CO₂ Concentrating Mechanisms in Algae: Mechanisms, Environmental Modulation, and Evolution

Mario Giordano,¹ John Beardall,² and John A. Raven³

¹Department of Marine Sciences, Università Politecnica delle Marche, 60121 Ancona, Italy; email: m.giordano@univpm.it
²School of Biological Sciences, Monash University, Clayton, Australia 3800; email: John.Beardall@sci.monash.edu.au
³University of Dundee at the Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom; email: j.a.raven@dundee.ac.uk

Key Words
carbonic anhydrase, carboxysome, cyanobacteria, inorganic carbon, photosynthesis, pyrenoid

Abstract
The evolution of organisms capable of oxygenic photosynthesis paralleled a long-term reduction in atmospheric CO₂ and the increase in O₂. Consequently, the competition between O₂ and CO₂ for the active sites of RUBISCO became more and more restrictive to the rate of photosynthesis. In coping with this situation, many algae and some higher plants acquired mechanisms that use energy to increase the CO₂ concentrations (CO₂ concentrating mechanisms, CCMs) in the proximity of RUBISCO. A number of CCM variants are now found among the different groups of algae. Modulating the CCMs may be crucial in the energetic and nutritional budgets of a cell, and a multitude of environmental factors can exert regulatory effects on the expression of the CCM components. We discuss the diversity of CCMs, their evolutionary origins, and the role of the environment in CCM modulation.
GENERAL INTRODUCTION

Why Do Some Algae Have CO₂ Concentrating Mechanisms?

Algal photosynthesis accounts for a large proportion (~50%) of the 111–117 Pg C yr⁻¹ global primary productivity (23). Although very few algae assimilate DIC from the environment via alternative pathways, most of this large carbon flux involves species using the C₃ pathway (the photosynthetic carbon reduction cycle or Calvin cycle) for DIC acquisition, fixing DIC directly via ribulose bisphosphate carboxylase/oxygenase (Rubisco, Equation 1). However, RUBISCO has a relatively low affinity for CO₂ and, for most species, is consequently less than half saturated under current CO₂ levels. The poor performance of RUBISCO as a CO₂-fixing enzyme is aggravated by its dual role as an oxygenase (Equation 2).

\[
\text{Ribulose-1,5-bisphosphate} + \text{CO}_2 + \text{H}_2\text{O} \rightarrow 2 \times \text{glycerate-3-P} \quad (1)
\]

\[
\text{Ribulose-1,5-bisphosphate} + \text{O}_2 \rightarrow \text{glycerate-3-P} + \text{glycolate-2-P} \quad (2)
\]

The phosphoglycolate produced by the oxygenase activity of RUBISCO inhibits RUBISCO carboxylase activity, but this inhibition is alleviated by the dephosphorylation of phosphoglycolate via the enzyme phosphoglycolate phosphatase. The glycolate is then available for further metabolism via photorespiration (19, 159) or can be lost from algal cells by excretion. Decarboxylation of intermediates during photorespiration adds to the overall inefficiency.
of net carbon assimilation based on RUBISCO (19, 159).

All known RUBISCOs have competitive carboxylase and oxygenase functions and have relatively low substrate-saturated carboxylase activities on a protein mass basis. The extent to which the two competitive reactions of RUBISCO occur in autotrophic cells depends on the O₂ and CO₂ concentrations at the RUBISCO active site and the molecular nature of the RUBISCO molecule involved. Equation 3 gives the selectivity factor defining the relative rates of carboxylase and oxygenase reactions,

\[ S_{rel} = \frac{K_{0.5}(O_2) \cdot k_{cat}(CO_2)}{K_{0.5}(CO_2) \cdot k_{cat}(O_2)} \]  

(3)

where \( K_{0.5}(CO_2) \) and \( K_{0.5}(O_2) \) are the half-saturation constants for the carboxylase and oxygenase functions, respectively, and \( k_{cat}(CO_2) \) and \( k_{cat}(O_2) \) are the corresponding substrate-saturated rates of catalysis. Different species of autotrophs possess different forms of RUBISCO. Thus, green algae, \( \beta \)-cyanobacteria, and higher plants have Form 1B, with \( S_{rel} \) values ranging from 35 to 90; rhodophytes, cryptophytes, haptophytes, and \( \alpha/\beta \)-proteobacteria have Form 1D RUBISCO (153), with much higher \( S_{rel} \); and the dinophytes and proteobacteria exhibit Form II RUBISCOs with low \( S_{rel} \) values of 9–15 for proteobacteria and \( \sim 30 \) for dinophytes. The dinophytes are the only eukaryotes with Form II RUBISCO, this having arisen by lateral gene transfer from a \( \delta \)-proteobacterium (7, 9, 146–148, 153). In general, a low \( K_{1/2}(CO_2) \) and a high \( S_{rel} \) correlate with a low \( k_{cat}(CO_2) \), and vice versa (153).

These biochemical properties of RUBISCO mean that for autotrophs dependent on diffusive CO₂ entry, the physiology of CO₂ assimilation shows inherent inefficiencies, such as significant inhibition of CO₂ fixation by oxygen, high CO₂ compensation points, and low affinities for external CO₂. However, all cyanobacteria examined, most algae and many aquatic plants have mechanisms that overcome the deficiencies of RUBISCO currently operating in what is essentially, in geological terms at least (20), a low-CO₂ environment. Collectively, these are referred to as CCMs but, as detailed below, the mechanisms involved are diverse.

Relatively few of the \( \sim 1500 \) described species of cyanobacteria, or \( \sim 53,000 \) described species of eukaryotic algae, have been examined for occurrence of a CCM. In the discussion below, we deal with each of the major lines (cyanobacteria, green algae, and red/brown algae) and consider what is known about inorganic C transport and CCM in each of them.

### Evidence for, and Mechanisms of, CCMs

Table 1 summarizes the mechanisms by which algae can accumulate CO₂. These range from biochemical C₄ and CAM mechanisms involving additional DIC fixation prior to that by RUBISCO, to biophysical processes involving either localized enhancement of external CO₂ concentration by acidification of the external medium, or the active transport of DIC across one or more cellular membranes.

Some of these mechanisms involve the primary use of HCO₃⁻ ions whereas others involve CO₂ uptake. Determining whether algae use HCO₃⁻ or CO₂ can be based on a number of approaches. (a) pH drift experiments are perhaps the simplest approach, with HCO₃⁻-using algae capable of raising the pH in the surrounding medium (as a function of decreasing dissolved CO₂ to the compensation point) to values in excess of those attained by species only able to use CO₂ (155, 156, 168). (b) Comparing the pH dependence of \( K_{0.5}(HCO_3^-) \) or \( K_{0.5}(CO_2) \) for photosynthesis is another option, with CO₂ users showing pH-independent values for \( K_{0.5}(CO_2) \) and, conversely, HCO₃⁻ users showing pH-independent values for \( K_{0.5}(HCO_3^-) \) (32, 212). (c) Demonstrating that the rate of photosynthesis can exceed the uncatalyzed rate of conversion of HCO₃⁻ to CO₂ in the medium indicates that cells can use HCO₃⁻ (111, 112, 155, 156, 168, 212). (d) Isotope disequilibrium
### Table 1  The major categories of CCM in terrestrial and aquatic phototrophs, their need for an energy input, and their necessity to elevate intracellular or intracompartmental DIC above extracellular levels

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Energy input</th>
<th>Necessity for mean DICᵢ or CO₂ᵢ to exceed DICₒ or CO₂ₒ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₄: inorganic C + C₃ → C₄ dicarboxylate in the cytosol → C₃ + CO₂ in plastid containing RUBISCO</td>
<td>In generation of C₃ acceptor (PEP)</td>
<td>Depends on relative volume of RUBISCO containing high-CO₂ compartment</td>
<td>(81, 87, 120, 163, 164)</td>
</tr>
<tr>
<td>CAM: inorganic C + C₃ → C₄ dicarboxylate in the cytosol at night; C₄ stored in vacuole until next day, released and decarboxylated with minimal CO₂ leakage (stomata closed in land plants)</td>
<td>In generation of C₃ acceptor and its conversion during decarboxylation to stored products. Also in transport of C₄ dicarboxylate to vacuole</td>
<td>Yes, at least in terrestrial CAM in decarboxylation phase</td>
<td>(86, 87)</td>
</tr>
<tr>
<td>HCO₃⁻ active influx, conversion to CO₂ by CA at RUBISCO site (often in carboxysome or pyrenoid)</td>
<td>In active influx of HCO₃⁻ at plasmalemma and/or plastid envelope</td>
<td>Yes for DICᵢ, if active transport is at the plasmalemma</td>
<td>(7, 9, 11, 83)</td>
</tr>
<tr>
<td>CO₂ active influx</td>
<td>In active influx of CO₂ at plasmalemma and/or plastid envelope</td>
<td>Yes, for CO₂, unless the compartment in which CO₂ is accumulated is relatively small</td>
<td>(7, 37, 83)</td>
</tr>
<tr>
<td>CO₂ passive influx at plasmalemma of cyanobacteria with conversion of CO₂ to HCO₃⁻ by NADHdh, then conversion to CO₂ by CA in carboxysome</td>
<td>In NADHdh, bringing about the unidirectional CA conversion of CO₂ to HCO₃⁻</td>
<td>Yes for DICᵢ</td>
<td>(9, 11, 83)</td>
</tr>
<tr>
<td>Acidified compartment to which HCO₃⁻ has access; conversion of HCO₃⁻ (using CA) to give high equilibrium level of CO₂, CO₂ diffusion to RUBISCO compartment</td>
<td>In producing and maintaining a low-pH compartment using H⁺ pumps at plasmalemma, thylakoid, and/or other (?) membranes</td>
<td>Yes for CO₂ if the compartment generating CO₂ and adjacent compartments are relatively large</td>
<td>(139–141, 145–147, 200, 205)</td>
</tr>
</tbody>
</table>

DIC, dissolved inorganic carbon  
Subscripts i and o, respectively, refer to the inside and the outside of the cell.

**CA**: carbonic anhydrase  
**CAₑₓₜ**: external (periplasmic) CA  

Techniques, following the kinetics of assimilation of inorganic carbon following supply of radioactively labeled HCO₃⁻ or CO₂, have also been applied to this question (e.g., 45, 89). The above techniques give no indication as to how HCO₃⁻ is utilized or whether CAₑₓₜ is involved. Careful use of CA inhibitors can, however, often elucidate this.  
(e) Recent developments using membrane-inlet mass spectroscopy have allowed major advances in determining the inorganic carbon species used by algae, with direct measurements of CO₂ transport (10); this is now a commonly used technique (2, 31, 40, 72, 170, 188, 190). Applying these various techniques shows that most algae examined can take up both HCO₃⁻ and CO₂, although, as detailed below, there are some chlorophytes and dinoflagellates and an eustigmatophyte, *Monodus subterraneus*, that will only take up CO₂ (37). In contrast, there is some evidence that two eustigmatophyte species can only actively transport HCO₃⁻ (37, 75).
Turning from evidence for use of different DIC species to evidence for possession of CCMs, all CCM mechanisms (Table 1) have the same physiological outcome, i.e., negligible inhibition of CO₂ fixation by oxygen, low CO₂ compensation points, and high affinities for external CO₂—features that are not characteristic of isolated RUBISCOs or intact cells of species that rely solely on CO₂ diffusion. Evidence for CCMs is thus based on physiological measurements of these parameters compared to those of the isolated RUBISCO and/or direct measurements of internal CO₂ pools using mass spectrometric or radioisotopic techniques (153). In addition, stable isotope measurements of the ¹³C/¹²C ratio of organic cellular material (relative to source DIC) can be used to indicate the presence of the CCM capacity of algae (153, 158). Isolated eukaryote RUBISCOs discriminate against ¹³C to the extent of ∼30‰, so species without CCMs may show isotopic discrimination ratios approaching this value. CCMs tend to reduce discrimination, so lower discrimination values suggest CCM activity. However, note that ¹³C/¹²C ratios are indicative rather than definitive (153, 158).

**CCMs Based on C₃ + C₁ Carboxylations (C₄ and CAM Metabolism)**

The first two mechanisms for CO₂ accumulation in Table 1 involve an additional carboxylation step, prior to that catalyzed by RUBISCO, which sees inorganic carbon derived from the external environment added to a C₁ carrier to form a C₄ intermediate that is decarboxylated at the site of RUBISCO, providing CO₂ to that enzyme’s active site (145, 146). The role of such a C₄ dicarboxylic acid intermediate is a biochemical transporter of DIC from a site with access to exogenous inorganic C to the site where RUBISCO is active. This is C₄ photosynthesis. In CAM the primary (C₁ + C₁) carboxylation occurs at night and the (C₄ – C₁) decarboxylation occurs during the day, so the dicarboxylate residence time is about 12 hours.

The presence of C₄ (or C₄-like) metabolism in some algae has been suggested in several instances, although in most cases the evidence is weak (18, 33, 69, 125, 145, 163). The evidence for C₄ metabolism comes from the time course of ¹⁴C incorporation into acid-stable compounds and the activity and location of (C₃ + C₁) carboxylases and (C₄ – C₁) decarboxylases. C₄-like metabolism is indicated if the first acid-stable product of ¹⁴C-inorganic C assimilation is a C₄ dicarboxylic acid rather than 3-phosphoglycerate, and especially if pulse-chase experiments show a transfer of label from a C₄ acid to phosphoglycerate. In terms of enzyme activity and enzyme compartmentation C₄ metabolism requires a (C₃ + C₁) carboxylase in a compartment accessible to external DIC and a (C₄ – C₁) decarboxylase in the compartment containing RUBISCO. In individual algal cells the cytosol is the potential carboxylation site and the plastid stroma is the decarboxylation site (164). The cytosolic carboxylase could be PEPc or PEPck, and the chloroplastic decarboxylase could be PEPck or NAD⁺ (or NADP⁺) malic enzyme (ME) (153). If PEPck is to be used as both carboxylase and decarboxylase then it must be regulated such that the carboxylase activity is favored in the cytosol but the decarboxylase activity is favored in the plastid. To date there have been convincing cases for C₄ photosynthesis made for only two algae, the green ulvophycean benthic macroalga *Udotea flabellum* (165; see 145) and the planktonic diatom *Thalassiosira weissflogii* grown under inorganic C-limited conditions (120, 163, 164), although for the latter some of the data are contentious (81, 153).

CAM has also been proposed as a contributor to photosynthetic inorganic C assimilation in brown macroalgae, albeit providing less than 10% of the total organic C (80, 146). The evidence here comes from the high-PEPck activity in brown algae and the overnight increase and daytime decrease in titratable acidity and malate measurable in algal homogenates (80, 146). However, as for some data on C₄ metabolism, the evidence is equivocal (e.g., see 86).
Figure 1
A schematic model for inorganic carbon transport and CO₂ accumulation in cyanobacterial cells. The model shown here incorporates low-affinity transport systems (shown in gray) and high-affinity systems (shown in black) at the plasmalemma and/or thylakoid membrane. Transporters whose characteristics are unknown are shown in white. Redrawn after Price et al. (138).

CCMs Based on Active Transport of Inorganic C Species
The mechanisms discussed above for DIC concentration at the RUBISCO active site can be thought of as biochemical CO₂ pumps. In contrast, the other mechanisms outlined in Table 1 represent biophysical CO₂ pumps. Most CCMs in cyanobacteria and algae are based on active transport of HCO₃⁻ and/or CO₂ across one or more of the membranes separating the bulk medium from RUBISCO. This requires that the membrane across which active transport occurs has a low permeability to the DIC species delivered to the side of the membrane closest to RUBISCO, otherwise active transport is short-circuited (153). This rules out the outer of the two plastid envelope membranes in eukaryotes as well as the gram-negative outer membrane of cyanobacteria because these have high densities of porins with no selectivity for molecules of Mᵣ less than ~800.

For cyanobacteria the CCM can be based on either CO₂ or HCO₃⁻ transport, either at the plasmalemma or thylakoid membrane (83, 88, 132, 153, 167). The various transporters deliver HCO₃⁻ to the cytosol regardless of the DIC species (CO₂ or HCO₃⁻) removed from the periplasm (Figure 1). The HCO₃⁻ then diffuses into the carboxysomes, which show the only CA activity in the cytosol (93, 138, 181, 187). The CO₂ generated by this CA builds up to a higher steady-state concentration in the carboxysomes than in the bulk medium, thus strongly favoring the carboxylase over the oxygenase activity of RUBISCO (83, 100).

For eukaryotic algae with CCMs active transport mechanisms for DIC could be based on the plasma membrane or the inner plastid envelope membrane or both (2, 83, 124, 153, 202, 212) (Figure 2). Equilibration between CO₂ and HCO₃⁻ in the various compartments (periplasmic space, chloroplast stroma, thylakoid lumen) of eukaryotic algal cells can involve a range of CAs (4, 119, 123, 202). Although it is tempting to draw correlations between the function of carboxysomes in cyanobacterial CCMs and the role of pyrenoids (regions of the plastid stroma where most, if not all, of the cellular complement of RUBISCO is localized in eukaryotes), the evidence is weak. Although all pyrenoid-containing algae have CCMs (7, 146, 147, 153), not all algae capable of expressing CCMs have pyrenoids (7, 121, 122, 146, 147, 153, 157, 158).
Figure 2

A schematic model for inorganic carbon transport and CO₂ accumulation processes in eukaryotic algal cells. The model incorporates the possibilities for DIC transport at the plasmalemma and/or chloroplast envelope as well as a putative C₄-like mechanism. CO₂ crosses membranes by diffusion, whereas active transport (shown by the shaded boxes) can be of CO₂ or HCO₃⁻. No attempt has been made to show the roles of the various internal CAs in the different compartments. For this the reader is referred to the text and Badger (6). Redrawn after Sulțemeyer (187) and Raven & Beardall (153).

CCMs Based on Enhancement of CO₂ Concentration Following Acidification in a Compartment Adjacent to RUBISCO

A final category of CCM concerns those species where acidification of a compartment containing high-HCO₃⁻ concentrations leads to enhanced levels of CO₂, which can then diffuse to the active site of RUBISCO at concentrations higher than would be expected by diffusion from the external bulk medium. The essential feature of this type of mechanism is that HCO₃⁻ from an alkaline medium_compartment is transported to a compartment that is maintained at a low pH by a proton pump (153). Here the equilibrium CO₂: HCO₃⁻ ratio is much higher than that in the first compartment. Raven (145) gives details of how this equilibrium may be maintained, but it is proposed to involve a relatively acid-stable CA or, in larger compartments, a proton-driven catalysis of the HCO₃⁻ to CO₂ conversion. The CO₂ produced in this acidic compartment then diffuses to RUBISCO in an adjacent, more alkaline (pH 7.5–8.0) compartment. Such mechanisms work most effectively if there is no CA in the compartment containing RUBISCO, at least in the cases where the RUBISCO compartment is closer to the medium than is the acidic compartment (e.g., a vacuole) (145). Walker et al. (205) first suggested and quantified, in its simplest forms, this model for the acid zones on the surface of internodal cells of characean freshwater macroalgae (see also 1, 53). A similar model for CO₂ concentration using the thylakoid lumen as the acidic compartment was later proposed by Pronina & Semenenko (141) and modified by Raven (145, 147).

For the variants of this mechanism that involve intracellular acid compartments there is a requirement for HCO₃⁻ transport from an adjacent alkaline (pH 7.0–8.0) compartment to the acid compartment. The alkaline compartment from which bicarbonate is transported is
supplied with bicarbonate from the medium via the plasmalemma and/or, when the acid compartment is the thylakoid, the chloroplast envelope membranes (see 145–147). The compartments into which $\text{HCO}_3^-$ is transported are the thylakoid lumen (141, 145–147), the vacuole (145–147), the aqueous compartment in the chloroplast endoplasmic reticulum of algae that obtained their plastids by secondary endosymbiosis (98, 99), and, for algae endosymbiotic in invertebrates, the perisymbiont space. Raven & Beardall (153) discuss in detail the (limited) evidence for these latter variants on the mechanism.

### Algae Lacking CCMs

In addition to the CCMs described above, a number of algal species do not possess any means of concentrating CO$_2$ at the RUBISCO active site and rely solely on CO$_2$ diffusion from the external environment. Species with low-$S_{\text{rel}}$ RUBISCOs would find it difficult to carry out net C assimilation under current CO$_2$ and O$_2$ levels without recourse to a CCM (7, 145, 146, 153). However, in at least some conditions, organisms with RUBISCOs with higher $S_{\text{rel}}$ values could rely on diffusive CO$_2$ supply to RUBISCO. Red algae lacking CCMs grow in freshwater with CO$_2$ levels several times that at air equilibrium (102, 152, 154, 203) and often in fast-flowing conditions, which minimize the diffusion boundary layer. Similarly, almost all freshwater chrysophyte and synurophyte (heterokont) algae lack CCMs (12). Other species that rely on diffusive CO$_2$ supply are certain lichenized green algae (*Coccomyxa*: 134) and some marine red algae, which mainly live at low light levels where a smaller CO$_2$ flux is required to satisfy the assimilatory needs of the cells (145, 157, 158, 174).

### CCMs IN CYANOBACTERIA

Several exhaustive reviews on cyanobacterial CCMs have been published recently (e.g., 11, 83); therefore, we refer the reader to these articles for more details. In the following paragraphs we describe the path of DIC toward the site of its fixation.

#### C$_i$ Acquisition

The uncharged CO$_2$ molecule can diffuse across the membrane at a rate sufficient to account for the rate of photosynthesis with CO$_2$ as C source; no active CO$_2$ transporter has yet been found in the plasmalemma of cyanobacteria. It is possible (192) that, as with other systems (28, 127), including some photosynthetic ones (197), CO$_2$ diffusion into the cells occurs through aquaporins that are possibly similar to the bacterial Aqpz (35). Bicarbonate permeation through the plasmalemma is negligible. Cyanobacteria, however, can actively take up this DIC species from the medium. The HCO$_3^-$ pumps afford intracellular concentrations up to three orders of magnitudes higher than those in the external medium (11, 83). Omata et al. (132) thoroughly characterized a low CO$_2$-induced ABC-type (68) HCO$_3^-$ uniporter, named BCT1, with a high affinity for HCO$_3^-$ . This transporter comprises four proteins encoded by the *cmp*ABCD operon (104, 105). The genes encoding BCT1 are exclusive to freshwater $\beta$-cyanobacteria and are absent from all sequenced genomes of marine $\alpha$- and $\beta$-cyanobacteria (11). This may reflect variable, and frequently low, availability of DIC in freshwater (e.g., 101). In *Synechocystis* 6803, inactivation of the *cmp*ABCD operon does not block HCO$_3^-$ transport; this is due to the presence of a Na$^+$-dependent HCO$_3^-$ transporter with a lower affinity for HCO$_3^-$ than BCT1 (175). This alternative HCO$_3^-$ transport system requires Na$^+$ for a HCO$_3^-$/Na$^+$ symport, probably energized by an ATP-powered Na$^+$/H$^+$ antiport. The Na$^+$ dependence of this transporter may, at least in part, explain the Na$^+$ requirement of many cyanobacterial strains, especially at high pH (116). Two genes are necessary for expressing this HCO$_3^-$ transport system: *sbtA* (or *slr 1512*) and *ntfJ* (175, 177). *SbtA* encodes the transmembrane protein responsible for the translocation of HCO$_3^-$, and its expression is triggered by exposure of cells...
to low CO₂, even if a small amount of transcript is also present in high-CO₂-grown cells (175). Two types of sbtA products, differing for the number of amino acids constituting the polypeptides, are present in cyanobacteria, with *Anabaena* PCC7120 possessing both of them (175). Homologues of sbtA have also been found in several β-cyanobacteria, but not in *Thermoymecoccus elongatus* and *Trichodesmium erythraeum* (11 and references therein); more distantly related homologues are also present in the marine α-cyanobacteria (9, 138, 175). The *ntpJ* gene is present in all organisms possessing sbtA; the exact function of the product of this gene is not known, but it has been proposed that it is involved in the primary Na⁺ pump (175). This Na⁺-dependent HCO₃⁻-transport may have great ecological relevance because it appears to be the only DIC-active uptake system present in α-cyanobacteria-like *Prochlorococcus*, possibly among the most abundant marine phytoplankters (9, 175). In these organisms, this HCO₃⁻ transport system is probably constitutive (11). In *P. marinus* PCC9511, high- and low-HCO₃⁻ uptake systems have been inferred from kinetic studies (133). The lack of molecular evidence for the presence of these systems, however, may be due to the fact that a biphasic system is encoded by a single gene (38). The constitutive nature of the HCO₃⁻ transport system may be related to the fact that *P. marinus* growth is presumably controlled by reduced N (NH₄⁺ and amino acids) availability (43, 169), whose intracellular concentrations could be maintained at relatively high levels by transporters such as those for NH₄⁺ present in *P. marinus* SS120 (43) and in *P. marinus* MIT9313 and MED4 (169). The fact that *P. marinus* cells can maintain relatively high-NH₄⁺ concentrations is also confirmed by the *Kₘ* of their glutamine synthetase, which is one order of magnitude higher than those of other cyanobacteria such as *Synechocystis* PCC 6301, *Calothrix* PCC 7601, and *Nostoc* PCC 7120 (44). In the presence of a control over growth exerted mostly by N, and of relatively constant intracellular N concentration, modulating C acquisition may not be necessary and the control mechanisms may have been “sacrificed” in the gene elimination process that presumably characterized the evolutionary path of *Prochlorococcus* (43). Because the N cell quota (26, 66) is probably highly regulated in the *Prochlorococcus* strains exclusively using NH₄⁺ and organic N (213), there is less need for a modulation in DIC acquisition to maintain a (near) optimal C/N ratio. The relative constancy of the C/N ratio in *Prochlorococcus* is also suggested by the nonphosphorylated state of the PII protein in *P. marinus* PCC9511 (133); phosphorylation and dephosphorylation, mediated by a protein serine kinase and a phosphatase (49, 78) and by the redox state of the cell (71), generally control the activity of the PII protein in cyanobacteria (49, 78, 96, 97, 126). The putatively permanently nonphosphorylated state of PII in *P. marinus* PCC9511 may betray the absence of a need to coordinate C and N metabolism in response to changes in availability of these two nutrients.

**Cytosolic DIC**

The form of DIC that accumulates in the cytosol is HCO₃⁻, irrespective of the form of DIC entering the cells. The conversion of HCO₃⁻ to CO₂ in the cytosol is slow owing to the absence of a cytosolic CA in cyanobacteria (204). Accumulation of HCO₃⁻ in the cytosol is confirmed by the finding that, if equilibration of HCO₃⁻ to CO₂ in the cytosol is accelerated by the expression of human CA, *Synechococcus* PCC7942 cells release CO₂ and cannot maintain a functional CCM (137). Because CO₂ influx across the external membrane occurs by passive diffusion, there is no CO₂ pump in the sense of a mechanism leading to a higher concentration (a surrogate for free energy for a neutral molecule) on the trans side of the plasmalemma than on the cis side, as a result of energizing the transmembrane flux of CO₂. An energized conversion of CO₂ to HCO₃⁻, however, occurs on the cytosolic side of the thylakoid membrane (103, 130, 131, 176). This conversion is energized by the electron transfer through NAD(P)H dehydrogenase.
(NADH-1 complex). Speculative modes of action for this system were illustrated by Kaplan & Reinhold (83) and Price et al. (138). According to Price et al. (138), the reduced intermediate generated within the NADH-1 complex by the electrons donated by NAD(P)H or ferredoxin converts Zn-H$_2$O$_2$ to Zn-OH at the active site of the complex. As in CA (180), the Zn-OH is then involved in hydrating CO$_2$ to HCO$_3^-$.

The residual proton is subsequently translocated into the lumen via proton channels of the complex. At least two systems have been described. One, named NADH-13 complex, is inducible, contains proteins encoded by the genes ndhF3, ndhD3, and chpY genes (103, 176). It was proposed that the chp proteins are involved in the CA-like reaction of CO$_2$ hydration (138), although these proteins have no sequence similarity with known CAs. The high-affinity CO$_2$ uptake system is absent from α-cyanobacteria genomes, and the low-affinity system is encoded by the ndhF4, ndhD4, and chpX genes (11, 36). On the contrary, both high- and low-affinity systems are present in β-cyanobacteria, with the sole exception of *Trichodesmium erythraeum*, which only has the low-affinity CO$_2$ uptake system (11 and references therein).

**CO$_2$ Production and Assimilation in the Carboxysome**

Because the intracellular movement of DIC is by diffusion, the concentration of HCO$_3^-$ in the carboxysome is presumably slightly lower than at the internal face of plasmalemma and the external face of the thylakoid. The HCO$_3^-$ accumulated in the cytosol then serves as the substrate for a fixation trap, where, with the help of a CA, CO$_2$ is locally produced and used by the proximally located primary photosynthetic carboxylase, RUBISCO. The trap can be physically identified with the carboxysome, where RUBISCO and CA are usually confined (11, 36). It has been suggested that the protein shell of the carboxysome represents a barrier to CO$_2$ back diffusion, facilitating carboxylation by RUBISCO and reducing short circuiting of the CCMs. However, little evidence is available on the existence of such a barrier to CO$_2$ diffusion. The closest analogue to the 3–4-nm thick protein shell of the carboxysome is possibly the protein envelope of cyanobacterial gas vacuoles (207). The gas vesicles of *Anabaena* are permeable to CO$_2$ and in general to gas molecules smaller than perfluorocyclobutane (C$_4$F$_8$), whose collision diameter is 0.63 mm (206). Thus, to efficiently act as a barrier to CO$_2$ diffusion, the maximum pore size in the carboxysome envelope should be substantially smaller than that in gas vesicles. It has been suggested that O$_2$ readily diffuses into the carboxysomes (179); if this is true, in the absence of presently undiscovered active selection mechanisms, the pores of the protein shell of carboxysomes would be larger than the collision diameter of O$_2$, but smaller than that of CO$_2$. However, Marcus et al. (107) reported that, in *Synechocystis* PCC6803, RUBISCO activity is less sensitive to O$_2$ in intact carboxysomes than in ruptured carboxysomes, suggesting that the carboxysome envelope is more permeable to CO$_2$ than O$_2$. In the absence of confirmation of the “intracarboxysomal” kinetics of RUBISCO reported by Marcus et al. (107), further investigations on the physical chemistry of CO$_2$ permeation across protein envelopes are required to determine the nature of the hypothesized carboxysomal diffusion barrier.

The traditional view of the CO$_2$ production in the carboxysome requires a carboxysomal CA, which would accelerate HCO$_3^-$ conversion to CO$_2$, providing the substrate for RUBISCO carboxylation at a relatively high rate. It has also been proposed that CA is located at the center of the carboxysome, and that the CO$_2$ that it generates is used up before it can diffuse across the thick intracarboxysomal protein arrangement (36). A carboxysomal CA is present in a number of β-cyanobacteria (6, 11), and a new type of CA (ε-CAs) was
identified in carboxysomes of the chemolithotroph *Halothiobacillus neapolitanus* (182). The gene encoding this ε-CA, named *csoS3*, has homologues in the α-cyanobacteria *Prochlorococcus* sp. MED4 and MIT9313 and *Synechococcus* WH8102 (182). The presence of a carboxysomal CA has not been confirmed for all cyanobacteria: The genes encoding specific carboxysome CAs are lacking from the genomes of the β-cyanobacteria *Trichodesmium erythreum* and *Thermosynechococcus elongatus* (6, 9, 11). Thus, if all cyanobacteria carboxysomes are involved in CCMs, the presence of a CA (at least from a known CA family) in the carboxysome is not an absolute requirement for efficient CO₂ fixation. Shively et al. (179) point out that producing protons by the carboxylation reaction of RUBISCO, especially in a closed compartment packed with the enzyme, may be sufficient to drive the dehydration of HCO₃⁻ at a rate sufficient to minimize the oxygenase reaction.

The CO₂ generated in the carboxysome of cyanobacteria serves as the substrate for Form 1A or 1B RUBISCOs. Their distribution strictly parallels the type of carboxysome: α-cyanobacteria contain α-carboxysomes with Form 1A RUBISCO; β-cyanobacteria contain β-carboxysomes with Form 1B RUBISCO (9). Whether each carboxysome-RUBISCO pair has evolved in parallel or is the result of a single lateral gene transfer is a matter of dispute (9). What advantages, disadvantages, or differences in activity are related to either type of carboxysome and RUBISCO are not yet clear due to the absence of adequate knowledge of the kinetics of Form 1A RUBISCO. Regardless of the type of RUBISCO, packing RUBISCO in carboxysomes is crucial for the efficiency of cyanobacterial photosynthesis (36), and packing may increase the RUBISCO activation state (173).

**CCMs IN THE CHLOROPHYTA, CHLORARACHNIOPHYTA, AND EUGLENOPHYTA**

In this section we consider the evidence for CCMs in the chlorophyte algae, including the euglenoids and chlorarachniophytes, which arose from chlorophytes following an endosymbiotic event with a flagellated protozoan and consequently have one and two additional chloroplast envelope membranes, respectively.

All chlorophytes examined have Form 1B RUBISCOs, with Sₜₑ₁ values in the range of 54 to 83; the highest value is for the lichen alga *Coccomyxa* sp. and it is similar to values for C₃ higher plants. Figure 2 shows a model for CCMs in eukaryotes, based mainly on experiments with chlorophytes such as *Chlamydomonas reinhardtii*.

**DIC Entry**

On the basis of evidence from isotope disequilibrium and mass spectrometric approaches, most chlorophyte algae can use both HCO₃⁻ and CO₂ as DIC sources (37). Notable exceptions are *Nannochloris atomus* and *N. maculata* (74, 76), which are only capable of using CO₂. *Coccomyxa* is unusual in that it shows no CCM activity and CO₂ entry is via diffusion (134).

Many green algae can use HCO₃⁻. However, although direct uptake of HCO₃⁻ was demonstrated by mass spectrometry (2, 136), in some cases concurrently with active CO₂ transport (189), in other instances it appears that HCO₃⁻ use involves the activity of a CAext. This enzyme activity is responsible for dehydrating HCO₃⁻ in the periplasmic space, increasing the potential for CO₂ uptake. In *Chlamydomonas reinhardtii*, in which both CO₂ and HCO₃⁻ are actively transported across the plasmalemma (although the former is the preferred species), there are two isozymes of CAext (50, 51, 162). These are pCA1 (the gene product of *Cah1*) and pCA2 (the gene product of *Cah2*). The former is highly expressed and inducible by limiting CO₂ and the latter is of unknown function and is repressed under low-CO₂ conditions and only weakly expressed under high CO₂ (186). A periplasmic CA was also reported in the charophytes *Chara zeylanica* and *Mongeotia* sp. (4).

Although pCA1 in *Chlamydomonas* certainly enhances the use of external HCO₃⁻ at neutral and alkaline pH, evidence from a null mutant
of Cab1 (cab1-1) suggests that pCA1 activity is not absolutely essential for growth in low CO$_2$ or for operation of the CCM (186, 201). Other species of microalgae have CCMs CA$_{ext}$ activity. In these cases, the major flux is likely HCO$_3^-$, except for the Nannochloris species mentioned above, where CO$_2$ is the only DIC species actively transported (37).

In some ulvophyte macroalgae, HCO$_3^-$ use based on CA$_{ext}$ and entry of CO$_2$ is supplemented, when the algae are exposed to the very low CO$_2$ levels associated with high-pH environments, by induction of an AE HCO$_3^-$ transporter. This system is characteristically inhibited by AE inhibitors such as DIDS, and under high-pH conditions both CA$_{ext}$ and DIDS-sensitive systems co-occur, although in normal environmental conditions the CA$_{ext}$ mechanism predominates. Not all green macroalgae show DIDS-sensitive HCO$_3^-$ use and other factors are required before the system is induced (95). Furthermore, much care is needed in the use and interpretation of data obtained using AE inhibitors such as DIDS (212), and the occurrence of an AE exchange HCO$_3^-$ transporter in macroalgae needs additional verification. DIDS-sensitive HCO$_3^-$ transport occurs in chlorophyte microalgae, although at least in Dunaliella tertiolecta, this is much less significant than the CA$_{ext}$ system is for HCO$_3^-$ use (212).

**DIC in the Cytosol and Plastid**

The DIC delivered from the external environment across the plasmalemma can be acted on in numerous ways. In contrast to the cyanobacteria, chlorophytes and other eukaryotic algae have the added complication of additional compartments, specifically the chloroplast and the included pyrenoid, which can be involved in CO$_2$ accumulation. Within the cytosol, DIC is present as both CO$_2$ and HCO$_3^-$. As yet, there is no evidence for a putative cytosolic CA except in Coccomyxa, which lacks a CCM (70), but chloroplasts of Chlamydomonas contain a stromal β-CA (Cah6) and a thylakoid lumen α-CA (Cah3), which catalyze the CO$_2$/HCO$_3^-$ equilibrium within those compartments (84, 119). How this stromal CA fits into models of CO$_2$ accumulation is unclear, although the model proposed by Mitra et al. (119) invokes not only active transport of DIC into the chloroplast but its transfer to the pyrenoid and the concentration of CO$_2$ based on localized acidification within the thylakoid lumen (see discussion below and figure 10 in 119).

There is considerable evidence in chlorophyte algae for a role for the plastid envelope in CCMs based on active transport of DIC. Photosynthetically active chloroplasts from high and low CO$_2$-grown chlorophyte cells possess low- and high-affinity DIC uptake systems respectively, as do intact cells (2, 10, 136). Such data are consistent with a major role for plastid envelope–based DIC transport in CCMs in these organisms. Soupene et al. (184) presented evidence that Chlamydomonas reinhardtii cells grown at high CO$_2$ show expression of the Rh1 (Rhesus) protein usually associated with red blood cells. Expression of rh1 is suppressed under low CO$_2$. In high-grown CO$_2$ cells, Rh1 is expressed as a CO$_2$ channel in the plastid envelope, maximizing CO$_2$ influx in the absence of active DIC transport systems. Its suppression under low-CO$_2$ conditions minimizes CO$_2$ leakage and short circuiting of CCMs (184; see also 82). To date, active DIC uptake (as both CO$_2$ and HCO$_3^-$) and CO$_2$ accumulation have been demonstrated in isolated chloroplasts of Chlamydomonas reinhardtii and Dunaliella tertiolecta (2) and in Tetraedon minimum and Chlamydomonas noitigamna (198). In contrast, intact cells of Chlorella ellipsoidea show a CCM, but isolated chloroplasts from this species are incapable of active CO$_2$ uptake and have very limited capacity for HCO$_3^-$ use, suggesting that the major site of DIC transport in this species is the plasmalemma (171, 172). Note that the possession of active transport of DIC at the chloroplast envelope does not rule out the option of active DIC transport at the plasmalemma (198).

The role of the pyrenoid in chlorophyte CCMs is unclear. Morita et al. (121) showed...
that species of Chloromonas can accumulate CO₂ and express a CCM even though they do not possess pyrenoids. However, in a later paper Morita et al. (122) showed that, for a range of Chlamydomonas and Chloromonas species, the size of the DIC pool was associated with pyrenoid form. Species that form typical spheroidal, electron dense pyrenoids with strong RUBISCO immuno-labeling had higher DIC pool sizes (150–250 µM), whereas species with atypical pyrenoids had lower DIC pools (13–31 µM) (121, 122). Given the significant number of algae of other lineages (see below) that lack pyrenoids yet express CCMs, the exact role of pyrenoids in CCM function requires further investigation.

**Evidence for Other Forms of CCM in Chlorophyte Algae**

**Evidence for a CCM based on thylakoid acidification.** The role of thylakoid acidification in CCM function derives from earlier work by Pronina and coworkers (139–141) and was developed by Raven (147). The model is based on the occurrence of an α-carbonic anhydrase (Cah3; 84) on the inner side of the thylakoid membrane. In Chlamydomonas, this carbonic anhydrase is necessary for growth of ambient CO₂ (84, 199). Following active transport of HCO₃⁻ to the compartment containing RUBISCO, a passive uniport involving HCO₃⁻ channels would transport HCO₃⁻ to the thylakoid lumen. Here it reacts with H⁺, accumulated in the lumen as a consequence of light-driven electron transport, and in the presence of Cah3 leads to localized elevated CO₂ concentrations in the lumen. The CO₂ then diffuses out of the lumen to the site of RUBISCO in the chloroplast stroma (or pyrenoid, where present). However, van Hunnik & Sültemeyer (200) were unable to demonstrate HCO₃⁻ influx (or CO₂ efflux) in isolated thylakoids of green algae. More work, including a search for bicarbonate channels or their functional equivalents, is needed to test this model. Involvement of Cah3 in CO₂ generation from HCO₃⁻ as part of the reaction sequence between exogenous inorganic C and RUBISCO does not preclude a role for the enzyme in photoactivation and function of the water-oxidizing complex of photosystem II (3).

However, it is clear that such a mechanism can account for cases where gas exchange physiology suggests the presence of a CCM yet the average inorganic C concentration in the cells is not greater than that in the medium. Furthermore, the compartmentation of well-established carbonic anhydrases in Chlamydomonas reinhardtii is consistent with Raven’s hypotheses (119, 145–147, 149).

A final role for carbonic anhydrase in CCMs in chlorophyte algae relates to the β-CA associated with mitochondria (187). This mtCA is proposed to function to convert CO₂, produced in the mitochondrial matrix by the TCA cycle and photorespiratory glycine decarboxylase activity, to HCO₃⁻, limiting the potential for CO₂ leakage through the plasmalemma (149). Giordano et al. (60) proposed a role for mtCA in the supply of HCO₃⁻ for anaplerotic carbon assimilation; mtCA is not only inducible under low CO₂ (46) but also by C:N ratio (60).

**C₄ mechanisms in the chlorophyta.** There is evidence for C₄ photosynthesis only in a single ulvophyte, the macroalga Udotea. The evidence is based on the kinetics of isotope labeling, including pulse-chase experiments and measurements of enzyme activity and localization. Udotea uses a cytosolic PEPck as the (C₃ + C₁) carboxylase, and, possibly, NAD ME as the stromal decarboxylase (146, 165). The kinetic data from labeling by ¹⁴C show transfer of ¹⁴C from malate to sugar phosphates during the chase period. Furthermore, the ¹⁴C label in malate relative to phosphoglycerate during the pulse labeling is higher than expected for C₃ biochemistry (165, 166). However, keep in mind that the shortest pulse-labeling times were 10 sec (165, 166) and Johnston (80) showed that labeling times as short as 1 sec were needed to show that phosphoglycerate, rather than a C₄ acid, was the initial product of photosynthetic DIC fixation in
the brown macroalga *Ascophyllum*. It would be especially useful to have more very short-term pulse-label data not only for *Udotea* but also for other algae that are presently believed to have C₃ biochemistry. Remember that CCMs based on active DIC (above) or H⁺ (below) transport would, like C₄-like metabolism, account for C₄-like gas exchange physiology (high CO₂ affinity; O₂ insensitivity), and that active DIC transport could make pulse-chase experiments more difficult to interpret because any ¹⁴CO₂ released from ¹⁴C-dicarboxylates would be less likely to be “chased” out of the cell and more likely to be refixed by RUBISCO even if this is not an obligate pathway from external DIC to RUBISCO. A low ¹³C/¹²C ratio in the organic C of the organism relative to source CO₂ can also be explained by a CCM based on DIC or H⁺ active transport as well as by CCMs based on C₄-like metabolism using PEPc as the (C₃ + C₁) carboxylase, and more readily than if PEPck is the carboxylase (146).

There is no evidence for CAM metabolism in green algae.

**CCMs IN RED AND CHROMIST ALGAE AND DINOFLAGELLATES**

All of the algae considered here have red algal plastids. The plastids are either within red algae or were transferred, by secondary endosymbiosis, to other unicellular heterotrophs. The secondary endosymbiotic event gave rise to the photosynthetic members of the Chromista (i.e., photosynthetic cryptophytes, haptophytes, and heterokonts) and of the Alveolata (i.e., photosynthetic dinoflagellates) (47). The ancestral RUBISCO in this group is Form 1D, which has the highest known selectivity for CO₂ over O₂. This Form 1D RUBISCO is found in all red algae, cryptophytes, haptophytes, and heterokonts that have been examined, but most dinoflagellates have a Form II RUBISCO with a much lower selectivity for CO₂ over O₂ (48, 153). A number of dinoflagellates have plastids from tertiary endosymbioses, and they variously have Form 1B (green symbiont) or Form 1D (chromistan symbiont); these organisms have not yet been examined with respect to inorganic carbon acquisition. Also not examined for DIC acquisition are the glaucocystophytes, a small group of freshwater unicells with plastids from the primary endosymbiosis. This is also the case for the related red algae and green algae plus higher plants, but they have retention of the Form 1B RUBISCO from the cyanobacterial plastid ancestor, as do the green algae and higher plants. These algae retain the peptidoglycan wall from the plastid ancestor around their plastid and have structures in the stroma that have been variously interpreted as carboxysomes and as pyrenoids, allowing speculation as to their DIC acquisition mechanism (150).

Much of the effort in investigating carbon acquisition in these organisms focused on diatoms (heterokonts) and coccolithophores and other haptophytes, which are important members of the freshwater (diatoms only) and marine phytoplankton, with less work on the experimentally less tractable dinoflagellates. There has also been considerable work on red and brown (heterokont) macroalgae, as well as on red microalgae. The evidence considered below indicates that the majority of the organisms examined have CCMs (153) but that, for various reasons, there is less mechanistic evidence on CCMs than on green algae and, especially, cyanobacteria. The availability of complete genome sequences for the marine diatom *Thalassiosira pseudonana* and for the thermoadophilic red alga *Cyanidioschyzon merolae* (113) should help in interpreting physiological and biochemical data on these and related organisms.

**Algae Apparently Lacking a CCM**

The CCM-less organisms include some red macroalgae, which grow in freshwater, some subtidal and low-intertidal marine red macroalgae, some marine red macroalgae, which grow at or above the high tide level or on the shaded side of intertidal rocks (157, 158), and almost all of the freshwater chrysophyte (heterokont) algae examined (12).
Algae with a CCM: Inorganic C Entry

The generation of a higher internal than external DIC, and of higher CO₂ concentration in cells than in the medium, has been demonstrated for one or more species of red alga, diatom, haptophyte, tribophyte, dinoflagellate, and eustigmatophyte (37, 153). The mass spectrometric method of Badger et al. (10) and the isotope disequilibrium technique show that many of the microscopic algae examined can take up both CO₂ and HCO₃⁻ (27, 31, 37, 111, 153, 170). The organisms concerned are a red alga, diatoms, haptophytes, and some eustigmatophytes. Other eustigmatophytes can take up either CO₂ or HCO₃⁻, whereas dinoflagellates can only take up CO₂ (see table 2 in 37, 40).

Some eustigmatophytes (Nannochloropsis spp.; marine) can take up HCO₃⁻, whereas Monodus subterraneus (freshwater/soil) only takes up CO₂ (37).

For the marine organisms with the capacity to take up both HCO₃⁻ and CO₂, growth at decreasing concentrations of CO₂ (and hence a lower CO₂: HCO₃⁻ ratio) in equilibrium with the seawater generally decreased the ratio of uptake of CO₂ to that of HCO₃⁻ in diatoms, but was less obvious in the haptophytes Phaeocystis globosa and, especially, Emiliania huxleyii (31, 111, 170). These experiments were carried out in the presence of dextran-bound sulfanilamide to inhibit extracellular CA activity. CO₂ is often the dominant form of DIC taken up under these conditions in air-equilibrated seawater, and would be even more important as the form entering the cells when CAext is not inhibited. The 5.4-fold slower rate of uncatalyzed dehydration of HCO₃⁻ in seawater than in freshwater (79, 111) means that extracellular CA activity is even more important in making the dominant (HCO₃⁻) form of DIC in seawater available to the CO₂ transport mechanism than is the case in fresh waters. In addition to its role as the major, or only, form of DIC entering the cell in most microalgal cells, CO₂ is the form of DIC whose concentration regulates the expression of the CCM components in some organisms, e.g., the marine diatom Phaeodactylum tricornutum (111).

Algae with a CCM: Role of the Plastid Envelope

Plastids isolated from the giant-celled marine red alga Griffithsia, and from two species of diatom, can photosynthesize at close to the in vivo

Algae with a CCM: Inorganic C in the Cytosol

The red alga Porphyridium and the diatom Phaeodactylum have intracellular β-CAs that may be cytosolic but have leader sequences consistent with a plastid location. If they are cytosolic then they would equilibrate cytosolic DIC species, facilitating uptake of DIC by plastids if the DIC species taken up by the plastids was not the one that was delivered to the cytosol (see below). Lack of understanding of the full-range plastid targeting sequences in diatoms means that there is uncertainty as to where other CAs detected in the Thalassiosira pseudonana genome (http://genome.jgi-psf.org.thaps1/thaps1.home.html), including γ-CAs, are expressed.

For the C₄-like photosynthetic C assimilation proposed by Reinfelder et al. (163, 164) and Morel et al. (120, cf. 80), for C₄- or Zn-limited cells of the diatom Thalassiosira weissflogii there is a clear role for cytosolic CA if CO₂ is the inorganic C species delivered to the cytosol. As in terrestrial C₄ plants, cytosolic CA is required to convert CO₂ into HCO₃⁻, the substrate for PEPc. The product of this reaction, oxaloacetate, is reduced to malate, which is transported into the plastids. There are still uncertainties in this proposed C₄-like pathway in a diatom, e.g., definitive data on the location of two of the enzymes involved, PEPc and PEPck. However, inhibitor evidence is consistent with a role for PEPc, and feeding of the proposed intermediates oxaloacetate and malate restricts the fixation of exogenous DIC while maintaining the rate of O₂ evolution (164). There is also evidence from silicone oil centrifugation experiments with a range of extraction-trapping solutions for a large fraction of the ¹⁴C taken up in 10 sec occurring in an organic acid rather than in DIC (164).

Algae with a CCM: Role of the Plastid Envelope

Plastids isolated from the giant-celled marine red alga Griffithsia, and from two species of diatom, can photosynthesize at close to the in vivo
rates on a chlorophyll basis, but there have been no studies of the mechanism(s) of DIC entry (145, 153). The isolated diatom plastids lack the two “chloroplast endoplasmic reticulum” membranes, as is typically the case for kleptoplasts from the tribophyte *Vaucheria litorea*, which can photosynthesize for many months in the saccoglossan mollusk *Elysia chlorotica* (63, 151, 161). The proposed C₄-like photosynthesis in diatoms requires importing malate into the plastids and exporting phosphoenolpyruvate or pyruvate to the cytosol.

**Algae with a CCM: Reactions in the Stroma**

Many of the algae with CCMs have pyrenoids containing much of the plastid complement of RUBISCO, although a significant number lack pyrenoids. The common assumption is that the pyrenoid also contains a CA, perhaps the β-CA, which may be targeted to the plastid in *Porphyridium* and *Phaeodactylum*. The most effective use of such a CA would be to produce CO₂ at the site of RUBISCO activity from HCO₃⁻ transported into the stroma. There is no obvious role for such a CA, or the pyrenoids, if CO₂ is the form of DIC that is concentrated in the plastid by a plasmalemma and/or a plastid envelope–located CO₂ pump, or for a C₄ acid decarboxylase such as PEPck in the case of the proposed C₄-like photosynthesis. However, such a CA activity would not be as damaging to the effective operation of the C₄ mechanism proposed by Reinfelder et al. (163, 164) as cytosolic CA expression in the bundle sheaths of terrestrial C₄ plants would be, unless there were HCO₃⁻-conducting channels on the plastid envelope membranes leaking HCO₃⁻ back to the cytosol.

**ENVIRONMENTAL MODULATION OF CCMs**

There have been numerous roles suggested for CCMs, namely (a) improving CO₂ supply and providing a competitive advantage when the DIC and/or CO₂ in the environment is decreased, (b) improving resource-use efficiency when nutrients such as N, P, Fe, S, etc. are in short supply, and (c) acting as a means of energy dissipation (14). Consequently, it is not surprising that environmental factors such as nutrient availability, energy supply, and CO₂ availability play a significant role in modulating CCM activity. A full discussion of environmental regulation of CCMs can be found in Beardall & Giordano (14).

**Inorganic Carbon Supply**

DIC transport and CCM capacity are downregulated by increasing CO₂ concentrations in the gas phase in equilibrium with the external medium. In *Chlamydomonas*, at least, high CO₂ suppresses expression of a high-affinity DIC state, but CO₂ accumulation can still occur as a low-affinity, constitutive mechanism (2, 136, 188, 194).

There appears to be a continuum in the degree to which the CCM is expressed in response to external DIC concentration, with higher concentrations leading to a greater degree of suppression of CCM activity (8, 14, 114, 117, 178). Many genes associated with aspects of CCM function are, in *Chlamydomonas reinhardtii*, controlled by DIC supply. These include the α-CA in the periplasmic space (Cab1), the β-CA associated with mitochondria (Mca1, Mca2), which are upregulated by low CO₂, and the other periplasmic α-CA (Cab2), which is downregulated. Low CO₂ also modulates the activity of a number of enzymes associated with photorespiratory and nitrogen metabolism (see 186 for a detailed review). Low-CO₂-grown cells of *Chlamydomonas reinhardtii* also show substantial changes in ultrastructure, including an increase in the starch sheath associated with the pyrenoid (92, 142) and shifts in mitochondrial distribution from the center of the cell to the periphery, between the plasmalemma and the chloroplast envelope (56).

In cyanobacteria, the HCO₃⁻ concentration in the external medium is the controlling signal governing CCM expression (114). However, in some eukaryotes, e.g., *Peridinium gatunense*
(25), *Chlorella ellipsoidea* (109, 110), *C. kessleri* (29), and *Phaeodactylum tricornutum* (111). CO₂ is the DIC species that controls CCM activity. Fukuzawa et al. (52) and Xiang et al. (210) identified a gene (named *ccm1* or *cia5*, respectively) that appears to code for a zinc finger transcription factor. Cia5 is clearly a key component in the regulation of genes controlled by low CO₂, although its precise mode of action is subject to debate (186).

Any environmental factor that affects inorganic carbon levels in the bulk medium around algal cells can thus affect the phenotypic expression of CCMs. In addition to the direct effect of levels of CO₂ in equilibrium with the bulk medium, factors such as pH, temperature, and salinity also alter CCM activity (14, 17).

### Light Availability

C₅ transport and accumulation is an active process, with evidence from several groups suggesting that the ATP necessary for carbon transport is derived from electron flow associated with PSI (128, 129, 135, 185), although some eustigmatophyte algae are unusual in having a CCM driven by respiratory ATP (73). Given that CO₂ fixation coupled to operation of a CCM is energetically costly (17), it might be expected, a priori, that limitations on energy supply (i.e., photon flux) could affect the activity of CCMs. In a number of species there is evidence that rates of inorganic carbon transport and CCM activity are greatest under high-photon flux densities (13), and acclimation to low-photon flux results in a decreased capacity and/or affinity for DIC transport in numerous algae (14, 90, 91, 178). However, in *Anabaena variabilis*, a decrease in CCM capacity was only found under severe light limitation (13).

In both *Chlamydomonas reinhardtii* (77) and *Synechocystis* sp. PCC6803 (115), high light regulates expression of a number of genes, including some of those that are low-CO₂ inducible and under the control of the Cia5 gene product (77), suggesting that any signaling mechanism modulating expression of CCM activity is not based solely on external DIC concentration (115).

### Other Nutrients

In most cases, CCM function depends on photosynthesis, and light energy harvesting can be compromised by macronutrient and iron deficiency (54, 55, 64). It is therefore important to understand the interactions between CCM activity, light, and nutrient availability. CCM function requires synthesis of specific proteins (106, 186, 193), which represents a demand for cellular nitrogen. The amount of resources that a cell invests in acquiring carbon through a CCM is likely to be coupled to the availability of other nutrients. Controlling cellular elemental ratios is fundamentally important for optimizing resources and maintaining the operational enzymic machinery necessary for survival and growth. Thus, it is expected that DIC acquisition interacts with nutrient acquisition, although, as we shall see, such interactions are complex.

For nutrients such as phosphorus and sulfur, uptake and assimilation mechanisms are induced by limitation by these elements (41, 59, 61). Unless limitation is mild, acclimation strategies are not sufficient to restore optimal growth and are employed in parallel with a downregulation of cell activities not related to acquiring the limiting nutrient (41, 61). For instance, because of the dependency of DIC acquisition on ATP supply, it seems possible that phosphorus limitation would affect CCMs (14, 17). There are some limited data for *Chlorella emersonii* that CCMs are downregulated under severe P limitation (21a).

Little information is available on the effect of sulfur limitation on regulating CCMs. In general terms, under sulfur limitation cells tend to reallocate their resources so that synthesis of low-sulfur proteins and degradation of high-sulfur proteins are favored (61). RUBISCO is a major reservoir of sulfur in the cells and therefore it is not surprising that sulfur limitation characteristically leads to a conspicuous
reduction in RUBISCO. This substantially reduces photosynthetic capacity (61) and also seems to impact the photosynthetic C-use efficiency (14). In *Chlamydomonas*, the reduced photosynthetic performance is a specific response controlled by the *sac1* gene product. *Sac1* mutants are unable to downregulate photosynthesis and rapidly die in the light when sulfur limited (42). However, in *Dunaliella salina*, periplasmic CA is not downregulated under sulfur-limited conditions (61), despite a reduction in affinity for inorganic carbon.

For nitrogen the situation is more complex. In *Chlorella emersonii* (15, 21) and *Dunaliella tertiolecta*, N limitation leads to an enhanced affinity for CO2 in whole cells (211a). In contrast, mildly N-limited cells of *Chlamydomonas reinhardtii* show a downregulation of the CCM and of mitochondrial β-CA (60). In *Chlamydomonas reinhardtii*, the photosynthetic CO2 use efficiency increases with increasing NH4+ (14). The stimulation of photosynthetic performance is thus somehow related to the concentration of N and might reflect a demand for carbon to maintain a correct C:N ratio, thereby ensuring optimal operational capacity for the cell. In species that show upregulation of CCMs under very low N availability, it has been suggested that this is a response to enhance the N-use efficiency of the cells (15, 21, 211a). If a possession of a CCM substantially favors the carboxylase function of RUBISCO, then the achieved rate of CO2 fixation per unit nitrogen in RUBISCO increases. Therefore, under N limitation, CCMs will increase the nitrogen efficiency of growth (rate of biomass production per unit algal nitrogen), provided the nitrogen allocated to the CCM components does not offset the nitrogen savings permitted by action of the CCM (160). At the same time, by possibly reducing losses of nitrogen through photorespiration, an active CCM in starved cells may limit the increase in the cell C:N ratio, somewhat moderating the decrease in size and functionality of the enzymatic machinery (14).

Cells of *Dunaliella salina* and *D. parva* grown on NH4+ have a higher affinity for CO2 than those grown on NO3 (14, 57, 58). This effect is largely independent of the external CO2 availability and seems to provide additional evidence that CCMs may be involved in the control of cellular elemental ratios (and specifically the C:N ratio) rather than simply in DIC acquisition (14). In this respect it is worth noting that Giordano et al. (60) showed that induction of mtCA is regulated as much by C:N ratio as by low CO2.

Iron availability, like that of nitrogen, can often compromise light-harvesting processes and photosynthesis (54, 55, 64). Large areas of the world’s oceans, such as the equatorial Pacific Ocean (22) and the Southern Ocean (195), are potentially iron limited. Nonetheless, there is little information available on the interaction between CCMs and iron limitation. In iron-limited, as in N-limited, chemostat cultures of *Dunaliella tertiolecta*, the affinity of cells for DIC was enhanced at lower growth rates (211, 211a), reflecting an increased investment in a CCM under these conditions. Maintaining high-affinity DIC acquisition under both Fe- and N-limited conditions in *Dunaliella tertiolecta* (211, 211a) may confer improved energy and resource use efficiency when photosynthetic energy-harvesting capacity is thus impaired. These findings suggest that maintaining efficient DIC-uptake kinetics could be a general response to energy limitation imposed by nutrient deficiency in this alga.

Zinc is used as the metal cofactor of α-, β-, and γ-CAs and is directly involved in the catalytic mechanism of these enzymes (123 and references therein). Thus, it can be expected that a reduction in Zn availability would affect CCMs and, in diatoms, the level of induction of Zn-CA would depend on the availability of Zn (93). However, Zn can be effectively substituted with cobalt (94). There are also indications of Cd-CA distinct from Zn-CA in the diatom *Thalassiosira weissglogii* (39), which is more abundant when cells are exposed to low-CO2 and -Zn concentrations. However, little is known of the physiological role of this Cd-CA.
**Other Environmental Factors**

Temperature has been proposed to regulate the CCM capacity of cells. At low temperatures, the need for a CCM is diminished due to the increased solubility of CO₂, CO₂:O₂ ratio, and pKa and the consequent greater availability of CO₂ (143, 157). However, Mitchell & Beardall (118) and Roberts & Beardall (168) showed that Antarctic micro- and macroalgae have similar DIC-acquisition capacities to their respective temperate-water counterparts. Changes in CA activity with temperature are inconsistent. Thus, CA₆ activity in *D. salina* is inversely related to temperature with a twofold decrease in CA₆ activity in cells grown at 25°C compared to cells grown at 10°C (B. Bernacchia & M. Giordano, unpublished), whereas the same authors observed a ~20% increase in CA₆ activity, for a similar temperature range, in the freshwater diatom *Asterionella formosa* and the marine coccolithophore *Emiliania huxleii* (14).

Although there are many reports on the effects of UV-B radiation on algal photosynthesis, there is virtually no information on the likely consequences of enhanced UV-B for CCM activity. In *Dunaliella tertiolecta*, UV-B inhibits carbon fixation but not transport, thereby causing intracellular DIC pools to be slightly elevated (16) and enhanced cellular affinity for CO₂ in photosynthesis. *Nannochloropsis gaditana*, which has active bicarbonate influx, showed more sensitivity to UV-B than when the CCM was repressed by growth with 1% CO₂ in air (183). In contrast, the CO₂-transporting species *Nannochloris atomus* showed no difference in UV-B sensitivity between treatments. This implies that the bicarbonate transporter may be UV-B sensitive but that the CO₂ transporter may be resistant. However, this requires further investigation.

**EVOlUTION**

Here we briefly consider evidence from extant organisms as to the phylogeny of CCMs, and also evidence from the Earth sciences on the occurrence of cyanobacteria and algae and their impact on the environment over the last four Gyr. For cyanobacteria, the two categories (α and β) identified by carboxysome proteomics, the occurrence of the 1A or the 1B form of RUBISCO, and the membrane-associated CCM components map reasonably well onto the phylogeny deduced from other molecular genetic markers (11). Thus, the α-cyanobacteria *Prochlorococcus marinus* and *Synechococcus* sp. WH8102 (and other marine strains of *Synechococcus*) are a relatively derived (apo-morphic) clade of cyanobacteria (169). Because cyanobacteria diversified prior to the endosymbiotic event leading to plastids (108, 196) it could be argued that at least β-carboxysomes and the associated components of the CCM evolved prior to the endosymbiotic origin of plastids. Badger & Price (11) suggest a later origin of CCMs and point out that the occurrence of both α- and β-carboxysomes in other kinds of bacteria means the evolution of cyanobacterial CCMs must take other bacteria, and the possibility of horizontal gene transfer, into account.

Hypotheses as to the origin(s) of CCMs in eukaryotes should not be divorced from the occurrence (or not) of a CCM in the β-cyanobacterial plastid ancestor (11). If the plastid ancestor had a CCM, then the presence of a CCM would have been the plesiomorphic state for photosynthetic eukaryotes, with absence of CCMs as derived states. A similar argument can be applied to the subsequent secondary and tertiary endosymbioses. The membrane-associated components of the CCM would have been initially located on membranes derived from the plasmalemma or thylakoids of the plastid ancestor, although the subsequent transfer of genes to the host nucleus and retargeting of their products is a common feature of the evolution of photosynthetic eukaryotes (108). This hypothesis, and others arguing for the pleisiomorphy of eukaryote CCMs, does not accord with the absence of reported homologues of cyanobacterial membrane-associated CCM components and the absence or rare occurrence of carboxysomes in eukaryotes.

A further problem is that phylogenetic analyses suggest that reacquisition of CCMs
after loss may have occurred quite frequently, although much of the evidence favoring this view comes from the occurrence of pyrenoids (157), and although pyrenoids are good indicators of the capacity to express a CCM in a (wild-type) organism, the reverse is not true. In the absence of genetic data on (a) component(s) of CCM, the various possibilities cannot be distinguished.

In considering how the evolution of CCMs relates to their environment, a key factor is the kinetics of RUBISCO. The ancestral RUBISCO was derived from an enzyme in a bacterial methionine salvage pathway (5) and probably had a low CO\(_2\) affinity and a low selectivity for CO\(_2\) over O\(_2\). At this time (perhaps \(\sim 3.8\) Gyr ago), a greater greenhouse effect was needed to account for the presence of liquid water on an Earth orbiting the “faint young sun,” which was only providing \(~75\%\) as much radiant energy as the sun does today (85). Mineralogical proxies for a 25°C surface temperature put a lower limit on CO\(_2\) partial pressure of 250 pascal (Pa) at 3.2 Gyr ago (67), and an upper limit of 1 kPa at 2.8 Gyr ago (85); the latter value requires another greenhouse gas (e.g., CH\(_4\)) to account for Earth’s surface temperature. O\(_2\) was very low at this time, and for more than 100 Myr after the origin of oxygenic photosynthesis, not later than 2.7 Gyr ago (24, 30, 85, 191), such a diffusive supply of CO\(_2\) to a phytoplankton cell would have been adequate even if its RUBISCO had a low CO\(_2\) affinity and a low CO\(_2\)/O\(_2\) selectivity. However, microbial mats and stromatolites (widespread from \(~2.3\) Gyr ago, at about the time that global oxygenation began) provided a high density of benthic (i.e., growing on a surface rather than free-floating) cells and could have caused a localized drawdown of CO\(_2\) and accumulation of O\(_2\).

Assuming a photosynthetic rate similar to extant microbial mats of 2 \(\mu\)mol m\(^{-2}\) s\(^{-1}\), and a bulk-phase seawater CO\(_2\) concentration (85) of 0.45 mM, a mean 0.4-mm diffusion distance to cyanobacterial cells yields an intracellular CO\(_2\) of 50 \(\mu\)M and O\(_2\) of 400 \(\mu\)M (cf. 144, which assumed higher CO\(_2\) levels). This would constitute a selective factor favoring high-CO\(_2\) affinity and higher CO\(_2\)/O\(_2\) selectivity of RUBISCO, and/or a CCM, in the benthic cyanobacteria. These effects would have been exacerbated by the rise (via surface seawater O\(_2\)) in atmospheric O\(_2\) which started later than 2.45 Gyr ago and had reached >0.02 Pa not later than 2.32 Gyr ago (24). The O\(_2\) increase probably occurred at a similar time to that of the origin of eukaryotes (30), and is within the time frame of the Huronian glaciations (24). Increased O\(_2\) would have converted the more potent greenhouse gas CH\(_4\) into the less potent CO\(_2\), so glaciations do not necessarily mean lower CO\(_2\) (24, 85; figure 4 in 47).

These arguments suggesting that there could have been selective pressures for CCMs as early as 2.3 Gyr ago do not necessarily apply to planktonic cyanobacteria, so further evolution of cyanobacteria, including the primary endosymbiotic event to yield photosynthetic eukaryotes, may not have involved cyanobacteria and photosynthetic eukaryotes with CCMs. Later, there were the glaciations, possibly as extreme as a “Snowball Earth,” in the Cryogenian/Ediacaran at 0.75 and 0.6 Gyr ago (85, 208). By 1.2 Gyr there were multicellular photosynthetic eukaryotes such as the red alga Bangiomorpha (34), with extant relatives that presumably survived the Snowball Earth in restricted, unfrozen areas of the ocean. These glaciations also could have involved very low CO\(_2\) levels, and thus have been a time favorable to the evolution of CCMs (157). Badger & Price (11) suggest the origin of cyanobacterial and algal CCMs in the Carboniferous-Permian glacial episode was \(~300\) Myr ago. This period was characterized by low (\(~40\) Pa) atmospheric CO\(_2\) and high- (up to 35 kPa) atmospheric O\(_2\) (87, 209); this was probably the time of origin of at least the submerged freshwater aquatic variant of CAM in vascular plants, although terrestrial C\(_4\) metabolism probably did not originate until 20–30 Myr ago during a more recent decline in atmospheric CO\(_2\) (87). Some examples of CCMs in algae also could have evolved at this time. The increase in the \(^{13}\)C/\(^{12}\)C ratio of organic sediments in the ocean over the last 30 Myr needs investigation in this respect.
Although many factors other than the occurrence of CCMs influence this isotope ratio (65). This brief survey shows that there were four times in Earth history (∼2.3 Gyr ago; 0.75, 0.6 Gyr ago; ∼300 Myr ago; from 30 Myr ago onward) that global conditions might have yielded selection pressures favoring CCMs. The earlier the suggested time of origin, the more problems there would have been in retaining the capacity for expressing CCMs during the intervening periods where only localized habitats would have favored the retention and expression of CCMs. Further advances in fixing the timing(s) of the origin(s) of CCMs may owe more to Earth sciences than to molecular clock (62) inputs.

CONCLUSIONS AND PROSPECTS

Since CCMs were last reviewed in this journal in 1999 there have been significant developments in our knowledge and understanding. There have been major advances in our understanding, at the molecular, mechanistic, and regulatory level, of the CCMs of β-cyanobacteria. However, complete genome sequences for several α-cyanobacteria have shown that these organisms from the oligotrophic ocean lack many of the components of the β-cyanobacterial CCMs without flagging up alternatives. The β-cyanobacterial genomic data have also not helped significantly in establishing the molecular basis for eukaryote CCMs, e.g., in Chlamydomonas reinhardtii. Another recent significant advance is the revival, with better evidence, of the hypothesis that diatoms have a CCM resembling the C₄ pathway of higher plants. This work on Thalassiosira weissflogii has not benefited as much as it might have from findings from the T. pseudonana genome project because we do not have a complete understanding of targeting sequences in diatoms to help establish the location of, for example, PEPck. Without playing down the advances that have been made, it is clear that much remains to be done to establish the mechanism(s), and regulation, of CCMs in many ecologically important groups of algae, such as the dinoflagellates.

As for environmental modulation, much has been achieved at the level of phenomena, but deeper understanding requires more knowledge of mechanisms and of regulation at the cellular and molecular level. This is an important area in view of the increasing CO₂ concentration, temperature, and acidity of the surface ocean and of inland water bodies. Increasing understanding of the mechanisms of CCMs, especially at the molecular level, will help our understanding of their evolution. Perhaps advances in our understanding of the phylogeny of algae might have predictive value in terms of the mechanisms of the CCM(s) of algae that have not previously been examined in detail. However, experience with α- and β-cyanobacteria suggests that caution is needed in the application of phylogeny as a predictive tool.

SUMMARY POINTS

1. CCMs evolved as a means of counteracting the effect of the increasing O₂/CO₂ ratio in the atmosphere on CO₂ fixation via RUBISCO.

2. The evolution of CCM is probably a polyphyletic process elicited by the selection pressure exerted in O₂/CO₂. Variations in this ratio occurred several times in the earth’s history. It is possible that the prokaryotic CCM evolved in β-cyanobacteria; however, the possibility of lateral gene transfer makes it difficult to ascertain this. If the evolution of CCMs occurred before the primary endosymbiotic event, the presence of CCMs would be the plesiomorphic (ancestral) state for photosynthetic eukaryotes, some of which lost CCM at a later stage. If the plastid ancestor did not have a CCM, eukaryotes acquired CCMs polyphyletically.
3. Largely as a result of the presence of CCMs, the growth of most photolithotrophs present in today’s aquatic environments is not limited by inorganic carbon availability.

4. CCMs can be based on biochemical mechanisms, such as C₄ photosynthesis and CAM, or on biophysical processes involving either localized enhancement of external CO₂ concentration by acidification of an external or internal compartment, or on the active transport of DIC across membranes. Different types of CCMs are present in almost all algal groups.

5. The carboxysome in prokaryotes and the pyrenoid in eukaryotes seem to play an important role in CCM (even though the absence of pyrenoids does not necessarily imply the absence of a CCM).

6. CCM activity can be modulated by environmental factors such as macro- and micro-nutrient supply, photosynthetically active and UV radiation, and temperature. CCMs likely play an important role in coupling DIC acquisition with the availability of energy and nutritional resources.

ACKNOWLEDGMENTS

John Beardall’s work on inorganic carbon acquisition is supported by the Australian Research Council. John Raven’s work on inorganic carbon acquisition is supported by the Natural Environment Research Council, United Kingdom. We are grateful to Ms. S. Stojkovic for her thorough and patient assistance in preparation of the manuscript.

LITERATURE CITED


100. Ludwig M, Sültemeyer D, Price GD. 2000. Isolation of *ccmKLMN* genes from the marine cyanobacterium *Synechococcus* sp. PCC7002 (cyanobacteria), and evidence that *ccmM* is essential for carboxysome assembly. *J. Phycol.* 36:1109–18


This paper describes the regulation, by high light, of genes involved in the CCM and suggests that any signaling mechanism modulating expression of CCM activity need not be based solely on external DIC concentration.


142. Ramazanov Z, Rawat M, Henk MC, Mason CB, Matthews SW, Moroney JV. 1994. The induction of the CO₂-concentrating mechanism is correlated with the formation of the starch sheath around the pyrenoid of *Chlamydomonas reinhardtii*. *Planta* 195:210–16


188. Sülttemeyer D, Amoroso G, Fock HP. 1995. Induction of intracellular carbonic anhydrases during the adaptation to low inorganic carbon concentrations in wild-type and *ca*-1 mutant cells of *Chlamydomonas reinhardtii*. *Planta* 196:217–24


Contents

Fifty Good Years
Peter Starlinger ......................................................... 1

Phytoremediation
Elizabeth Pilon-Smits ................................................... 15

Calcium Oxalate in Plants: Formation and Function
Vincent R. Franceschi and Paul A. Nakata ................................ 41

Starch Degradation
Alison M. Smith, Samuel C. Zeeman, and Steven M. Smith .................. 73

CO₂ Concentrating Mechanisms in Algae: Mechanisms,
Environmental Modulation, and Evolution
Mario Giordano, John Beardall, and John A. Raven ....................... 99

Solute Transporters of the Plastid Envelope Membrane
Andreas P.M. Weber, Rainer Schrake, and Ulf-Ingo Flügge .................. 133

Abscisic Acid Biosynthesis and Catabolism
Eiji Nambara and Annie Marion-Poll .................................... 165

Redox Regulation: A Broadening Horizon
Bob B. Buchanan and Yves Balmer ........................................ 187

Endocytotic Cycling of PM Proteins
Angus S. Murphy, Anindita Bandyopadhyay, Susanne E. Holstein,
and Wendy A. Peer .................................................... 221

Molecular Physiology of Legume Seed Development
Hans Weber, Ljudmilla Borisjuk, and Ulrich Wobus ......................... 253

Cytokinesis in Higher Plants
Gerd Jürgens .............................................................. 281

Evolution of Flavors and Scents
David R. Gang ............................................................ 301
Biology of Chromatin Dynamics

Tsung-Fu Hsieh and Robert L. Fischer .................................................. 327

Shoot Branching

Paula McSteen and Ottoline Leyser .................................................. 353

Protein Splicing Elements and Plants: From Transgene Containment to Protein Purification

Thomas C. Evans, Jr., Ming-Qun Xu, and Sribarsa Pradhan ....................... 375

Molecular Genetic Analyses of Microsporogenesis and Microgametogenesis in Flowering Plants

Hong Ma .......................................................... 393

Plant-Specific Calmodulin-Binding Proteins

Nicolas Bouché, Ayelet Yellin, Wayne A. Snedden, and Hillel Fromm ............... 435

Self-Incompatibility in Plants

Seiji Takayama and Akira Isogai .................................................. 467

Remembering Winter: Toward a Molecular Understanding of Vernalization

Sibum Sung and Richard M. Amasino .................................................. 491

New Insights to the Function of Phytopathogenic Bacterial Type III Effectors in Plants

Mary Beth Mudgett .......................................................... 509

INDEXES

Subject Index ............................................................................. 533

Cumulative Index of Contributing Authors, Volumes 46–56 ....................... 557

Cumulative Index of Chapter Titles, Volumes 46–56 .................................. 562

ERRATA

An online log of corrections to Annual Review of Plant Biology chapters may be found at http://plant.annualreviews.org/