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The biodiversity of carbon assimilation

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A B S T R A C T

As all plastids that have been investigated so far can be traced back to endosymbiotic uptake of cyanobacteria by heterotrophic host cells, they accordingly show a high similarity regarding photosynthesis, which includes both the photosystems and the biochemical reactions around the CO_2 fixation via the Calvin–Bassham cycle. Major differences between the different algal and plant groups may include the presence or absence of carbon concentrating mechanisms, pyrenoids, Rubisco activases, carbonic anhydrases as well as differences in the regulation of the Calvin–Bassham cycle. This review describes the diversity of primary carbon fixation steps in algae and plants and the respective regulatory mechanisms.

Keywords:
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Rubisco

Contents

Introduction

All organismic life on earth is based on carbon molecules. Accordingly the enzymatic fixation of atmospheric or dissolved CO_2 into larger organic molecules was the evolutionary invention that allowed the production of biomass required for life. The first product of CO_2 fixation usually is 3-PGA which is converted into different types of carbohydrates which subsequently can be transformed into other substances that are essential for the cell, like lipids or amino acids. The carbohydrates themselves can be used either as low-osmotic polymeric storage products (Oren, 2007), as components of cell walls, or simply being secreted. Although archeae and eubacteria developed a number of different ways to fix CO_2 biochemically, it is the reductive pentose phosphate pathway in plants and algae, employing Rubisco as the key enzyme, which is mostly used for primary production (Berg, 2011). As Rubisco is a very ancient enzyme, one could expect that during the long evolutionary periods, there was sufficient time for evolution to optimize this enzyme regarding turnover rate and substrate specificity. However, research in the recent decades revealed a rather poor performance of this enzyme type, including a low turnover rate, a low affinity for CO_2 (Spreitzer, 1999), together with a tendency to react with oxygen (Ogren and Bowes, 1971) during an oxygenase reaction, resulting in subsequent energy-consuming photorespiration reactions (Ogren, 2003; Sage, 2013; Sage and Stata, 2015). Thus algae and plants had to develop strategies to overcome the limitations of Rubisco's enzymatic properties. One possible way to achieve this goal, is to increase the cellular enzymatic activity. Accordingly most organisms generate large amounts of Rubisco protein per cell to increase the total enzymatic activity (Ellis, 1979). Furthermore there are Rubisco types with varying catalytic and regulatory properties in different organismal groups (Tabita et al.,

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2008). Another obstacle especially for aquatic prokaryotic and eukaryotic organisms is that they may have to cope with potentially CO_2 limited conditions. The availability of dissolved inorganic carbon (DIC: CO_2 plus bicarbonate (HCO_3^-)) may depend for instance on the water depth or the water composition. While CO_2 can easily diffuse in air, CO_2 dissolved in water has a limited diffusibility. In seawater DIC mostly appears as bicarbonate (HCO_3^-), while in fresh water these ratios can be more variable (Zhang et al., 2014). As CO_2, and not bicarbonate, is the substrate of Rubisco, facilitated enzymatic conversion of HCO_3^- into CO_2 by carbonic anhydrases (CAs) is one way to increase CO_2 fixation by Rubisco, while the additional utilization of other carboxylating enzymes with either a higher affinity for CO_2 or specificity for bicarbonate represents another option. A detailed study on this option is given by Maberly et al. (2015). Processes involving carbonic anhydrases and a pre-fixation of CO_2 have been termed carbon concentrating mechanisms (CCMs) and they can already be found in the prokaryotic cyanobacteria (see Giordano et al., 2005; Meyer and Griffiths, 2013; Reinfelder, 2011). This review presents a general overview of the diversity of key elements of carbon fixation in plants and algae: the carboxylating enzymes (Rubisco), and the presence or absence of CO_2 concentrating mechanisms and pyrenoids in the different groups of oxygenic photoautotrophic organisms.

CO_2 fixation as a key process for the establishment of life on earth

There are numerous theories on how life on earth has evolved (Cavalier-Smith, 2001; Miller, 1953). The most recent theory proposes that the first cells originated from hot hydrothermal vents in the oceans in porous mineral material consisting of metal sulfides serving as redox catalysts (Martin and Russell, 2003; Russell et al., 2010). Important issues for the successful establishment of the earliest cells the creation of reactions chambers separated by biological membranes as well as the production of biomass allowing an increase of biological material by growth and division of cells (Lane et al., 2013). As reduced carbon is the most essential backbone of biological systems, the development of the first enzymes that allow the fixation of the – at that time (about up to 2–2.5 Ga ago, Raven et al., 2012) – highly abundant carbon dioxide must have been a major breakthrough. Two types of organisms capable of autotrophic CO_2-fixation are known, chemoautotrophic and photoautotrophic organisms (Erb, 2011). The first group uses chemical energy directly, while the second group utilizes the energy of sunlight in order to fix CO_2. Up to now a number of biochemical CO_2 fixing processes have been described that all perform different reactions. If the theory holds true that cells originated in hydrothermal vents as described above (Martin and Russell, 2003), then it is likely that the first carboxylases might have been related to those that perform the bacterial Wood–Ljungdahl (or reductive acetyl-CoA) pathway (Huber and Wächtershäuser, 1997; Wood, 1991). The respective enzymes of this pathway (carbon monoxide dehydrogenase/acetyl-CoA synthase) use H_2 to reduce carbon dioxide to carbon monoxide which in a second step is fixed to a methyl group forming acetyl-CoA (Ragsdale, 2008). This reaction is the only one of the known carboxylating reactions with a positive net ATP balance (Ragsdale, 2008). Other, probably later developed, pathways to fix CO_2 are the reductive citric acid cycle (Arnon–Buchanan cycle; Evans et al., 1966), the 3-hydroxypropionate bicycle (Zarzycki and Brecht, 2009), the hydroxypropionate/hydroxybutyrate cycle (Berg et al., 2007), the dicarboxylate/hydroxybutyrate cycle (Huber et al., 2008), and finally the Calvin–Bassham –Benson cycle (Bassham and Calvin, 1957), which is the essential pathway for photoautotrophic reactions. In addition to these metabolic enzymes, a number of other enzymes have been described that include assimilatory carboxylases (that merely have the function to introduce functional groups), anaplerotic carboxylases (like for instance within the TCA-cycle), biosynthetic carboxylases (generating building blocks for instance in fatty acid synthesis) and redox balancing carboxylases (in some bacteria) (see Erb, 2011). Although nature apparently developed the principle of creating biomass via carboxylases several times independently (Schada von Borzyskowski et al., 2013), the process of CO_2 fixation via the Calvin cycle using the enzyme ribulose-bisphosphate carboxylase (Rubisco) apparently had the strongest impact on the development of life on this planet especially when combined with oxygenic electron transport, like we do find it in cyanobacteria as well as in plastids of eukaryotic algae and land plants.

Evolution of eukaryotic photoautotrophs

Fossil records of bacterial biofilms indicate that unicellular organisms related to modern cyanobacteria might have been among the first organisms that coupled light driven biomass formation and CO_2 fixation with the cleavage of water resulting in a release of oxygen (Rasmussen et al., 2008). After the establishment of oxygenic photosynthesis the oxygen content of the oceans and the atmosphere did not increase instantly. Indeed it took another 1.5 billion years until the released oxygen was not instantly captured by reductive substances in the oceans and a net release of oxygen was possible (Blank and Sanchez-Baracaldo, 2010). Although CO_2 concentrations during that time of the early earth development, when cyanobacteria were the dominant oxygenic photoautotrophs, were still rather high (Raven et al., 2011), the modern cyanobacteria possess sophisticated CO_2 concentrating mechanisms that allow trapping of CO_2 within the cells and thus enhanced Rubisco efficiency (see below). There is a large biodiversity of cyanobacteria (Schirrmeister et al., 2013), but considering their restrictions as prokaryotes with regard to an asexual lifestyle and the absence of higher organized life forms, endosymbiotic processes involving cyanobacteria probably were the key events during evolution that allowed the development of higher organisms (Keeling, 2013). Photosynthesis as we know it from land plants thus obviously is an invention of the cyanobacteria (Archibald, 2009) and apparently has never been developed de novo in eukaryotes (at least that we know of from extant organisms). Instead, eukaryotic cells took up cyanobacteria via an endosymbiotic process (Fig. 1) and converted them into organelles (Bhattacharya et al., 2007; Cavalier-Smith, 2013). It is more or less consensus today, that all studied plastids in algae and plants (with one exception, *Paulinella chromatophora* (Nowack et al., 2008)) can be derived from a primary endosymbiosis in which a eukaryotic host cell took up a cyanobacterium and converted it into an organelle (Delwiche and Palmer, 1997). Additionally, secondary and tertiary endosymbioses occurred in which eukaryotic algae, either ancestors of green algae or red algae, or even diatoms, haptophytes, or cryptophytes had been taken up by eukaryotic host cells (see Fig. 1 and also Keeling, 2013 for a current view on these processes). Accordingly plastids have been transmitted again and again during the endosymbiotic processes, thus it is not too surprising to see that all the plastids in general show very similar photosystems and photosynthetic reactions. Smaller differences here are mostly due to adaptations to environmental conditions or to genetic or biochemical modifications. Regarding for instances the regulation of Rubisco and the subsequent redox regulation of the reactions of the Calvin–Bassham cycle, there may be substantial differences in different algal groups (see Wilhelm et al., 2006; Michelet et al., 2013; Mekhalfi et al., 2014).

Rubisco as the key player of photosynthesis

In total, about 10^{17} g (100 Gt) of CO_2 are supposed to be converted into biomaterials and organic compounds per year (Field et al., 1998). All oxygenic photosynthetic organisms utilize Rubisco enzyme for CO_2 fixation instead of the other five autotrophic

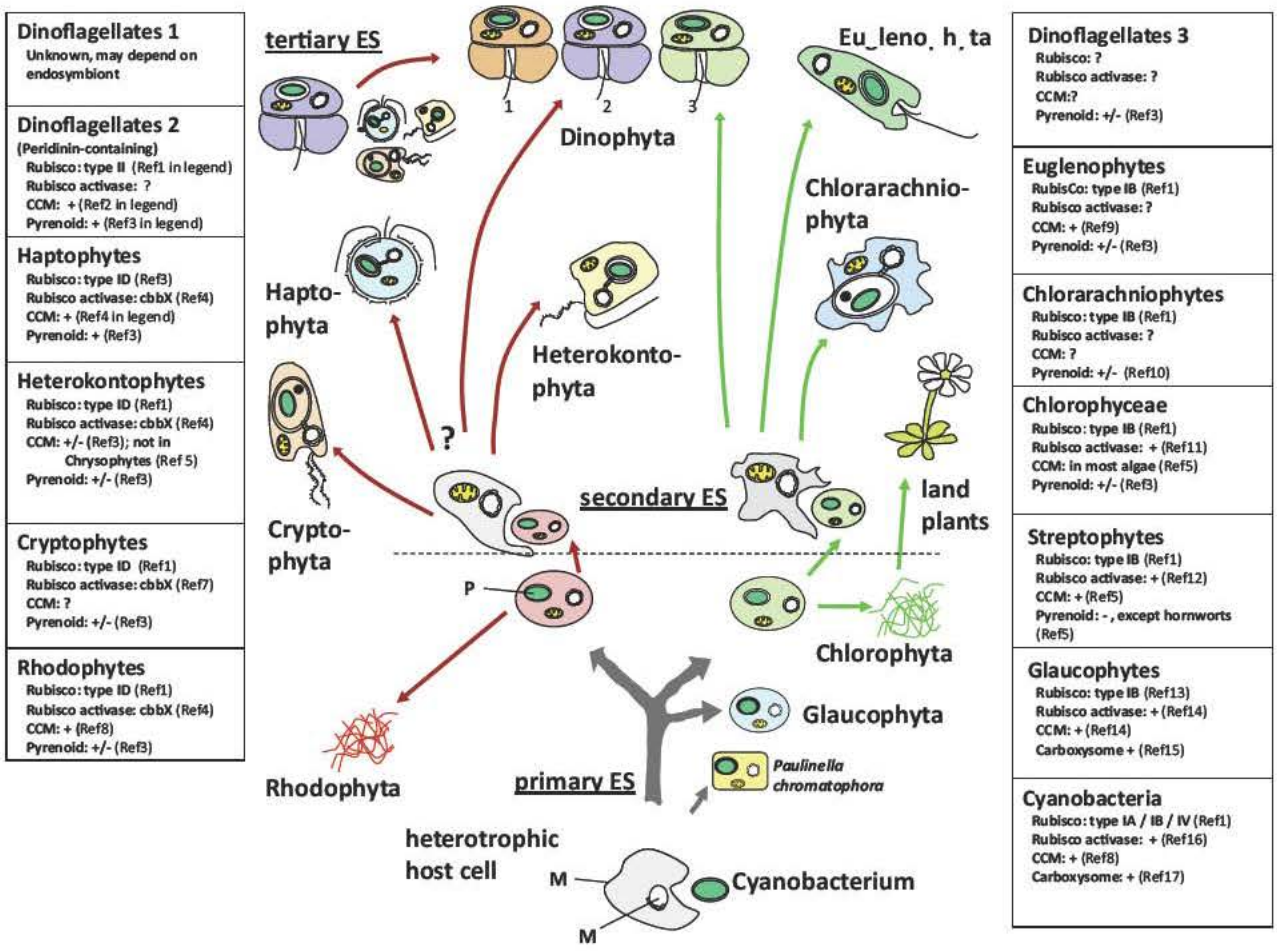

Fig. 1. Schematic scenario of major endosymbiotic events in plastid evolution and the respective algal groups. Primary plastids originated via the endosymbiotic integration of a cyanobacterium into a heterotrophic host cell (bottom of the figure), resulting in plastids with two surrounding membranes in glaucophytes (which retained their peptidoglycan wall between the two plastid membranes), red algae, green algae, and their close relatives, the land plants. Both, ancestors of modern red algae and green algae, served as endosymbionts for secondary endosymbioses in which eukaryotic host cells took up the respective eukaryotic algae. The number of secondary endosymbioses is under debate, it is unclear yet whether for instance the cryptomonad and haptophyte plastids originated from the same endosymbiosis as those of stramenopiles and alveolates (the latter consisting of ciliates, apicomplexans, and dinoflagellates), which is pointed out by the question mark labeling the secondary endosymbiosis step (see also Keeling, 2013). Dinoflagellates consist of three different groups that possibly all originally possessed red type plastids (Peridinin-containing dinoflagellates, labeled group 2), some of which apparently have been subsequently replaced by either endosymbiotic haptophytes, cryptophytes or diatoms (group 1) or by a green alga (group 3). On the, those algae are shown that have evolved by uptake of a green alga, inducing the euglenophytes and the chlorarachniophytes. There so far is a single example for an independent primary endosymbiotic origin of plastids in the amoeba *Paulinella chromatophora* (Nowack et al., 2008). For the major groups of algae and streptophytes the following parameters are shown: type of Rubisco, presence/absence of Rubisco activase, presence of absence of CCM in that group, presence of absence of a pyrenoid. References in Fig. 1: Ref1: Tabita et al. (2008); Ref2: Ratti et al. (2007); Ref3: Badger et al. (1997); Ref4: Fujita et al. (2008); Ref5: Meyer and Griffiths (2013); Ref6: Maberly et al. (2009); Ref7: Maier et al. (2000); Ref8: Raven et al. (2012); Ref9: Diaz and Maberly (2009); Ref10: Ota et al. (2009); Ref11: Pollock et al. (2003); Ref12: Carmo-Silva and Salvucci (2013); Ref13: Uchino and Yokota (2003); Ref14: Bhattacharya et al. (2014); Ref15: Raven (2003); Ref16: Li et al. (1999); Ref17: Badger and Price (2003). Abbreviations: ES: endosymbiosis; M: mitochondrium; P: plastid; N: nucleus.

pathways described above. Accordingly more than 99% of all organic carbon is fixed by Rubisco (Berg, 2011). Here, catalyzed by Rubisco, the tautomeric enediol form of the sugar bisphosphate ribulose 1,5-bisphosphate (RubP) first accepts a molecule of CO_2 resulting in intermediate 2-carboxy-3-keto-arabinitol,1,5-bisphosphate. After hydratization, this intermediate forms two molecules of 3 phophoglycerate. At least four different types of RubisCo enzyme complexes are known and termed forms I, II, III, IV. They can be found in cyanobacteria as well as in algal and land plant chloroplasts, but also in different types of eubacteria and archaea. The differentiation into different forms is based on differences in their primary sequences and their type of multi subunit complexes (Tabita et al., 2008; Tabita, 1999).

The enzymes of most algal and higher plant chloroplasts have been termed form I-B and like all type I enzymes, they consist of eight large and eight small subunits (L_8S_8) (Spreitzer and Salvucci, 2002; Tabita et al., 2008). This Ib enzyme apparently has evolved directly from the respective cyanobacterial form I enzyme (Badger et al., 1997). Some cyanobacteria also possess a green form IA Rubisco which is related to proteobacteria. Red algae and those algal groups that developed via secondary endosymbiosis by uptake of a red alga (like the chrysophytes, brown algae, diatoms, haptophytes or some dinoflagellates), possess a form ID Rubisco which is phylogenetically related to α/β purple bacteria like *Ralstonia eutropha*. However, in most dinoflagellates, such as *Amphidinium carterae*, a much more different type of Rubisco is found which is termed type II (Morse et al., 1995) and which apparently was obtained by lateral gene transfer. The respective complexes here consist of large subunits only. The Rubisco types III and IV have been discovered recently in archaea and some green sulphur phototrophic bacteria (Finn and Tabita, 2003). While the type III Rubisco apparently has a similar functionality as I and II enzymes, type IV Rubisco is not capable of RuBP dependent CO_2 fixation (Watson et al., 1999), accordingly they have also been

termed Rubisco-like proteins (Tabita et al., 2007). Rubisco enzymes in cyanobacteria, algae and land plants can be regulated on different levels (Spreitzer and Salvucci, 2002), among others via (i) ions like Mg^{2+}, which during illumination are moved out the thylakoid lumen into the stroma, (ii) the presence of carbon dioxide, (iii) pH, and (iv) Rubisco activase (Sage et al., 2008), which together with Rubisco can be activated by reduced thioredoxin (Mueller-Cajar et al., 2014), and a number of metabolites. In land plants, genes encoding the large and the small subunits are located in different compartments: *RbcL*, encoding the large subunit is on the plastid genome, while *RbcS* is found in the nucleus. In red algae and algae deriving from red algae by secondary endosymbiosis both genes are still on the plastid genome forming an operon (Hwang and Tabita, 1991). Interestingly, no genes for a Rubisco activase have been found so far in these algae, while genes encoding CbbX proteins have been identified instead. The CbbX protein, an AAA+ ATPase unrelated to Rubisco activase, originally has been discovered to have Rubisco activase activity in the α-proteobacterium *Rhodobacter sphaeroides* (Mueller-Cajar et al., 2011).

Carbon concentrating mechanisms

CO_2 is one basic substrate required for photosynthesis. Plants dwelling on land and algae living in water have different problems regarding their CO_2 supply. Most plants can easily take up CO_2 from the atmosphere via their stomata. However, during water limitation, due to closure of the stomata, CO_2 entry can be limited (Meyer and Griffiths, 2013). Algae have a different problem. Living submerged, the accumulation of inorganic carbon from the water by algae is limited by the diffusion rate of carbon dioxide (CO_2) in water, the potentially limited availability of CO_2 in surface waters with high photosynthesis rates and the dehydration kinetics of bicarbonate to CO_2 (Reinfelder, 2011). Interestingly plants and algae both evolved inorganic carbon (or CO_2) concentrating mechanisms (CCMs) which may increase the supply of CO_2 to Rubisco. Here, basically the first step of carbon fixation involves the formation of a C4 compound or organic acid using enzymes like the phosphoenolpyruvate carboxylase (PEPC), which have a higher substrate affinities than Rubisco as well as no oxygenase properties. In plants, some specialized CCMs are found, being termed C4 and CAM (crassulacean acid metabolism) (Wang et al., 2014), based on a spatial (mesophyll and bundle sheath cells) or a temporal (day and night) separation of the individual carbon fixing reactions, respectively. Both processes, C4 photosynthesis and CAM, rely on a first fixation of HCO_3^- to an organic molecule and a subsequent decarboxylation and release of CO_2 in close proximity to Rubisco, where it may increase Rubisco activity (Bräutigam et al., 2014). In C4 plants, with the exception of single-cell C4 photosynthesis (Edwards et al., 2004), this CO_2-concentrating mechanism usually requires compartmentalized photosynthetic reactions in two distinct cell types. The product generated by PEPC, a C4 acid, is subsequently converted to either malate or aspartate in bundle sheath cells and then translocated to mesophyll cells where it is decarboxylated in close proximity to Rubisco. Certain variations of this process are known in different plants (Wang et al., 2014). In algae biophysical or biochemical CCMs may vary between different algae and may involve a combination of inorganic carbon transporters, which require ATP, NADPH or an ion gradient as energy source, carbonic anhydrases (CAs) and confinement of Rubisco to a micro-compartment within the plastid (Raven et al., 2012). The transporters are required to build up intracellular pools of bicarbonate, which by CAs can be converted to CO_2 based on the equilibrium distribution. In cyanobacteria, the Rubisco is mostly confined to carboxysomes, small proteinaceous structures that are surrounded by polyhedral protein shells about 80 to 140 nanometres in diameter (Rae et al., 2013). In a number of eukaryotic algae, but not in all, Rubisco can be found in one or more pyrenoids (Raven et al., 2012) within the plastids (see below). Interestingly, for the unicellular diatoms, it was reported that they may possess a biochemical C4 pathway (Reinfelder et al., 2000). There is, however, still some disagreement on how relevant this process may be in this algal group. There is experimental evidence that a C4-type CCM operates in at least one marine diatom, *Thalassiosira weissflogii* (Roberts et al., 2007b), while for other diatoms there are contradictory results (Granum et al., 2009; McGinn and Morel, 2008; Roberts et al., 2007a). In *Phaeodactylum tricornutum* silencing of the enzyme pyruvate-orthophosphate dikinase (PPDK), usually involved in C4 pathways, did not affect the photosynthetic properties of the alga (Haimovich-Dayan et al., 2013). However, due to the limited number of species investigated and methodological differences between the studies direct comparisons are complicated. Although diatoms may contain a number of carboxylating enzymes in different compartments (Kroth et al., 2008), no decarboxylating enzyme has yet been identified in diatom plastid stroma, thus in the same compartment as the Rubisco. In a single group algae, the chrysophytes, no CCM was detected at all, probably restricting the distribution of these algae to niches with sufficient CO_2 availability (Maberly et al., 2009). Generally, it is interesting to observe that different kinds of CCMs with different operational modes can be found within nearly all groups of photosynthetic organisms (Fig. 1). This raises the question whether the poor enzymatic performance of Rubisco might have triggered the development of these processes or whether environmental changes, both on the global or the long term scale, but also microclimate conditions may have induced them, thereby increasing the competitiveness of those organisms that possess such a CCM.

Pyrenoids

Pyrenoids are crystal-like proteinaceous structures that are found in the plastids of a number of eukaryotic algae and in a single group of land plants, the hornworts. Interestingly they are no indicators for specific groups as they have been found in all major algal taxa, while they may be missing in a number of organisms from the same lineage (see Badger et al., 1997). A recent study implies five to six origins and an equal number of subsequent losses of pyrenoids in hornworts (Villarreal and Renner, 2012). Pyrenoids have been studied on the morphological level mostly by electron microscopy but their biochemical structure and the function of the pyrenoids are still largely unknown. It has been suggested that they may play an important role in carbon concentrating mechanisms (Moroney and Ynalvez, 2007) by separating Rubisco from the CA in the stroma of the chloroplast. Pyrenoids can have very diverse morphologies, from simple aggregates of Rubisco to more complicated structures like thylakoids spanning through the pyrenoid body. For instance in the marine diatom *P. tricornutum* the structure of the pyrenoid has been reported as a lens-shaped body residing in the central part of the stroma with two layered thylakoid membranes that penetrate the center of the pyrenoid. Recently it was demonstrated that in *Chlamydomonas reinhardtii* the small subunit of Rubisco is involved in pyrenoid formation (Meyer et al., 2012). The unifying feature of pyrenoids is the presence of large amounts of Rubisco, for instance in *C. reinhardtii* most of Rubisco protein is located in the pyrenoid (Borkhsenious et al., 1998). Similarly, pyrenoids of *Chlorella pyrenoidosa* (McKay and Gibbs, 1989) or *Euglena gracilis* (Osafune et al., 1990) have been shown very early to represent the major amount of Rubisco within the plastids. It seems obvious that other proteins may also be found in pyrenoids, although a proteomic inventory is still lacking. However, in addition to Rubisco, also nitrate reductase (NR) (Lopez-Ruiz et al., 1991), Rubisco activase (McKay et al., 1991), and the LCIB/C complex (Wang and Spalding, 2006; Yamano et al., 2010) have been localized in the pyrenoids of *C. reinhardtii*. The LCI (low-CO_2 (LC)-inducible) genes have recently been demonstrated to be present in

a number of different eukaryotic algae including diatoms (Yamano et al., 2010). Two of the respective proteins LCIB and C have been shown to interact and furthermore have been localized in the vicinity of the pyrenoid structure of *C. reinhardtii* (Yamano et al., 2010), possibly shielding the pyrenoid from CO_2 leakage or recapturing leaking CO_2 from the pyrenoid (Mitra et al., 2004; Yamano et al., 2010).

Furthermore recently two plastidic fructose-1,6-bisphosphate aldolases (FBAs) have been shown to reside in the pyrenoid of the diatom *P. tricornutum* (Allen et al., 2011). As the enzymatic FBA reaction is one of the primary rate limiting factors of the Calvin cycle in higher plants this indicates that diatom pyrenoids also play a central role in controlling carbon metabolism. Although an involvement of pyrenoidal carbonic anhydrases (CA) in photosynthesis has been proposed earlier in algae with CCMs, the CAs PtCA1 and 2 are the first CAs to be verified in pyrenoids (Kitao et al., 2008; Matsuda and Kroth, 2014).

Conclusions

Considering the rich biodiversity of photosynthetic organisms from bacteria and unicellular algae to multicellular plants and from picoplankton to huge and massive trees, the process of carbon fixation and of photosynthesis is astonishingly well conserved. Starting from the orientation and functionality of the dual photosystem arrangements over Rubisco functionality to the reactions of the reductive pentose phosphate pathway, there is a high general similarity of the processes. Although it appears obvious that certain components, like the CbbX protein (replacing the Rubisco activase in red algae and diatoms), and the type II Rubisco found in some dinoflagellates, are substituting original proteins, these changes do not severely affect the general principle of photosynthesis. Considering that both, cyanobacteria and plastids, over the long period since primary endosymbiosis developed independently and under completely different conditions, this impressively demonstrates how highly developed both the functionality and the efficiency of the photosynthetic apparatus in the cyanobacteria must have been already before plastids have been established.

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