolic counterpart would be expected to participate in protein glycosylation. The complete set of enzymes that are involved in UDP-*N*-acetylmuramate biosynthesis as well as the peptide side-chain adding enzymes and the alanine (Alr) and glutamate (MurI) racemases are encoded on the nuclear genome of the alga. The IEM-bound or associated MraY and MurG proteins complete this compilation.

Genes for enzymes of PG biosynthesis were transferred twice into Archaeplastida during the course of evolution – from the more ancient donor of the mitochondrion and from the subsequent cyanobacterial ancestor of plastids. These genes retain a high sequence similarity in *Arabidopsis thaliana* (few genes) and the moss

Physcomitrella patens (almost complete set), but their functions are likely to have changed. As long as chemical and structural proof is lacking, (pleiotropic) effects of antibiotics or gene knockouts on plastid division do not provide sufficient evidence to claim the presence and biosynthesis of PG in the plastids of bryophytes (Takano and Takechi 2010). FtsZ in (cyano)bacteria and muroplasts (derived from a single gene) is assumed to recruit the divisome proteins forming the peptidoglycan septum. In rhodoplasts and chloroplasts, the FtsZ ring is thought to instead recruit the outer PD ring and the dynamin ring to perform the constriction of the OEM. The *C. paradoxa* genome does not encode any of the host cell-derived plastid division proteins, whereas *P. patens* encodes three DRP5B dynamins (Miyagishima et al. 2014b).

With the present state of knowledge, glaucophyte PG – in the sense of a contiguous, stress-bearing layer between the envelope membranes – appears unique among Archaeplastida. In the rhizarian testate amoeba *Paulinella chromatophora*, the situation is different: there is also PG in this eukaryote, but all genes necessary for its biosynthesis are encoded on the endosymbiont (i.e., "chromatophore," photosynthetic organelle) genome which exceeds the size of plastid genomes by a factor of five to ten (Nowack et al. 2008). Unlike their counterparts in *C. paradoxa*, these genes retain their prokaryotic character; i.e., they were not transferred to the nuclear genome, and thus no import of precursor proteins is required for biosynthesis of the sacculus in photosynthetic *Paulinella* species.

The finding of more than one gene to a given function is not uncommon among cyanobacteria. For example, one of two genes with high sequence similarity to *murG* is more closely related to MGDG synthases, the likely function of "MurG" in plants. In an analogous fashion, *murD*-like genes might instead play a role in folate biosynthesis. Thus, one should expect modified functions for "mur-like" genes, e.g., "MurE" of *Arabidopsis* is involved in chloroplast development but not in chloroplast division (Garcia et al. 2008). However, should it become possible to demonstrate PG in bryophyte chloroplasts through novel, highly sensitive detection methods, as in the case of the cell wall-less bacterium *Chlamydia trachomatis* (Liechti et al. 2014), the chloroplast division apparatus of *P. patens* will have to be reevaluated. A first step in that direction was reported very recently (Hirano et al. 2016).

The Photosynthetic Apparatus of *Cyanophora paradoxa* Muroplasts

The first comprehensive investigation of the components of photosynthesis in *C. paradoxa* was performed by Burnap and Trench (1989). These authors purified ferredoxin, cytochrome b_6, and cytochrome c_6 and verified the absence of plastocyanin from muroplasts. They also isolated photochemically active PSI complexes and could resolve five subunits ranging from 66 kDa to 11 kDa. Further data included the preparation of PSII core particles and of phycobilisomes. More than 10 years later another round of research papers on this topic emerged after the muroplast genome sequence was published. This certainly was very helpful since more than 50% of the thylakoid proteins are contained therein. Shibata et al. (2001) prepared oxygen-

evolving thylakoid membranes and solubilized PSII particles. These contained PsbO and PsbV (cytochrome c_{550}; muroplast encoded), but PsbU was lost from the preparation. Enami et al. (2005) described PsbO, PsbV, and PsbU as the extrinsic proteins of the oxygen-evolving complex (OEC) of cyanobacteria and *C. paradoxa* muroplasts. PsbO, PsbV (rhodoplast encoded), PsbU, and PsbQ' were assigned to *C. merolae* rhodoplasts (Enami et al. 2005), whereas the chloroplasts of green algae and land plants were long known to harbor the OEC components PsbO, PsbP, and PsbQ, all of them as the products of nuclear genes. PSI preparations now allowed the identification of ten subunits, whereby N-terminal protein sequencing was adopted. Sequence alignments in some cases yielded higher similarity to cyanobacterial homologs, in other cases to the counterparts from plants and green algae (Koike et al. 2000). In a comparison of supercomplex organization (where unicellular cyanobacteria possess a PSI trimer), the filamentous N_2-fixing *Anabaena* sp. PCC 7120 and *C. paradoxa* had a PSI tetramer and dimer instead, and the lack of LHCI, likely in all glaucophytes (in contrast to all other phototrophic eukaryotes), was corroborated. On the other hand, PSI monomers only were reported for the extremophilic rhodophyte *C. merolae*. Thus, with respect to PSI, glaucophytes are closer to cyanobacteria than to rhodophytes which also are distinct from the former through their LHCI antennae (Watanabe et al. 2011).

The next quantum leap to come was the *Cyanophora* Genome Project that stimulated a number of related investigations, e.g., on the muroplast proteome of *C. paradoxa* (Facchinelli et al. 2013). A total of 510 polypeptides were identified, among them the proteins of the photosynthesis apparatus with few exceptions, e.g., AtpA. Meanwhile, the state of the art with respect to cyanobacterial OEC components has changed to PsbO, PsbV, PsbU, PsbQ', and PsbP' (the latter two with sequence similarity to chloroplast PsbQ and PsbP), PsbP' being present in substoichiometric amounts with a presumed function in assembly/stability of PSII (Bricker et al. 2012). Muroplast proteomics confirmed PsbO and PsbV and identified PsbP' as an additional component (Facchinelli et al. 2013). PsbU is known to be encoded on the *Cyanophora* genome. The precursor contains the twin-arginine motif in the signal sequence and is one of the candidate passengers for the Tat translocase (Steiner et al. 2005a). Muroplast prePsbP' (also equipped with the RR signature) was proven to be imported into the thylakoid lumen via the Tat pathway in heterologous and homologous import experiments (Kleiner 2014), in analogy to PsbP from land plants. Very recently, a contig representing a PsbQ' homolog (J.M. Steiner, unpublished) completed the list for *C. paradoxa* resulting in a very similar OEC subunit structure for cyanobacteria, glaucophytes, and red algae. Thus, the "primitive" muroplasts and rhodoplasts differ from chloroplasts not only with respect to their extrinsic PBS antennae on the stromal side but also with respect to the extrinsic OEC proteins on the lumenal side of the thylakoid membranes. Chloroplasts have lost PsbV and PsbU in the course of evolution, whereas the gene for PsbP expanded to a small multigene family in land plants (Bricker et al. 2012).

Cyanophora RuBisCO belongs to form IB (as in cyanobacteria and chloroplasts) whereas rhodoplasts contain form ID. Common to muroplasts and rhodoplasts is the *rbcL-rbcS* transcription unit on the respective plastomes and the concentration and

compaction of RuBisCO into a microcompartment, the pyrenoid (see below). Calvin cycle enzymes corresponded to major transcripts (frequent in EST collections) and grouped among abundant stromal proteins with respect to spectral counts (Facchinelli et al. 2013). Again, canonical STPs were found throughout. Ferredoxin-NADP+ oxidoreductase (FNR) of *Cyanophora paradoxa* was characterized at the protein and cDNA level (Gebhart et al. 1992; Jakowitsch et al. 1993). The 34 kDa protein showed high amino acid sequence similarity to land plant counterparts and lacked the C-terminal extension of the cyanobacterial homologs responsible for binding to phycobilisomes. The availability of the ^{35}S-labeled precursor was important for the establishment of an efficient muroplast in vitro import system (see section on Protein Import into Muroplasts). A NAD(P)-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was purified from a muroplast extract of *C. paradoxa* as a 142 kDa homotetramer with features similar to the cyanobacterial counterpart (Serrano and Löffelhardt 1994). This is in agreement with the postulated duplication of the *GapA* gene early in streptophyte evolution (Petersen et al. 2006).

The gene for the CP12 protein involved in the formation of inactive complexes of Calvin cycle enzymes during night was also characterized (Petersen et al. 2006). A muroplast-localized fructose-1,6-bisphosphate aldolase of class II was fractionated from *C. paradoxa* extracts as a 85 kDa protein and was shown to be bifunctional for fructose-1,6-bisphosphate and sedoheptulose-1,7-bisphosphate cleavage (Flechner et al. 1999). The cDNA of pre-transketolase was sequenced. In a neighbor-net graph, the *Cyanophora* enzyme occupied a position intermediate to the plastid and cyanobacterial homologs (Ma et al. 2009). The single copy gene was downregulated upon shift to low CO_2 conditions, typical for Calvin cycle enzymes (Burey et al. 2007).

Photorespiration: The oxygenase activity of RuBisCO inevitably leads to photorespiration (in different variations) in cyanobacteria and in all oxygenic phototrophs (where peroxisomes and mitochondria are involved in addition to plastids). The *Cyanophora* Genome Project inspired a study about evolution and phylogeny of this pathway in the earliest branching phototrophic eukaryote (Kern et al. 2013). The outcome was that some cyanobacterial genes (originally obtained through endosymbiotic gene transfer [EGT]) were lost, as for glycerate-3-kinase, or later replaced by α-proteobacterial homologs, as for glycine decarboxylase. Only phosphoglycolate phosphatase appears to be derived from Archaea. Glycolate oxidase was described to be of cyanobacterial origin in *Cyanophora* and all other algae/plants. A cyanobacterial origin was also postulated for serine:glyoxylate aminotransferase of *C. paradoxa*, whereas the counterparts from red algae and green algae/plants were found to be derived from proteobacteria through HGT. A similar situation is assumed for hydroxypyruvate reductase. Taken together, *Cyanophora* seems to have retained more cyanobacterial genes of the C_2 pathway than other algae and land plants in accordance with the predicted basal position of glaucophytes among Archaeplastida (Kern et al. 2013). Certainly, more biochemical research in this field is needed, as the lack of glycerate-3-kinase points toward some changes in the C_2 pathway of glaucophytes. Proteomics confirmed the muroplast localization of phosphoglycolate

phosphatase that showed the canonical transit sequence at the gene level (Facchinelli et al. 2013). There is but one experimental paper investigating glycolate metabolism in *C. paradoxa* that revealed glycolate oxidase and glycolate dehydrogenase activity. Furthermore, multiple forms of hydroxypyruvate reductase were shown, whereas serine:glyoxylate aminotransferase could not be detected. This was also taken at that time to indicate some deviations from the glycolate metabolism observed in leaves of land plants (Betsche et al. 1992).

The potential C_4 pathway of CO_2 fixation in algae including *C. paradoxa* was assessed in the light of emerging genome data: most of the respective enzymes, if present at all, appear to be derived from archaea/proteobacteria rather than from cyanobacteria, which are assumed to lack a complete C_4 pathway. Nevertheless, some algae, e.g., diatoms, seem to contain the enzymes necessary for the C_4 pathway, whereas *C. paradoxa*, lacking pyruvate:phosphate dikinase, malic enzyme, and alanine amino transferase, is not likely to perform C_4 photosynthesis (Chi et al. 2014). After all, a pyrenoidal CCM is operative in glaucophytes to cope with low CO_2 conditions (see below).

The Phycobilisomes of *Cyanophora paradoxa*

Phycobilisomes (PBS) are the primary light-harvesting pigment complexes of cyanobacteria, red algae, and glaucophytes and are attached to the stromal surface of the thylakoids (for review see, e.g., Adir 2008). These high molecular weight protein complexes with multiple functions consist of 400–700 subunits originating from more than 20 individual polypeptides with 600–2,000 covalently linked chromophores. Sequential assembly, conformational flexibility, and interaction between the chromophore and protein components are the main features of this complex network. Linker polypeptides play a central role in all of these processes, modulate the spectral characteristics of the phycobiliprotein chromophores, and mediate the attachment of the PBS to the photosynthetic membrane. Two structural domains, the central core complex and the peripheral rods, form this superstructure. For PBS without phycoerythrin, the core is composed of three cylinders, each formed by four allophycocyanin (APC) trimers $(\alpha\beta AP)_3$ with additional minor phycobiliprotein components and core-specific linker proteins. The rods radiate from the core and consist of three to four hexameric phycocyanin (PC)-rod linker (L_R) complexes $(\alpha\beta PC)_6L_R$. The rods are connected to specific domains of the core via rod-core linker polypeptides. In rhodophytes and the model glaucophyte *Cyanophora paradoxa*, the phycobiliprotein genes reside on the plastid genome, while the colorless linker proteins are encoded by the nucleus (Egelhoff and Grossman 1983). In cyanobacteria, the PBS most likely undergo a self-assembly process mediated by the amount of PBS assembly interaction partners and assisted by chaperones and processing enzymes (Anderson and Toole 1998). In muroplasts, where the PBS components are genetically separated, transcription events in the nucleus followed by translation in the cytosol and subsequent protein import must be coordinated with transcription events of muroplast-encoded subunits and in organello biosynthesis. In

Table 5 Components of purified, intact muroplast phycobilisomes

Apparent MW (kDa)	Abundance	Phycobiliprotein	Correlated cyanobacterial gene	Function
98	Medium	Yes	*apcE*	Core-membrane linker
55	Medium	No	n. m. (*cpcK1*)	Rod linker
53	Medium	No	n. m. (*cpcK2*)	Rod linker
38	Low	No	*cpcG2*	Rod-core linker
31	Low	No	*cpcG1*	Rod-core linker
18–20	High	Yes	*cpcA*[a],*B*[a]	Phycocyanin subunits
17–18	High	Yes	*apcA*[a],*B*[a],*D*[a],*F*[a]	Allophycocyanin subunits
10	Low	No	*apcC2*	Core linker (ApcD associated)
9	Low	No	*cpcD*	Terminal rod linker
8	Low	No	*apcC1*	Core linker

n. m. No orthologous match to rod linkers from phycocyanin-PBS, size comparable to red algal linkers and to an unusual chromophorylated phycoerythrin linker from *Synechococcus* sp. WH 8102, see Six et al. (2005)
[a]Muroplast encoded

vitro PBS assembly could be shown after import of the radiolabeled small core linker precursor protein preApcC1 from *Cyanophora paradoxa* into isolated muroplasts and subsequent isolation of the PBS (Steiner et al. 2003).

Phycobilisome components: *Cyanophora* PBS are of dual genetic origin, as are plastid microcompartments in general. The gene distribution is clear-cut: The seven phycobiliproteins including the "core-membrane linker" ApcE are muroplast encoded, whereas the non-chromophorylated linker polypeptides are nuclear encoded (Table 5; Steiner and Löffelhardt 2011; Watanabe et al. 2012). All these precursors show the canonical transit sequence containing a phenylalanine residue in the N-terminal domain. The functional assignments are based on MS measurements, on 2D gel electrophoresis of purified intact PBS, and on PBS dissociation studies followed by sucrose density gradient fractionation and SDS-PAGE (Steiner et al., manuscript in preparation). The genes encoding all non-chromophorylated PBS subunits in *C. paradoxa* could be identified from abundant ESTs (and later in the Genome Project; Price et al. 2012) and by research conducted in parallel by others (Watanabe et al. 2012).

The two large rod linkers, CpcK1 and CpcK2 (Watanabe et al. 2012), were shown to result from tandem duplications of the *cpcG* (rod-core linker) gene and are – in that respect – not related to the large linker polypeptides from red algae. There is but one similar special case among cyanobacterial PBS: a 59 kDa chromophorylated phycoerythrin linker originating from a fusion of two smaller linkers (Six et al. 2005). Further, two additional truncated *cpcG* genes were found adding up to a third version (*cpcG3*) – up to four genes were reported for filamentous cyanobacteria.

CpcG3 might be part of a rudimentary PSI antenna (consisting of a rod only) as was reported for cyanobacteria (Kondo et al. 2007) and red algae (Busch et al. 2010). The three small linkers are interpreted as follows: The two core linkers, ApcC1 and ApcC2, form complexes with ApcA,B and ApcA,B,D, respectively. In cyanobacteria, just one core linker is common, whereas in red algae also two core linkers are reported. The third small linker is the terminal rod linker CpcD that determines rod length.

A typical cyanobacterial rod linker protein CpcC consists of two domains, an N-terminal pfam00427 (PBS linker domain) and a C-terminal pfam01383 (CpcD/APC linker domain). The two CpcK linker proteins from *Cyanophora* consist of two pfam00427 domains in tandem, while the pfam01383 domain is missing. Two competing models exist for the location of linker proteins in the PBS rod. Novel "skeleton-like" structures have been described in the phycobilisomes of *C. paradoxa* (Watanabe et al. 2012). The authors showed, via native polyacrylamide gel electrophoresis (PAGE), two subcomplexes (ApcE/CpcK1/CpcG2/ApcA/ApcB/CpcD and ApcE/CpcK2/CpcG1/ApcA/ApcB) that may serve as a scaffold for the whole PBS assembly. CpcK1 and CpcK2 correspond to the large pfam00427 (PBS linker domain) tandem-duplicated rod linkers. However, data obtained by different types of native PAGE combined with limited proteolysis (Steiner et al., manuscript in preparation) suggest that these "skeleton-like" structures are most likely protein aggregates originating from phycobilisome degradation. When appropriate protease inhibitors are used, isolated subcomplexes showed a more "classical" pattern in native PAGE where the main APC core particle was complexed to ApcC1, the smaller of the two tandem-duplicated rod linkers (CpcK2) migrated in a complex together with phycocyanin and the terminal rod linker (CpcD), whereas the larger tandem-duplicated rod linker (CpcK1) migrated in a complex with phycocyanin only (Fig. 10; Weisser 2012). Since both complexes show a molecular weight of about 460 kDa, an association of cpcK1 and cpcK2 with three PC trimers (one trimer about 120 kDa without linkers) seems reasonable. Moreover, CpcG2 could be shown to form a separate complex with PC, APC, and ApcC2 (Maluck 2012). Limited proteolysis followed by native and SDS-PAGE allowed to estimate the amount of protected linker protein fragments and therefore the size of the different phycobilisome subparticles. Altogether a model is favored where the two tandem-duplicated rod linkers are part of the same rod with CpcK1 being the core-proximal hexamer rod linker and CpcK2 being the core-distal hexamer rod linker (Fig. 11, right; Steiner et al., manuscript in preparation) as opposed to the model with only one of the large linkers per rod, in more stretched conformation (Fig. 11, left; Watanabe et al. 2012). A schematic view of the *Cyanophora* PBS as a whole is given in Fig. 12.

The Nature of the RuBisCO-Containing Microcompartment of Muroplasts

The conspicuous, electron-dense central body of *C. paradoxa* muroplasts was shown to contain the bulk of RuBisCO (Mangeney and Gibbs 1987) and has been denoted

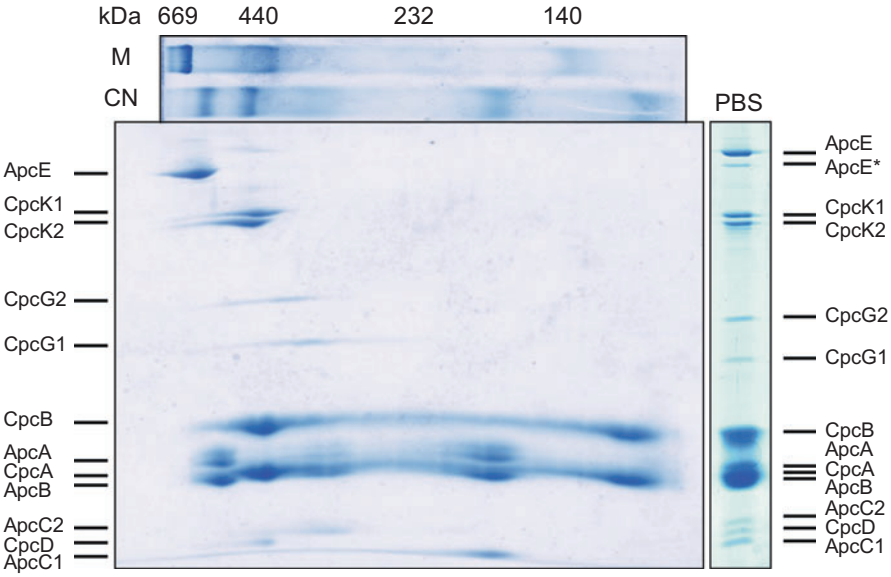

Fig. 10 Colorless native (CN)-PAGE of isolated *Cyanophora* phycobilisomes followed by SDS-PAGE: *upper* horizontal panel (M), high molecular weight marker; *upper* horizontal panel (CN), first dimension (CN-PAGE). *Lower* panel, second dimension (SDS-PAGE). Right lane, SDS-PAGE of intact phycobilisomes; ApcE*, typical degradation product of ApcE

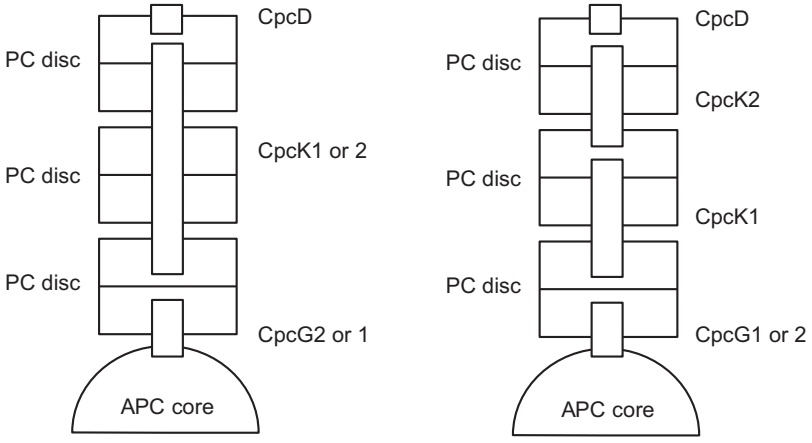

Fig. 11 Comparison of *Cyanophora* phycobilisome substructure models: *left*, skeleton-like structure (Watanabe et al. 2012); *right*, model proposed by Steiner et al., manuscript in preparation

the "carboxysome" in most publications. Despite the fact that eukaryotes contain pyrenoids (Meyer and Griffiths 2013) functioning in the carbon-concentrating

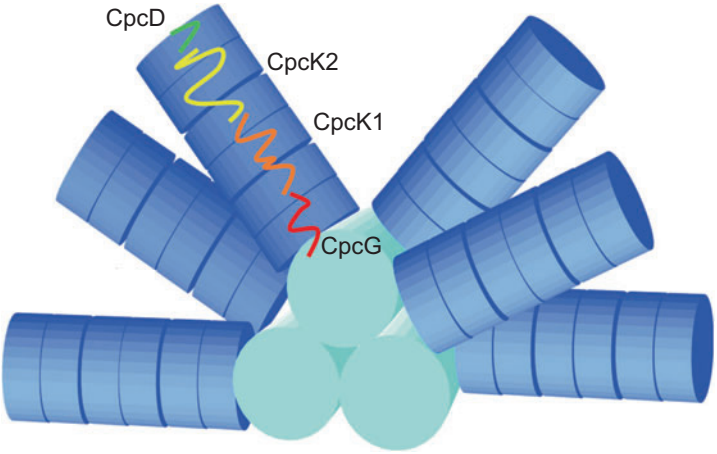

Fig. 12 Complete model of the *Cyanophora* phycobilisome according to Steiner et al. *Blue*, phycocyanin rods; *cyan*, allophycocyanin core; *green*, *yellow*, *orange*, and *red*, linker proteins

mechanism (CCM), this coinage emphasized the often-postulated transitional position of glaucophytes between plastids and cyanobacteria. Further, the hypothesis of Raven (2003) that muroplasts had retained the peptidoglycan wall for osmotic protection since these were the only plastids that had also retained carboxysomes was quite appealing: A carboxysomal CCM (Badger and Price 2003) would lead to a much higher accumulation of bicarbonate in the stroma than a pyrenoidal CCM. However, all attempts to identify genes for carboxysomal shell proteins corresponding to cyanobacterial ccmKLMNO in the *C. paradoxa* genome have failed (Price et al. 2012) as did proteomic studies on isolated muroplast central bodies (Fathinejad et al. 2008). Indeed, it might be problematic to harbor shell protein genes in the nucleus, because they have high affinities to each other and likely self-assemble as carboxysomal prestructures (Kinney et al. 2011), thereby interfering with protein import into muroplasts. On the other hand, evidence was obtained (Table 6; Bhattacharya et al. 2014) for a number of genes (e.g., *LCIB* and *LCIC*) with functions in the pyrenoidal CCM of *Chlamydomonas reinhardtii* (Yamano et al. 2010). LCIB and LCIC were shown to form a hexameric complex (ca. 360 kDa) close to the pyrenoid under light and low [CO_2]. A role for this complex is assumed in trapping of CO_2 that has escaped from the pyrenoid via interaction with the carbonic anhydrase CAH6. Alternatively, Yamano et al. (2010) envisage physical blockage of CO_2 from escaping the pyrenoid (somehow analogous to the function of the carboxysomal shell). Some putative cyanobacterial plastid ancestors – given their filamentous nature (*Lyngbya*) or capability of producing a starch-like reserve carbohydrate (*Cyanothece*) – contain *LCIB* and *LCIC*. These cyanobacteria might use mechanisms of the type discussed above that are superimposed on their carboxysomal CCM. At present, the more recent *Paulinella chromatophora* "plastid" origin (ca. 100 Ma) constitutes the only proven example of "eukaryotic carboxysomes." Here, the necessary genes remain on the genome of

Table 6 Genes for proteins potentially involved in the CCM of *Cyanophora paradoxa*

Gene[a]	Function	Comments
LCIA[b]	Bicarbonate transport	TP, complete
LCIA	Bicarbonate transport	TP, complete
LCIB[b]	CCM	TP, complete
LCIB	CCM	TP, complete
LCIB, LCID?	CCM	TP, 3′-truncated
LCIB, LCIC?	CCM	fragment
Rca[b]	RuBisCO activase	TP, complete
CAH8 [b]	Carbonic anhydrase	Beta-CA superfamily, periplasmic
CAH4 [b]	Carbonic anhydrase	Beta -CA superfamily, mitochondrial
CAH5[b]	Carbonic anhydrase	Beta -CA superfamily, mitochondrial
?	Carbonic anhydrase	Gamma-CA family, cytosolic?
?	Carbonic anhydrase	Gamma-CA family, cytosolic

[a]Nomenclature corresponding to the homologs from C. reinhardtii
[b]CO2-responsive gene; *TP* muroplast transit peptide, containing phenylalanine in the N-terminal region

the cyanelle (photosynthetic organelle; Nowack et al. 2008), interestingly originating from HGT (Marin et al. 2007). If carboxysomes were transferred to early plastids via endosymbiosis, the separation between carboxysomal and pyrenoidal CCM could have occurred within the phylum Glaucophyta, i.e., *C. paradoxa* and *Glaucocystis nostochinearum* already progressed toward a pyrenoidal CCM, whereas *Gloeochaete wittrockiana* (Fig. 5; Kies 1976) and *Cyanoptyche gloeocystis* (Kies 1989), with their polyhedral microcompartments confined by an electron-dense, shell-like layer (both features missing in the two former species), might have retained the carboxysomal CCM (Fathinejad et al. 2008). Under such a scenario, the *ccmKLMNO* genes would be expected to reside on the muroplast genomes of *G. wittrockiana* and *C. gloeocystis*. The PG wall, though no longer necessary, was retained for unknown reasons in the plastids of *C. paradoxa* and *G. nostochinearum*. Table 6 includes two genes encoding the putative bicarbonate transporter LCIA (Yamano et al. 2015) and several genes with strong sequence similarity to genes for LCIB, LCIC, and LCID from *C. reinhardtii*. Because these are closely related, an exact assignment is difficult. However, whenever the N-termini are intact, unequivocal muroplast presequences were found for these proteins.

A key enzyme of the CCM is carbonic anhydrase (CA), either co-packaged with RuBisCO in cyanobacterial carboxysomes or located in the lumen of thylakoids traversing the pyrenoid of *C. reinhardtii* (Karlsson et al. 1998). The number of CAs can vary among algae, e.g., from 9 in *C. reinhardtii* to 13 in some diatoms (Tachibana et al. 2011). Five CAs from *C. paradoxa* are shown in Table 6. Two of these belong to the gamma-CA family with high sequence similarity to homologs in plants. The other three contain the conserved Zn-binding site (VCGHSHCGAMKG) of (cyano)bacterial beta-CAs. In the case of the putative mitochondrial CAs, high sequence similarities to *C. reinhardtii* CAH4 and CAH5 are observed. The third beta-CA resembles the periplasmic CAH8. A bona fide muroplast CA (e.g., the

stromal CAH6 or the lumenal CAH3 of *C. reinhardtii*) is missing from this compilation. In a recent data mining effort among 15 microalgae, Meyer and Griffiths (2013) revealed two additional bicarbonate transporters in the *Cyanophora* database via sequence similarity to *Chlamydomonas* homologs: the plasma-membrane-localized ABC transporter HLA3 (Yamano et al. 2015) and CCP1 in the plastid envelope. As a consequence, if we assume a pyrenoidal CCM in *C. paradoxa*, the organism must utilize a mechanism different from that in *C. reinhardtii* (Meyer and Griffiths 2013). There is no evidence in *C. paradoxa* of a muroplast microcompartment traversed by thylakoid membranes. A recent high-resolution ultrastructural study of the *C. reinhardtii* cell (Engel et al. 2015) posits that the thylakoid-derived pyrenoid tubules contain several minitubules thought to transport ATP, RubP, etc. across the starch sheath to the RuBisCO in the pyrenoid interior. Because starch is stored in the cytosol of glaucophytes, such a function may not be necessary here. In the diatom *Phaeodactylum tricornutum*, the carbonic anhydrase CA-1 (CO_2 responsive) is co-packaged with pyrenoidal RuBisCO and does not reside in the lumen of the traversing thylakoid (Tachibana et al. 2011). Mass spectrometric analysis of central body proteins from *C. paradoxa* did not reveal a CA-like protein either. The only outcome of these studies (in addition to RuBisCO LSU and SSU) was RuBisCO activase that was also corroborated by Western blotting and assembly studies after in vitro import into isolated muroplasts (Fathinejad et al. 2008). *C. paradoxa* activase, whereas showing high sequence similarity to both cyanobacterial and plant homologs, lacks the C-terminal extension typical for filamentous cyanobacteria but shows the N-terminal extension present in plant homologs only. Taken together, the domain structure of RuBisCO activase from *C. paradoxa* does not support the carboxysome concept either. Several genes listed in Table 6 were shown to be CO_2 responsive in the closely related *C. cuspitata* SAG 45.84 (Kies strain) underlining their postulated role in the CCM (Burey et al. 2007).

Other Metabolic Pathways in Muroplasts

The *C. paradoxa* genome project, in combination with the muroplast proteome (Facchinelli et al. 2013) and some biochemical investigations, allows interesting insights into the metabolism of a primitive plastid in comparison to the abundant data on chloroplast metabolism.

Glycolysis: With respect to glycolysis, significant deviations from the known chloroplast pathways were found: Phosphoglyceromutase and enolase are present in the muroplast stroma in contrast to the situation in chloroplasts, allowing direct production of PEP from photosynthetically generated 3-phosphoglycerate. On the other hand, hexokinase and phosphofructokinase are missing from muroplasts. Fructose-1,6-bisphosphatase and phosphoglucomutase are sufficient to generate glucose-6-phosphate, the metabolite to be exported to the cytosol (see below).

Glucose-6-phosphate dehydrogenase was purified from a *C. paradoxa* muroplast extract (Fester et al. 1996). The 59 kDa protein forms enzymatically active dimers and tetramers. 6-Phosphogluconate dehydrogenase was identified in the stroma

through proteomics. The corresponding gene showed a canonical muroplast STP (Facchinelli et al. 2013). This points toward a muroplast-localized oxidative pentose phosphate pathway.

Isoprenoid lipid biosynthesis: Proteomics yielded very conclusive results concerning isoprenoid metabolism. With one exception, all enzymes of the 1-deoxy-xylulose-5-phosphate/2-C-methylerythritol-4-phosphate (MEP) pathway of isopentenyl diphosphate synthesis were demonstrated in the muroplast stroma (Facchinelli et al. 2013) but none of the mevalonate pathway. Proteomics also corroborated the muroplast localization of other enzymes of the prenyl lipid pathway (Facchinelli et al. 2013) as geranyl-geranyl diphosphate reductase (phytol biosynthesis), geranyl-geranyl diphosphate synthase (CrtE, muroplast encoded), solanesyl diphosphate synthase (PreA, muroplast encoded), and homogentisate solanesyl transferase (plastoquinone biosynthesis). In most of these cases, nucleus-encoded muroplast proteins possess canonical transit sequences (with F replaced by Y or W in a few instances).

Amino acid biosynthesis: As plastids, muroplasts are the main contributors to amino acid biosynthesis. This became apparent from the genome data where muroplast STPs preceded the respective genes and also from the analysis of the muroplast proteome (Facchinelli et al. 2013).

Photooxidative stress management: *Cyanophora paradoxa* is known to prefer low light intensities for growth (Löffelhardt and Bohnert 2001). A recent survey showed that *C. paradoxa* does not use the ascorbate/ascorbate peroxidase system that plays an important role in coping with reactive oxygen species (ROS), which is unparalleled among phototrophs (Wheeler et al. 2015). Furthermore, *C. paradoxa* is devoid of glutathione reductase (Serrano and Löffelhardt 1994). However, *C. paradoxa* contains catalase, glutathione peroxidase, and peroxiredoxins, and its muroplasts harbor the unusual peroxidase symerythrin (Cooley et al. 2011). In glaucophytes, the low levels of ascorbate synthesized by the unusual enzyme gulonolactone oxidase (land plants and green algae use a gulonolactone dehydrogenase) might have a role as enzyme cofactor but neither in the ascorbate/glutathione antioxidative pathway nor in the xanthophyll cycle (Wheeler et al. 2015).

Miscellaneous: The NADP-dependent malate dehydrogenase of *C. paradoxa* was shown not to be responsive to reductive activation (Ocheretina et al. 2000) as red algal enzymes, in contrast to enzymes of the "green" lineage. Accordingly, attempts to demonstrate thioredoxin *m* in *C. paradoxa* were unsuccessful (Dai et al. 1992). A muroplast pyrophosphatase (sPPase I) was isolated from *C. paradoxa* and characterized by N-terminal sequencing and MW determination via MALDI-TOF mass spectrometry (Gómez-García et al. 2006). The monomeric 30 kDa protein is more related to PPases from heterotrophic eukaryotes than to the smaller cyanobacterial enzymes. This also applies to the plastid enzymes from other algae, e.g., *C. reinhardtii*, and from plants. Taken together, this means that early in plastid evolution, the endosymbiont gene was lost and the product of a host cell gene was relocalized to the organelle.

Genome Analysis of Glaucophytes

Glaucophyte genome-wide analyses are relatively scarce when compared to plants and green algae. This is explained by the limited expressed sequence tag (EST) and complete genome data available from these taxa. For many years, the only sources of EST data were from the Pringsheim (Reyes-Prieto et al. 2006) and Kies strains of *Cyanophora paradoxa* and from *Glaucocystis nostochinearum* (http://tbestdb.bcm.umontreal.ca/searches/login.php). The complete plastid genome sequence of *C. paradoxa* (Pringsheim strain; Stirewalt et al. 1995; Loeffelhardt et al. 1997) was also available (see section on the Muroplast Genome; Table 7). Uses of the EST data from *C. paradoxa* included assessment of the divergence position of glaucophytes within Plantae/Archaeplastida (Reyes-Prieto and Bhattacharya 2007a; Deschamps and Moreira 2009) and estimation of the contribution of cyanobacterial genes to the nuclear genome of glaucophytes via EGT (e.g., Timmis et al. 2004; Reyes-Prieto et al. 2006). Bioinformatic analyses suggested that 6–11% of *C. paradoxa* nuclear genes owed their origin to EGT from the endosymbiont (Reyes-Prieto et al. 2006; Qiu et al. 2013a). The *C. paradoxa* plastid genome has been invaluable to many researchers who have used it to infer the phylogenetic history of this organelle, its gene content, and gene order (e.g., Stirewalt et al. 1995; Rodriguez-Ezpeleta et al. 2005; Sato et al. 2005; Janouškovec et al. 2010; Qiu et al. 2013a). A recent biochemical characterization of the *C. paradoxa* plastid proteome that identified a partial list of 586 non-redundant proteins (Facchinelli et al. 2013) demonstrated their complex evolutionary histories. Maximum likelihood analysis of these proteins by Qiu et al. (2013a) showed that 25% were plastid encoded, 12% were derived from EGT candidates encoded in the nucleus, 7% were of non-cyanobacterial (HGT) origin, and the remaining (56%) were derived from the host or were of ambiguous provenance based on analysis of current data. The phylogenetic origins of non-redundant plastid proteins in *C. paradoxa*, *Chlamydomonas reinhardtii* (1,057 proteins), and *Arabidopsis thaliana* (1,660 proteins) are shown in Fig. 13 (Qiu et al. 2013a). More recently, mitochondrial genomic data have been analyzed from seven different glaucophytes and used to test (and validate) Archaeplastida monophyly (Jackson and Reyes-Prieto 2014). These organelle genomes have a highly conserved gene content but show significant variation in gene order across taxa (Jackson and Reyes-Prieto 2014).

Genome data: A significant step forward for the field of glaucophyte genomics came in 2012 with the publication of the draft genome assembly from the *C. paradoxa* Pringsheim strain CCMP329 (SAG 29.80; Price et al. 2012). This work was supported by the United States National Science Foundation and resulted in the generation of 8.3 billion base pairs (Gbp) of Roche 454 and Illumina GAIIx sequence data that were co-assembled with 279 Mbp of random-shear Sanger sequence from this taxon. The resulting assembly comprised 60,119 contigs, totaling 70.2 Mbp. More recent sequencing of this strain using the long-read PacBio platform suggests that the genome size is closer to 120 Mbp based on the initial assembly output. Pulsed-field gel electrophoresis suggests the existence of at least seven chromosomes in *C. paradoxa* with the smallest being less than 3 Mbp in size

(Price et al. 2012). Given the initial Sanger/Roche/Illumina and the later PacBio genome data, we posit that the Price et al. (2012) assembly likely captured most of the gene inventory in the gene-rich regions (see below), whereas assembly of the complex (e.g., repeated or with strong nucleotide bias, such as homopolymers) DNA regions was only possible with the PacBio long-read technology. Generation and analysis of a hybrid Illumina/PacBio genome assembly are underway in the Bhattacharya and Andreas P.M. Weber labs. Interestingly, the PacBio results are more in line with previous fluorescence-activated cell sorting (FACS) work that suggested the haploid genome size in *C. paradoxa* to be ca. 140 Mbp (Löffelhardt et al. 1997).

Consistent with these observations, genome analysis done by Price et al. (2012) demonstrated an unusually high G + C content in *C. paradoxa* (83.8% at third codon positions) that likely explains the highly fragmented, initial assembly. Nonetheless, BLASTN analysis using 3,900 Sanger-derived EST unigenes from the glaucophyte against the draft assembly showed that 99% of the ESTs had hits (at *e*-value $\leq$1E-10), suggesting that the vast majority of expressed genes were present in these genome data. Given this promising result, 15 Gbp of Illumina mRNA-seq data were used to train ab initio gene predictors to generate 27,921 gene models for downstream analysis (Price et al. 2012). Below we will discuss some of the insights that were gained through analysis of the *C. paradoxa* genome data generated by Price et al. (2012), recognizing that the PacBio results will likely lead to additional novel insights.

Phylogenomic analysis test Archaeplastida monophyly: Given that many multigene (i.e., concatenated protein dataset) phylogenies have provided conflicting topologies regarding the monophyly of Archaeplastida in the eukaryote tree of life (e.g., Burki et al. 2007; Baurain et al. 2010; Parfrey et al. 2010; Yabuki et al. 2014; Jackson et al. 2015) and have failed to reach any consensus on this important question, Price et al. (2012) took another approach. Rather than joining proteins, often with uncertain histories into a single dataset, they analyzed each protein separately using maximum likelihood (ML) phylogeny reconstruction and tabulated the overall signal for Archaeplastida monophyly. In their analysis, a total of 4,628 proteins had significant BLASTP hits (*e*-value $\leq$1E-10) to sequences in a comprehensive local database that were used for comparative analysis (e.g., Moustafa et al. 2009; Chan et al. 2011). Using an automated approach (Chan et al. 2011), they generated 4445 ML trees for *C. paradoxa* proteins that had significant database hits. Only trees containing $\geq$3 phyla were considered and a minimum number of terminal taxa (N) that ranged progressively from 4 to 40 (Fig. 14a). Using this approach they found that >60% of all trees supported (at bootstrap value $\geq$90%) a sister-group relationship between glaucophytes and red and/or green algae. The glaucophytes were most often positioned as sister to Viridiplantae in trees that excluded non-Archaeplastida algae, a result that was found even though a large number of trees favored glaucophyte-red-green (Archaeplastida) monophyly (44, 40, 32, 18, and 16 trees at $N = 4$, 10, 20, 30, and 40, respectively), and they had substantial red algal genome data in the database. Most of the trees showed *C. paradoxa* to be monophyletic with other Archaeplastida in a clade ("shared") that also included

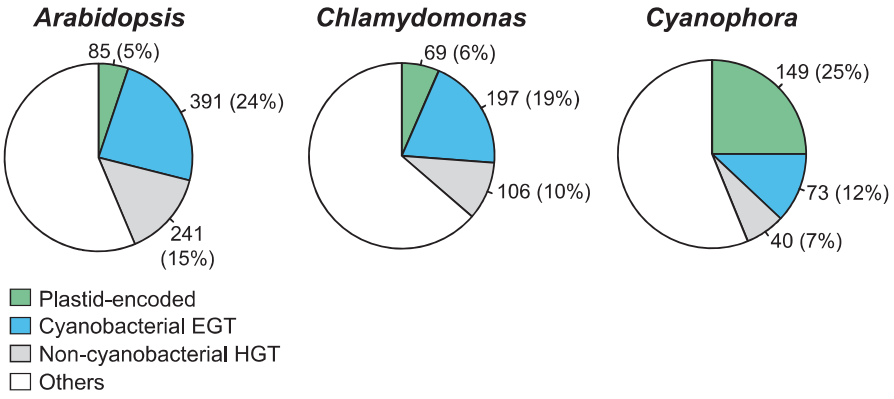

Fig. 13 Results of phylogenetic analysis of single proteins represented as pie charts that show the relative contribution of cyanobacterial and non-cyanobacterial sources to Archaeplastida plastid proteomes (for details, see Qiu et al. 2013a)

non-Archaeplastida phyla (GlR/GlVi/GlRVi in Fig. 14). When they sorted the phylogenomic output using the red or green algae as the query to test Archaeplastida monophyly, these results also identified Archaeplastida as the most frequently recovered clade. Expectedly, red and green algae showed far more gene sharing than glaucophytes because they, unlike glaucophytes, are involved in secondary endosymbioses (Harper and Keeling 2003; Moustafa et al. 2009; Baurain et al. 2010; Chan et al. 2011; Bhattacharya et al. 2013). These results demonstrate a highly complex phylogenetic history for glaucophyte and algal genome data in general, showing that EGT and HGT have moved genes between disparate lineages leaving a highly reticulate signal within their genomes. Regardless, the single protein trees overall strongly support a single origin of Archaeplastida and likely a single primary plastid endosymbiosis in their common ancestor (Fig. 14; Price et al. 2012). Future genome projects that add more glaucophytes and other poorly sampled Archaeplastida lineages (e.g., prasinophytes) to the analysis are needed to validate the hypothesis of Archaeplastida monophyly.

Given the extent of gene sharing among algae, Price et al. (2012) investigated the "footprint" of non-cyanobacterial, prokaryotic HGT in the nuclear genomes of Archaeplastida. For this analysis, they constructed a database that included sequences from NCBI Refseq, *C. paradoxa* and the red algae *Calliarthron tuberculosum* and *Porphyridium purpureum* (Bhattacharya et al. 2013). These data were then queried using each *C. paradoxa*, *C. tuberculosum*, and *P. purpureum* protein, as well as those derived from two Viridiplantae (i.e., *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*). The top five bacterial hits (BLASTP *e*-value $\leq$1E-10) were retained for each Archaeplastida query sequence and used as input for an automated phylogenetic tree-building pipeline (for details of procedure, see Price et al. 2012, supplement). Inspection of the maximum likelihood-generated trees turned up 444 non-cyanobacterial gene families shared by prokaryotes and Archaeplastida. Of these, 15 were present in all three Archaeplastida phyla. One

such ancient HGT resulted in the transfer of a thiamine pyrophosphate-dependent pyruvate decarboxylase family protein involved in alcohol fermentation. This analysis turned up 60 other genes that are present in only two of the three phyla (i.e., 24, 10, and 26 genes in Glaucophyta-Viridiplantae, Glaucophyta-Rhodophyta, and Rhodophyta-Viridiplantae, respectively). More recent work has shown that HGT plays a key role in adaptation of algae to their environment and the impacts of this process will likely become more widely appreciated as additional complete algal genomes are analyzed (Qiu et al. 2013b; Schönknecht et al. 2013; Foflonker et al. 2015).

Evolution of the plastid translocon and metabolite transport: Important innovations that have been the subject of much study in algae and plants are the evolution of the protein import system for the plastid and the emergence of metabolic connections between the captured cyanobacterial endosymbiont and the host cell. A fundamental outcome of the cyanobacterium-to-plastid evolutionary transition in the Archaeplastida primary endosymbiosis was the establishment of protein translocons for protein targeting into the organelle (e.g., Gross and Bhattacharya 2008, 2009; Reumann et al. 2005; Sommer and Schleiff 2014). Components of the translocons at the outer and inner envelope membranes of chloroplasts (Toc and Tic, respectively) were known in other Archaeplastida and in chromalveolates (McFadden and van Dooren, 2004). The existence of an equivalent protein import system in *C. paradoxa* was suggested by immunological detection of epitopes in this alga using plant Toc75 and Tic110 antibodies and heterologous protein import assays (see section on Protein Import into Muroplasts; Steiner et al. 2005a; Yusa et al. 2008). Analysis of the genome of *C. paradoxa* turned up homologs of Toc75 and Tic110 that are OEM (outer envelope membrane) and IEM (inner envelope membrane) protein conducting channels, respectively, two Toc34-like receptors, as well as homologs of the plastid Hsp70 and Hsp93 chaperones and stromal processing peptidase (Price et al. 2012). These are likely to have formed the primordial protein translocation system in the Archaeplastida ancestor (Gross and Bhattacharya 2008, 2009). In summary, analysis of *C. paradoxa* genome data revealed the presence of the conserved core of translocon subunits derived from the cyanobacterial endosymbiont (i.e., Toc75, Tic20, Tic22), suggesting that the Toc/Tic system was likely to have been in place in the Archaeplastida common ancestor. Toc75 of glaucophytes and likely also of rhodophytes is closer than the homolog of Viridiplantae to the ancestral Omp85 of cyanobacteria in recognizing phenylalanine in the N-terminal part of the transit peptides (see also section on Protein Import into Muroplasts; Wunder et al. 2007). A dual function as receptor and pore is assumed (Steiner and Löffelhardt 2005). This phenylalanine requirement is no longer found in chloroplast import: Toc75 in Chlorophyta and Streptophyta has only retained the pore function, whereas the receptor function is taken over by a small family of proteins, e.g., Toc159. Likely, this went along with the need for import of certain abundant proteins (RuBisCO SSU, LHCPII). In addition, the Tic translocon appears to be more elaborate in land plants: a 1 Mda complex contains Tic20 (pore?), Tic56, Tic100, and Tic214 (Nakai 2015).

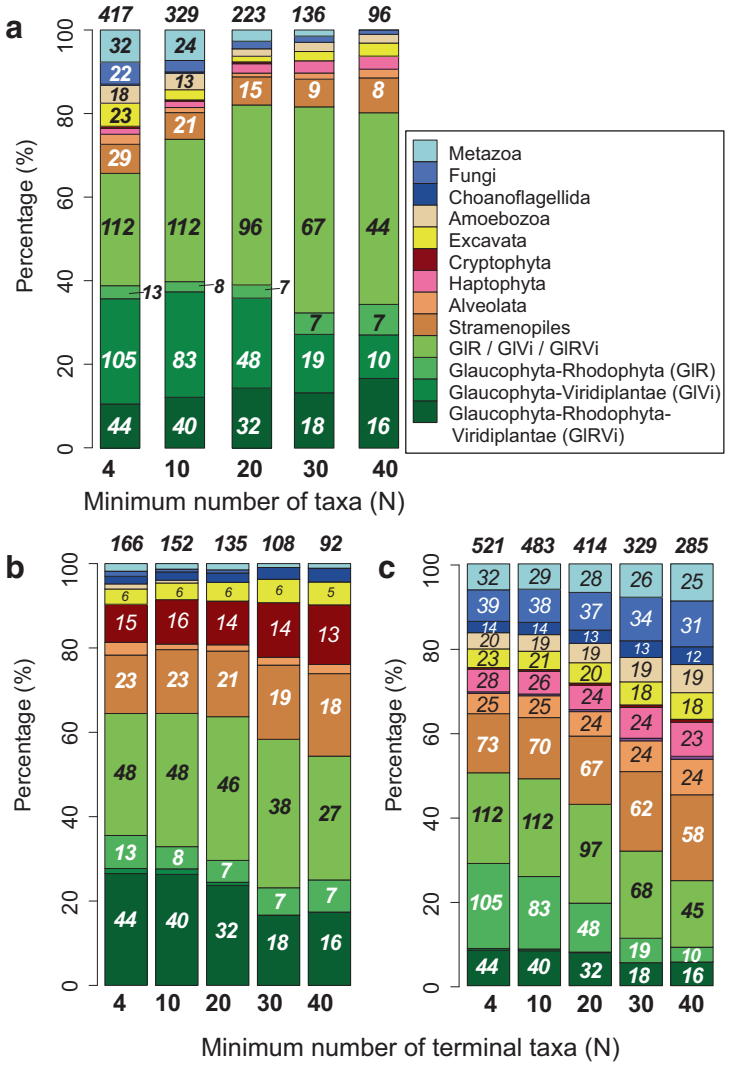

Fig. 14 Maximum likelihood analysis of single proteins derived from the *C. paradoxa* genome assembly (for details, see Price et al. 2012). **a** Percentage of single protein maximum likelihood trees (raw numbers shown in the bars) at bootstrap cutoff >90% that support the monophyly of glaucophytes solely with other Archaeplastida or in combination with non-Archaeplastida taxa that interrupt this clade. These latter groups of trees are explained by *red/green* algal EGT into the nuclear genome of chromalveolates (e.g., diatoms, haptophytes) and euglenids, respectively. For each of these algal lineages, the set of trees with different numbers of taxa (N) ≥4, ≥10, ≥20, ≥30, and ≥40 and distinct phyla ≥3 in a tree are shown. The Archaeplastida-only groups are Glaucophyta-Rhodophyta (GlR), Glaucophyta-Viridiplantae (GlVi), and Glaucophyta-Rhodophyta-Viridiplantae (GlRVi). Trees with evidence of EGT are shown as the single group, GlR/GlVi/GlRVi. **b** The same analysis done with *red* algae as the query to search for support for Archaeplastida monophyly. **c** The same analysis done with *green* algae as the query to search for support for Archaeplastida monophyly

Another landmark trait linked to plastid establishment is the coordination of carbon metabolism between the host and plastid that relies on sugar-phosphate transporters. Previous work had shown that plastid-targeted sugar transporters evolved from existing host endomembrane nucleotide sugar transporters (NSTs) through gene duplication, divergence, and retargeting to the photosynthetic organelle (Weber et al. 2006; Colleoni et al. 2010). Analysis of the *C. paradoxa* genome turned up a surprising result in this respect. Price et al. (2012) found that although six endomembrane-type NST genes existed in *C. paradoxa*, there were no genes for plastid-targeted phosphate translocator (PT) proteins. The search for the missing genes turned up two candidates that encode homologs of bacterial UhpC-type hexose-phosphate transporters. These genes were also found in other algal members of the Archaeplastida, but lost in plants. Both *C. paradoxa* UhpC homologs encode an N-terminal extension that could serve as a plastid targeting sequence. Surprisingly, both of these UhpC genes were derived via HGT in the Archaeplastida ancestor from parasites related to *Chlamydiae* and *Legionella* (Price et al. 2012). Support for the absence of typical NST-derived sugar transporters in the plastid of *C. paradoxa* was found in the analysis of the plastid permeome from this species. Using YFP-fusion constructs in *Nicotiana benthamiana*, Facchinelli et al. (2013) validated the capacity of the UhpC transit peptide to target to the chloroplast inner membrane in *N. benthamiana*, as well as the localization of the complete protein to this site for both *Chlamydiae*-derived transporters in *C. paradoxa*, as predicted by Price et al. (2012). Subsequent work done by Karkar et al. (2015), using the same approach, showed that the UhpC homologs in the red algae *Galdieria sulphuraria* and *C. merolae* are also targeted to the chloroplast inner membrane in *N. benthamiana*. These results demonstrate that the diversification of the PT gene family occurred in the red-green algal ancestor, with the glaucophytes relying on UhpC, a gene that is also retained by algal members of the Rhodophyta and Viridiplantae. Whether these data prove an early divergence of glaucophytes within Archaeplastida is unclear because PT gene loss in this lineage could also explain the current distribution. Regardless, these results bring to a close an intriguing, open question in Archaeplastida evolution and suggest that UhpC could have been the primordial sugar transporter in this supergroup (for details, see Karkar et al. 2015).

Small RNAs in Cyanophora paradoxa: RNAi (RNA interference) is a strategy found among eukaryotes to protect their genomes from the spread of self-replicating genetic entities such as transposable elements and viruses (e.g., Mallory and Vaucheret 2010). This pathway relies on the production of small RNAs (sRNAs) from double-stranded RNA (dsRNA). The initial RNAi signal may be amplified by the generation of multiple secondary sRNAs from a targeted mRNA. This reaction is catalyzed by RNA-dependent RNA polymerases (RdRPs), a phenomenon known as transitivity (Calo et al. 2012) that is particularly important in plants to limit the spread of viruses (Chen et al. 2010). The RNAi process in which sRNAs formed from perfect dsRNAs acting in *cis* by pairing to their cognate producing transcripts is referred to as the small interfering RNA (siRNA) pathway (Obbard et al. 2009). , microRNAs (miRNAs) also represent a class of sRNAs widespread in eukaryote genomes that probably evolved from the ancestral siRNA pathway (Piriyapongsa

and Jordan 2008). Gross et al. (2013) generated extensive sRNA data from *C. paradoxa* to characterize their genome-wide distribution and to gain insights into their potential functions. Given the monophyly of glaucophytes and Viridiplantae within the Archaeplastida, it was postulated that *C. paradoxa* could represent an ancestral form of the highly developed RNAi system found in plants such as *Arabidopsis thaliana*.

To establish the presence of a putative RNAi pathway in *C. paradoxa*, BLASTP analysis of the glaucophyte genome was done using, as queries, homologs of the *A. thaliana* Dicer and Argonaut proteins. These sequences were found as were several putative homologs of *A. thaliana* RdRP. Bioinformatic analysis of 4,739,151 sRNA reads derived from four *C. paradoxa* cDNA libraries showed that sequences had a predominant size of 21 nt (Fig. 15a) with overrepresentation of adenine and uracil in the first nucleotide (Fig. 15b) (Gross et al. 2013). Because *C. paradoxa* sRNAs mapped to over 70% of the EST contigs and to 75% of the predicted CDSs (Fig. 15c), Gross et al. (2013) concluded that sRNA production in this species was primarily associated with mRNA (exonic) sequences. A possible explanation for the significant levels that were found of transcript-derived sRNAs is through the production of secondary siRNA by RdRPs (present in the glaucophyte) during amplificatory cascades of the RNAi signal (for details, see Gross et al. 2013). This intriguing finding has however not been validated due to the lack of genetic tools in *C. paradoxa*. Given the postulated transitivity in *C. paradoxa* and its known presence in the fungus *Mucor circinelloides* (Calo et al. 2012), it is likely that a complex RNAi system was present in the ancestor of all eukaryotes.

In summary, nuclear genome data from glaucophytes have provided a myriad of important insights into the evolution of Archaeplastida. However, much of what has been learned is gleaned from a single draft assembly and several EST databases. As the *C. paradoxa* genome assembly improves, it will provide a valuable reference source for other glaucophyte complete genome projects. These are underway in different labs and become increasingly more tenable as sequencing costs continue to fall and better, long-read technologies are developed. Although we have touched upon some key aspects of glaucophyte genome evolution, we did not address several others that are rapidly advancing. One of these is the work led by the lab of J. Clark Lagarias on phytochrome function and evolution in algae. Analysis of glaucophyte phytochromes demonstrates that *C. paradoxa* (CparGPS1) has an unusual blue/far-red photocycle, whereas *Gloeochaete wittrockiana* (GwitGPS1) has a red/blue photocycle (Rockwell et al. 2014). This is in stark contrast to classical plant phytochromes that are associated with red/far-red photoreception that regulates gene expression for developmental pathways and the shade avoidance response (Rockwell et al. 2006). The surprising diversity of phytochromes in algae (Duanmu et al. 2014; Anders and Essen 2015), and in particular in glaucophytes, indicates that much still needs to be learned about how algae tune their light response to ambient conditions. In this regard, the sequence of a genomic clone of cyanophoropsin, a highly conserved homolog of fungal and bacterial rhodopsins, was described by Frassanito et al. (2010). This trait seems to be unrelated to the known photophobic response of *C. paradoxa* (Häder 1985) because uniform immuno-decoration of the

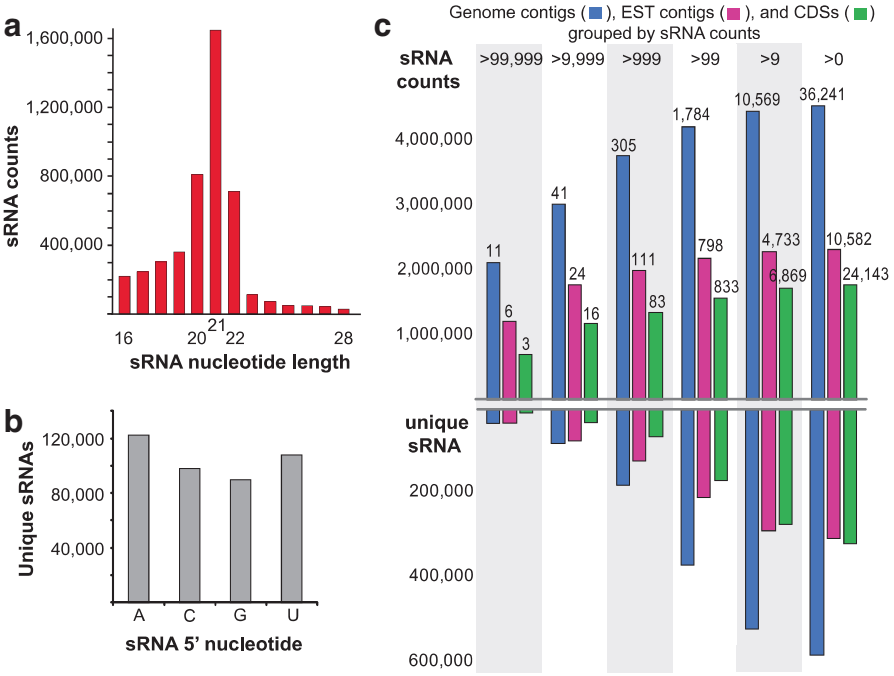

Fig. 15 Analysis of sRNAs from *C. paradoxa*. (**a**) Size distribution of redundant sRNAs in *C. paradoxa* showing the predominance of the 21 nt length class. (**b**) Composition of the 5′ nucleotide of unique sRNAs in *C. paradoxa*. (**c**) The results of mapping redundant (above the x-axis) and unique (below the x-axis) sRNAs to genomic contigs, EST contigs, and CDSs from *C. paradoxa*. The numbers on the top of the colored bars correspond to the number of genomic contigs (*blue*), EST contigs (magenta), and CDSs (*green*) that are associated with the sRNA counts shown at the *top* of the panel

muroplast envelope was achieved using specific antisera directed against an N-terminal recombinant peptide. Therefore, Frassanito et al. (2010) suggest the role of a light-driven proton pump, possibly in conjunction with bicarbonate import into the muroplasts (see section on CCM). Several, but not all, amino acid positions thought to be essential for this function are conserved. Interestingly, corresponding ESTs were overrepresented in low [CO_2] cDNA libraries, indicating that the cyanophoropsin gene is CO_2 responsive (Burey et al. 2007). In addition, a second form of cyanophoropsin was purified as a recombinant protein (Frassanito et al. 2013). Opsins localize to the muroplast envelope; the corresponding genes lack both N-terminal phenylalanine and a canonical stroma-targeting peptide as revealed by terminal amine labeling of substrates (TAILS; Köhler et al. 2015). It is therefore clear that glaucophyte genomes will provide exciting and novel insights into the broader story of algal evolution and help us understand how these taxa thrive in highly variable environments.

The 135.6 kb Muroplast Genome of *Cyanophora paradoxa* SAG 29.80

The list of genes of the completely sequenced muroplast genome of *C. paradoxa* (Stirewalt et al. 1995; Löffelhardt et al. 1997), given in Table 7, contains more than 60 genes that are nuclear encoded or missing in land plants. This gene content is typical for primordial plastids, i.e., those from algae devoid of chlorophyll *b*. The 192 muroplast genes rank between the 174 genes present on the 120 kb plastome from the diatom, *Odontella sinensis*, and the 251 genes found on the 191 kb plastome from the red alga *Porphyra purpurea* (Reith 1995). With some exceptions, e.g., the *ndh* genes and *infA* (missing from all algal plastomes investigated thus far), *atpI*, or *accD*, the muroplast genome contains the standard set of chloroplast-encoded genes. In addition, the muroplast genome encodes many more ribosomal proteins, several enzymes involved in anabolic pathways other than photosynthesis, chaperones, (putative) transcription factors, and components of ABC transporters and the Sec preprotein translocase (Table 7). The most conspicuous feature of the gross organization of the muroplast genome is the 11.3 kb inverted repeat (IR), which corresponds to about half the size of land plant chloroplast IRs. Another obvious feature is the small intergenic spacer regions between muroplast genes. In a few cases (*orf299/orf244*, *ycf16/ycf24*, *atpD/atpF*, *psbD/psbC*) adjacent genes have been found to overlap by 3–16 bp. Moreover, only few noncoding regions that extend over several hundred bp are observed. Just one single intron has been identified: the 232 bp group I intron in the anticodon loop of $trnL^{UAA}$. These three features explain why muroplasts encodes around 50 genes more than land plant chloroplasts most of which even have slightly larger genomes. A restriction map of muroplast DNA from *C. cuspitata* (Kies-isolate; SAG 46.84) showed significant differences in size (about 10 kbp) and restriction pattern. However, the overall sequence identity to the Pringsheim isolate (SAG 29.80) was above 85% and 18 protein gene loci and the rDNA regions appeared to be conserved (Löffelhardt et al. 1997).

RNA genes: About half of the IR regions are occupied by the two rDNA units. The rDNA spacer is small, as is typical for chlorophyll *b*-less algae, and harbors *trnI* and *trnA* as in most plastids and prokaryotes (Löffelhardt et al. 1997). The *rnpB* gene, also present on the *P. purpurea* plastome, specifies the essential RNA component of RNaseP, a ribonucleoprotein responsible for 5′-processing of plastid tRNAs. This marks another distinction between primitive plastids and chloroplasts. In land plant chloroplasts, the enzyme activity is protein based only, whereas in *C. paradoxa* muroplasts an RNA component with strong similarity to bacterial counterparts is present – the protein component, if any, has not been found yet. In contrast to red algal RnpB, an RNA-only activity (as shown for bacteria) has been demonstrated for the muroplast RNA (Li et al. 2007). Addition of RnpA protein from *E. coli* considerably enhanced the activity, indicating a certain conformational instability of muroplast RnpB. A tmRNA combining properties of tRNAs and mRNAs that ameliorates problems arising from stalled ribosomes was also found to be encoded by a muroplast gene and shown to be processed by RNaseP (Gimple and Schön 2001). This is again typical for primitive organelles whose tmRNAs are examples of

Table 7 Muroplast genes from *Cyanophora paradoxa*. Gene nomenclature follows the guidelines for chloroplast genes (Stoebe et al. 1998). Genes marked with an asterisk are not found on any other plastid genome. Genes underlined are absent from the chloroplast genomes of land plants

Ribosomal RNAs (3): *rrsA, rrlA, rrfA*
Transfer RNAs (36)
Other RNAs (2): *rnpB,* tmRNA
Ribosomal proteins (37): *rpl1, rpl2, rpl3, rpl5, rpl6, rpl7, rpl11, rpl14, rpl16, rpl18, rpl19, rpl20, rpl21, rpl22, rpl28, rpl33, rpl34, rpl35, rpl36, rps2, rps3, rps4, rps5, rps6, rps7, rps8, rps9, rps10, rps11, rps12, rps13, rps14, rps16, rps17, rps18, rps19, rps20*
RNA polymerase subunits (4): *rpoA, rpoB, rpoC1, rpoC2*
Phycobiliproteins (7): *apcA, apcB, apcD, apcE, apcF, cpcA, cpcB*
Photosystem I and II proteins (27): *psaA, psaB, psaC, psaE, psaF, psaI, psaJ, psaM, psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbV, psbX, psbY, psbZ, psb30*
ATP synthase subunits (7): *atpA, atpB, atpD, atpE, atpF, atpG, atpH*
Cytochrome b_6/f subunits and ferredoxin (8): *petA, petB, petD, petG, petL, petN, petX, petF*
Anabolic enzymes (13): *rbcL, rbcS, chlB, chlI, chlL, chlN, acpP, nadA*, preA, crtE*, hemA*, hisH, trpG*
Peptidoglycan biosynthesis/muroplast division (2): *ftsW, sepF*
Proteases (2): *clpP1, clpP2*
Chaperones (3): *dnaK, groEL, groES**
Translation factor: *tufA*
Preprotein translocase: *secY*
ORFs with unknown or putative function (37): *ycf3*[a], *ycf4*[a], *ycf5*[b], *ycf16*[c], *ycf17*[d], *ycf21, ycf23, ycf24*[e], *ycf27*[f], *ycf29*[f], *ycf30*[g], *ycf33*[h], *ycf34, ycf35, ycf36, ycf37*[i], *ycf38*[j], *ycf39*[k], *orf27, orf48, orf77, orf91, orf102, orf108, orf163, orf179, orf180**[l], *orf182, orf188, orf206, orf244*, orf299*, orf333*[m]

[a]Role in PS I assembly
[b]Role in PS I function
[c]ABC transporter subunit, ortholog to bacterial sufC, involved in [Fe-S] cluster biogenesis
[d]CAB/ELIP/HLIP superfamily protein
[e]ABC transporter subunit, ortholog to bacterial sufB, involved in [Fe-S] cluster biogenesis
[f]Response regulator of PS I genes (rpaB)
[g]Transcription factor (RuBisCo operon)
[h]Role in cyclic electron transport
[i]PSI stability or assembly
[j]ABC transporter
[k]Photosystem II assembly factor
[l]Symerythrin
[m]Role in assembly/stability of PSII

reductive evolution compared to their bacterial counterparts (de Novoa and Williams 2004). An RNA component of the algal plastid SRP, encoded on all sequenced rhodoplast genomes, could not be detected on muroplast DNA.

Muroplast gene expression: The codon bias of muroplast genes, likely a selection for translation efficiency, is more pronounced than that of other algae or land plants (Morton 1998). Putative promoter motifs can often be observed that are similar in both sequence and spacing to the canonical sequences from *E. coli* and other

eubacteria. Three muroplast ORFs (*ycf27*, *ycf29*, and *ycf30*), that are conserved among primitive plastid genomes, show significant sequence similarity to prokaryotic transcription regulatory factors of the OmpR and LysP classes. The occurrence of these putative regulators suggests that some transcriptional regulation occurs in muroplasts. *Ycf27* homologous response regulator genes (*rpaB*) appear to be confined to phycobiliprotein-containing organisms. Many genes show short poly-purine stretches complementary to the 3′ end of the cyanelle 16S rRNA (−CCUCCUUU-3′OH) at a distance of 7–12 bases upstream of the initiation codon. Typical ribosome binding sites (Shine-Dalgarno sequences) are AAGG, AGGA, GGAG, and GAGG. The gene arrangements observed suggest a predominance of polycistronic transcripts as reported for chloroplasts (e.g., the large ribosomal protein gene cluster) and cyanobacteria (e.g., phycobiliprotein gene clusters) which could be proven in several cases. Processing of the primary transcripts to smaller mRNAs seems to be rather common (Löffelhardt et al. 1997). The widespread distribution of a specific gene cluster (5′-*rpoB-rpoC1-rpoC2-rps2-atpH-atpG-atpF-atpD-atpA*-3′) strongly supports the hypothesis of a common origin of all plastid types. Three transcription units (*rpoBC1C2*, *rps2-tsf*, and *atpIHFGDAC*) that are widely separated on cyanobacterial genomes seem to have been fused together after the endosymbiotic event. This cluster is found with some variation in gene content, but never in gene order, in muroplasts and rhodoplasts as well as in land plant chloroplasts. The existence of this "diagnostic" cluster in plastids of different evolutionary levels can only be explained when a single primary endosymbiotic event is assumed (Kowallik 1994; Reith 1995; Löffelhardt 2014). In *O. sinensis*, this cluster is bipartite, and it is completely disintegrated in *Chlamydomonas reinhardtii*, which shows that there is no particular selection pressure to maintain or to reach this kind of gene arrangement.

A signature of primitive plastids devoid of chlorophyll *b* is that both subunits of RuBisCO are plastome encoded as first shown for *C. paradoxa* (Heinhorst and Shively 1983) and cotranscribed (Starnes et al. 1985). Interestingly, the *rbcLS* and *atpBE* genes are adjacent and divergently transcribed in muroplasts and land plant chloroplasts.

There are a few cases where the muroplast genome contains cyanobacterial genes and transcription units that are absent from the *P. purpurea* rhodoplast genome in spite of the 30% surplus in size and gene content of the latter. One of them is *groES-groEL*: the chaperonin-10 homolog is nucleus encoded in the red alga. Other examples are *crtE* (specifying geranyl-geranyl pyrophosphate synthase), *hemA* (glutamyl-tRNA reductase), and *orf244-orf299* encoding two components of an ABC transporter, likely for manganese, based on the significant sequence similarity to the cyanobacterial *mntA* and *mntB* genes (Bartsevich and Pakrasi 1995). The *orf333* upstream from muroplast *psbE* is found in this position in cyanobacteria, too, but is absent from all other plastid genomes. ORF333 is the product of a nuclear gene (*hcf136*) in *Arabidopsis thaliana* and is absolutely required for assembly/stability of functional PSII units (Meurer et al. 1998). A special case is *orf180* found only on muroplast DNA (in the *petA-psaM* intergenic region) and on the genome of the peculiar cyanobacterium, *Gloeobacter violaceus*. The gene product, symerythrin,

belongs to the ferritin-like superfamily (FLSF, Cooley et al. 2011). While its in vivo functions are still unknown, the recombinant protein displays oxidase and peroxidase activity. Other members of the FLSF (e.g., the rubrerythrins) have six or seven ligands to the diiron metallocenter, whereas symerythrin has eight ligands. Other unique features comprise the high internal symmetry of the crystal structure and the spontaneously formed carbon-carbon cross-link between a valine and a phenylalanine side chain. This led the authors to assume an ancestral role for this fold in the evolution of FLSF (Cooley et al. 2011). Recently, the muroplast DNA of *G. nostochinearum* was sequenced (B.F. Lang and G. Burger, unpublished) and was found to resemble that of *C. paradoxa* both in size and gene outfit. Interestingly, *orf180* was also detected, almost identical in sequence to the *Cyanophora* counterpart. In summary, such features of the plastome lend support to the often claimed "living fossil" status of glaucophytes, whereas the mosaic structure of the gene-rich nuclear genome of *Cyanophora* rather seems to contradict this view (Price et al. 2012).

Glaucophyte Mitochondrial Genomes

The complete mitochondrial DNAs (mtDNAs) of *C. paradoxa* (51.6 kbp) and *G. nostochinearum* (34.1 kbp) have been sequenced (Price et al. 2012). Glaucophyte mtDNAs do not stand out as particularly large or gene rich. Repetitive regions and larger intergenic distances in the *Cyanophora* metagenome account for the size difference. They encode the basic set of genes typical for animals and fungi, plus those characteristic of many protists and plants (i.e., close to a dozen coding for ribosomal proteins, a few extra subunits of the NDH and SDH complexes, and 5S rRNA). Recently, the mtDNA sequences of *Gloeochaete wittrockiana* (36 kbp) and *Cyanoptyche gloeocystis* (33.2 kbp) were published (Jackson and Reyes-Prieto 2014) with coding capacities strongly resembling those of the other two glaucophytes. Red and green algae share mtDNA-encoded TatC, a protein translocase component (see section on "Conservative Sorting"), and *ccm* genes specifying ABC transporters involved in cytochrome *c* biogenesis (Verissimo and Daldal 2014). Both these gene classes are absent from glaucophyte mtDNAs. In turn, green and glaucophyte algae share *rpl2*, *nad7*, and *nad9*, which are not present in red algal mtDNAs. Finally, glaucophytes possess a mitochondrion-encoded *nad11* that was lost by the two other groups. In conclusion, there is nothing at the level of mitochondrial gene complement that would specifically unite two of the three lineages.

Despite earlier claims likely caused by bacterial contaminants (Kiefel et al. 2004), no genes for mitochondrial division proteins of prokaryotic origin were found on the *C. paradoxa* genome. This is paralleled in green algae and plants, whereas mtMinD, mtMinE, and mtFtsZ were reported for rhodophytes and chromophytes (Leger et al. 2015). There is a single gene specifying (muroplast-targeted) TatC in the genome of *C. paradoxa* indicating the absence of the mitochondrial Tat pathway as, e.g., in land plants, where the AAA-ATPase Bcs1 assists mtRieske Fe-S protein in IM translocation and assembly into the cytochrome bc_1 complex (Wagener et al. 2011).

Interestingly, a contig for a Bcs1 homolog with a predicted mitochondrial localization was detected in the *Cyanophora* genome database (J.M. Steiner, unpublished). This would mean an advanced aspect of *Cyanophora* mitochondria, as primordial mitochondria (e.g., of jakobids) retained the proteobacteria-derived Tat pathway (Wagener et al. 2011).

Metabolic Pathways in the Cytosol of *Cyanophora paradoxa*

Starch metabolism: Early diverging phototrophic eukaryotes seem to play an important role in the conversion of cyanobacterial glycogen into the starch of green algae and land plants during evolution (Deschamps et al. 2008). Reserve carbohydrate granules have long been known to reside in the cytosol of glaucophytes (Kies 1992) and also of rhodophytes and algae derived through red algal secondary endosymbiosis. *C. paradoxa* starch showed a (high) amylose and amylopectin content with chain length distributions and crystalline organization similar to green algae and land plants that use ADP-glucose as the activated monomer for starch synthesis and temporary storage in the chloroplasts (Plancke et al. 2008). However, several starch synthase activities were found in *C. paradoxa* utilizing UDP-glucose, this time in analogy to rhodophytes that also synthesize their (more amylopectin-related) floridean starch in the cytosol. In addition, a multimeric isoamylase complex and multiple starch phosphorylases were demonstrated and of isoamylase: There is a correlation between the presence of starch and the debranching activity of isoamylase; those alpha-1,6-branches that impede the attainment of a crystalline structure are removed (Cenci et al. 2014). These results were obtained at the zymogram level and in some cases also at the gene level (Plancke et al. 2008). Transcription of a granule-bound starch synthase (responsible for amylose formation) was shown to be upregulated upon shift to low [CO_2] (Burey et al. 2007). Furthermore, the cytosolic transglucosidase DPE2 (disproportionating enzyme 2), transferring one glucose moiety from maltose (resulting from starch degradation by beta-amylase) to a cytosolic heteroglucan, could be demonstrated on *C. paradoxa* zymograms (Fettke et al. 2009). The *Cyanophora* Genome Project (http://dblab.rutgers.edu/cyanophora/home.php) (Price et al. 2012) allowed the identification of numerous putative carbohydrate metabolism enzymes using the Carbohydrate-Active enZymes (CAZy) database (Cantarel et al. 2009): about 84 glycoside hydrolases (GHs) and 128 glycosyl transferases (GTs), significantly more than in the green microalga *Ostreococcus lucimarinus* or the extremophilic red alga *Cyanidioschyzon merolae*, but less than in land plants. Many *C. paradoxa* CAZymes are involved in starch metabolism. Synthesis of the polysaccharide within Viridiplantae plastids relies on ADP-glucose-dependent enzymes of the GT5 family associated with glycogen synthesis in bacteria. The major *C. paradoxa* enzyme is phylogenetically related to the UDP-glucose-specific enzyme of heterotrophic eukaryotes (Cantarel et al. 2009) and has been partially purified from this alga (Plancke et al. 2008). This suggests the absence of ADP-glucose pyrophosphorylase in *C. paradoxa*. Surprisingly, another gene was found in the glaucophyte genome whose product is related to the SSIII-

SSIV (GT5) type of starch synthases in Viridiplantae. This gene is phylogenetically related to glucan synthases in chlamydiae, cyanobacteria, and some proteobacteria and is hypothesized to have played a key role in linking the biochemistry of the host and the endosymbiont. The SSIII-SSIV enzyme uses ADP-glucose in bacteria and land plants, suggesting that *C. paradoxa* or, rather, the common ancestor of Viridiplantae and glaucophytes may have used both types of nucleotide sugars for starch synthesis at the onset of the endosymbiosis. Cytosolic ADP-glucose is thought to arise from the cyanobacterial endosymbiont at that time via a sugar nucleotide transporter of host origin (Weber et al. 2006). A third player is thought to have contributed to this merging of the reserve carbohydrate synthesis pathways of host cell and endosymbiont: Chlamydiae, known for their intracellular lifestyle, might have supplied crucial enzymes and transporters to the cytosol and the endosymbiont/phagosome membranes during an earlier long-term, but transitory, infection. This "ménage a trois" could have been instrumental for the transition from glycogen of the heterotrophic host to starch of the eukaryotic phototroph (Ball et al. 2013): SSIII-SSIV (GlgA), isoamylase (presumably after gene duplication and some change in function of the bacterial direct debranching enzyme GlgX), and (at a later stage) the glucose-6-phosphate transporter UhpC likely represent the contributions (via HGT) from chlamydiae. Granule-bound starch synthase is of cyanobacterial origin (EGT), whereas the other enzymes stem from the metabolic repertoire of the host cell. New developments necessitated due to the glycogen-starch transition are glucan, water dikinase (GWD) and phosphoglucan, water dikinase (PWD), genes for which are also found on the *Cyanophora* genome. Degradation of the quasicrystalline starch granules by beta-amylases and phosphorylases is only possible after previous action of GWD and PWD (Cenci et al. 2014). Readers should note that the impact of *Chlamydiales* on Archaeplastida evolution and the validity of the ménage a trois hypothesis are considered controversial by some parties (e.g., Dagan et al. 2013; Deschamps 2014; Domman et al. 2015). More recent biochemical, phylogenetic, and genomic data however provide strong support for this model of Archaeplastida primary plastid establishment (see Ball et al. 2016a, b; Cenci et al. 2017; Gehre et al. 2016).

Biosynthesis of long-chain fatty acids and isoprenoids: De novo biosynthesis of fatty acids is compartmentalized in muroplasts as in plant chloroplasts. Elongation beyond C_{16} occurs in the cytosol with acetyl coenzyme A provided by the action of ATP citrate lyase (ACL). The long-assumed plastid localization of ACL was falsified for *C. paradoxa*, and, for the first time, a heterodimeric structure as in fungi and the prokaryote *Chlorobium tepidum* was proposed (Ma et al. 2001). This now applies for all plants as opposed to the large monomer observed in metazoa. cDNA and genomic sequencing of the gene for the catalytic subunit provided information about intron structure of nuclear genes: introns are numerous, in the size range of 53–65bp, with conserved border and (putative) branch point nucleotides (Ma et al. 2001; Bhattacharya and Weber 1997). The regulatory subunit is also present as evidenced by ESTs (http://tbestdb.bcm.umontreal.ca/searches/login.php).

The mevalonate pathway (missing in green algae), also dependent on acetyl coenzyme A provided by ACL, seems to be restricted to the cytosol of

C. paradoxa as shown by amplification of four selected genes (Grauvogel and Petersen 2007) and was confirmed later through the genome project.

Anaerobic Energy Metabolism

C. paradoxa was long considered an obligatory phototroph, and attempts to grow it on carbon sources as glucose or acetate were unsuccessful (Trench 1982). Therefore, it came as a surprise that the genome project revealed the potential for various fermentative metabolic pathways (Price et al. 2012). The respective gene repertoire is almost as extensive as that of *C. reinhardtii*, the best known model for this trait among green algae, and even exceeds that of picochlorophyta, whereas such genes are rare in red algae (Atteia et al. 2013). It remains to be seen if the corresponding enzyme activities, e.g., acetate:succinate CoA-transferase, hydrogenase (and maturation factors), pyruvate:formate lyase (and activating enzyme), and pyruvate: $NADP^+$ oxidoreductase, can be demonstrated in the appropriate compartments of the *Cyanophora* cell. Cytosol, plastids, and mitochondria are known to be involved in the anaerobic energy metabolism of algae (Atteia et al. 2013). The complex fermentative capabilities conserved between the distant relatives *C. paradoxa* and *C. reinhardtii* likely represent an evolutionarily advantageous combination of anoxic enzymes from the eukaryotic host and the cyanobacterial endosymbiont (Price et al. 2012).

Evolutionary History

No fossil remnants of glaucophytes are known, but their origin among the Archaeplastida is thought to date back to the Mesoproterozoic/Neoproterozoic boundary as that of rhodophytes (Butterfield 2000). Apart from the common possession of multilayered structures in members of the three algal groups mentioned, glaucophytes differ from prasinophycean green algae (flagella with scales, pellicular lacunae absent, intraplastidial starch), green algae (different kinetids and flagella movement, pellicular lacunae absent, intraplastidial starch), and euglenids (different pellicular structure, different type of mitosis, paramylon instead of starch as reserve polyglycan).

Phylogenetic Relationships

The phylum Glaucophyta as one of the three groups containing primary plastids contains all genera described by Kies (1992) based on morphological criteria (Table 2) and the presence of muroplasts. A concatenated phylogenetic analysis of plastid-encoded genes placed *C. paradoxa* and thus the glaucophytes on the first branch after the single primary endosymbiotic event (Martin et al. 1998; Rodríguez-Ezpeleta et al. 2005). This was corroborated by concatenated nuclear genes

(Rodríguez-Ezpeleta et al. 2005; Reyes-Prieto and Bhattacharya 2007a, b). Phylogenomics, made possible through the *Cyanophora* Genome Project, gave additional support (see above). Thus glaucophytes can be considered as direct descendants of the most ancient phototrophic eukaryotes, at least among the species known at present.

Phylogenetic relationships within the Glaucophyta have been investigated thoroughly in two independent studies (Chong et al. 2014; Takahashi et al. 2014). Based on concatenated and single genes of plastid (*psbA*) and mitochondrial (*cob* and *cox1*) origin, and the nuclear internal transcribed spacer (ITS) region, Chong et al. (2014) revealed that strains of *Glaucocystis nostochinearum* (or *Glaucocystis* species complex) were divided into six clades that possibly correspond to individual species (Fig. 8). The monophyletic group of *Glaucocystis* sp. complex was clustered together with the monophyletic *Gloeochaete wittrockiana* and *Cyanoptyche gloeocystis* clade. Five *Cyanophora* species were separated from the rest of the glaucocystophycean clade (see Fig. 8). Within the *Cyanophora* clade, *C. sudae* and *C. biloba* were clustered strongly and separated from the remaining *C. paradoxa* + *C. kugrensii* + *C. cuspidata* clades based on the *psa*B and ITS phylogenies (Takahashi et al. 2014). Although three new *Cyanophora* species were suggested based on morphological and molecular data (Takahashi et al. 2014), it still is a challenge to delimitate species in glaucophytes, because of the lack of authentic (Type) strain(s) and the simple morphology prevailing. However, using a combination of molecular and morphological data, the latter made possible through advanced EM methodology, Takahashi et al. (2016) confirmed the *Glaucocystis* clades proposed by Chong et al. (2014) and delineate six individual species (Fig. 8). Subtle, but significant differences in the peripheral ultrastructure of the cells, i.e., in the vesicle system underlying the plasma membrane (lacunae, cf. Fig. 7), were the key to this problem.

Acknowledgments The authors thank B. Franz Lang, Hideya Fukuzawa, and Steven Ball for helpful comments. W.L. is grateful to the Austrian Research Fund for 25 years of support.

References

Adir, N. (2008). Structure of the phycobilisome Antennae in Cyanobacteria and red algae. In P. Fromme (Ed.), *Photosynthetic protein complexes: A structural approach* (pp. 243–274). Weinheim: Wiley-VCH.

Adl, S. M., Simpson, A. G. B., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. A., Bowser, S. S., Bragerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., & Taylor, M. F. J. R. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology, 52*, 399–451.

Aitken, A., & Stanier, R. Y. (1979). Characterization of peptidoglycan from the cyanelles of *Cyanophora paradoxa*. *Journal of General Microbiology, 112*, 219–223.

Aldridge, C., Spence, E., Kirkilionis, L., Frigerio, M. A., & Robinson, C. (2008). The Tat-dependent targeting of Rieske iron-sulfur proteins to both the plasma and thylakoid membranes in the cyanobacterium *Synechocystis* PCC6803. *Molecular Microbiology, 70*, 140–150.

Anders, K., & Essen, L. O. (2015). The family of phytochrome-like photoreceptors: Diverse, complex and multi-colored, but very useful. *Current Opinion in Structural Biology, 35*, 7–16.

Anderson, L. K., & Toole, C. M. (1998). A model for early events in the assembly pathway of cyanobacterial phycobilisomes. *Molecular Microbiology, 30*, 467–474.

Atteia, A., van Lis, R., Tielens, A. G. M., & Martin, W. F. (2013). Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. *Biochimica et Biophysica Acta, 1827*, 210–223.

Badger, M. R., & Price, G. D. (2003). CO_2 concentrating mechanisms in cyanobacteria: Molecular components, their diversity and evolution. *Journal of Experimental Botany, 54*, 609–622.

Ball, S. G., Subtil, A., Bhattacharya, D., Moustafa, A., Weber, A. P. M., Gehre, L., Colleoni, C., Arias, M. C., Cenci, U., & Dauvillée, D. (2013). Metabolic effectors secreted by bacterial pathogens: Essential facilitators of plastid endosymbiosis? *Plant Cell, 25*, 7–21.

Ball, S. G., Bhattacharya, D., Qiu, H., & Weber, A. P. (2016a). Commentary: Plastid establishment did not require a chlamydial partner. *Frontiers in Cellular and Infection Microbiology, 6*, 43.

Ball, S. G., Bhattacharya, D., & Weber, A. P. (2016b). Evolution. Pathogen to powerhouse. *Science, 351*, 659–660.

Barreteau, H., Kovac, A., Boniface, A., Sova, M., Gobec, S., & Blanot, D. (2008). Cytoplasmic steps of peptidoglycan biosynthesis. *FEMS Microbiology Reviews, 32*, 168–207.

Bartsevich, V. V., & Pakrasi, H. (1995). Molecular identification of an ABC transporter complex for mangenese. Analysis of a cyanobacterial mutant strain impaired in the photosynthetic oxygen evolution process. *EMBO Journal, 14*, 1845–1853.

Baudelet, P.-H., Gagez, A.-L., Bérard, J.-B., Juin, C., Bridiau, N., Kaas, R., Thiéry, V., Cadoret, J.-P., & Picot, L. (2013). Antiproliferative activity of *Cyanophora paradoxa* pigments in melanoma, breast and lung cancer cells. *Marine Drugs, 11*, 4390–4406.

Baurain, D., Brinkmann, H., Petersen, J., Rodríguez-Ezpeleta, N., Stechmann, A., Demoulin, V., Roger, A. J., Burger, G., Lang, B. F., & Philippe, H. (2010). Phylogenomic evidence for separate acquisition of plastids in cryptophytes, haptophytes, and stramenopiles. *Molecular Biology and Evolution, 27*, 1698–1709.

Berenguer, J., Rojo, F., de Pedro, M. A., Pfanzagl, B., & Löffelhardt, W. (1987). Penicillin-binding proteins in the cyanelles of *Cyanophora paradoxa*, an eukaryotic photoautotroph sensitive to beta-lactam antibiotics. *FEBS Letters, 224*, 401–405.

Betsche, T., Schaller, D., & Melkonian, M. (1992). Identification and characterization of glycolate oxidase and related enzymes from the endocyanotic alga *Cyanophora paradoxa* and from pea leaves. *Plant Physiology, 98*, 887–893.

Bhattacharya, D., & Schmidt, H. A. (1997). Division glaucocystophyta. *Plant Systematics and Evolution [Suppl.], 11*, 139–148.

Bhattacharya, D., & Weber, K. (1997). Analysis of the coding region of the actin gene of the glaucocystophyte *Cyanophora paradoxa*: Analysis of the coding region and introns, and an actin phylogeny of eukaryotes. *Current Genetics, 31*, 439–446.

Bhattacharya, D., Helmchen, T., Bibeau, C., & Melkonian, M. (1995). Comparisons of nuclear-encoded small subunit ribosomal RNAs reveal the evolutionary position of the Glaucocystophyta. *Molecular Biology and Evolution, 12*, 415–420.

Bhattacharya, D., Price, D. C., Chan, C. X., Qiu, H., Rose, N., Ball, S., Weber, A. P., Arias, M. C., Henrissat, B., Coutinho, P. M., Krishnan, A., Zäuner, S., Morath, S., Hilliou, F., Egizi, A., Perrineau, M. M., & Yoon, H. S. (2013). Genome of the red alga *Porphyridium purpureum*. *Nature Communications, 4*, 1941.

Bhattacharya, D., Price, D. C., Chan, C. X., Gross, J., Steiner, J. M., & Löffelhardt, W. (2014). Analysis of the genome of *Cyanophora paradoxa*: An algal model for understanding primary endosymbiosis. In W. Löffelhardt (Ed.), *Endosymbiosis* (pp. 135–150). Heidelberg: Springer.

Bouhss, A., Trunkfield, A. E., Bugg, T. H., & Mengin-Lecreulx, D. (2008). The biosynthesis of peptidoglycan lipid-linked intermediates. *FEMS Microbiology Reviews, 32*, 208–233.

Bricker, T.M., Roose, J.L., Fagerlund, R.D., Frankel, L.K., Eaton-Rye, J.J. (2012). The extrinsic proteins of photosystem II. Biochimica et Biophysica Acta, 1817, 121–142.

Bruce, B. D. (2000). Chloroplast transit peptides: Structure, function and evolution. *Trends in Cell Biology, 10*, 440–447.

Burey, S. C., Fathi-Nejad, S., Poroyko, V., Steiner, J. M., Löffelhardt, W., & Bohnert, H. J. (2005). The central body of the cyanelles of *Cyanophora paradoxa*: An eukaryotic carboxysome? *Canadian Journal of Botany, 83*, 758–764.

Burey, S. C., Poroyko, V., Ozturk, Z. N., Fathi-Nejad, S., Schüller, C., Ohnishi, N., Fukuzawa, H., Bohnert, H. J., & Löffelhardt, W. (2007). Acclimation to low [CO2] by an inorganic carbon concentrating mechanism in *Cyanophora paradoxa*. *Plant, Cell & Environment, 30*, 1422–1435.

Burki, F., Shalchian-Tabrizi, K., & Pawlowski, J. (2007). Phylogenomics reshuffles the eukaryotic supergroups. *PLoS ONE, 2*, e790.

Burnap, R. L., & Trench, R. K. (1989). The Biogenesis of the cyanellae of *Cyanophora paradoxa*. I. Polypeptide composition of the cyanellae. Proceedings of the Royal Society of London. Series B, 238, 53–72.

Busch, A., Nield, J., & Hippler, M. (2010). The composition and structure of photosystem I associated antenna from *Cyanidioschyzon merolae*. *Plant Journal, 62*, 886–897.

Butterfield, N. J. (2000). *Bangiomorpha pubescens* n. gen. n. sp.: Implications for the evolution of sex, multicellularity, and the mesoproterozoic/neoproterozoic radiation of eukaryotes. *Palaeobiology, 26*, 386–404.

Calo, S., Nicolas, F. E., Vila, A., Torres-Martinez, S., & Ruiz-Vazquez, R. M. (2012). Two distinct RNA-dependent RNA polymerases are required for initiation and amplification of RNA silencing in the basal fungus *Mucor circinelloides*. *Molecular Microbiology, 83*, 379–394.

Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., & Henrissat, B. (2009). The carbohydrate-active enZymes database (CAZy): An expert resource for glycogenomics. *Nucleic Acids Research, 37*(Database issue), D233–D238.

Cenci, U., Nitschke, F., Steup, M., Minassian, B. A., Colleoni, C., & Ball, S. G. (2014). Transition from glycogen to starch metabolism in Archaeplastida. *Trends Plant Science, 19*, 18–28.

Cenci, U., Bhattacharya, D., Weber, A.P.M., Colleoni, C., Subtil, A., & Ball, S.G. (2017). Biotic host-pathogen interactions as major drivers of plastid endosymbiosis. *Trends in Plant Science*, 22, in press. DOI: http://dx.doi.org/10.1016/j.tplants.2016.12.007.

Chan, C. X., Yang, E. C., Banerjee, T., Yoon, H. S., Martone, P. T., Estevez, J. M., & Bhattacharya, D. (2011). Red-and-green algal monophyly and extensive gene sharing found in a rich repertoire of red algal genes. *Current Biology, 21*, 328–333.

Chen, H. M., Chen, L. T., Patel, K., Li, Y. H., Baulcombe, D. C., & Wu, S. H. (2010). 22-Nucleotide RNAs trigger secondary siRNA biogenesis in plants. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 15269–15274.

Chi, S., Wu, S., Yu, J., Wang, X., Tang, X., & Liu, T. (2014). Phylogeny of C-4-Photosynthesis enzymes based on algal transcriptomic and genomic data supports an archaeal/proteobacterial origin and multiple duplication for most C-4-related genes. *PLoS ONE, 9*, e110154.

Chong, J., Jackson, C., Kim, J. I., Yoon, H. S., & Reyes-Prieto, A. (2014). Molecular markers from different genomic compartments reveal cryptic diversity within glaucophyte species. *Molecular Phylogenetics and Evolution, 76*, 181–188.

Cline, K., & Dabney-Smith, C. (2008). Plastid protein import and sorting: Different paths to the same compartments. *Current Opinion in Plant Biology, 11*, 585–592.

Colleoni, C., Linka, M., Deschamps, P., Handford, M. G., Dupree, P., Weber, A. P. M., & Ball, S. G. (2010). Phylogenetic and biochemical evidence supports the recruitment of an ADP-glucose translocator for the export of photosynthate during plastid endosymbiosis. *Molecular Biology and Evolution, 27*, 2691–2701.

Cooley, R. B., Arp, D. J., & Karplus, P. A. (2011). Symerythrin structures at atomic resolution and the origins of rubrerythrins and the ferritin-like superfamily. *Journal of Molecular Biology, 413*, 177–194.

Croft, M. T., Lawrence, A. D., Raux-Deery, E., Warren, M. J., & Smith, A. G. (2005). Algae acquire vitamin B_{12} through a symbiotic relationship with bacteria. *Nature, 438*, 90–93.

Dagan, T., Roettger, M., Stucken, K., Landan, G., Koch, R., Major, P., Gould, S. B., Goremykin, V. V., Rippka, R., Tandeau de Marsac, N., Gugger, M., Lockhart, P. J., Allen, J. F., Brune, I., Maus, I., Pühler, A., & Martin, W. F. (2013). Genomes of Stigonematalean cyanobacteria (subsection V) and the evolution of oxygenic photosynthesis from prokaryotes to plastids. *Genome Biology and Evolution, 5*, 31–44.

Dai, H. P., Kentemich, T., Schmitz, K., Müller, B., & Bothe, H. (1992). Distribution of thioredoxins in heterocysts and vegetative cells of cyanobacteria. *Journal of Photochemistry and Photobiology, 16*, 285–295.

de Novoa, P. G., & Williams, K. P. (2004). The tmRNA website. Reductive evolution of tmRNA in plastids and other endosymbionts. *Nucleic Acids Research, 32*, D104–D108.

Deschamps, P. (2014). Primary endosymbiosis: Have cyanobacteria and Chlamydiae ever been roommates? *Acta Societatis Botanicorum Poloniae, 83*, 291–302.

Deschamps, P., & Moreira, D. (2009). Signal conflicts in the phylogeny of the primary photosynthetic eukaryotes. *Molecular Biology and Evolution, 26*, 2745–2753.

Deschamps, P., Colleoni, C., Nakamura, Y., Suzuki, E., Putaux, J.-L., Buléon, A., Haebel, S., Ritte, G., Steup, M., Falcón, L. I., Moreira, D., Löffelhardt, W., Raj, J. N., Plancke, C., d'Hulst, C., Dauvillée, D., & Ball, S. (2008). Metabolic symbiosis and the birth of the plant kingdom. *Molecular Biology and Evolution, 25*, 536–548.

Domman, D., Horn, M., Embley, T. M., & Williams, T. A. (2015). Plastid establishment did not require a chlamydial partner. *Nature Communications, 6*, 6421.

Duanmu, D., Bachy, C., Sudek, S., Wong, C. H., Jiménez, V., Rockwell, N. C., Martin, S. S., Ngan, C. Y., Reistetter, E. N., van Baren, M. J., Price, D. C., Wei, C. L., Reyes-Prieto, A., Lagarias, J. C., & Worden, A. Z. (2014). Marine algae and land plants share conserved phytochrome signaling systems. *Proceedings of the National Academy of Sciences USA, 111*, 15827–15832.

Egelhoff, T., & Grossman, A. R. (1983). Cytoplasmic and chloroplast synthesis of phycobilisome polypeptides. *Proceedings of the National Academy of Sciences USA, 80*, 3339–3343.

Enami, I., Suzuki, T., Tada, O., Nakada, Y., Nakamura, K., Tohri, A., Ohta, H., Inoue, I., & Shen, J. R. (2005). Distribution of the extrinsic proteins as a potential marker for the evolution of photosynthetic oxygen-evolving photosystem II. *FEBS Journal, 272*, 5020–5030.

Engel, B. D., Schaffer, M., Kuhn Cuellar, L., Villa, E., Plitzko, J. M., & Baumeister, W. (2015). Native architecture of the *Chlamydomonas* chloroplast revealed by in situ cryo-electron tomography. *eLife, 4*, e04889.

Facchinelli, F., & Weber, A. P. M. (2015). Analysis of *Cyanophora paradoxa* tells important lessons on plastid evolution. *Perspectives in Phycology, 2*, 3–10.

Facchinelli, F., Pribil, M., Oster, U., Ebert, N. J., Bhattacharya, D., Leister, D., & Weber, A. P. M. (2013). Proteomic analysis of the *Cyanophora paradoxa* muroplast provides clues on early events in plastid endosymbiosis. *Planta, 237*, 637–651.

Fathinejad, S., Steiner, J. M., Reipert, S., Marchetti, M., Allmaier, G., Burey, S. C., Ohnishi, N., Fukuzawa, H., Löffelhardt, W., & Bohnert, H. J. (2008). A carboxysomal CCM in the cyanelles of the "coelacanth" of the algal world, *Cyanophora paradoxa*? *Physiologia Plantarum, 133*, 27–32.

Fester, T., Volke, E., & Schenk, H. E. A. (1996). Purification and partial characterization of the cyanoplast glucose-6-phosphate dehydrogenase in *Cyanophora paradoxa*. *Endocytobiology Cell Research, 11*, 159–176.

Fettke, J., Hejazi, M., Smirnowa, J., Höchel, E., Stage, M., & Steup, M. (2009). Eukaryotic starch degradation: Integration of plastidial and cytosolic pathways. *Journal of Experimental Botany, 60*, 2907–2922.

Flachmann, R., Michalowski, C. B., Löffelhardt, W., & Bohnert, H. J. (1993). SecY, an integral subunit of the bacterial preprotein translocase is encoded by a plastid genome. *Journal of Biological Chemistry, 268*, 7514–7519.

Flechner, A., Gross, W., Martin, W. F., & Schnarrenberger, C. (1999). Chloroplast class I and II aldolases are bifunctional for fructose-1,6-bisphosphate and sedoheptulose-1,7-bisphosphate cleavage in the Calvin cycle. *FEBS Letters, 447*, 200–202.

Foflonker, F., Price, D. C., Qiu, H., Palenik, B., Wang, S., & Bhattacharya, D. (2015). Genome of the halotolerant green alga *Picochlorum* SENEW3 reveals strategies for thriving under fluctuating environmental conditions. *Environmental Microbiology, 17*, 412–426.

Frassanito, A. M., Barsanti, L., Passarelli, V., Evangelista, V., & Gualtieri, P. (2010). A rhodopsin-like protein in *Cyanophora paradoxa*: Gene sequence and protein immunolocalization. *Cellular and Molecular Life Sciences, 67*, 965–971.

Frassanito, A. M., Barsanti, L., Passarelli, V., Evangelista, V., & Gualtieri, P. (2013). A second rhodopsin-like protein in *Cyanophora paradoxa*: Gene sequence and protein expression in a cell-free system. *Journal of Photochemistry and Photobiology B: Biology, 125*, 188–193.

Garcia, M., Myouga, F., Takechi, K., Sato, H., Nabeshima, K., Nagata, N., Takio, S., Shinozaki, K., & Takano, H. (2008). An Arabidopsis homolog of the bacterial peptidoglycan synthesis enzyme MurE has an essential role in chloroplast development. *Plant Journal, 53*, 924–934.

Gebhart, U. B., Maier, T. L., Stefanovic, S., Bayer, M. G., & Schenk, H. E. A. (1992). Ferredoxin-NADP oxidoreductase of *Cyanophora paradoxa*: Purification, partial characterization, and N-terminal amino acid sequence. *Protein Expression and Purification, 3*, 228–235.

Gehre, L., Gorgette, O., Perrinet, S., Prevost, M. C., Ducatez, M., Giebel, A. M., Nelson, D. E., Ball, S. G., & Subtil, A. (2016). Sequestration of host metabolism by an intracellular pathogen. *eLife, 5*, e12552.

Geitler, L. (1959a). Syncyanosen. In W. Ruhland (Ed.), *Handbuch der Pflanzenphysiologie* (Vol. 11, pp. 530–545). Berlin/Göttingen/Heidelberg: Springer.

Geitler, L. (1959b). Eine neue Endocyanose, *Cyanoptyche dispersa* n.sp., und Bemerkungen über ähnliche Syncyanosen. *Österreichische Botanische Zeitschrift, 106*, 464–471.

Gimple, O., & Schön, A. (2001). In vitro and in vivo processing of cyanelle tmRNA by RNaseP. *Biological Chemistry, 382*, 1421–1429.

Gómez-García, M. R., Losada, M., & Serrano, A. (2006). A novel subfamily of monomeric inorganic pyrophosphatases in photosynthetic eukaryotes. *Biochemical Journal, 395*, 211–221.

Gould, S. B., Sommer, M. S., Hadfi, K., Zauner, S., Kroth, P. G., & Maier, U.-G. (2006). Protein targeting into the complex plastids of cryptophytes. *Journal of Molecular Evolution, 62*, 674–681.

Grauvogel, C., & Petersen, J. (2007). Isoprenoid biosynthesis authenticates the classification of the green alga *Mesostigma viride* as an ancient streptophyte. *Gene, 396*, 125–133.

Gross, J., & Bhattacharya, D. (2008). Revaluating the evolution of the Toc/Tic protein translocons. *Trends in Plant Science, 14*, 13–20.

Gross, J., & Bhattacharya, D. (2009). Mitochondrial and plastid evolution in eukaryotes: An outsider's perspective. *Nature Reviews Genetics, 10*, 495–505.

Gross, J., Wajid, S., Price, D. C., Zelzion, E., Li, J., Chan, C. X., & Bhattacharya, D. (2013). Evidence for widespread exonic small RNAs in the glaucophyte alga *Cyanophora paradoxa*. *PLoS ONE, 8*, e67669.

Häder, D.-P. (1985). Photomovement in *Cyanophora paradoxa*. *Archives of Microbiology, 143*, 100–104.

Hamoen, L. W., Meile, J. C., de Jong, W., Noirot, P., & Errington, J. (2006). SepF, a novel FtsZ-interacting protein required for a late step in cell division. *Molecular Microbiology, 59*, 989–999.

Harper, J. T., & Keeling, P. J. (2003). Nucleus-encoded, plastid-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) indicates a single origin for chromalveolate plastids. *Molecular Biology and Evolution, 20*, 1730–1735.

Heimann, K., Reize, I. B., & Melkonian, M. (1989). The flagellar developmental cycle in algae: Flagellar transformation in *Cyanophora paradoxa (Glaucocystophyceae)*. *Protoplasma, 148*, 106–110.

Heimann, K., Becker, B., Harnisch, H., Mukherjee, K. D., & Melkonian, M. (1997). Biochemical characterization of plasma membrane vesicles of *Cyanophora paradoxa*. *Botanica Acta, 110*, 401–410.

Heinhorst, S., & Shively, J. M. (1983). Encoding of both subunits of ribulose-1,5-bisphosphate carboxylase by organelle genome of *Cyanophora paradoxa*. *Nature, 304*, 373–374.

Helmchen, T. A., Bhattacharya, D., & Melkonian, M. (1995). Analyses of ribosomal RNA sequences from glaucocystophyte cyanelles provide new insights into evolutionary relationships of plastids. *Journal of Molecular Evolution, 41*, 203–210.

Hirano, T., Tanidokoro, K., Shimizu, Y., Kawarabayasi, Y., Ohshima, T., Sato, M., Tadano, S., Ishikawa, H., Takio, S., Takechi, K., & Takano, H. (2016). Moss chloroplasts are surrounded by a peptidoglycan wall containing D-amino acids. *Plant Cell, 28*, 1521–1532.

Hoshaw, R. W., & Rosowski, J. R. (1973). Methods for microscopic algae. In J. R. Stein (Ed.), *Handbook of phycological methods, vol. 1, culture methods and growth measurements* (pp. 53–68). Cambridge, UK: Cambridge University Press.

Iino, M., & Hashimoto, H. (2003). Intermediate features of cyanelle division of *Cyanophora paradoxa* (Glaucocystophyta) between cyanobacterial and plastid division. *Journal of Phycology, 39*, 561–569.

Jackson, C. J., & Reyes-Prieto, A. (2014). The mitochondrial genomes of the glaucophytes *Gloeochaete wittrockiana* and *Cyanoptyche gloeocystis*: Multilocus phylogenetics suggests a monophyletic Archaeplastida. *Genome Biology and Evolution, 6*, 2774–2785.

Jackson, C., Clayden, S., & Reyes-Prieto, A. (2015). The glaucophyta: The blue-green plants in a nutshell. *Acta Societatis Botanicorum Poloniae, 84*, 149–165.

Jakowitsch, J., Bayer, M. G., Maier, T. L., Lüttke, A., Gebhart, U. B., Brandtner, M., Hamilton, B., Neumann-Spallart, C., Michalowski, C. B., Bohnert, H. J., Schenk, H. E. A., & Löffelhardt, W. (1993). Sequence analysis of pre-ferredoxin-NADP+-reductase cDNA from *Cyanophora paradoxa* specifying a precursor for a nucleus-encoded cyanelle polypeptide. *Plant Molecular Biology, 21*, 1023–1033.

Jakowitsch, J., Neumann-Spallart, C., Ma, Y., Steiner, J. M., Schenk, H. E. A., Bohnert, H. J., & Löffelhardt, W. (1996). *In vitro* import of pre-ferredoxin-NADP+−oxidoreductase from *Cyanophora paradoxa* into cyanelles and into pea chloroplasts. *FEBS Letters, 381*, 153–155.

Janouskovec, J., Horák, A., Oborník, M., Lukes, J., & Keeling, P. J. (2010). A common red algal origin of the apicomplexan, dinoflagellate, and heterokont plastids. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 10949–10954.

Kalanon, M., & McFadden, G. I. (2008). The chloroplast protein translocase complexes of *Chamydomonas reinhardtii*: A bioinformatics comparison of Toc and Tic components in plants, green algae and red algae. *Genetics, 179*, 95–112.

Karkar, S., Facchinelli, F., Price, D. C., Weber, A. P. M., & Bhattacharya, D. (2015). Metabolic connectivity as a driver of host and endosymbiont integration. *Proceedings of the National Academy of Sciences USA, 112*, 10208–10215.

Karlsson, J., Clarke, K. A. K., Chen, Z. Y., Huggins, S. Y., Park, Y. I., Husic, H. D., Moroney, J. V., & Samuelsson, G. (1998). A novel alpha-type carbonic anhdrase associated with the thylakoid membrane in *Chlamydomonas reinhardtii* is required for growth at ambient CO_2. *The EMBO Journal, 10*, 1208–1216.

Kern, R., Eisenhut, M., Bauwe, H., Weber, A. P. M., & Hagemann, M. (2013). Does the *Cyanophora paradoxa* genome revise our view on the evolution of photorespiratory enzymes? *Plant Biology, 15*, 759–768.

Kiefel, B. R., Gilson, P. R., & Beech, P. L. (2004). Diverse eukaryotes have retained mitochondrial homologues of the bacterial division protein FtsZ. *Protist, 155*, 105–115.

Kies, L. (1976). Untersuchungen zur Feinstruktur und taxonomischen Einordnung von *Gloeochaete wittrockiana*, einer apoplastidalen capsalen Alge mit blaugrünen Endosymbionten (Cyanellen). *Protoplasma, 87*, 419–446.

Kies, L. (1979). Zur systematischen Einordnung von *Cyanophora paradoxa, Gloeochaete wittrockiana* und *Glaucocystis nostochinearum*. *Berichte der Deutschen Botanischen Gesellschaft, 92*, 445–454.

Kies, L. (1989). Ultrastructure of *Cyanoptyche gloeocystis f. dispersa* (Glaucocystophyceae). *Plant Systematics and Evolution, 164*, 65–73.

Kies, L. (1992). Glaucocystophyceae and other protists harboring prokaryotic endosymbionts. In W. Reisser (Ed.), *Algae and symbioses* (pp. 353–377). Bristol: Biopress.

Kies, L., & Kremer, B. P. (1986). *Typification of the Glaucocystophyta. Taxon, 35*, 128–135.

Kies, L., & Kremer, B. P. (1990). Phylum glaucocystophyta. In L. Margulis, J. O. Corliss, M. Melkonian, & D. J. Chapman (Eds.), *Handbook of protoctista* (pp. 152–166). Boston: Jones & Bartlett.

Kilian, O., & Kroth, P. G. (2005). Identification and characterization of a new conserved motif within the presequences of proteins targeted into complex diatom plastids. *Plant Journal, 41*, 175–183.

Kinney, J. N., Axen, S. D., & Kerfeld, C. A. (2011). Comparative analysis of carboxysome shell proteins. *Photosynthesis Research, 109*, 21–32.

Kleiner, F. (2014). Analyse eines "c6-like" Cytochroms von Cyanophora paradoxa. MSc Thesis, MLU Halle-Wittenberg.

Kneip, C., Voss, C., Lockhart, P. J., & Maier, U. G. (2008). The cyanobacterial endosymbiont of the unicellular alga *Rhopalodia gibba* shows reductive genome evolution. *BMC Evolutionary Biology, 8*, 30.

Köhler, D., Dobritzsch, D., Hoehenwarter, W., Helm, S., Steiner, J., & Baginsky, S. (2015). Identification of protein N-termini in *Cyanophora paradoxa* cyanelles: Transit peptide composition and sequence determinants for precursor maturation. *Frontiers in Plant Science, 6*, 559.

Koike, H., Shibata, M., Yasutomi, K., Kashino, Y., & Sato, K. (2000). Identification of photosystem I components from a glaucocystophyte, *Cyanophora paradoxa*: The PsaD protein has an N-terminal stretch homologous to higher plants. *Photosynthesis Research, 65*, 207–217.

Koike, H., Ikeda, Y., Yusa, F., Kashino, Y., & Sato, K. (2007). Isolation and characterization of outer and inner envelope membranes of cyanelles from a glaucocystophyte, *Cyanophora paradoxa*. *Photosynthesis Research, 93*, 45–53.

Kojima, S., Ko, K.-C., Takatsuka, Y., Abe, N., Kaneko, J., Itoh, Y., & Kamio, Y. (2010). Cadaverine covalently linked to the peptidoglycan mediates the interaction between the peptidoglycan and periplasm-exposed SLH domain of major outer membrane protein Mep45 in *Selenomonas ruminantium*. *Journal of Bacteriology, 192*, 5953–5961.

Kondo, K., Ochiai, Y., Katayama, M., & Ikeuchi, M. (2007). The membrane-associated CpcG2-phycobilisome in *Synechocystis*: A new photosystem I antenna. *Plant Physiology, 144*, 1200–1210.

Korshikov, A. A. (1924). Protistologische Beobachtungen. I. Cyanophora paradoxa n. g. et sp. *Russisches Archiv für Protistologie, 3*, 57–74.

Kowallik, K. (1994). From endosymbionts to chloroplasts: Evidence for a single prokaryotic/eukaryotic endocytobiosis. *Endocytobiosis & Cell Research, 10*, 137–149.

Kugrens, P. (2001). Structure and phylogeny of *Cyanophora* species. In J. Seckbach (Ed.), *Symbiosis* (pp. 257–270). Dordrecht: Kluwer Academic Publishers.

Leger, M. M., Petru, M., Žárský, V., Eme, L., Vlček, Č., Harding, T., Lang, B. F., Eliáš, M., Doležal, P., & Roger, A. J. (2015). An ancestral bacterial division system is widespread in eukaryotic mitochondria. *Proceedings of the National Academy of Sciences USA, 112*, 10239–10246.

Li, D., Willkomm, D. K., Schön, A., & Hartmann, R. K. (2007). RNase P of the *Cyanophora paradoxa* cyanelle: A plastid ribozyme. *Biochimie, 89*, 1528–1538.

Liechti, G. W., Kuru, E., Hall, E., Kalinda, A., Brun, Y. V., Vannieuwenhze, M., & Maurelli, A. T. (2014). A new metabolic cell wall labelling method reveals peptidoglycan in *Chlamydia trachomatis*. *Nature, 506*, 507–510.

Löffelhardt, W. (2010). Low CO_2 stress: Glaucocystophytes may have found an unique solution. In J. Seckbach & M. Grube (Eds.), *Symbioses and stress: Joint ventures in biology* (Vol. 17, pp. 83–94). Dordrecht/Heidelberg/London/New York: Springer.

Löffelhardt, W. (2014). The single primary endosymbiotic event. In W. Löffelhardt (Ed.), *Endosymbiosis* (pp. 39–52). Heidelberg/New York: Springer.

Löffelhardt, W., & Bohnert, H. J. (2001). The cyanelle (muroplast) of *Cyanophora paradoxa*: A paradigm for endosymbiotic organelle evolution. In J. Seckbach (Ed.), *Symbiosis* (pp. 111–130). Dordrecht: Kluwer Academic Publishers.

Löffelhardt, W., Bohnert, H. J., & Bryant, D. A. (1997). The complete sequence of the *Cyanophora paradoxa* cyanelle genome (*Glaucocystophyceae*). *Plant Systematics and Evolution, 11*, 149–162.

Ma, Y., Jakowitsch, J., Maier, T. L., Bayer, M. G., Müller, N. E., Schenk, H. E. A., & Löffelhardt, W. (2001). ATP citrate lyase of the glaucocystophyte alga *Cyanophora paradoxa* is a cytosolic enzyme: Characterisation of the large subunit at the cDNA and genomic level. *Molecular Genetics and Genomics, 266*, 231–238.

Ma, Y., Jakowitsch, J., Deusch, O., Henze, K., Martin, W., & Löffelhardt, W. (2009). Transketolase from *Cyanophora paradoxa*: In vitro import into cyanelles and pea chloroplasts and a complex history of a gene often, but not always, transferred in the context of secondary endosymbiosis. *Journal of Eukaryotic Microbiology, 56*, 568–576.

Mackle, M. M., & Zilinskas, B. A. (1994). Role of signal peptides in targeting of proteins in cyanobacteria. *Journal of Bacteriology, 176*, 1857–1864.

Mallory, A., & Vaucheret, H. (2010). Form, function, and regulation of ARGONAUTE proteins. *Plant Cell, 22*, 3879–3889.

Maluck, E. (2012). Charakterisierung der Phycobiliprotein-Linkerkomplexe der Phycobilisomen aus Cyanophora paradoxa. BSc Thesis, MLU Halle-Wittenberg.

Mangeney, E., & Gibbs, S. P. (1987). Immunocytochemical localization of ribulose-1,5-bisphosphate carboxylase/oxygenase in the cyanelles of *Cyanophora paradoxa* and *Glaucocystis nostochinearum*. *European Journal of Cell Biology, 43*, 65–70.

Marbouty, M., Mazouni, K., Saguez, C., Cassier-Chauvat, C., & Chauvat, F. (2009). Characterization of the *Synechocystis* strain PCC 6803 penicillin-binding proteins and cytokinetic proteins FtsQ and FtsW and their network of interactions with ZipN. *Journal of Bacteriology, 191*, 5123–5133.

Margulis, L. (1981). *Symbiosis in cell evolution*. San Francisco: W. H. Freeman and Company.

Margulis, L., & Sagan, D. (2003). *Acquiring genomes: A theory of the origin of species*. New York: Basic Books.

Marin, B., Nowack, E. C. M., Glöckner, G., & Melkonian, M. (2007). The ancestor of the *Paulinella* chromatophore obtained a carboxysomal operon by horizontal gene transfer from a *Nitrococcus*-like gamma-proteobacterium. *BMC Evolutionary Biology, 7*, 85.

Martin, W., Stoebe, B., Goremykin, V., Hansmann, S., Hasegawa, M., & Kowallik, K. (1998). Gene transfer to the nucleus and the evolution of chloroplasts. *Nature, 393*, 162–165.

McFadden, G. I., & van Dooren, G. G. (2004). Evolution: Red algal genome affirms a common origin of all plastids. *Current Biology, 14*, R514–R516.

Melkonian, M. (1983). Evolution of green algae in relation to endosymbiosis. In H. E. A. Schenk & W. Schwemmler (Eds.), *Endocytobiology II* (pp. 1003–1007). Berlin: W. de Gruyter.

Mereschkowsky, C. (1905). Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Biologisches Zentralblatt, 25*, 593–604.

Meurer, J., Plücken, H., Kowallik, K. V., & Westhoff, P. (1998). A nuclear-encoded protein of prokaryotic origin is essential for the stability of photosystem II in *Arabidopsis thaliana*. *EMBO Journal, 17*, 5286–5297.

Meyer, M., & Griffiths, H. (2013). Origins and diversity of eukaryotic CO_2-concentrating mechanisms: Lessons for the future. *Journal of Experimental Botany, 64*, 769–786.

Miyagishima, S., Suzuki, K., Okazaki, K., & Kabeya, Y. (2012). Expression of the nucleus-encoded chloroplast division genes and proteins regulated by the algal cell cycle. *Molecular Microbiology, 29*, 2957–2970.

Miyagishima, S., Kabeya, Y., Sugita, C., Sugita, M., & Fujiwara, T. (2014a). DipM is required for peptidoglycan hydrolysis during chloroplast division. *BMC Plant Biology, 14*, 57.

Miyagishima, S., Nakamura, M., Uzuka, A., & Era, A. (2014b). FtsZ-less prokaryotic cell division as well as FtsZ- and dynamin-less chloroplast and non-photosynthetic plastid division. *Frontiers in Plant Science, 5*, 459.

Morton, B. R. (1998). Selection on the codon bias of chloroplast and cyanelle genes in differing plant and algal lineages. *Journal of Molecular Evolution, 46*, 449–459.

Moustafa, A., Beszteri, B., Maier, U. G., Bowler, C., Valentin, K., & Bhattacharya, D. (2009). Genomic footprints of a cryptic plastid endosymbiosis in diatoms. *Science, 324*, 1724–1726.

Münch, D., Roemer, T., Lee, S. H., Engeser, M., Sahl, H. G., & Schneider, T. (2012). Identification and in vitro analysis of the GatD/MurT enzyme-complex catalyzing lipid II amidation in *Staphylococcus aureus*. *PLoS Pathogens, 8*, e1002509.

Nakai, M. (2015). The TIC complex uncovered: The alternative view on the molecular mechanism of protein translocation across the inner envelope membrane of chloroplasts. *Biochimica et Biophysica Acta, 1847*, 957–967.

Nakai, M., Sugita, D., Omata, T., & Endo, T. (1993). SecY protein is localized in both the cytoplasmic and thylakoid membranes in the cyanobacterium *Synechococcus* PCC 7942. *Biochemical and Biophysical Research Communications, 193*, 228–234.

Nakayama, T., Kamikawa, R., Tanifuji, G., Kashiyama, Y., Ohkouchi, N., Archibald, J. M., & Inagaki, Y. (2014). Complete genome of a non-photosynthetic cyanobacterium in a diatom reveals recent adaptations to an intracellular lifestyle. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 11407–11412.

Nowack, E. C. M., Melkonian, M., & Glöckner, G. (2008). Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Current Biology, 18*, 410–418.

Obbard, D. J., Gordon, K. H., Buck, A. H., & Jiggins, F. M. (2009). The evolution of RNAi as a defence against viruses and transposable elements. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 364*, 99–115.

Ocheretina, O., Haferkamp, I., Tellioglu, H., & Scheibe, R. (2000). Light-modulated NADP-malate dehydrogenases from mossfern and green algae: Insights into evolution of the enzyme's regulation. *Gene, 258*, 147–154.

Ossenbühl, F., Inaba-Sulpice, M., Meurer, J., Soll, J., & Eichacker, L. (2006). The *Synechocystis* sp. PCC 6803 Oxa1 homolog is essential for membrane integration of reaction center precursor protein pD1. *Plant Cell, 18*, 2236–2246.

Paila, Y. D., Richardson, L. G., & Schnell, D. J. (2015). New insights into the mechanism of chloroplast protein import and its integration with protein quality control, organelle biogenesis and development. *Journal of Molecular Biology, 427*, 1038–1060.

Parfrey, L. W., Grant, J., Tekle, Y. I., Lasek-Nesselquist, E., Morrison, H. G., Sogin, M. L., Patterson, D. J., & Katz, L. A. (2010). Broadly sampled multigene analyses yield a well-resolved eukaryotic tree of life. *Systematic Biology, 59*, 518–533.

Pascher, A. (1929). Studien über Symbiosen. I. Über einige Endosymbiosen von Blaualgen in Einzellern. *Jahrbücher für Wissenschaftliche Botanik, 71*, 386–462.

Patron, N. J., & Waller, R. F. (2007). Transit peptide diversity and divergence: A global analysis of plastid targeting signals. *BioEssays, 29*, 1048–1058.

Petersen, J., Teich, R., Becker, B., Cerff, R., & Brinkmann, H. (2006). The GapA/B gene duplication marks the origin of the streptophyta (Charophytes and land plants). *Molecular Biology and Evolution, 23*, 1109–1118.

Pfanzagl, B., & Löffelhardt, W. (1999). *In vitro* synthesis of peptidoglycan precursors modified with N-acetylputrescine by *Cyanophora paradoxa* cyanelle envelope membranes. *Journal of Bacteriology, 181*, 2643–2647.

Pfanzagl, B., Allmaier, G., Schmid, E. R., de Pedro, M. A., & Löffelhardt, W. (1996a). N-Acetylputrescine as a characteristic constituent of cyanelle peptidoglycan in glaucocystophyte algae. *Journal of Bacteriology, 179*, 6994–6997.

Pfanzagl, B., Zenker, A., Pittenauer, E., Allmaier, G., Martinez-Torrecuadrada, J., Schmid, E. R., de Pedro, M. A., & Löffelhardt, W. (1996b). Primary structure of cyanelle peptidoglycan of *Cyanophora paradoxa*: A prokaryotic cell wall as part of an organelle envelope. *Journal of Bacteriology, 178*, 332–339.

Pickett-Heaps, J. (1972). Cell division in *Cyanophora paradoxa*. *New Phytologist, 71*, 561–567.

Piriyapongsa, J., & Jordan, I. K. (2008). Dual coding of siRNAs and miRNAs by plant transposable elements. *RNA, 14*, 814–821.

Pittenauer, E., Allmaier, G., Schmid, E. R., Pfanzagl, B., Löffelhardt, W., Quintela Fernandez, C., de Pedro, M. A., & Stanek, W. (1993). Structural characterization of the cyanelle peptidoglycan of *Cyanophora paradoxa* by ^{252}Cf-plasma desorption mass spectrometry and fast atom bombardment/tandem mass spectrometry. *Biological Mass Spectrometry, 22*, 524–536.

Plaimauer, B., Pfanzagl, B., Berenguer, J., de Pedro, M. A., & Löffelhardt, W. (1991). Subcellular distribution of enzymes involved in the biosynthesis of cyanelle murein in the protist *Cyanophora paradoxa*. *FEBS Letters, 284*, 168–172.

Plancke, C., Colleoni, C., Deschamps, P., Dauvillée, D., Nakamura, Y., Haebel, S., Steup, M., Buléon, A., Putaux, J.-L., Dupeyre, D., d'Hulst, C., Ral, J.-P., Löffelhardt, W., Maes, E., & Ball, S. G. (2008). The pathway of starch synthesis in the model glaucophyte *Cyanophora paradoxa*. *Eukaryotic Cell, 7*, 247–257.

Price, D. C., Chan, C. X., Yoon, H. S., Yang, E. C., Qiu, H., Weber, A. P. M., Schwacke, R., Gross, J., Blouin, N. A., Lane, C., Reyes-Prieto, A., Durnford, D. G., Neilson, J. A. D., Lang, B. F., Burger, G., Steiner, J. M., Löffelhardt, W., Meuser, J. E., Posewitz, M. C., Ball, S., Arias, M. C., Henrissat, B., Coutinho, P. M., Rensing, S. A., Symeonidi, A., Doddapaneni, H., Green, B. R., Rajah, V. D., Boore, J., & Bhattacharya, D. (2012). *Cyanophora paradoxa* genome elucidates origin of photosynthesis in algae and plants. *Science, 335*, 843–847.

Pringsheim, E. G. (1958). *Organismen mit blaugrünen Assimilatoren* (pp. 165–184). Praha: Studies in Plant Physiology.

Qiu, H., Price, D. C., Weber, A. P., Facchinelli, F., Yoon, H. S., & Bhattacharya, D. (2013a). Assessing the bacterial contribution to the plastid proteome. *Trends in Plant Science, 18*, 680–687.

Qiu, H., Price, D. C., Weber, A. P., Reeb, V., Yang, E. C., Lee, J. M., Kim, S. Y., Yoon, H. S., & Bhattacharya, D. (2013b). Adaptation through horizontal gene transfer in the cryptoendolithic red alga *Galdieria phlegrea*. *Current Biology, 23*, R865–R866.

Raven, J. A. (2003). Carboxysomes and peptidoglycan walls of cyanelles: Possible physiological functions. *European Journal of Phycology, 38*, 47–53.

Reith, M. (1995). Molecular biology of rhodophyte and chromophyte plastids. *Annual Review Plant Physiology Plant Molecular Biology, 46*, 549–575.

Reumann, S., Inoue, K., & Keegstra, K. (2005). Evolution of the general import pathway of plastids. *Molecular Membrane Biology, 22*, 73–86.

Reyes-Prieto, A., & Bhattacharya, D. (2007a). Phylogeny of nuclear-encoded plastid-targeted proteins supports an early divergence of glaucophytes within plantae. *Molecular Biology and Evolution, 24*, 2358–2361.

Reyes-Prieto, A., & Bhattacharya, D. (2007b). Phylogeny of Calvin cycle enzymes supports Plantae monophyly. *Molecular Phylogenetics and Evolution, 45*, 384–391.

Reyes-Prieto, A., Hackett, J. D., Soares, M. B., Bonaldo, M. F., & Bhattacharya, D. (2006). Cyanobacterial contribution to algal nuclear genomes is primarily limited to plastid functions. *Current Biology, 16*, 2320–2325.

Robinson, D. G., & Preston, R. D. (1971). Studies on the fine structure of *Glaucocystis nostochinearum* Itzigs. I. Wall structure. *Journal of Experimental Botany, 22*, 635–643.

Rockwell, N. C., Su, Y. S., & Lagarias, J. C. (2006). Phytochrome structure and signaling mechanisms. *Annual Review of Plant Biology, 57*, 837–858.

Rockwell, N. C., Duanmu, D., Martin, S. S., Bachy, C., Price, D. C., Bhattacharya, D., Worden, A. Z., & Lagarias, J. C. (2014). Eukaryotic algal phytochromes span the visible spectrum. *Proceedings of the National Academy of Sciences USA, 111*, 3871–3876.

Rodríguez-Ezpeleta, N., Brinkmann, H., Burey, S. C., Roure, B., Burger, G., Löffelhardt, W., Bohnert, H. J., Philippe, H., & Lang, B. F. (2005). Monophyly of primary photosynthetic eukaryotes: Green plants, red algae and glaucophytes. *Current Biology, 15*, 1325–1330.

Rogers, C. E., Stewart, K. D., & Mattox, K. R. (1981). Comparison of the flagellar apparatuses of *Cyanophora paradoxa* and *Glaucocystis nostochinearum*: A characterization of the Glaucophyceae. *Journal of Phycology, 17* , 12.Supplement, Abstract 59

Sato, N., Ishikawa, M., Fujiwara, M., & Sonoike, K. (2005). Mass identification of chloroplast proteins of endosymbiont origin by phylogenetic profiling based on organism-optimized homologous protein groups. *Genome Informatics, 16*, 56–68.

Sato, M., Mogi, Y., Nishikawa, T., Miyamura, S., Nagumo, T., & Kawano, S. (2009). The dynamic surface of dividing cyanelles and ultrastructure of the region directly below the surface in *Cyanophora paradoxa*. *Planta, 229*, 781–791.

Sauvage, E., Kerff, F., Terrak, M., Ayala, J. A., & Charlier, P. (2008). The penicillin-binding proteins: Structure and role in peptidoglycan biosynthesis. *FEMS Microbiology Reviews, 32*, 234–258.

Schenk, H. E. A. (1970). Nachweis einer lysozymempfindlichen Stützmembran der Endocyanellen von *Cyanophora paradoxa* Korschikoff. Zeitschrift für Naturforschung, 25b, 640–656.

Schenk H. E. A. (1994). Cyanophora paradoxa: Anagenetic model or missing link of plastid evolution? *Endocytobiosis & Cell Research, 10*, 87–106.

Schnepf, E. (1965). Struktur der Zellwände und Zellulosefibrillen bei *Glaucocystis*. *Planta, 67*, 213–224.

Schnepf, E., Koch, W., & Deichgräber, G. (1966). Zur Cytologie und taxonomischen Einordnung von *Glaucocystis*. *Archiv für Mikrobiologie, 55*, 149–174.

Schönknecht, G., Chen, W. H., Ternes, C. M., Barbier, G. G., Shrestha, R. P., Stanke, M., Bräutigam, A., Baker, B. J., Banfield, J. F., Garavito, R. M., Carr, K., Wilkerson, C., Rensing, S. A., Gagneul, D., Dickenson, N. E., Oesterhelt, C., Lercher, M. J., & Weber, A. P. (2013). Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. *Science, 339*, 1207–1210.

Scott, O. T., Castenholz, R. W., & Bonnett, H. T. (1984). Evidence for a peptidoglycan envelope in the cyanelles of *Glaucocystis nostochinearum* Itzigsohn. *Archives of Microbiology, 139*, 130–138.

Sensen, C. W., Heimann, K., & Melkonian, M. (1993). The production of clonal and axenic cultures of microalgae using fluorescence-activated cell sorting. *European Journal of Phycology, 28*, 93–97.

Serrano, A., & Löffelhardt, W. (1994). Identification of 2 different glyceraldehyde-3-phosphate dehydrogenases (phosphorylating) in the photosynthetic protist *Cyanophora paradoxa*. *Archives of Microbiology, 162*, 14–19.

Shibata, M., Kashino, Y., Sato, K., & Koike, H. (2001). Isolation and characterization of oxygen-evolving thylakoid membranes and photosystem II particles from a glaucocystophyte, *Cyanophora paradoxa*. *Plant Cell Physiology, 42*, 733–741.

Six, C., Thomas, J.-C., Thion, L., Lemoine, Y., Zal, F., & Partensky, F. (2005). Two novel phycoerythrin-associated linker proteins in the marine cyanobacterium *Synechococcus* sp. strain WH8102. *Journal of Bacteriology, 187*, 1685–1694.

Skalitzky, C. A., Martin, J. R., Harwood, J. H., Beirne, J. J., Adamczyk, B. J., Heck, G. R., Cline, K., & Fernandez, D. E. (2011). Plastids contain a second Sec translocase system with essential functions. *Plant Physiology, 155*, 354–369.

Skuja, H. (1954). Glaucophyta. In H. Melcher & E. Werdermann (Eds.), *Syllabus der Pflanzenfamilien* (Vol. 1, pp. 56–57). Berlin: Borntraeger.

Skuja, H.: Taxonomische und biologische Studien über das Phytoplankton schwedischer Binnengewässer. Nova Acta Regiae Societatis Scientiarum Urpsaliensis Ser. 4, Vol. 4 (3), 1–404 (1956).

Smeekens, S., Weisbeek, P., & Robinson, C. (1990). Protein transport into and within chloroplasts. *Trends in Biochemical Sciences, 15*, 73–76.

Sommer, M. S., & Schleiff, E. (2014). Evolution of the protein translocons of the chloroplast envelope. In W. Löffelhardt (Ed.), *Endosymbiosis* (pp. 81–110). Heidelberg: Springer.

Starnes, S. M., Lambert, D. H., Maxwell, E. S., Stevens, S. E., Porter, R. D., & Shively, J. M. (1985). Cotranscription of the large and small subunit genes of ribulose-1,5-bisphosphate carboxylase/oxygenase in *Cyanophora paradoxa*. *FEMS Microbiology Letters, 28*, 165–169.

Steiner, J. M., Serrano, A., Allmaier, G., Jakowitsch, J., & Löffelhardt, W. (2000). Cytochrome *c*6 from *Cyanophora paradoxa*: Characterization of the protein and the cDNA of the precursor and import into isolated cyanelles. *European Journal of Biochemistry, 267*, 4232–4241.

Steiner, J. M., & Löffelhardt, W. (2002). Protein import into cyanelles. *Trends in Plant Sciences, 7*, 72–77.

Steiner, J. M., Pompe, J. A., & Löffelhardt, W. (2003). Characterization of *apcC*, the nuclear gene for the phycobilisome core linker polypeptide $L_c^{7.8}$ from the glaucocystophyte alga *Cyanophora paradoxa*. Import of the precursor into cyanelles and integration of the mature protein into intact phycobilisomes. *Current Genetics, 44*, 132–137.

Steiner, J. M., & Löffelhardt, W. (2005). Protein translocation into and within cyanelles. *Molecular Membrane Biology, 22*, 123–132.

Steiner, J. M., Yusa, F., Pompe, J. A., & Löffelhardt, W. (2005a). Homologous protein import machineries in chloroplasts and cyanelles. *Plant Journal, 44*, 646–652.

Steiner, J. M., Berghöfer, J., Yusa, F., Pompe, J. A., Klösgen, R. B., & Löffelhardt, W. (2005b). Conservative sorting in a primitive plastid: the cyanelle of *Cyanophora paradoxa*. *FEBS Journal, 272*, 987–998.

Steiner, J. M., & Löffelhardt, W. (2011). The Photosynthetic Apparatus of the Living Fossil, *Cyanophora paradoxa*. In G. A. Peschek, C. Obinger, & G. Renger (Eds.), *Bioenergetic processes of cyanobacteria* (pp. 71–87). Dordrecht: Springer.

Steiner, J. M., Bhattacharya, D., & Löffelhardt, W. (2012). Conservative sorting in the muroplasts of *Cyanophora paradoxa*: A reevaluation based on the completed genome sequence. *Symbiosis, 58*, 127–133.

Stirewalt, V. L., Michalowski, C. B., Löffelhardt, W., Bohnert, H. J., & Bryant, D. A. (1995). Nucleotide sequence of the cyanelle genome from *Cyanophora paradoxa*. *Plant Molecular Biology Reporter, 13*, 327–332.

Stoebe, B., Martin, W., & Kowallik, K. V. (1998). Distribution and nomenclature of protein–coding genes in 12 sequenced chloroplast genomes. *Plant Molecular Biology Reporter, 16*, 243–255.

Struyvé, M., Moons, M., & Tommassen, J. (1991). Carboxy-terminal phenylalanine is essential for the correct assembly of a bacterial outer membrane protein. *Journal of Molecular Biology, 218*, 141–148.

Tachibana, M., Allen, A. E., Kikutani, S., Endo, Y., Bowler, C., & Matsuda, Y. (2011). Localization of putative carbonic anhydrases in two marine diatoms, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. *Photosynthesis Research, 109*, 205–221.

Takahashi, T., Nishida, T., Tuji, A., Saito, C., Matsuzaki, R., Sato, M., Toyooka, K., Yasuda, H., & Nozaki, H. (2016). Delineation of six species of the primitive algal genus *Glaucocystis* based on *in situ* ultrastructural characteristics. *Scientific Reports, 6*, 29209.

Takahashi, T., Sato, M., Toyooka, K., Matsuzaki, R., Kawafune, K., Kawamura, M., Okuda, K., & Nozaki, H. (2014). Five Cyanophora (Cyanophorales, Glaucophyta) species delineated based on morphological and molecular data. *Journal of Phycology, 50*, 1058–1069.

Takano, H., & Takechi, K. (2010). Plastid peptidoglycan. *Biochimica et Biophysica Acta, 1800*, 144–151.

Timmis, J. N., Ayliffe, M. A., Huang, C. Y., & Martin, W. (2004). Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nature Reviews Genetics, 5*, 123–135.

Tissier, C., Woolhead, C. A., & Robinson, C. (2002). Unique structural determinants in the signal peptides of 'spontaneously' inserting thylakoid membrane proteins. *European Journal of Biochemistry, 269*, 3131–3141.

Trench, R. K. (1982). Physiology, biochemistry, and ultrastructure of cyanelles. In F. E. Round & D. J. Chapman (Eds.), *Progress in phycological research* (Vol. 1, pp. 257–288). Amsterdam: Elsevier.

Verissimo, A. F., & Daldal, F. (2014). Cytochrome *c* biogenesis system I: An intricate process catalyzed by a maturase supercomplex? *Biochimica et Biophysica Acta, 1837*, 989–998.

Voulhoux, R., & Tommassen, J. (2004). Omp85, an evolutionarily conserved bacterial protein involved in outer-membran-protein assembly. *Reseach in Microbiology, 155*, 129–135.

Wagener, N., Ackermann, M., Funes, S., & Neupert, W. (2011). A pathway of protein translocation in mitochondria mediated by the AAA-ATPase Bcs1. *Molecular Cell, 44*, 191–202.

Watanabe, M., Kubota, H., Wada, H., Narikawa, R., & Ikeuchi, M. (2011). Novel supercomplex organization of photosystem I in *Anabaena* and *Cyanophora paradoxa*. *Plant Cell Physiology, 52*, 162–168.

Watanabe, M., Sato, M., Kondo, K., Narikawa, R., & Ikeuchi, M. (2012). Phycobilisome model with novel skeleton-like structures in a glaucocystophyte *Cyanophora paradoxa*. *Biochimica et Biophysica Acta, 1817*, 1428–1435.

Weber, A. P. M., Linka, M., & Bhattacharya, D. (2006). Single ancient origin of a plastid metabolite translocator family in Plantae from an endomembrane-derived ancestor. *Eukaryotic Cell, 5*, 609–612.

Weisser, A. (2012). Charakterisierung der Phycobilisomen-Architektur von Cyanophora paradoxa. MSc Thesis, MLU Halle-Wittenberg.

Wheeler, G., Ishikawa, T., Pornsaksit, V., & Smirnoff, N. (2015). Evolution of alternative biosynthetic pathways for vitamin C following plastid acquisition in photosynthetic eukaryotes. *eLife, 4*, e06369.

Wunder, T., Martin, R., Löffelhardt, W., Schleiff, E., & Steiner, J. M. (2007). The invariant phenylalanine of precursor proteins discloses the importance of Omp85 for protein translocation into cyanelles. *BMC Evolutionary Biology, 7*, 236.

Yabuki, A., Kamikawa, R., Ishikawa, S. A., Kolisko, M., Kim, E., Tanabe, A. S., Kume, K., Ishida, K., & Inagaki, Y. (2014). *Palpitomonas bilix* represents a basal cryptist lineage: Insight into the character evolution in Cryptista. *Scientific Reports, 4*, 4641.

Yamano, T., Tsujikawa, T., Hatano, K., Ozawa, S., Takahashi, Y., & Fukuzawa, H. (2010). Light and low-CO2 -dependent LCIB–LCIC complex localization in the chloroplast supports the carbon-concentrating mechanism in *Chlamydomonas reinhardtii*. *Plant Cell Physiology, 51*, 1453–1468.

Yamano, T., Sato, E., Iguchi, H., Fukuda, Y., & Fukuzawa, H. (2015). Characterization of cooperative bicarbonate uptake into chloroplast stroma in the green alga *Chlamydomonas reinhardtii*. *Proceedings of the National Academy of Sciences USA, 112*, 7315–7320.

Yang, Y., Glynn, J. M., Olson, B. J., Schmitz, A. J., & Osteryoung, K. W. (2008). Plastid division: Across time and space. *Current Opinion in Plant Biology, 11*, 577–584.

Yusa, F., Steiner, J. M., & Löffelhardt, W. (2008). Evolutionary conservation of dual Sec translocases in the cyanelles of *Cyanophora paradoxa*. *BMC Evolutionary Biology, 8*, 304.