Aquatic photosynthetic microorganisms account for almost 50% of the world’s photosynthesis (19). These organisms face several challenges in acquiring CO2 from the environment. The first challenge is presented by the properties of ribulose bisphosphate carboxylase-oxygenase (Rubisco). Rubisco is an unusually slow enzyme with a low affinity for CO2. At atmospheric levels of CO2, Rubisco can function at only about 25% of its catalytic capacity because the concentration of dissolved CO2 is less than the $K_m$ (CO2) of Rubisco and due to the relatively high concentration of O2 which competes with CO2. A second challenge these organisms face is that the diffusion of CO2 in an aqueous solution is 10,000 times slower than the diffusion of CO2 in air. Thus, the ability to scavenge CO2 as quickly as it becomes available is highly advantageous to aquatic photosynthetic organisms. Third, algae often experience significant fluctuations in inorganic carbon (Ci) levels and pH, which change the availability of CO2 and HCO3- for photosynthesis. At an acidic pH, the vast majority of Ci is in the form of CO2, while at an alkaline pH, Ci is mostly in the form of HCO3-, with CO2 making up only a small fraction of the available Ci (8, 25).

Algae have adapted to these challenges through the development of a CO2 concentrating mechanism (CCM). The CCM is a biological adaptation to low carbon dioxide concentrations in the environment. It is a mechanism which augments photosynthetic productivity in algal cells by increasing levels of inorganic carbon many times over the environmental concentration of carbon dioxide. In this minireview, we aim to provide an update on the CCM and present a model on how the green alga Chlamydomonas reinhardtii concentrates CO2.

**TYPES OF CCMs**

CCMs can be based on biochemical mechanisms such as C4 photosynthesis and crassulacean acid metabolism (CAM), on active transport of Ci across membranes, or on processes involving localized enhancement of the CO2 concentration by acidification of a particular cellular compartment (28). The role of the CCM is to increase the concentration of CO2 for Rubisco, the enzyme responsible for the initial fixation of CO2. While three different mechanisms are discussed below, it is likely that aquatic photosynthetic organisms display a variety of ways to concentrate CO2. Algae comprise a very diverse group of organisms and have been adapting to the slow diffusion of inorganic carbon in the water for a long time.

**C4 mechanism.** C4 photosynthesis and CAM in terrestrial higher plants were the first photosynthetic CCMs to be described in detail. They involve a spatial (C4) or temporal (CAM) separation of the fixation of CO2 by phosphoenolpyruvate (PEP) carboxylase to produce a four-carbon dicarboxylic acid which is transported and decarboxylated, increasing the CO2 available to Rubisco (44, 74). In higher plants, the CCM is dependent on a specialized operation and the interaction of leaf mesophyll and bundle sheath photosynthetic cells. The primary CO2 capture mechanism is through PEP carboxylase located in the cytosol of the mesophyll cells. PEP carboxylase uses HCO3- as its primary substrate for fixation of CO2 into oxaloacetate, so CO2 entering from the external environment must be hydrated rapidly by a carbonic anhydrase (CA) and converted to HCO3-. Thus, in C4 plants, the predominant CA activity is found in the mesophyll cell cytosol in order to make this HCO3-, in contrast to C3 plants, where the highest levels of CA activity are associated with the stroma of the mesophyll cell chloroplasts (6, 13, 40, 59). C4 carboxylic acids such as malate or aspartate formed in the mesophyll cell cytosol serve as the intermediate CO2 pool.

The presence of C4- or CAM-like metabolism has been observed in submerged aquatic plants and algae. Examples include Isoetes howelli and Sagittaria subulata (39), the green ulophycean benthic macroalga Udotea flabelum (79, 80), and the planktonic diatom Thalassiosira weissflogii (77, 78) grown under inorganic CO2-limited conditions. Evidence of a CAM-like mechanism has also been proposed for brown macroalgae, where high levels of PEP carboxykinase and diel fluctuations in titratable acidity and malate have been observed (33, 72).

**Active transport of inorganic carbon.** Examples of active transport of HCO3- come primarily from studies using cyanobacteria. Cyanobacteria have a sophisticated CCM which involves a variety of active CO2 and HCO3- uptake systems and an internal microcompartment, the carboxysome (7, 63). At least five distinct Ci transport systems are known for cyanobacteria (Fig. 1). An interesting feature of the cyanobacterial CCM is the induction of multiple transporters under Ci limitation. Cyanobacteria appear to utilize pairs of Ci transporters with complementary kinetics for the same Ci species. For example, two complementary HCO3- transporters are present in Synechococcus PCC7002. The BicA transporter has
the CO2 concentration above ambient levels. This mechanism, first suggested by Semenenko, Pronina, and colleagues, re-
about 6.3 (HCO$_3^-$ elevates HCO$_3^-$ to CO$_2$, which has a pH of between 4 and 5. This pH differential is significant
in the cytosol of the cell and converts this 
accumulated C$_i$ back to CO$_2$ in the carboxysome, the location
must be a transport protein or complex that allows HCO$_3^-$ to enter the thylakoid lumen. This model predicts that CO$_2$ accu-
cumulation would not occur in the dark, as light-driven photosynthetic electron transport is required to set up these pH
gradients. As discussed below, evidence for this type of CCM comes primarily from work using the model eukaryotic green alga Chlamydomonas reinhardtii.

CHLAMYDOMONAS REINHARDTII CCM

The C. reinhardtii CCM. A proposed model for concentrat-
ing CO$_2$ in C. reinhardtii is shown in Fig. 2. In this model, the
CCM can be divided into two phases. The first phase involves acquiring inorganic carbon from the environment and delivering CO$_2$ and HCO$_3^-$ to the chloroplast. The components of this part of the CCM would include CAs in the periplasmic space (CAH1 and possibly CAH8) and a CA in the cytoplasm (CAH9) as well as HCO$_3^-$ transporters and CO$_2$ channels on both the plasma membrane and the chloroplast envelope. The second part of the proposed model entails the generation of elevated levels of HCO$_3^-$ in the chloroplast stroma, utilizing the pH gradient across the thylakoid membrane. This part of the CCM includes the CA located in the chloroplasm stroma (CAH6) and the CA located within the thylakoid lumen (CAH3) as well as a proposed but still hypothetical HCO$_3^-$ transporter on the thylakoid membrane.

It should be emphasized that C. reinhardtii has a strictly C$_3$ biochemistry, since unlike the C$_4$ pathway, wherein trans-
ported carbon is stored as organic C$_4$, C. reinhardtii accumu-
lates inorganic carbon, specifically HCO$_3^-$, in the chloroplast stroma. In addition, while experiments indicate that the marine diatom Thalassiosira weissflogii has a C$_4$-like pathway, the same researchers concluded that a C$_4$-like pathway is unlikely to operate in green algae (78). Although C. reinhardtii has two PEP carboxylase genes, CRPPC1 and CRPPC2 (45), the PEP carboxylase activity in C. reinhardtii is never higher than 20%

FIG. 1. Model for inorganic carbon acquisition by cyanobacteria. This model is based on the article by Woodger et al. (102). Most cyanobacteria do not contain all of the transporters depicted. In addition, any given cyanobacteria would have only one type of carboxy-
some (alpha or beta), although both pathways are shown in this figure.

FIG. 2. Model of the CCM of Chlamydomonas reinhardtii. The figure depicts an algal cell with a single chloroplast containing a single pyrenoid. As indicated by the size of the lettering, the concentrations of bicarbonate and carbon dioxide within the chloroplast and pyrenoid are higher than those in the external environment. CAH1, CAH3, CAH6, CAH8, and CAH9 stand for specific CA isoforms. PGA, 3-phosphoglyceric acid; PM, plasma membrane; CE, chloroplast en-
velope; TM, thylakoid membrane. The filled circles indicate possible bicarbonate (or C$_i$) transporters, and the closed diamonds indicate the photosynthetic electron transport chain.
Physiological evidence for CO₂ uptake in *C. reinhardtii*. The physiological evidence that *C. reinhardtii* can accumulate CO₂ and enhance CO₂ fixation is twofold. First, *C. reinhardtii* has the ability to efficiently fix CO₂ even when the external CO₂ concentration is well below the *Kₘ* of Rubisco (4, 5, 55). For example, whole-cell photosynthesis rates are saturated at about 2 to 3 mM CO₂, while the *Kₘ* of *C. reinhardtii* Rubisco is about 20 μM (34). In addition, CO₂ uptake has been measured directly in a number of laboratories (3, 4, 6, 55, 92, 93), and the C₅ concentration inside the cell is higher than can be accounted for by diffusion alone.

Further evidence for the existence of a CCM in *C. reinhardtii* comes from mutant studies. In these studies, mutantized cells were screened for growth on high (5% CO₂ in air) and low (air containing strains of *C. reinhardtii* have an anaplerotic, nonphotosynthetic role in *C. reinhardtii* (45).

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algal CAs and have three His residues coordinating the Zn ion. CAs are usually monomeric. In contrast, Zn.

A number of CAH genes have been identified in plants. These genes are often clustered and the catalytic function of PSII reaction centers (60, 67, 90). Putting the wild-type CAH3 gene back into these strains restores normal photosynthesis (24, 37). Strains defective in CAH3 also accumulate large pools of C4, but are unable to use C4 efficiently for photosynthesis (58, 90). Therefore, CAH3 appears to convert accumulated HCO3− to CO2, the form of C4 that Rubisco can use. Its location suggests that it may be important for the CCM. CAH3 is expressed under limiting CO2 conditions, where the CCM is operational (23).

CAH3, the third CA gene described for C. reinhardtii, codes for an α-CA that has a leader sequence consistent with targeting CAH3 to the thylakoid lumen (24, 38). Immunoblot studies using antibodies raised against CAH3 demonstrated that CAH3 is associated with the thylakoid membrane (37). More specifically, immunolocalization studies indicated that CAH3 is localized on the luminal side of the thylakoids and inside the pyrenoid tubules (47). The evidence that CAH3 plays an essential role in the CCM is persuasive. C. reinhardtii strains defective in CAH3 cannot grow in air levels of CO2 even though they grow normally on elevated levels of CO2 (37, 58, 67, 90). Putting the wild-type CAH3 gene back into these strains restores normal photosynthesis (24, 37). Strains defective in CAH3 also accumulate large pools of C4, but are unable to use C4 efficiently for photosynthesis (58, 90). Therefore, CAH3 appears to convert accumulated HCO3− to CO2, the form of C4 that Rubisco can use. Its location suggests that CAH3 catalyzes the formation of CO2 from HCO3− in the acid lumen of thylakoids and that this CO2 diffuses through the thylakoid membrane to the pyrenoid, where the CO2 will be fixed by Rubisco (5, 53, 54, 60, 71). CAH3 is expressed under both high- and low-CO2 growth conditions, although there is a twofold increase in message abundance under low-CO2 conditions.

CAH3 has also been proposed to be associated with photosystem II (PSII) and to help to stabilize the PSII manganese cluster and the catalytic function of PSII reaction centers (60, 99). This hypothesis is reinforced by the evidence that at low C4 concentrations, the cah3 mutant, cia3, is impaired in maintaining high rates of electron transport and/or coupling the residual electron transport to ATP formation (31). However, sub-

### Table 2. CAs in *C. reinhardtii*

| CA   | Gene family | Location    |
|------|-------------|-------------|
| CAH1 | α           | Periplasm   |
| CAH2 | α           | Periplasm   |
| CAH3 | α           | Thylakoids  |
| CAH4 | β           | Mitochondria|
| CAH5 | β           | Mitochondria|
| CAH6 | β           | Chloroplast stroma |
| CAH7 | β           | Chloroplast |
| CAH8 | β           | Periplasm   |
| CAH9 | β           | Cytosol?    |

CAH1 has been shown to be controlled by two regulatory regions, namely, a silencer region, which represses transcription under high-CO2 conditions or in the dark, and an enhancer region, which activates it under low-CO2 conditions in the light (42). These sites may be important cis-acting elements that constitutively bind one or more proteins that assist in the regulated transcription of CAH1 (43). LCR1 has also been identified as a regulatory gene of CAH1. LCR1 is a Myb transcription factor that functions in amplification and maintenance of CAH1 mRNA levels in response to limiting CO2 (105).

CAH2 is also a periplasmic α-CA but is not thought to have an important role in the CCM. CAH2 is an active CA (75, 95) but is poorly expressed. In fact, CAH2 expression is downregulated under limiting CO2 conditions, the growth conditions under which the CCM is operational (75). CAH2 is only 1.4 kb away from the CAH1 gene (20) and may be the result of a recent gene duplication.

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sequent studies with the *Chlamydomonas cah3* mutant have shown that as CO$_2$ becomes limiting, the chloroplast ribulose 1,5-bisphosphate pool is increased compared with that in the wild type, which indicates a CO$_2$ supply limitation rather than a PSII energy supply defect (30).

*C. reinhardtii* contains identical mitochondrial β-CAs (mtCAs), CAH4 and CAH5, that exhibit a pattern of expression which correlates with the expression of the CCM. The genes encoding CAH4 and CAH5 are adjacent to each other in the *C. reinhardtii* genome (18). They are highly induced at both the transcriptional and translational levels under low-CO$_2$ conditions (17, 18, 26, 49) and may have an important role in the acclimation of *C. reinhardtii* to low-CO$_2$ conditions. However, the exact role of these CAs is still not clear. One suggested function of mtCAs is to buffer the mitochondrial matrix, since prior to the complete induction of the CCM, photorespiratory glycine decarboxylation produces equivalent amounts of NH$_3$ and CO$_2$. The mtCA might serve to catalyze the hydration of CO$_2$, producing H$^+$, which would prevent alkalinization in the mitochondrial matrix as a result of the generation of NH$_3$ by glycine decarboxylation (18). Alternatively, the mtCAs have been proposed to play a role in converting the CO$_2$ generated by respiration and photorespiration to HCO$_3^-$. This would effectively "recapture" the CO$_2$ generated by the photorespiratory pathway (73). More recently, it has been shown that even under low-CO$_2$ conditions, but with increasing NH$_4^+$ concentrations in the growth medium, the expression of mtCAs decreases at both the transcriptional and translational levels. Thus, it has been proposed that mtCAs are involved in supplying HCO$_3^-$ to PEP carboxylase for NH$_4^+$ assimilation under certain conditions (27). As of this writing, there are no mutants of *C. reinhardtii* missing these mtCAs.

CAH6 is a constitutively expressed β-CA in the chloroplast stroma (47, 48). This CA might be involved in recapturing CO$_2$ as it effluxes from the thylakoid lumen and in helping to maintain a high concentration of inorganic carbon in the stroma. Likewise, it might be another CA responsible for supplying CO$_2$ for Rubisco. It might shuttle HCO$_3^-$ to CO$_2$ in the stroma as CO$_2$ is depleted by the action of Rubisco. This is the same role proposed for chloroplast CAs of higher plants. The generation of mutants of *CAH6* could help to confirm the physiological role of CAH6 in photosynthesis and the CCM.

Two additional β-CAs, designated CAH7 and CAH8, are closely related CAs with 63% similarity. They are constitutively expressed at moderate levels in *C. reinhardtii*. An interesting feature of these two CAs is the presence of long, relatively hydrophobic C-terminal extensions that are unusual among β-CAs described to date. CAH7 has been localized to the chloroplast, while CAH8 has been localized to the periplasmic space close to the cell membrane (R. A. Ynalvez et al., unpublished data). The location of CAH8 at or near the plasma membrane suggests that it might help to facilitate C$_i$ entry into the cell. The most recently discovered CA is CAH9. This β-CA has no leader sequence and has tentatively been assigned to be localized to the cytoplasm. An interesting point is that the sequence of this β-CA aligns more closely with bacterial CAs than with the other *C. reinhardtii* β-CAs (D. Deb and J. V. Moroney, unpublished results). The role, if any, of CAH9 in CO$_2$ acquisition remains to be determined.

**Putative transporters.** While a number of CAs have been shown to be part of the CCM in *C. reinhardtii*, no transporter has been linked definitively to the CCM. However, some promising candidate genes and proteins have been identified, and it is likely that one or more of the following proteins may participate in C$_i$ uptake in *C. reinhardtii*. The candidate proteins are CCP1, CCP2, LCI1, NARI1.2 (LC1A), LCI8, HLA3, RH1, and YCF10. All of these proteins are encoded in the nucleus, with the exception of YCF10, which is encoded by the chloroplast genome. Most of these proteins, or the corresponding genes, were first identified because the protein or mRNA dramatically increases in abundance when *C. reinhardtii* is grown under limiting CO$_2$ growth conditions. For example, CCP1, CCP2, LCI1, NARI1.2, LCI8, and HLA3 are all strongly induced when *C. reinhardtii* is making a functional CCM. In addition, mutations in the putative transcription factor CIAS/CCM1 (22, 103) reduce the expression of many of these proteins (15, 46, 49, 57).

Very few mutants that affect the expression of the genes encoding putative C$_i$ transport proteins have been found. However, the *pmp1* mutant does have a mutation in *LCI8*, and this mutant is defective in C$_i$ transport (91, 97). Recently, the allele of *ad1*, air dier 1, was also described, and this strain also cannot grow in low CO$_2$ (350 ppm) but can grow either in high CO$_2$ (5% CO$_2$) or in very low CO$_2$ (200 ppm). The fact that the *pmp1/ad1* mutant fails to grow on air levels of CO$_2$ but manages to survive on very low levels of CO$_2$ has been interpreted as indicative of the existence of multiple C$_i$ transport systems in *C. reinhardtii* corresponding to multiple CO$_2$ level-dependent acclimation states (89, 98, 100). This would be similar to the multiple C$_i$ uptake systems seen in cyanobacteria. *pmp1/ad1* was found to be identical to the previously identified CO$_2$-responsive gene *LCI8* (49). LCI8 does not have any significant homology to proteins from other organisms, but its predicted amino acid sequence has similarity with the predicted amino acid sequence of three genes, *LC1C*, *LC1D*, and *LC1E*, in the *C. reinhardtii* genome. *LC1C* and *LC1D* are also upregulated under low-CO$_2$ conditions. While these observations point to a role for LCI8 in the adaptation to low CO$_2$, it is unlikely that LCI8 is a transport protein by itself, as it lacks any hydrophobic transmembrane domains. Therefore, LCI8 more likely has a regulatory role or might be part of a complex that transports C$_i$ (97).

Another promising candidate protein to be a C$_i$ transporter is LCI1. The *LCI1* gene was first identified as being very highly expressed in cells growing under low-CO$_2$ conditions (14). LCI1 contains four predicted transmembrane helices and also shows very little homology to any other protein in the NCBI database. Recent work with strains showing reduced expression of *LCI1* due to the presence of an *LCI1*-RNA interference (*LCI1*-RNAi) insert showed reduced growth on low CO$_2$ (Mason and Moroney, unpublished observations), but the physiological role of LCI1 remains to be determined.

Two other genes encoding putative C$_i$ transport proteins are *CCP1* and *CCP2*. These genes encode the low-CO$_2$-inducible proteins LIP-36 G1 and LIP-36 G2 (26). These two proteins are 96% identical, have six transmembrane domains, are localized in the chloroplast envelope (54, 69), and have a high degree of similarity to the mitochondrial carrier family of proteins (15). When the abundance of *CCP1* and *CCP2* messages
was reduced using RNAi, the resultant strains grew poorly with low CO₂ levels but normally with elevated levels of CO₂ (62). However, C₅ uptake was normal in these strains (61). This might indicate that CCP1 and CCP2 are transporters of metabolic intermediates of photorespiration or transporters of other metabolic intermediates (61) or that these proteins are part of a redundant system of C₅ transport, as seen in cyanobacteria.

Another putative C₅ transporter, LCIA, was also first discovered using expression analysis (49). LCIA is also called NAR1.2. LCIA/NAR1.2 was first annotated as a nitrite transporter and has strong similarity to the bacterial nitrite/formate family of transporters. NAR1.2 belongs to a gene family consisting of six NAR genes in C. reinhardtii, and surprisingly, these genes have no obvious homolog in Arabidopsis. The expression of NAR1.2 is induced under low-CO₂ conditions and is partially under the control of CIA5, a transcription factor that is required for the expression of other CCM genes (49). NAR1.2 is predicted to be localized to the chloroplast thylakoid or chloroplast envelope and has six transmembrane domains. The functional expression of NAR1.2 in Xenopus oocytes has shown that the presence of NAR1.2 increases the bicarbonate entry into oocytes twofold compared to that of the control (46). These features suggest that NAR1.2 is an attractive candidate to be a bicarbonate transporter.

Three other proteins suggested to be part of the C₅ uptake system include HLA3 (32), RH1 (86), and YCF10 (81). HLA3 was first identified as a gene showing expression when C. reinhardtii cells were exposed to high light. Subsequent work showed that HLA3 expression is also controlled by the CO₂ concentration. HLA3 has strong sequence similarity to an ABC transporter and was first predicted to be localized to the chloroplast membrane (32). However, more recent versions of the prediction servers give much less clear predictions as to the location of HLA3. HLA3 might be a potential transporter in the acclimation of cells to low CO₂ or might be involved in redox control and only indirectly involved in the control of CCM expression (32). Another chloroplast envelope protein that has been implicated in C₅ uptake is the product of the ycf10 gene. It can form two or three transmembrane domains and has been localized in the inner chloroplast envelope membrane (82). Disruption of the open reading frame affected the uptake of inorganic carbon (81). These observations raise the possibility that this protein is a C₅ transporter. However, subsequent experiments provided evidence that YCF10 may not be involved directly in C₅ uptake but rather may regulate the C₅ transport system. It could be associated with a system in the chloroplast envelope involved in HCO₃⁻ and/or CO₂ uptake (81).

RH1 has been implicated in CO₂ transport because it is very similar to bacterial proteins shown to be ammonia and/or CO₂ channels (86). However, the expression of this protein is not consistent with it being part of the CCM, as RH1 is expressed at high levels of CO₂ when cells are grown on elevated CO₂ and not when cells are grown on low CO₂. In addition, when RH1 expression is reduced by mutation, C. reinhardtii can still grow on low levels of CO₂ but shows reduced growth on elevated levels of CO₂ (87). Likewise, RH1 is not regulated by CIA5 (101). The possible physiological role of this protein is to facilitate CO₂ entry into the cell when the CO₂ level is high. The role of RH1 in CO₂ transport remains a very interesting question in this field.

**CHALLENGES AND FUTURE DIRECTIONS**

The biggest challenge facing researchers studying the CCMs in eukaryotic algae is identifying the transport components involved in inorganic carbon accumulation. This is especially true for the proposed thylakoid HCO₃⁻ transporter. In the case of the thylakoid, experiments need to be done to demonstrate whether HCO₃⁻ can cross the membrane at all, as the only report on HCO₃⁻ transport was negative (31). In an effort to identify additional components of the CCM in C. reinhardtii, a number of insertion mutations have been generated (Table 1). While a number of candidate transport proteins have been identified in C. reinhardtii, none of these proteins has been proven conclusively to be an essential part of the CCM. One issue that may be hampering these efforts is that there may be a number of transporter proteins and eliminating only one through mutation may not lead to an obvious growth phenotype. This is the case for cyanobacteria. One frustrating point has been that none of the transport proteins identified in cyanobacteria aligns well with an annotated gene product in C. reinhardtii. This lack of homology underscores both the evolutionary distance between green algae and cyanobacteria and the possibility that the CCM may have evolved independently in these different lineages.

In contrast, the number and location of the CA isoforms are becoming clearer. While mutants exist for only two of the CA genes (CAH1 and CAH3), RNAi studies should help to clarify the physiological roles of the other isoforms. It will be interesting to see if the mtCAs are important to the CCM. The two mitochondrial proteins, CAH4 and CAH5, dramatically increase in abundance when C. reinhardtii is in a low-CO₂ environment. This induction implies that CAH4 and CAH5 are important to the cells' acclimation to limiting CO₂ conditions. However, whether these mitochondrial proteins are important in CO₂ recapture, the photorespiratory pathway, or some other anaplerotic function remains to be established.

The role of the pyrenoid remains another important topic of research. In C. reinhardtii, there is a dramatic rearrangement of starch granules when the cells are shifted from high- to low-CO₂ growth conditions (Fig. 3). When the cells experience high CO₂, the starch granules are evenly distributed throughout the chloroplast stroma. When they are switched to low CO₂, the starch strongly associates with the pyrenoid, forming a “shell” or “sheath” around the pyrenoid (11). Since almost all of the Rubisco is contained within the pyrenoid, that means that all of the Rubisco is encased in this carbohydrate shell (41, 52). This observation has evoked the speculation that the starch sheath might be an important acclimation to low-CO₂ growth conditions. However, when mutants unable to make starch were tested for growth with low CO₂, they were still able to grow at a rate indistinguishable from that of wild-type cells (Mason and Moroney, unpublished observations). For cyanobacteria, mutations that disrupt the carboxysome or cause Rubisco not to package in the carboxysome (64, 83) cause the bacteria to grow slowly on low levels of CO₂. To date, no mutations that disrupt the pyrenoid structure in C. reinhardtii
are known, except for mutations in rbcL, itself which eliminate Rubisco and also eliminate the pyrenoid altogether (76).

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