

# Functional Plant Biology

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## Contents

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### CO<sub>2</sub> Concentrating Mechanisms in Aquatic Photosynthetic Organisms

Contributed papers originating from presentations at the IVth International Symposium on Inorganic Carbon Utilisation by Aquatic Photosynthetic Organisms, Palm Cove, Queensland, Australia, August 2001

(See photo of participants at CCM2001)

#### Overview

Advances in understanding how aquatic photosynthetic organisms utilize sources of dissolved inorganic carbon for CO<sub>2</sub> fixation

**G. Dean Price and Murray R. Badger**      117–121

In this short article, the co-convenors of CCM2001 outline the significant advances achieved in aquatic CCM research that appear in this Special Issue.

#### Cyanobacteria

Two CO<sub>2</sub> uptake systems in cyanobacteria: four systems for inorganic carbon acquisition in *Synechocystis* sp. strain PCC6803

**Mari Shibata, Hiroshi Ohkawa, Hirokazu Katoh, Masaya Shimoyama and Teruo Ogawa**      123–129

The cyanobacterium *Synechocystis* sp. strain PCC6803 possesses two CO<sub>2</sub> uptake systems, plus two types of HCO<sub>3</sub><sup>-</sup> transporter. These authors have generated mutants of *Synechocystis* PCC6803 in which one, or a combination, of these inorganic carbon acquisition mechanisms is disrupted. The response of these mutants to growth under various CO<sub>2</sub> and pH regimes is discussed.

Modes of active inorganic carbon uptake in the cyanobacterium, *Synechococcus* sp. PCC7942

**G. Dean Price, Shin-ichi Maeda, Tatsuo Omata and Murray R. Badger**      131–149

This review focuses on the various modes of inorganic carbon transport in the model cyanobacterium, *Synechococcus* sp., and highlights their functional redundancy. A model is proposed in which two unique proteins (ChpX and ChpY) possessing CO<sub>2</sub> hydration activity, contribute to CO<sub>2</sub> uptake when coupled to photosynthetic electron transport through specialized NDH-1 complexes.

Structure, function and regulation of the cyanobacterial high-affinity bicarbonate transporter, BCT1

**Tatsuo Omata, Yukari Takahashi, Osamu Yamaguchi and Takashi Nishimura**      151–159

Active transport of HCO<sub>3</sub><sup>-</sup> is one method by which cyanobacteria accumulate inorganic carbon. This article summarizes current knowledge on BCT1, a primary-active HCO<sub>3</sub><sup>-</sup> transporter belonging to the ABC superfamily of transporters.

Evolution and diversity of CO<sub>2</sub> concentrating mechanisms in cyanobacteria

**Murray R. Badger, David Hanson and G. Dean Price**      161–173

Here, the authors capitalize on the recent explosion in the availability of complete cyanobacterial genomes to consider various gene components of photosynthesis and CO<sub>2</sub> concentrating mechanisms. Distinct forms of carboxysome, carbonic anhydrase, and inorganic carbon uptake system are identified, and related in an evolutionary context.

*Cover illustration:* The schematic depicts BCT1, a HCO<sub>3</sub><sup>-</sup> transporter of *Synechococcus* PCC7942, which is encoded by the *cmpABCD* operon and transcribed when CO<sub>2</sub> is limiting. A *cmpA* deletion mutant appears yellowish-green, relative to wild type, when cultured under low-CO<sub>2</sub> conditions (lower left; see Omata *et al.* pp. 151–159). The middle panel shows immunolocalization of carbonic anhydrase (green fluorescence) in cells of *Thalassiosira weissflogii* (see Morel *et al.* pp. 301–308). Important phototrophic symbioses are found in coral reef systems (see Leggat *et al.* pp. 309–322). The right panel shows a view of the Agincourt Reef system off Port Douglas in the Great Barrier Reef National Park (photo courtesy of Dr Eddy van Hunnik; see van Hunnik and Sültemeyer pp. 243–249).

Carboxysome genomics: a status report  
**Gordon C. Cannon, Sabine Heinhorst,  
Christopher E. Bradburne and Jessup M. Shively**  
175–182

The fixation of CO<sub>2</sub> by Rubisco is enhanced by the enzyme's sequestration into polyhedral inclusion bodies called carboxysomes. Here, the authors compare and contrast the arrangement of genes for Rubisco and carboxysome-shell proteins in a range of species, and conclude that two types of carboxysome operons can be distinguished based on these arrangements.

Characterization of the C-terminal extension of carboxysomal carbonic anhydrase from *Synechocystis* sp. PCC6803  
**Anthony K-C. So, Swan S-W. Cot and George S. Espie**  
183–194

Carbonic anhydrase catalyzes the reversible cycle of hydration and dehydration between CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>. Here, the authors use recombinant DNA and yeast two-hybrid technology to characterize the C-terminal extension of carboxysomal carbonic anhydrase from the cyanobacterium *Synechocystis*, in order to shed light on its role in the function of this key photosynthetic enzyme.

Functionally distinct NAD(P)H dehydrogenases and their membrane localization in *Synechocystis* sp. PCC6803  
**Hiroshi Ohkawa, Masatoshi Sonoda, Natsu Hagino,  
Mari Shibata, Himadri B. Pakrasi and Teruo Ogawa**  
195–200

By disrupting *ndhD* genes in the model cyanobacterial species, *Synechocystis*, Ohkawa *et al.* have made an important contribution to our understanding of the role of the NAD(P)H dehydrogenase complex in CCM. Their data provides information on the effect of *ndh* mutation on the transport of inorganic carbon, and the cellular location of NAD(P)H dehydrogenase complexes.

Hypothesis: versatile function of ferredoxin-NADP<sup>+</sup> reductase in cyanobacteria provides regulation for transient photosystem I-driven cyclic electron flow  
**Hans C. P. Matthijs, Robert Jeanjean,  
Nataliya Yeremenko, Jef Huisman, Françoise Joset  
and Klaas J. Hellingwerf**  
201–210

The high-CO<sub>2</sub> requiring phenotype of the *Synechocystis* sp. PCC6803 mutant, M55, is partially relieved by salt stress, which enhances cyclic electron transfer in PSI. A mutant created in the M55 background that expresses a truncated form of ferredoxin-NADP<sup>+</sup> reductase cannot adapt to salt stress. These authors therefore propose that ferredoxin-NADP<sup>+</sup> reductase is involved in the regulation of cyclic electron transfer in PSI.

### ***Chlamydomonas***

Regulation of a carbon concentrating mechanism through CCM1 in *Chlamydomonas reinhardtii*  
**Kenji Miura, Tsutomu Kohinata, Satoshi Yoshioka,  
Kanji Ohyama and Hideya Fukuzawa**  
211–219

Genes involved in the carbon concentrating mechanism are induced in *C. reinhardtii* in response to limiting CO<sub>2</sub>. In this brief review, the role of the regulatory gene, *Ccm1*, in this CO<sub>2</sub>-responsive induction pathway is examined, with emphasis given to the regulation of carbonic anhydrase and photorespiratory enzymes.

Acclimation of *Chlamydomonas* to changing carbon availability  
**Martin H. Spalding, Kyujung Van, Yingjun Wang and  
Yoshiko Nakamura**  
221–230

*C. reinhardtii* has the ability to acclimate to limiting concentrations of inorganic carbon, with the induction of a CO<sub>2</sub> concentrating mechanism being a key component of this response. Elucidation of the signalling cascade that transduces the low carbon signal has been aided by mutational analyses, which have identified genes such as the putative transcription factor, *Cia5*.

Use of the bleomycin resistance gene to generate tagged insertional mutants of *Chlamydomonas reinhardtii* that require elevated CO<sub>2</sub> for optimal growth  
**Sergio L. Colombo, Steve V. Pollock, Karla A. Eger,  
Ashley C. Godfrey, James E. Adams, Catherine B. Mason  
and James V. Moroney**  
231–241

Previously, the use of insertional mutagenesis to pinpoint genes that allow *C. reinhardtii* to grow at very low concentrations of CO<sub>2</sub> has been hampered by the difficulty of identifying the location of inserted DNA. These authors show that use of the bleomycin resistance gene to 'tag' DNA inserts makes it easier to identify the gene disrupted by insertional mutagenesis.

A possible role for carbonic anhydrase in the lumen of chloroplast thylakoids in green algae  
**Eddy van Hunnik and Dieter Sültemeyer**  
243–249

These authors isolated thylakoids from various green algal species, including mutants lacking cell walls or lumen carbonic anhydrase, to investigate the function of this enzyme. Results indicate that lumen carbonic anhydrase does not function in the CO<sub>2</sub> concentrating mechanism, but may contribute to the establishment of a proton gradient across the thylakoid membrane.

Analysis of *Chlamydomonas* mutants with abnormal expression of CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> uptake systems  
**Christoph Thyssen, Eddy van Hunnik, Marie Teresa Navarro, Emilio Fernández, Aurora Galván and Dieter Sültemeyer** 251–260

Here, the authors use insertional mutagenesis of *Chlamydomonas* to study genes involved in inorganic carbon uptake and the signal transduction pathway via which cells acclimate to limiting carbon availability. Data is also presented which suggests a close connection between high-affinity uptake of HCO<sub>3</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>.

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## Phytoplankton

The diversity of inorganic carbon acquisition mechanisms in eukaryotic microalgae  
**Brian Colman, I. Emma Huertas, Shabana Bhatti and Jeffrey S. Dason** 261–270

Eukaryotic microalgae employ a diverse range of mechanisms to facilitate uptake of inorganic carbon. Individual species may actively take up either CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup> or both, or passively take up CO<sub>2</sub>, with or without the involvement of carbonic anhydrase. Here, the specific mechanisms employed by both marine haptophytes and dinoflagellates are examined.

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Inorganic carbon acquisition and its energization in eustigmatophyte algae  
**I. Emma Huertas, Brian Colman and George S. Espie** 271–277

The eustigmatophyceans are primitive unicellular algae that occur primarily in waters with a high nutrient load. Here, the authors use mass spectrometry to compare the carbon acquisition strategies employed by marine and freshwater eustigmatophytes.

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Regulation of the expressions of HCO<sub>3</sub><sup>-</sup> uptake and intracellular carbonic anhydrase in response to CO<sub>2</sub> concentration in the marine diatom *Phaeodactylum* sp.  
**Yusuke Matsuda, Keiichi Satoh, Hisashi Harada, Dan Satoh, Yasutaka Hiraoka and Takumi Hara** 279–287

These authors present data showing that CO<sub>2</sub> limitation induces both internal carbonic anhydrase and HCO<sub>3</sub><sup>-</sup> uptake activity in the marine diatom, *P. tricornutum*, and suggest that this acclimation process is governed by the concentration of CO<sub>2</sub> rather than HCO<sub>3</sub><sup>-</sup>. The occurrence of a specific carbonic anhydrase (β-type) is also discussed.

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Calcification and inorganic carbon acquisition in coccolithophores  
**Lorraine Berry, Alison R. Taylor, Uwe Lucken, Keith P. Ryan and Colin Brownlee** 289–299

The precise purpose of the precipitation of calcite within intracellular vesicles of certain coccolithophorid phytoplankton remains enigmatic. Here, the authors summarize current knowledge of the calcification process, and relate this to the hypothesis that calcification plays a role in the supply of carbon for photosynthesis.

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Acquisition of inorganic carbon by the marine diatom *Thalassiosira weissflogii*  
**François M. M. Morel, Elizabeth H. Cox, Anne M. L. Kraepiel, Todd W. Lane, Allen J. Milligan, Irene Schaperdoth, John R. Reinfelder and Philippe D. Tortell** 301–308

Here, the authors review their most recent findings on the mechanism of inorganic carbon acquisition by marine diatoms, an area which has received little attention relative to the uptake of other major inorganic nutrients. Important contradictions between their own observations and those of other researchers are discussed.

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Dinoflagellate symbioses: strategies and adaptations for the acquisition and fixation of inorganic carbon  
**William Leggat, Elessa M. Marendy, Brett Baillie, Spencer M. Whitney, Martha Ludwig, Murray R. Badger and David Yellowlees** 309–322

In the symbiotic relationship between the dinoflagellate, *Symbiodinium*, and the giant clam, the transport of inorganic carbon from seawater to *Symbiodinium* is an essential function of the host, which in turn relies on dinoflagellate photosynthesis for production of its respiratory substrates. Here, the authors present a model for inorganic carbon transport within this system that involves passive diffusion of inorganic carbon into the host and an algal CCM.

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<sup>13</sup>C discrimination patterns in oceanic phytoplankton: likely influence of CO<sub>2</sub> concentrating mechanisms, and implications for palaeoreconstructions  
*Edward A. Laws, Brian N. Popp, Nicolas Cassar and Jamie Tanimoto* 323–333

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The carbon isotopic composition ( $\delta^{13}\text{C}$ ) of marine organic matter reflects palaeoenvironmental conditions. Photosynthetic organisms discriminate strongly against  $\delta^{13}\text{C}$ , and are therefore potentially valuable indicators of past atmospheric CO<sub>2</sub> concentrations. These authors examine the features of phytoplankton that influence the relationship between CO<sub>2</sub> concentration and  $\delta^{13}\text{C}$  in order to assess the robustness of this correlation.

Ecological implications of microalgal and cyanobacterial CO<sub>2</sub> concentrating mechanisms, and their regulation  
*John Beardall and Mario Giordano* 335–347

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The ability of algae to concentrate CO<sub>2</sub> at the active site of Rubisco is influenced by a variety of environmental factors, such as light, temperature and nutrient availability. These authors examine the complex interactions between these regulatory factors. In addition, the ecological consequences of algal CCMs are discussed, with particular reference to global climate change.

### Seaweeds, angiosperms and bryophytes

Inorganic carbon utilization in marine angiosperms (seagrasses)  
*Sven Beer, Mats Bjork, Frida Hellblom and Lennart Axelsson* 349–354

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Traditionally, the systems for utilization of inorganic carbon by seagrasses have been considered less efficient than those of macroalgae. Here, new experimental data, generated using modern techniques such as PAM fluorometry, is reviewed, and the validity of the original paradigm is questioned.

Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses  
*John A. Raven, Andrew M. Johnston, Janet E. Kübler, Rebecca Korb, Shona G. McInroy, Linda L. Handley, Charlie M. Scrimgeour, Diana I. Walker, John Beardall, Mathew Vanderklift, Stein Fredriksen and Kenneth H. Dunton* 355–378

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The natural abundance of carbon isotopes is a useful tool for understanding carbon acquisition by plants. This overview examines the benefits and problems associated with use of  $\delta^{13}\text{C}$  values, with particular emphasis given to marine macroalgae. The comprehensive Appendix of  $\delta^{13}\text{C}$  values is sure to be a useful tool for researchers in this area.

C<sub>4</sub> mechanisms in aquatic angiosperms: comparisons with terrestrial C<sub>4</sub> systems  
*George Bowes, Srinath K. Rao, Gonzalo M. Estavillo and Julia B. Reiskind* 379–392

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Of the total number of plants that utilize C<sub>4</sub> photosynthesis, only a few aquatic species have so far been described. In this review, the physiology, biochemistry and anatomy of aquatic C<sub>4</sub> species are discussed with reference to their terrestrial counterparts. Emphasis is given to the submersed monocot, *Hydrilla*, in which the C<sub>4</sub> and Calvin cycles operate uniquely, without Kranz anatomy, in the same cell.

Freshwater angiosperm carbon concentrating mechanisms: processes and patterns  
*Stephen C. Maberly and Tom V. Madsen* 393–405

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Photosynthesis in aquatic angiosperms is limited by CO<sub>2</sub> supply and low light intensity. This paper reviews the structural, morphological, physiological, and biochemical features of freshwater macrophytes as they relate to maximization of carbon uptake, and discusses how inorganic carbon may influence macrophyte ecology.

Variability of the pyrenoid-based CO<sub>2</sub> concentrating mechanism in hornworts (Anthocerotophyta)  
*David Hanson, T. John Andrews and Murray R. Badger* 407–416

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The pyrenoid of hornworts is an aggregation of Rubisco, traversed by thylakoid lamellae. Here, evidence for a correlation between the presence and structure of pyrenoids and CCM activity is presented. A combined fluorometer/mass spectrometer technique was used to compare the CCM function of hornworts, both possessing and lacking pyrenoids, with a C<sub>3</sub> liverwort.