# Chapter 2
# The Photosynthetic Apparatus of the Living Fossil, *Cyanophora paradoxa*

**Jürgen M. Steiner and Wolfgang Löffelhardt**

Muroplasts, the peculiar plastids (previously called cyanelles) of glaucocystophyte algae did retain—with some modifications—the peptidoglycan wall of their cyanobacterial ancestor. This is not only a convincing proof of the endosymbiotic theory, but earns glaucocystophytes the status of living fossils, as peptidoglycan is found nowhere else among eukaryotes. Muroplasts show even more cyanobacterial features than other primitive plastids, e.g., rhodoplasts. Almost all data available at present come from one species, *Cyanophora paradoxa*. The plastome, containing a surplus of 50 protein genes compared to chloroplast genomes (Table 2.1) and about 30% of the transcriptome (ESTs) of this organism are sequenced and a genome project is in progress. While *Cyanophora* does not offer such possibilities for genetic analysis as the *Chlamydomonas* system, due to its reasonable growth rate it is amenable to biochemical investigations.

The light-harvesting antennae, the pathways of protein sorting into and within plastids, the genetic compartmentation of photosynthesis genes, the composition of the oxygen-evolving complex, and the nature of the carbon-concentrating mechanism will be compared for muroplasts, rhodoplasts and (green algal) chloroplasts. Preliminary data on the biosynthesis of starch in *C. paradoxa* will be put in the context of reserve carbohydrate nature and localization in various oxygenic autotrophs. Based on sequence alignments and phylogenetic analyses, *Cyanophora* occupies a bridge position between cyanobacteria, red algae and green algae, in some cases taking sides with the ancestors, in other cases with the green lineage or the red lineage, respectively.

W. Löffelhardt (✉)
Max F. Perutz Laboratories, Department of Biochemistry, University of Vienna, Dr. Bohrgasse 9, 1030 Vienna, Austria
e-mail: wolfgang.loeffelhardt@univie.ac.at

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**Table 2.1** Muroplast genes from *Cyanophora paradoxa* specifying proteins of the photosynthetic apparatus

| |
|---|
| Phycobiliproteins (7): *apcA, apcB, apcD, apcE, apcF, cpcA, cpcB* |
| Photosystem I proteins: *psaA, psaB, psaC, psaE, psaF, psaI, psaJ, psaM* |
| Photosystem II proteins: *psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbV, psbX, psbY, psbZ, psb30* |
| ATP synthase subunits: *atpA, atpB, atpD, atpE, atpF, atpG, atpH* |
| Subunits of the cytochrome $b_6/f$ complex & ferredoxin: *petA, petB, petD, petG, petL, petN, petX; petF* |
| Calvin cycle enzymes: *rbcL, rbcS* |
| Chlorophyll biosynthesis: *hemA*[*], *chlB, chlI, chlL, chlN* |
| Carotenoid & prenylquinone biosynthesis: *crtE*[*], *preA* |
| ORFs with putative function in photosynthesis: *ycf3*[a], *ycf4*[a], *ycf5*[b], *ycf16*[c], *ycf24*[d], *ycf27*[e], *ycf33*[f], *orf333*[g] |

Gene nomenclature according to 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 higher plants

[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] ABC transporter subunit, ortholog to bacterial *sufB*, involved in [Fe-S] cluster biogenesis

[e] Response regulator of PS I genes (*rpaB*)

[f] Role in cyclic electron transport

[g] Role in assembly/stability of PS II (*hcf136*)

## 2.1 Introduction

Phototrophic eukaryotes emerged from the so-called primary endosymbiotic event, about 1.5 billion years ago (Fig. 2.1). It is generally accepted that cyanobacteria gave rise to plastids after massive gene transfer from the endosymbiont genome to the nucleus of the heterotrophic host cell. Most, but not all researchers in the field consider the primary endosymbiotic event a single one, i.e., the immensely complicated establishment of a hereditary symbiosis leading to a photosynthetic organelle happened only once (Martin et al. 1998; Rodríguez-Ezpeleta et al. 2005; Reyes-Prieto et al. 2007a), between a suitable host cell and a suitable—likely filamentous and nitrogen-fixing—cyanobacterium (Deusch et al. 2008). This view implies monophyly of the kingdom "Plantae": all plastid types, regardless of their morphology and pigmentation are derived from an ancestral organelle, the "protoplastid". This primordial organelle had already performed 90% of the total gene transfer events to the nucleus, was still armored by a peptidoglycan wall, and had developed a specific protein import apparatus at its envelope. The Archaeplastida (glaucocystophytes, rhodophytes, chlorophytes and streptophytes) are phototrophic eukaryotes containing primary plastids, surrounded by two envelope membranes (Adl et al. 2005). The phagosomal membrane was lost early in plastid evolution as evidenced by two much more recent endosymbioses: the photosynthetic chromatophores (cyanelles) from the thecamoeba *Paulinella chromatophora* (Nowack et al.

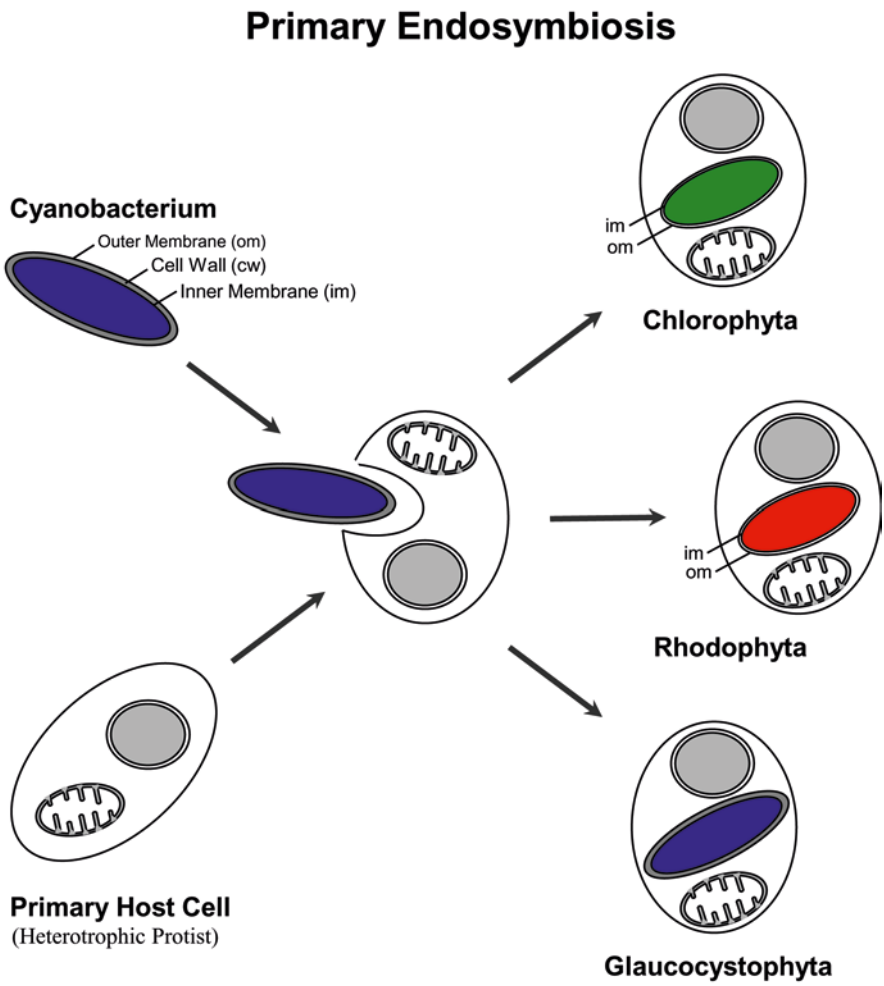


**Fig. 2.1** The single primary endosymbiotic event. (Modified from Stoebe and Maier 2002)

2008; derived from a *Synechococcus*-like ancestor, estimated age 60 My) are surrounded by two membranes only, whereas the nitrogen-fixing spheroid bodies from the diatom *Rhopalodia gibba* (Kneip et al. 2008; derived from a *Cyanothece*-like cyanobacterium, estimated age 25–35 My) are still surrounded by three membranes (Kovacevic et al. 2009). The inner (IEM) and outer (OEM) envelope membranes of the Archaeplastida clearly correspond to the respective membranes of the ancestral cyanobacterium. The majority of algal phyla arose from superimposed (multiple and polyphyletic) secondary endosymbiotic events where hetrotrophic protists engulfed red algae or green algae, i.e. phototrophic eukaryotes. The resulting secondary or complex plastids are surrounded by three or four membranes (Stoebe and Maier 2002).

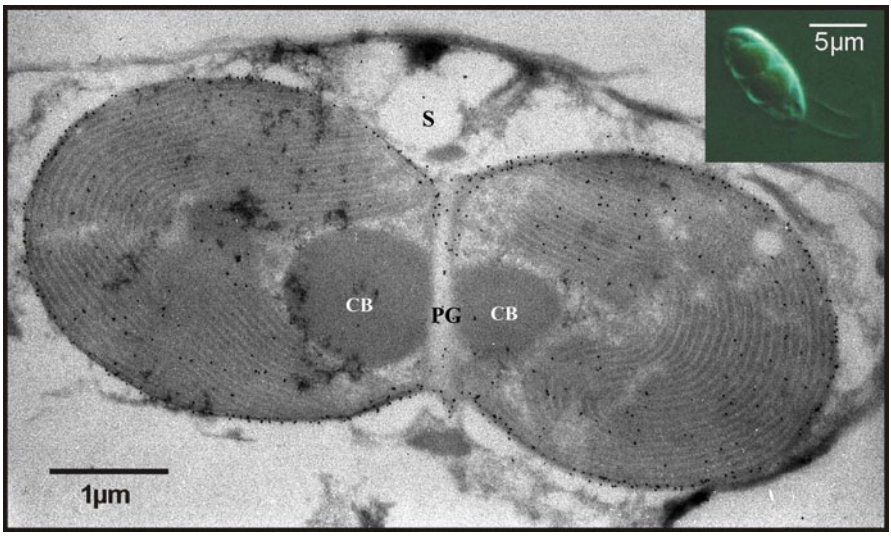


**Fig. 2.2** Eukaryotic peptidoglycan in the muroplast envelope of *Cyanophora paradoxa* visualized via immunogold electron microscopy. Primary antibodies are directed against peptidoglycan from *Escherichia coli*. *PG*, peptidoglycan septum; *CS*, putative carboxysome

Most phylogenetic analyses hold the glaucocystophytes as the most ancient phototrophic eukaryotes known to date (Rodríguez-Ezpeleta et al. 2005; Reyes-Prieto et al. 2007b) and their muroplasts (Fig. 2.2) occupy a bridge position between plastids and free-living cyanobacteria. It is the purpose of this review to elaborate these special features mimicking early events in plastid evolution.

## 2.2 The Phycobilisome Antenna System

This major light harvesting system of cyanobacteria (MacColl 1998), rhodoplasts, and glaucocystophyte muroplasts was studied in some detail in *C. paradoxa* (Table 2.2). While its cyanobacterial origin is obvious, modifications typical for eukaryotic phycobilisomes (PBS) are encountered: some of the putative rod linker polypeptides are of unusual size, i.e., 50–60 kDa, as also found in rhodoplasts (Egelhoff and Grossman 1983; Liu et al. 2008a). In contrast to cyanobacterial PBS, no binding of FNR to the outer segments of rods (Jakowitsch et al. 1993; Steiner et al. 2003) was observed. Unlike red algae, there is no phycoerythrin (PE) and the genetic compartmentalization is more rigorous: the chromophorylated phycobiliproteins are all muroplast-encoded whereas all colorless linker polypeptides are products of nuclear genes and thus major import products into muroplasts (Table 2.2). However, there are PBS components with features from both groups as the chromophore-bearing, linker-like γ–phycoerythrin from the red alga *Aglaothamnion neglectum* (Apt et al. 1993), which is nucleus-encoded. On the other hand, the "genuine" rod-core linker

**Table 2.2** Components of purified, intact muroplast PBS (Steiner et al. 2003)

| Apparent MW (kDa) | Abundance | Phycobiliprotein | Correlated gene | Assignment |
|---|---|---|---|---|
| 98 | Medium | Yes | *apcE*[a] | Core-membrane linker |
| 55 | Medium | No | | Rod linker? |
| 53 | Medium | No | | Rod linker? |
| 38 | Low | No | | Rod linker[b]? |
| 31 | Low | No | *cpcG1, cpcG2*[b] | Rod-core linker |
| 19–21 | High | Yes | *apcA*[a],*B*[a],*D*[a],*F*[a] | Allophycocyanin subunits |
| 16–18 | High | Yes | *cpcA*[a],*B*[a] | Phycocyanin subunits[b] |
| 9 | Low | No | | Terminal rod linker? |
| 8 | Low | No | *apcC* | Core linker |

[a] Muroplast-encoded
[b] Potential components of a PS I associated PBS subcomplex

CpcG is encoded by the plastid genome in *A. neglectum* (Apt and Grossman 1993). The accessory allophycocyanins ApcD and ApcF have functions in energy transfer from the PBS to PS I and PS II, respectively, in cyanobacterial (Dong et al. 2009) and, likely, also in glaucocystophyte PBS. Recently, evidence was obtained that PBS serve both photosystems in rhodoplasts (Liu et al. 2008a; Busch et al. 2010).

In analogy to cyanobacteria (Wang et al. 2004), the expression of the rod-core linker gene *cpcG1* is downregulated upon shift of the cultures from high to low [$CO_2$] indicating a reduction of antenna size during $CO_2$-limitation (Burey et al. 2007). The 7.8 kDa core linker ApcC (45–51% sequence identity to cyanobacterial counterparts of almost the same size) was expressed through *in vitro* transcription/translation of the cloned gene and imported into isolated muroplasts where its fast assembly into PBS could be demonstrated (Steiner et al. 2003). This is convincing evidence for the dynamic behavior of PBS. ApcC from *C. paradoxa* shows an insertion of four amino acids (AKKT) between the highly conserved positions P-11 and S-12 of cyanobacterial homologs which is not found in the corresponding ESTs from red algae. Partial cDNA sequence as well as peptide sequence information is available for the other linker polypeptides of Table 2.2 with the exception of the 9 kDa protein (J. Steiner, unpublished). The small linker polypeptides described for the unicellular red alga, *Porphyridium cruentum*, are of higher MW (13 kDa and 14 kDa; Liu et al. 2008a). Interestingly, the large linkers (Table 2.2) seem to have arisen from a duplication of the *cpcG* gene, which itself exists in two variants, *cpcG1* and *cpcG2* (J. Steiner, unpublished). A similar gene fusion event has been reported for an unusual, chromophore-bearing phycoerythrin linker protein from a marine *Synechococcus* sp. (Six et al. 2005). Recently, CpcG2 was shown to be part of a novel photosystem I antenna, consisting of a rod only, in *Synechocystis* sp. PCC 6803 (Kondo et al. 2007). It remains to be seen if this can be generalized to muroplast CpcG2, the C-terminus of which is less hydrophobic than that of the cyanobacterial counterpart. However, there is convincing evidence for a dual PSI antenna in the red alga *C. merolae* consisting of crescent-shaped Lhcr proteins and a PBS subcomplex of the *Synechocystis* type (Busch et al. 2010). There is only one

gene for CpcG in the (streamlined) genome of the extremophile, that also lacks a hydrophobic stretch at the C-terminus. Taken together, a bridge position seems to emerge for PSI of glaucocystophytes: no extrinsic chlorophyll antenna (unlike red algae) but a (reduced) special PBS antenna. CpcG2, a fraction of the alpha- and beta-phycocyanins, and the putative 38 kDa rod linker (substoichiometric in Table 2.2) could function as subunits thereof.

The prevalent muroplast pigments are chlorophyll *a*, β-carotene, zeaxanthin, β-cryptoxanthin, allophycocyanin, and C-phycocyanin. Carotenoids typical for cyanobacteria such as echinenone and myxoxanthophyll are absent. A carotenoid–rich protein (CRP) was isolated from *C. paradoxa* through its cross-reactivity with LHCP (Rissler and Durnford 2005). However, the 28 kDa protein appeared to be a peripheric rather than an integral thylakoid membrane protein with a preference for zeaxanthin binding. Elucidation of its function, likely photoprotective as in cyanobacteria (Liu et al. 2008b), must await further studies.

## 2.3 Photosystem II

The photosynthetic apparatus of cyanobacteria (including primitive plastids) and chloroplasts (from green algae and higher plants) show some differences in spite of their high overall similarity. The oxygen evolving complex (OEC) in cyanobacteria (Shen et al. 1998) consists of three peripheral membrane proteins: a 33 kDa protein (OE33, the product of the *psbO* gene), cytochrome $c_{550}$ (*psbV*), and a 9–12 kDa protein (*psbU*). Chloroplasts possess only one of these components: the 33 kDa protein. An unrelated 23 kDa protein, OE23 (*psbP*), fulfills the function of cytochrome $c_{550}$, whereas PsbU is replaced by an unrelated 16 kDa protein, OE16 (*psbQ*). Primitive plastids comprise the muroplasts of *Cyanophora paradoxa*, the rhodoplasts of e.g., *Porphyra purpurea* and *Cyanidium caldarium* and, likely, those complex plastids (e.g., of the cryptomonad *Guillardia theta* and the diatom *Odontella sinensis*) that arose from a red algal secondary endosymbiont. Evidence for their cyanobacterial type OEC is the *psbV* gene which resides on the plastid genomes in all known cases (Kowallik et al. 1995; Reith and Munholland 1995; Löffelhardt et al. 1997; Douglas and Penny 1999; Glöckner et al. 2000) and the nuclear *psbU* genes of e.g., *C. caldarium* (Ohta et al. 1999) and *C. paradoxa* (Steiner et al. 2005b) which show bipartite presequences. In addition, the photosystem II and OEC complex was characterized at the protein level for *C. caldarium* (Enami et al. 1995) and *C. paradoxa* (Shibata et al. 2001). The nucleus-encoded *psbP* and *psbQ* genes of higher plants and green algae display "twin-arginine" motifs in the thylakoid transfer domains of their bipartite presequences, which is an indication for the usage of the ΔpH or Tat pathway for thylakoid translocation. This pathway is employed by lumenal proteins unable to translocate via the alternative SecA/Y/E pathway because of folding in the stroma with or without cofactors (Dalbey and Robinson 1999). At present, there is no evidence for any cofactors of OE23 and OE16. On the other hand, the twin-arginine motif is missing from the presequences of *psbU* genes from cyanobacteria.

Muroplast SecA appeared to be quite susceptible to inhibition by sodium azide, but not to the Tat pathway inhibitor Nigericin, during import experiments with homologous precursors: 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 muroplast lysis and thylakoid isolation, for the first time protease protection of the mature protein inside of phycobilisome-bearing thylakoids. Recently, a psbQ-like protein corresponding to ORF *sll*1638 has been identified by proteomic analysis of a highly active PSII preparation from *Synechocystis* sp. PCC6803 (Kashino et al. 2002). In the red alga *Cyanidium caldarium* the OEC is of "primitive" type consisting of PsbO, PsbU and PsbV (cyt $c_{550}$), but additionally contains a 20 kDa protein with significant sequence similarity to PsbQ, now named PsbQ′, which has been shown to be able to functionally replace the green algal 17 kDa protein on binding to green algal, but not higher plant PSII and restore oxygen evolution (Ohta et al. 2003). Later on, PsbQ′ was established as an essential component of the cyanobacterial OEC (Roose et al. 2007). A crystalline preparation of *C. caldarium* PS II dimers with excellent resolution of protein bands (Adachi et al. 2009) also revealed the additional extrinsic (lumenal) protein of 20 kDa (PsbQ′). In the dBEST data base, a *Cyanophora* EST with sequence similarity (http://amoebidia.bch.umontreal.ca/pepdb/pepdb.html) to PsbQ′ is contained, pointing towards even greater analogies between the OECs from cyanobacteria and primitive algae than previously expected.

PSII reaction center core and intrinsic subunits are very similar for all oxygenic phototrophs. The muroplast genome encodes in total 19 subunits of PS II (Table 2.1), the same applies for rhodoplasts (Reith and Munholland 1995). Shibata et al. (2001) investigated the subunit composition of purified oxygen-evolving PS II particles of *C. paradoxa*: more than 30 protein bands were resolved including PsbO and cytochrome $c_{550}$ whereas PsbU obviously was lost during the preparation.

## 2.4 Cytochrome $b_6/f$ Complex and Electron Donors to Photosystem I

As in the case of PSII, the muroplast genome is a treasure trove containing seven genes for membrane-bound subunits (Table 2.1). Pre-cytochrome *f*(*petA*) contains a signal sequence with a highly hydrophobic core domain. The only nuclear-encoded subunit known to date is Rieske iron sulfur protein (PetC). About five aa of its N-terminus with a noncleavable signal-anchor domain protrude into the stroma, followed by one trans-membrane helix and the lumenally exposed bulk of the protein containing the $Fe_2S_2$ cluster. The "twin arginine" motiv of cyanobacterial PetC is replaced by RK: nevertheless there is good evidence that the Tat translocase assists in thylakoid membrane translocation/insertion of this folded holoprotein that has to

attain its prosthetic group in the stroma (Steiner et al. 2005b). There are no data at present on purification of the cytochrome $b_6/f$ complex and subunit characterization at the protein level for *C. paradoxa* or red algae.

Cytochrome $c_6$ (PetJ) of *C. paradoxa* was characterized at the protein and cDNA level (Steiner et al. 2000). The bipartite presequence of the nuclear-encoded precursor shows a signal peptide with a core domain of moderate hydrophobicity, unlike the muroplast-encoded pre-cytochrome *f* and pre-cytochrome $c_{550}$. The intermediate form, i-cytochrome $c_6$ accumulated in the stroma after import into isolated cyanelles and transit peptide cleavage when sodium azide was added to the incubation buffer (Steiner et al. 2005b). As expected c-type cytochromes attain their heme in the thylakoid lumen and are translocated across the thylakoid membrane as unfolded Sec passengers. PetJ is solely responsible for electron transport between the cytochrome $b_6$/f complex and PS II as there is no plastocyanin in *C. paradoxa*. PetJ is present in the periplasmic space as well as in the thylakoid lumen of muroplasts and might play a role as electron donor to yet unknown terminal oxidases in the inner envelope membrane (G. Peschek, personal communication). There is no indication for a continuity of IEM and TM in muroplasts, a question which is also answered in the negative for cyanobacteria (Liberton et al. 2006). Nevertheless, under freeze-thaw conditions muroplasts are completely drained of cytochrome $c_6$ whereas no loss of phycobiliproteins was observed (Steiner et al. 2000).

## 2.5 Photosystem I

At the present state of knowledge the very well investigated PS I of cyanobacteria is trimeric, whereas PS I of algae is a monomer (good evidence for green and red algae, by analogy for glaucocystophytes). The contribution of the muroplast genome—eight genes –is less pronounced than for PS II (Table 2.1). PS I preparations from *C. paradoxa* revealed, in addition to the major polypeptides, a number of smaller subunits. PsaD is nuclear-encoded in contrast to red algae and its N-terminus resembled the counterparts from the “green lineage”. On the other hand, the N-terminal sequence of PsaL was closer to the cyanobacterial homologs. (Koike et al. 2000). In this study, immunodetection of Lhca proteins was unsuccessful. Thus, in contrast to other algal phyla, the lack of an extrinsic chlorophyll antenna in PS I must be assumed for *C. paradoxa*, a parallel to cyanobacteria. It remains to be seen upon the completion of the *C. paradoxa* genome sequence if PsaGHK (involved in Lhca and Lhcr binding) are absent.

Muroplast ferredoxin was the first plastid-encoded ferredoxin reported (Table 2.1, Löffelhardt et al. 1997). 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 shows high amino acid sequence similarity to higher plant counterparts and lacks the C-terminal extension of cyanobacterial enzymes (around 45 kDa) responsible for binding to phycobilisomes. The availability of the $^{35}S$-labeled precursor was important for the establish-

ment of a muroplast *in vitro* import system (Jakowitsch et al. 1996). Muroplasts and likely rhodoplasts appear to import precursors displaying a phenylalanine residue in the N-terminal domain of the transit sequences via a primordial Toc translocon with postulated receptor and pore functions. The bulk import products of chloroplasts, Rubisco SSU and Lhcb, which necessitated the development of specific receptors, are not relevant here (Steiner et al. 2005a; Steiner and Löffelhardt 2005).

## 2.6 Calvin Cycle Enzymes

A NAD(P)-dependent glyceraldehyde-3-phosphate dehydrogenase 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). In addition, the NAD-dependent cytosolic enzyme involved in glycolysis was purified (Serrano and Löffelhardt 1994).

The gene for CP12 protein involved in the formation of inactive complexes of Calvin cycle enzymes during night was also characterized (Petersen et al. 2006). Cytosolic and muroplast-localized fructose-1,6-bisphosphate aldolases of class II were fractionated from *C. paradoxa* extracts as 90 and 85 kDa proteins, respectively (Gross et al. 1994). The latter was shown to be bifunctional for fructose-1,6-bisphosphate and sedoheptulose-1,7-bisphosphate cleavage (Flechner et al. 1999). The gene for the cyanelle enzyme was subject to phylogenetic analysis. It proved to belong to type B, hitherto not found in plastids, with *Synechocystis* sp. as the nearest neighbor (Nickol et al. 2000). The cDNA of pre-transketolase from *C. paradoxa* was sequenced. In a neighbor-net graph, the *Cyanophora* enzyme occupied a position intermediate to the plastid and cyanobacterial homologs. *In vitro* import of the precursor into muroplasts and pea chloroplasts was equally efficient (Ma et al. 2009). The single copy gene was downregulated upon shift to low $CO_2$ conditions (Burey et al. 2007)

## 2.7 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 higher plants during evolution (Deschamps et al. 2008). *C. paradoxa* starch showed a (high) amylose and amylopectin content with chain length distributions and crystalline organization similar to green algae and land plants. However, several starch synthase activities were found utilizing UDP-glucose, this time in analogy to rhodophytes that also synthesize starch in the cytosol. In addition, a multimeric isoamylase complex (in support of the proposed correlation between the presence of starch and of isoamylase) and multiple starch phosphorylases were demonstrated. 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). Recently, the transglucosidase DPE2 (disproportionating enzyme 2, transferring one glucose moiety from maltose to a cytosolic heteroglucan) from *C. paradoxa* could be demonstrated on zymograms. Despite the differential location of starch in the two cases, DPE2 is a cytosolic enzyme as in *Arabidopsis* (Fettke et al. 2009).

The properties of a number of transporters were studied in the muroplast inner envelope membrane: a phosphate translocator (TPT) similar to the chloroplast homolog and a glucose transporter (Schlichting and Bothe 1993) as well as a glutamine carrier with unusual features (Kloos et al. 1993) whereas a malate/oxaloacetate shuttle and an ATP/ADP translocator could not be demonstrated (Schlichting et al. 1990). The efficient triose phosphate (TP)/phosphate exchange resembles that observed for rhodophytes: in both cases starch is synthesized and stored in the cytosol, necessitating rapid export of photosynthate from the plastids, whereas the low-affinity plant TPTs allow for sufficient TP levels for starch biosynthesis inside of the chloroplasts (Linka et al. 2008). An interesting hypothesis postulates the insertion of host cell transporters into the endosymbiont plasma membrane as a primordial step in the establishment of endosymbioses: candidates are the TPT (Weber et al. 2006) and a transporter for ADP-glucose required for relocation of starch synthesis from the endosymbiont into the host cytosol (Deschamps et al. 2008).

## 2.8 The Case of the Eukaryotic Carboxysome

All cyanobacteria (Badger and Price 2003) and most algae (Giordano et al. 2005) possess an inorganic carbon-concentrating mechanism (CCM) that involves a microcompartment—carboxysomes in prokaryotes, pyrenoids in eukaryotes—harboring the bulk of cellular (plastidic) Rubisco. Inorganic carbon (bicarbonate) is concentrated at the very site where the bulk of Rubisco is involved in efficient $CO_2$ fixation, i.e., superior to the rate that soluble Rubisco acting below substrate saturation could achieve. Carbonic anhydrase (CA) is important for converting the "storage form" bicarbonate into the Rubisco substrate $CO_2$. CA is co-packaged with Rubisco in cyanobacterial carboxysomes whereas it localizes to the lumen of thylakoids traversing the pyrenoid in *C. reinhardtii*. The two microcompartment types also differ in size, number and morphology. Typically, one pyrenoid (diameter 500 nm–1 μm) of rounded shape without distinct contours is seen per plastid. In cyanobacteria, several smaller (diameter 100–200 nm) polyhedral carboxysomes with a hexagonal cross-section are found in the centroplasm, i.e., distant from the thylakoid membranes. Carboxysomes are never penetrated by thylakoids. At higher magnification, the quasicrystalline arrangement of the Rubisco matrix and the surrounding proteinaceous layer consisting of the shell proteins CcmK-N (Kerfeld et al. 2005) become apparent.

For *C. paradoxa*, the operation of a CCM was a matter of debate. Recently, microarray data revealing 142 $CO_2$-responsive genes (induced or repressed through a

shift from high to low $CO_2$ conditions), gas exchange measurements, and measurements of photosynthetic affinity provided strong support for a CCM in the muroplasts (Burey et al. 2007). The microarray results compared well with corresponding data on *Synechocystis* PCC6803 (Wang et al. 2004) and *C. reinhardtii* (Miura et al. 2004). Photosynthesis genes specifying subunits of photosystem I and II and Calvin cycle enzymes were downregulated, whereas genes for proteins expected to be involved in the CCM as carbonic anhydrases, the putative bicarbonate transporter LciA, Rubisco activase, and granule-bound starch synthase were upregulated (Table 2.3; Burey et al. 2007).

A recent hypothesis claims that glaucocystophyte muroplasts as the closest cousins to cyanobacteria among plastids contain "eukaryotic carboxysomes": bicarbonate enrichment within muroplasts should be considerably higher than in chloroplasts with their pyrenoid-based CCM. Thus, the stress-bearing function of the peptido-

**Table 2.3** A selection of $CO_2$-responsive genes in *Cyanophora paradoxa* (From Burey et al. 2007)

| Function | Gene | Protein |
|---|---|---|
| 1. Up-regulated upon shift to low [$CO_2$] | | |
| Photosynthesis (CCM) | *rca* | Rubisco activase[a] |
| | | Granule-bound starch synthase[a] |
| CCM | *CAH3-1?* | beta-CA (mitochondrial)[a] |
| | *CAH3-2?* | beta-CA (mitochondrial)[a] |
| | | CA (cytosolic?) |
| | LciA | Bicarbonate transporter[a] |
| ROS inactivating enzymes | Prdx1 | Peroxiredoxin1 |
| | cat1 | Catalase |
| | | Glutaredoxin |
| Protein degradation | UBC4 | Ubiquitin-conjugating enzyme E2 |
| | | Ubiquitin/ribosomal protein S27a |
| Chaperones | | Protein disulfide-isomerase |
| | | Peptidyl-propyl cis-trans-isomerase |
| 2. Down-regulated upon shift to low [$CO_2$] | | |
| Phycobilisome antenna | *cpcG* | Phycobilisome rod-core linker[b] |
| Calvin cycle | | Sedoheptulose-1,7-bisphosphatase[a,b] |
| | pgk | Phosphoglycerate kinase[ab] |
| | tktC | Transketolase[a,b] |
| Photosynthetic electron transport | psaC | PS I reaction center subunit II[a,b] |
| | psaK | PS I reaction centre subunit X[a,b] |
| | | PS I reaction center subunit XI[a,b] |
| | petJ | Cytochrome $c_6$ |
| Protein synthesis | | Translation elongation factor 1 beta-2[a] |
| | | Translation elongation factor 1 alpha[a] |
| Cytoskeleton | | Tubulin alpha-2[a] |
| | | Tubulin beta-1[a] |
| | | Tubulin gamma[a] |

[a] Paralleled in *C. reinhardtii* (Miura et al. 2004)
[b] Paralleled in *Synechocystis* sp. PCC6803 (Wang et al. 2004)

glycan layer (Pfanzagl et al. 1996), the other unique cyanobacterial heritage, would be indispensable (Raven 2003). An isolation method for muroplast "carboxysomes" was developed and the protein components other than Rubisco analysed by mass spectrometry. Rubisco activase was identified for the first time in a carboxysome-like microcompartment and corroborated by western blotting. Rubisco activase incorporation into isolated muroplast carboxysomes after *in vitro* import followed a time course and increased from approximately 15% at 7 min to 25% at 25 min (Burey et al. 2005; Fathinejad et al. 2008). Other protein bands yielded good mass spectra but no match to existing databases was found—the incomplete knowledge on *C. paradoxa* nuclear genes certainly is an obstacle.

The geological record indicates a decrease in [$CO_2$] and a concomitant increase in [$O_2$] during the Phanerozoic period about 400 million years ago, which certainly posed a problem for Rubisco-dependent $CO_2$ fixation. This led Badger and Price (2003) to propose a scenario different from Raven's: independent origin of the carboxysomal CCM in cyanobacteria and the pyrenoidal CCM in algae during that period.

Glaucocystophyte muroplasts contain a conspicuous, electron-dense aggregate of Rubisco (Mangeney and Gibbs 1987) of pyrenoid size in the center (only in *G. nostochinearum* at a polar position) of the coccoid organelles surrounded by multiple layers of concentric thylakoids (Kies 1992). For *C. paradoxa*, the muroplast nucleoid was shown to occupy the narrow space between the central body and the innermost thylakoids (Löffelhardt and Bohnert 2001). In no known case are these microcompartments traversed or penetrated by thylakoid membranes (Figs. 2.2, 2.3 and 2.4). They remain stable during muroplast division and are neatly bisected

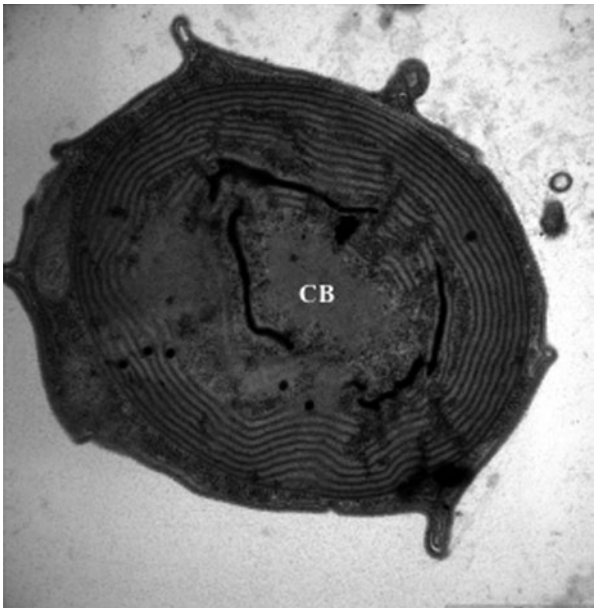


**Fig. 2.3** Transmission electron micrograph of a *Cyanophora* cell grown under high [$CO_2$] Electron-dense regions around the innermost thylakoids are notable. (scale bar, 1 μM). Chemically fixed and epoxy resin embedding. *CB*, central bodies; *Env*, envelope. Under high [$CO_2$] the contours of the CBs are not clearly defined. Electron-dense material is abundant among thylakoids proximal to the center. Cyanelle ribosomes appear around the central body (for Rubisco synthesis) and between the thylakoids (for phycobiliprotein synthesis)

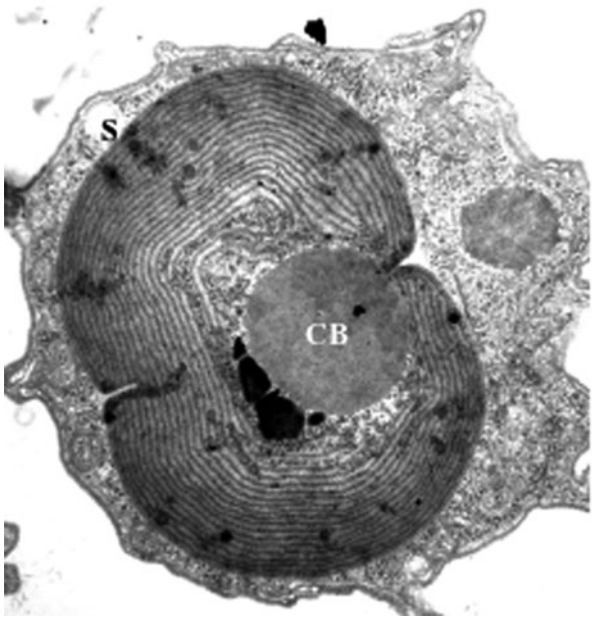


**Fig. 2.4** Transmission electron micrograph of a *Cyanophora* cell grown under low [$CO_2$] (scale bar, 1 μM). Chemically fixed and epoxy resin embedding. *CB*, central body; *S*, starch granule; The onset of septum growth is observed. Indication for a polyhedral shape of CB (*arrows*)

by the growing peptidoglycan septum (Fig. 2.2). In *C. paradoxa* and *G. nostochinearum* the microcompartment has a rounded shape though some polyhedral elements become apparent, especially under low $CO_2$ conditions. Then, the central bodies also appear to be enlarged (Fig. 2.4; Fathinejad et al. 2008). Ribosomes are now concentrated around the central body (involved in Rubisco synthesis, Fig. 2.4) whereas under high $CO_2$ conditions they are also abundant in the thylakoid area (involved in phycobiliprotein synthesis, Fig. 2.3). This may be consistent with a dynamic structure for the microcompartment which expands and shrinks according to $CO_2$ availability. In *Gloeochaete wittrockiana* and *Cyanoptyche gloeocystis* the single, large central bodies clearly are polyhedral (with a pentagonal cross-section) and are confined by a distinct, electron-dense layer (Kies 1992). This structure is suggestive of a proteinaceaous shell as found in cyanobacterial carboxysomes. According to Raven (2003), earlier low $CO_2$ episodes could have occurred beyond the 600 million years covered by the geological record. An acquisition of the CCM by cyanobacteria prior to the primary endosymbiotic event could explain the retainment of carboxysomes solely in the muroplasts of the most ancient glaucocystophytes whereas all other algae shifted towards a pyrenoidal CCM and lost the plastid peptidoglycan wall. A final decision between carboxysome or pyrenoid must await the identification of muroplast CA and, especially, the demonstration of shell proteins. Also, the internal accumulation of Ci within muroplasts under low $CO_2$ conditions has to be determined: is the concentration factor in the range of >1,000 (carboxysomal CCM) or just around 70 (pyrenoidal CCM)? Intermediate stages between carboxysomes and pyrenoids can also be envisaged for the muroplast microcompartments.

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