How do algae concentrate CO$_2$ to increase the efficiency of photosynthetic carbon fixation?

James V. Moroney  
*Louisiana State University*

Aravind Somanchi  
*Louisiana State University*

Follow this and additional works at: [https://digitalcommons.lsu.edu/biosci_pubs](https://digitalcommons.lsu.edu/biosci_pubs)

**Recommended Citation**  

This Article is brought to you for free and open access by the Department of Biological Sciences at LSU Digital Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Digital Commons. For more information, please contact ir@lsu.edu.
How Do Algae Concentrate CO₂ to Increase the Efficiency of Photosynthetic Carbon Fixation?¹

James V. Moroney* and Aravind Somanchi

Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803

The ability of photosynthetic organisms to use CO₂ for photosynthesis depends in part on the properties of Rubisco. Rubisco has a surprisingly poor affinity for CO₂, probably because it evolved in an atmosphere that had very high CO₂ levels compared with the present atmosphere. In C₃ plants the Kₘ(CO₂) of Rubisco ranges between 15 and 25 μM. In cyanobacteria Rubisco has an even lower affinity for CO₂ and the Kₘ(CO₂) can be greater than 200 μM. In comparison, the concentration of CO₂ in water in equilibrium with air is approximately 10 μM. From these numbers it becomes apparent that Rubisco is operating at no more than 30% of its capacity under standard atmospheric conditions. This is one of the reasons that C₃ plants contain such large amounts of Rubisco. Exacerbating this situation is the fact that O₂ is a competitive substrate with respect to CO₂.

In the atmosphere, where the O₂ level is 21% and the CO₂ level is 0.035%, the competition by O₂ accounts for as much as 30% of the reactions catalyzed by Rubisco. A number of photosynthetic organisms have developed ways to increase the level of CO₂ at the location of Rubisco in the plant. This results in an increase in CO₂ fixation and a decrease in the deleterious oxygenation reaction. An excellent example of a CO₂-concentrating mechanism in higher plants is C₄ photosynthesis, which has arisen independently in a number of plant families. Aquatic photosynthetic organisms such as the microalgae have also adapted to low CO₂ levels by concentrating CO₂ internally. This Update will focus on CO₂-concentrating mechanisms in the microalgae. For more detailed reviews of the CO₂ concentration by algae, the reader is referred to the special issue of the Canadian Journal of Botany (1998, Vol. 76) and the article by Raven (1997).

**TYPES OF CO₂-CONCENTRATING MECHANISMS AND THE PROBLEM OF LEAKAGE OF ACCUMULATED CO₂**

C₄ plants are the best-studied organisms that concentrate CO₂ to enhance the carboxylation reaction of Rubisco. They have high levels of PEPC carboxylase in leaf mesophyll cells, whereas Rubisco is located primarily in the bundle-sheath cells. CA within the mesophyll converts CO₂ entering the leaf into HCO₃⁻, which is the substrate for PEPC carboxylase. The advantages that PEPC carboxylase has over Rubisco are its high affinity for HCO₃⁻ and its insensitivity to O₂. At physiological CO₂ levels and pH, the HCO₃⁻ concentration in the cytoplasm of mesophyll cells is about 50 μM, whereas the Kₘ(HCO₃⁻) of PEPC carboxylase is estimated to be about 8 μM. Therefore, in contrast to Rubisco, PEPC carboxylase is saturated for HCO₃⁻ at ambient CO₂ levels. To finish the CO₂-concentrating effect of C₄ metabolism, the C₄ acid generated in the mesophyll cells is then transported to the bundle-sheath cells and decarboxylated, creating an elevated CO₂ level specifically within these cells.

The problem faced by all photosynthetic organisms that concentrate CO₂ is that it can easily diffuse through biological membranes. How can such a slippery substance be accumulated? In C₄ plants CO₂ is concentrated in specific bundle-sheath cells within the leaf. These are the only cells containing significant amounts of Rubisco. Here the thickened cell walls of the bundle sheath prevent the diffusion of the CO₂ generated by decarboxylation reactions. Microalgae face an additional problem in that they are composed of only one or a few cells, all with ready access to the environment; therefore, they must prevent the diffusion of CO₂ out of the cell while allowing the entry of other nutrients.

Microalgae overcome the problem of CO₂ diffusion by accumulating HCO₃⁻. Being a charged species, HCO₃⁻ diffuses through membranes much more slowly than CO₂. However, because CO₂ is the substrate required by Rubisco, the accumulated HCO₃⁻ must be converted to CO₂ before C₁ fixation takes place. This appears to be accomplished by packaging Rubisco within the algal cell and generating the CO₂ at that location through the action of a CA. A locally elevated CO₂ environment is thereby created in which CO₂ can out-compete O₂ at the active site of Rubisco. This allows the CO₂ to be used for photosynthesis before it can diffuse out of the cell. Thus, microalgae that concentrate CO₂ package Rubisco in a very specific location, have a means of concentrating HCO₃⁻, and have a means of converting the accumulated HCO₃⁻ to CO₂ rapidly at the location of Rubisco.

*Corresponding author; e-mail btmoro@unix1.sncc.lsu.edu; fax 1-504-388-8499.

Abbreviations: ABC, ATP-binding cassette; CA, carbonic anhydrase; C₄, inorganic carbon.

¹ This work was supported by National Science Foundation grant no. IBN-9632087.
THE LOCATION OF RUBISCO IN MICROALGAE

In higher plants Rubisco appears to act largely as a soluble protein that is distributed throughout the chloroplast stroma. By analogy, one might expect eukaryotic algae to have Rubisco throughout their chloroplast stroma and cyanobacteria to contain Rubisco throughout their cytoplasm, but this is clearly not the case. In most microalgae Rubisco is concentrated in a specific location: in carboxysomes in cyanobacteria and in the pyrenoid in algae (Fig. 1; Table I). Recent studies support the hypothesis that Rubisco localization is required for efficient acquisition of environmental CO₂.

Carboxysomes are electron-dense particles that are surrounded by a protein shell. Evidence that they contain large amounts of Rubisco is extensive. In fact, isolated carboxysomes have been found to be composed mostly of Rubisco (Price et al., 1992). Immunolocalization studies using antibodies raised against Rubisco indicate that the carboxysome is the primary location in cyanobacteria (McKay et al., 1993). A mutation that causes a 30-amino acid extension of the Rubisco small subunit leads to a Rubisco that does not pack into the carboxysome, which leaves the carboxysome empty (Schwarz et al., 1995). Mutations in any of the genes affecting the assembly, functioning, or shape of the carboxysome result in cells that cannot grow on air levels of CO₂ (Price et al., 1998).

Rubisco is also packaged in microalgae, where it is the major protein component of the pyrenoid. Pyrenoids have been purified from both Eremosphera (Okada, 1992) and Chlamydomonas reinhardtii (Kuchitsu et al., 1991), and in both cases they consisted primarily of Rubisco. In addition, C. reinhardtii cells with a mutation of the rbcL gene (Rubisco large subunit) that leads to a truncation of the large subunit of Rubisco have no pyrenoids (Rawat et al., 1996). Although it is accepted that Rubisco is the major constituent of the pyrenoid, there are conflicting findings regarding what percentage of the cell’s Rubisco is in the pyrenoid. A recent report by Borksenious et al. (1998) demonstrated that in C. reinhardtii the amount of Rubisco in the stroma varies with growth conditions.

In all published immunolocalization studies the pyrenoid is densely labeled when an anti-Rubisco antibody is used as the primary probe (Borksenious et al., 1998). An example of this immunogold labeling is shown in Figure 1D. In these studies the amount of Rubisco in each subcellular location was estimated by multiplying the density of particles (particles per area) in that location by the average volume of the pyrenoid (2.4 mm³) or the stroma (35.6 mm³) (Lacoste-Royal and Gibbs, 1987). However, this still leaves a fairly broad range of estimates for the amount of Rubisco in the pyrenoid, from 50% to 99%. These differences could be attributed to the growth regime used by the various
research groups. Borkhsenious et al. (1998) found that the amount of Rubisco in the stroma varied with growth conditions: about 50% of the Rubisco was localized to the pyrenoid in *C. reinhardtii* cells grown on elevated CO₂ (5%, v/v). In contrast, they reported that when *C. reinhardtii* cells were grown under low CO₂ (ambient levels of CO₂ are considered low) more than 90% of the Rubisco was located in the pyrenoid. These results are consistent with those of Morita et al. (1997), who reported that 99% of the Rubisco was located in the pyrenoid in cells grown with ambient levels of CO₂.

*C. reinhardtii* concentrates CO₂ only when it is grown under low-CO₂ conditions. Because more than 90% of the Rubisco is localized to the pyrenoid under low-CO₂ conditions, one question is whether pyrenoidal Rubisco is active in CO₂ fixation or whether the pyrenoid is a storage body. In vitro measurements of Rubisco activity imply that the enzyme in the pyrenoid must be active to account for the levels of CO₂ fixation observed in *C. reinhardtii*. A specific localization of Rubisco to the pyrenoid is also compatible with the view that organisms that have CO₂-concentrating mechanisms specifically package Rubisco. In lichens and bryophytes there is a good correlation between the operation of a CO₂-concentrating mechanism and the presence of a pyrenoid (Smith and Griffiths, 1996). In cyanobacteria it appears that the CO₂ level is elevated within the carboxysome (Price et al., 1998), thus favoring carboxylation activity over the oxygenation activity of Rubisco. The pyrenoid may serve a similar function in *C. reinhardtii* and other microalgae.

### The Accumulation of HCO₃⁻

The physiological evidence for the existence of CO₂ concentration in microalgae is 2-fold. First, algae are very efficient at pulling CO₂ out of the environment. They are much more efficient than would be expected, with cells showing an apparent affinity for CO₂ of about 1 μM versus the *Kₐ*₈(CO₂) of Rubisco of about 20 μM. In some cases the growth conditions of the alga influences the cell’s affinity for CO₂. Some species of algae, when grown on elevated CO₂ concentrations (10 times higher than ambient), are not efficient in their acquisition of CO₂ (Matsuda et al., 1998). However, if these same algae are grown on limiting CO₂ they become very efficient in CO₂ uptake and fixation. This implies that there are inducible transport mechanisms, because the amount of Rubisco does not change during adaptation from high- to low-CO₂ conditions.

Second, the accumulation of CO₂ within the cell can be measured directly. In the light, cyanobacteria can concentrate HCO₃⁻ within the cell more than 100-fold (Miller et al., 1990). Eukaryotic algae are not as efficient but can accumulate HCO₃⁻ at least 20-fold over ambient CO₂ levels. CO₂ transporters and CA's may enable the cells to accumulate HCO₃⁻ within the cell. The exact identity of the CO₂ transporters is still unknown, but recent work has identified some transporters that may play a significant role in the accumulation of CO₂ (Okamura et al., 1997).

In cyanobacteria difficulty in obtaining CO₂ and HCO₃⁻-transport mutants has been proposed to indicate the presence of multiple transporters for CO₂ and HCO₃⁻. There is physiological evidence for three types of transporters: (a) a Na⁺-independent HCO₃⁻ transporter, (b) a Na⁺-dependent HCO₃⁻ transporter, and (c) a CO₂ transporter.

Na⁺-independent HCO₃⁻ transport under extreme CO₂ limitation (Espie and Kandasamy, 1992) and a difference in the magnitude of the requirement of Na⁺ for CO₂ transport versus CO₂ transport (Miller et al., 1990) have been detected in *Synechococcus PCC 7942*. These data indicate the presence of either a Na⁺/HCO₃⁻ symporter (Espie and Kandasamy, 1994) or the regulation of pH through Na⁺/H⁺ antiport mechanisms.

A mutant of *Synechococcus PCC 7942*, M42, has been shown to have a reduced affinity for HCO₃⁻. The mutation in M42 has been shown to be in the gene cluster *tnpABCD*, which codes for a Na⁺-independent, high-affinity HCO₃⁻ transporter induced under low CO₂ (Okamura et al., 1997). This is the first reported primary transporter for HCO₃⁻, and belongs to the subfamily of ABC transporters also known as traffic ATPases (Higgins, 1992). The presence of an ABC-type transporter indicates that at high pH, when HCO₃⁻ is taken up, ATP may be the energy source for CO₂ uptake. A high-CO₂-requiring mutant of *Synechococcus PCC 7942* has recently been characterized; it has a lesion in the gene *dc14* (Ronen-Tarazi et al., 1998), which encodes a putative Na⁺-dependent HCO₃⁻ transporter. This transporter may be responsible for the fast induction response to low CO₂ reported from *Synechococcus PCC 7942* and *Synechocystis PCC 7002* (Süldtemeyer et al., 1997).

Much less is known about the transport of CO₂ in microalgae. Extracellular CO₂ has to pass through at least two membrane systems to reach the site of carboxylation, which makes transport more complex than in cyanobacteria. At least two types of CO₂ uptake can be observed in microalgae. There is evidence for both direct transport of HCO₃⁻ and CA-facilitated diffusion of CO₂ across the membrane. The two membranes that we will consider as possible sites of CO₂ transport are the plasma membrane and the chloroplast envelope.

### Table I. Location of Rubisco in organisms with different types of photosynthesis

<table>
<thead>
<tr>
<th>Photosynthesis Type</th>
<th>Ability to Concentrate CO₂</th>
<th>Rubisco Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₃ photosynthesis (higher plants)</td>
<td>No</td>
<td>Chloroplast stroma of most cells in leaf</td>
</tr>
<tr>
<td>C₄ photosynthesis (higher plants)</td>
<td>Yes</td>
<td>Chloroplast stroma of bundle-sheath cells</td>
</tr>
<tr>
<td>Eukaryotic microalgae</td>
<td>Yes</td>
<td>Pyrenoid of the chloroplast</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Yes</td>
<td>Carboxysomes</td>
</tr>
</tbody>
</table>
At the plasma membrane there is evidence for both HCO$_3^-$ uptake and CA-facilitated diffusion. In Scenedesmus obliquus there is very good evidence that HCO$_3^-$ is taken up directly by the cell (Thielmann et al., 1990). These cells can photosynthesize even when the pH is greater than 10 and HCO$_3^-$ and CO$_2^-$ are the major C$_i$ species. Chlorella saccharophila also appears to take up HCO$_3^-$, although CO$_2$ is its preferred C$_i$ source (Williams et al., 1995).

The other major process by which microalgae take up C$_i$ is through the uptake of CO$_2$. Many microalgae produce large amounts of CA when grown on limiting CO$_2$ (Raven, 1997). CA is a zinc metalloprotein, often located in the periplasmic space of the cell, that catalyzes the interconversion of CO$_2$ and HCO$_3^-$ according to the following formula:

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \]

Genes encoding periplasmic CAs have been identified in both Dunaliella salina and C. reinhardtii (Fujiwara et al., 1990). CA1, the periplasmic CA, has been identified as one of the prominent low-CO$_2$-inducible proteins in C. reinhardtii. The ability of microalgal cells to use external HCO$_3^-$ for photosynthesis has been correlated with the presence of periplasmic CA. The presence of external CA inhibitors decreased the use of external C$_i$ for photosynthesis (Moroney et al., 1985). The periplasmic CA probably increases the efficiency with which the cells can take in external C$_i$. This includes both the supply of CO$_2$ for diffusion across the plasma membrane and the supply of HCO$_3^-$ for the plasma membrane’s HCO$_3^-$-transport system.

The chloroplast envelope is another possible location of HCO$_3^-$ accumulation (Beardall, 1981). Intact chloroplasts isolated from C. reinhardtii and Dunaliella tertiolecta retain the ability to accumulate HCO$_3^-$ when grown on low CO$_2$ and have the ability to concentrate CO$_2$. At low CO$_2$, C. reinhardtii induces the synthesis of LIP-36, a transport protein that is localized to the chloroplast envelope (Chen et al., 1997). LIP-36 belongs to a family of transport proteins that often act as exchangers (e.g. ATP for ADP transporters). It is possible that LIP-36 plays a role in HCO$_3^-$ accumulation by the chloroplast, because chloroplasts with LIP-36 accumulate HCO$_3^-$ and those without LIP-36, isolated from high-CO$_2$-grown cells, do not. The fact that LIP-36 is encoded by two separate genes (Chen et al., 1997) has made it difficult to obtain mutants devoid of this protein.

THE GENERATION OF CO$_2$ AT THE LOCATION OF RUBISCO

The generation of CO$_2$ at the location of Rubisco is accomplished through the action of a CA located at or near Rubisco. In cyanobacteria a CA is localized to the carboxysome (Price et al., 1992). Carboxysomes purified from Synechocystis species have significant CA activity. In Synechocystis 6803, for which the complete genome has been sequenced, only one CA gene has been identified. The role of this CA is the dehydration of accumulated HCO$_3^-$ to form a localized, elevated concentration of CO$_2$ in the carboxysome. Loss of the carboxysomal CA through mutation leads to a cell that cannot grow well on limiting levels of CO$_2$ (Fukuzawa et al., 1992). In addition, cells missing the carboxysomal CA actually accumulate HCO$_3^-$ to higher levels than wild-type cells, presumably because the cell can no longer convert the HCO$_3^-$ to CO$_2$ for photosynthesis. In these CA-deficient cells, the CO$_2$-concentrating mechanism is still operational, but the final conversion of HCO$_3^-$ to CO$_2$ is too slow.

It is noteworthy that CA activity is not found in the cytoplasm of cyanobacteria. Price and Badger (1989) demonstrated that transforming Synechococcus species with a human CA actually “short-circuits” HCO$_3^-$ accumulation, and this transformant requires high CO$_2$ for growth. The human CA was localized to the cytoplasm and converted the accumulated HCO$_3^-$ to CO$_2$. The CO$_2$ thus formed then leaked from the cell and could not be used efficiently for photosynthesis. From these studies it appears that the location of the internal CA is as important as the packaging of Rubisco.

In eukaryotic algae CA is often found inside the cell and in the periplasmic space. It is now clear that C. reinhardtii has at least five genes that encode CAs. Two of these genes, Cah1 and Cah2, encode CAs that are directed to the periplasmic space (Fujiwara et al., 1990). Two more genes encode mitochondrial CAs (Eriksson et al., 1996). Recently, a fifth gene, Cah3, was found to encode a chloroplast CA (Karlsson et al., 1998). This CA has a leader sequence that directs the protein into the lumen of the thylakoid membrane. Pharmacological and genetic evidence indicates that Cah3 is essential in generating an elevated CO$_2$ concentration for Rubisco. It appears to play a role similar to that of the carboxysomal CA of cyanobacteria. This thylakoid CA is sensitive to sulfonamides, pharmaceuticals often used to inhibit mammalian CAs. Treatment of C. reinhardtii with sulfonamides can enter the cell results in repression of CO$_2$ fixation (Moroney et al., 1985). Sulfonamides also severely inhibit photosynthesis in many other algae at low CO$_2$ concentrations, indicating that this thylakoid CA may be found in many algae. Furthermore, mutant strains of Cah3 are unable to grow at low CO$_2$, although the ability of these strains to accumulate HCO$_3^-$ is not impaired. The thylakoid CA is thought to increase the concentration of CO$_2$ in the chloroplast by dehydrogenation of the high concentration of HCO$_3^-$ the cell accumulates there.

Chloroplast CAs from higher plants are quite different from the Cah3 protein of C. reinhardtii. Cah3 does not share any sequence similarity with higher-plant chloroplast CAs. The higher-plant enzymes are of the b-type and are found in the chloroplast stroma (Badger and Price, 1994). In contrast, Cah3 is of the α-type and is found in the thylakoid lumen (Karlsson et al., 1998). At this point no stromal CA has been found in an algal species that actively concentrates CO$_2$. It appears that a stromal CA might short-circuit the active accumulation of HCO$_3^-$. If CA were present in the chloroplast stroma it might convert accumulated HCO$_3^-$ back to CO$_2$, allowing it to leak out of the cell before being fixed by Rubisco.
A MODEL FOR CO₂ CONCENTRATION

Even though the types of cells that possess CO₂-concentrating abilities are very different, they have certain properties in common that allow them to use CO₂ efficiently. The first property is the ability to accumulate HCO₃⁻ in some fashion. For most cyanobacteria and many eukaryotic algae, HCO₃⁻ can be transported into the cell directly. For other eukaryotic algae, particularly those that live in acidic environments, where the concentration of HCO₃⁻ is low, CO₂ is the Cᵢ species that enters the cell and is accumulated in the chloroplast. A second property is that Rubisco is usually packaged in a very specific way within the photosynthetic cell. Although it is possible that not every microalgal cell that concentrates CO₂ has a carboxysome or a pyrenoid, most cyanobacteria have carboxysomes that not every microalgal cell that concentrates CO₂ has a carboxysome. A similar situation occurs in the cyanobacteria through loss of the carboxysomal shell protein. The third property that appears to be common among these types of cells is the presence of a CA near the location of Rubisco. The CA supplies the Rubisco with CO₂ from the pool of HCO₃⁻. In Figure 2 three different types of transporters are shown that encode subunits of a NAD(P)H dehydrogenase. Deletions of these ndh genes lead to cells that require high CO₂ for photoautotrophic growth. The explanation for these mutants is that cyclic electron transport is disrupted in these cells such that too little ATP is made to support HCO₃⁻ transport. Mi et al. (1992) have also provided evidence that cyclic electron transport around PSI is required for HCO₃⁻ uptake.

Because Rubisco uses CO₂ and not HCO₃⁻, the HCO₃⁻ accumulated by the cyanobacteria must be converted to CO₂ for fixation. As indicated in Table II, any disruption of the proper localization of Rubisco to the carboxysome in cyanobacteria leads to a cell that requires high CO₂ for photoautotrophic growth. One example of this is the loss of carboxysomes through loss of the carboxysomal shell proteins (Orús et al., 1995), in which case Rubisco is distributed in the cytoplasm. A similar situation occurs in the mutant EK6, which contains a 30-amino acid extension of Rubisco and has empty carboxysomes (Schwarz et al., 1995). Even though the kinetics of this extension of Rubisco small subunit results

![Figure 2](https://academic.oup.com/plphys/article/119/1/9/6098645)

**Figure 2.** A model for CO₂ concentration in cyanobacteria. The font sizes of CO₂ and HCO₃⁻ indicate the relative concentrations of these Cᵢ species. PGA, 3-Phosphoglyceric acid.

| Table II. High-CO₂-requiring strains and constructs of cyanobacteria |
|-------------------------|---------------------------|-------------------------------|---------------------|
| Strain or Construct     | Mutant Phenotype          | Process Disrupted             | Explanation               |
| M3 and D₄              | Lack of carboxysomes      | Rubisco packaging            | Carboxysomes fail to form; Rubisco is located in cytoplasm |
| Strain with *Rhodospirillum rubrum* Rubisco             | Rubisco in cytoplasm       | Rubisco packaging            | Bacterial Rubisco does not locate to carboxysome |
| Extension of Rubisco small subunit              | Empty carboxysomes         | Rubisco packaging            | Rubisco cannot package into carboxysome |
| Cmp deletions            | ABC transporter lost      | HCO₃⁻ accumulation            | High-affinity HCO₃⁻ transport lost |
| HCA II transformant      | CA in cytoplasm           | HCO₃⁻ accumulation            | Accumulated HCO₃⁻ leaks out as CO₂ |
| IctA deletion            | Loss of carboxysome CA    | CO₂ generation                | HCO₃⁻ not converted to CO₂ in carboxysome |
| Numerous ndh deletions  | Loss of NADH dehydrogenase| Energy mutations             | Cyclic electron flow is disrupted |

*Orús et al. (1995).*  
*b Pierce et al. (1989).*  
*c Schwarz et al. (1995).*  
*d Okamura et al. (1997); Price et al. (1998).*  
*e Price and Badger (1989).*  
*f Fukuzawa et al. (1992).*  
*g Ogawa et al. (1998); Price et al. (1998).*
Rubisco appears normal, this strain requires high concentrations of CO₂ for normal growth. Again, the explanation appears to be that without an elevated CO₂ supply, the Rubisco is not packaged correctly into the carboxysome and ends up in the cytoplasm. Finally, the substitution of a bacterial Rubisco in place of the normal enzyme (Pierce et al., 1989) results in Rubisco free in the cytoplasm and in cells that require high CO₂ for growth.

The location of CA in cyanobacteria is also critical to the operation of the CO₂-concentrating mechanism. If the carboxysomal CA is inhibited or lost through mutation, the cell loses its ability to grow on low CO₂ concentrations. Therefore, the CA indicated in the carboxysome in Figure 2 is essential for the CO₂-concentrating mechanism, and its packaging is as important as the packaging of Rubisco.

A model of CO₂ concentration in eukaryotic algae is shown in Figure 3. This system is less understood because eukaryotic algae have more cellular compartments, are a very diverse group of organisms, and there are a limited number of systems in which molecular and genetic tools are available. However, the overall scheme of CO₂ concentration retains many similarities to the cyanobacterial model of active HCO₃⁻ accumulation, Rubisco packaging, and HCO₃⁻ dehydration in the chloroplast. In Figure 3 we have indicated both active uptake of HCO₃⁻ and diffusion of CO₂ across the plasma membrane, an uptake facilitated by the periplasmic CA. Microalgae also package their Rubisco in the pyrenoid, and deletion of the rbcL gene results in a strain without a pyrenoid (Table III).

The generation of CO₂ for Rubisco is also catalyzed by a specific CA, Cah3. Mutations in the gene encoding this chloroplastic CA require high CO₂ for photoautotrophic growth, and these mutants can be complemented by transforming the strain with the wild-type gene (Funke et al., 1997; Karlsson et al., 1998). In Figure 3 this CA is shown in the pyrenoid near Rubisco, but its exact location in relation to the pyrenoid has not been clearly established.

One important difference between algae, which concentrate CO₂₂, and C₃ plants, which do not, is the amount of CA activity in the stroma of the chloroplasts. In C₃ plants, there is a highly active, β-type CA in the chloroplast stroma (Badger and Price, 1994). In C. reinhardtii and other green algae there is very little, if any, stromal CA activity. In fact, the only chloroplast CA known is located in the thylakoid lumen (Karlsson et al., 1998). If the chloroplast is analogous to the cyanobacterial cytoplasm, a stromal CA might short-circuit the CO₂-concentrating mechanism. In cyanobacteria the insertion of human CA in the cytoplasm defeated the activity of the HCO₃⁻-accumulation system (Price and Badger, 1989). One prediction of the model shown in Figure 3 is that the presence of a CA in the chloroplast stroma might result in a cell that requires high CO₂ for growth.

### AREAS OF CURRENT INTEREST

From the discussion above it is clear that there are still many unanswered questions about the mechanism by which microalgae accumulate Cᵢ. The first challenge is to identify the other transport components of cyanobacteria and microalgae. In cyanobacteria a combination of better screening strategies for insertional mutants and the availability of the complete genome database for *Synechocystis* PCC 6803 should facilitate identification of the other Cᵢ transporters and their mechanisms of operation. In microalgae the role of LIP-36 is being investigated. The recent development of several positive selectable markers for insertional mutagenesis in *C. reinhardtii* provides a powerful tool that will aid these studies. Insertional mutagenesis may be used not only for the identification of the Cᵢ transporter, but also for the identification of other components involved in HCO₃⁻ accumulation, as well as the characterization of the roles played by these proteins.

A second important area of future interest is the role of the carboxysome and pyrenoid in CO₂ concentration. Carboxysomes are relatively well characterized in terms of their constituents, the genes that encode the proteins involved, and the effect of inactivation of these genes. However, the current evidence for the role of the pyrenoid in CO₂ concentration is circumstantial. The identification of mutants with disrupted or aberrant pyrenoids would help to clarify this issue.

A third area is the cost of CO₂ concentration. There is strong evidence for a light requirement in this process

### Table III. High-CO₂-requiring strains and Rubisco mutants of *C. reinhardtii*

<table>
<thead>
<tr>
<th>Strain or Construct</th>
<th>Mutant Phenotype</th>
<th>Process Disrupted</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroplast <em>rbcL</em> mutations&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Lack of pyridin</td>
<td>Rubisco packaging</td>
<td>Loss of Rubisco; no pyridin formed</td>
</tr>
<tr>
<td><em>pmp</em> mutant&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No HCO₃⁻ accumulation</td>
<td>HCO₃⁻ accumulation</td>
<td>Possible loss of transporter</td>
</tr>
<tr>
<td><em>ca-1</em> alleles&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Loss of chloroplastic CA</td>
<td>CO₂ generation</td>
<td>HCO₃⁻ not converted to CO₂ in chloroplast</td>
</tr>
<tr>
<td><em>cia-5</em>&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Poor growth on low CO₂</td>
<td>Regulatory mutants</td>
<td>Does not adjust to low-CO₂ conditions</td>
</tr>
</tbody>
</table>

<sup>a</sup> Spreitzer et al. (1985); Rawat et al. (1996).  
<sup>b</sup> Spalding et al. (1983).  
<sup>c</sup> Funke et al. (1997); Karlsson et al. (1998).  
<sup>d</sup> Moroney et al. (1989).
(Raven, 1997). In cyanobacteria mutant analysis and antibody studies provide evidence for the energization of C₄ accumulation through the NAD(P)H-dependent PSI cyclic electron flow (Mi et al., 1992). In microalgae the light requirement for CO₂ concentration may be attributable in part to the acidification of the lumen, because that is the location of Cah3 and, presumably, the site of the generation of CO₂ for Rubisco. As specific transport proteins are identified, the energy costs can be better estimated. It will be interesting to compare the cost of this process with C₃ and C₄ photosynthesis.

The regulation of the CO₂-concentrating mechanism is also an interesting challenge for future research. It is clear that the synthesis of many of the components of the CO₂-concentrating mechanism increases under low-CO₂ conditions (Beardall et al., 1998). Current evidence indicates that algal cells can “sense” the CO₂ level in the environment (Matsuda et al., 1998). The existence of mutants that fail to respond to low CO₂ (Table III) indicates that this signal transduction pathway can be identified through insertional mutagenesis studies. In addition, there are mutants of Chlorella ellipsoidea that express the CO₂-concentrating mechanism constitutively (Matsuda et al., 1998). A different approach has been taken by investigators who have linked the promoter regions for genes that respond to low-CO₂ conditions to reporter genes in C. reinhardtii (Eriksson et al., 1998). These chimeric genes respond to CO₂, and mutants have been found that fail to induce the reporter gene.

Another important current research topic is how organisms with a CO₂-concentrating mechanism will respond to increasing atmospheric CO₂ levels. For example, how will marine phytoplankton respond? If these organisms already possess an active CO₂-concentrating mechanism, then only a small increase in photosynthesis would be expected. On the other hand, if an algal species does not express the CO₂-concentrating mechanism under present atmospheric conditions, the increase in CO₂ might enhance its growth and photosynthesis. It is known that most algae, including coccoliths, diatoms, and cyanobacteria, have the ability to concentrate CO₂; however, little is known about whether these organisms express the CO₂-concentrating mechanism in their native environments.

In conclusion, although much progress has been made in this field of study in the past few years, we are still a long way from complete characterization. The development of new tools and strategies will contribute to further progress in the elucidation of the C₄-accumulation mechanism in the microalgae.

ACKNOWLEDGMENTS

The authors thank Olga Borkhsenious for her help with the electron micrographs and James Adams, Sergio Colombo, Catherine Mason, and Patricia Moroney for critically reading the manuscript.

Received August 11, 1998; accepted October 12, 1998.

LITERATURE CITED


