C₃ and C₄ Pathways of Photosynthetic Carbon Assimilation in Marine Diatoms Are under Genetic, Not Environmental, Control

Karen Roberts, Espen Granum, Richard C. Leegood*, and John A. Raven

Plant Research Unit, University of Dundee at Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom (K.R., J.A.R.); and Robert Hill Institute and Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, United Kingdom (E.G., R.C.L.)

Marine diatoms are responsible for up to 20% of global CO₂ fixation. Their photosynthetic efficiency is enhanced by concentrating CO₂ around Rubisco, diminishing photorespiration, but the mechanism is yet to be resolved. Diatoms have been regarded as C₃ photosynthesizers, but recent metabolic labeling and genome sequencing data suggest that they perform C₄ photosynthesis. We studied the pathways of photosynthetic carbon assimilation in two diatoms by short-term metabolic ¹⁴C labeling. In Thalassiosira weissflogii, both C₃ (glyceraldehyde-P and triose-P) and C₄ (mainly malate) compounds were major initial (2–5 s) products, whereas Thalassiosira pseudonana produced mainly C₃ and C₆ (hexose-P) compounds. The data provide evidence of C₂-C₃ intermediate photosynthesis in T. weissflogii, but exclusively C₄ photosynthesis in T. pseudonana. The labeling patterns were the same for cells grown at near-ambient (380 μL L⁻¹) and low (100 μL L⁻¹) CO₂ concentrations. The lack of environmental modulation of carbon assimilatory pathways was supported in T. pseudonana by measurements of gene transcript and protein abundances of C₄-metabolic enzymes (phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase) and Rubisco. This study suggests that the photosynthetic pathways of diatoms are diverse, and may involve combined CO₂-concentrating mechanisms. Furthermore, it emphasizes the requirement for metabolic and functional genetic and enzymic analyses before accepting the presence of C₄-metabolic enzymes as evidence for C₄ photosynthesis.

Marine planktonic diatoms are responsible for up to 20% of primary production on earth, fixing more than 10 billion tons of inorganic carbon each year (Falkowski and Raven, 2007). Diatoms achieve this, despite CO₂-limiting conditions in the oceans, by using CO₂-concentrating mechanisms (CCMs) actively to increase the steady-state CO₂ concentration around Rubisco, the principal photosynthetic carboxylase (Giordano et al., 2005; Roberts et al., 2007). By increasing the ratio of CO₂ to O₂, this diminishes the wasteful process of photorespiration. Despite their great ecological impact, photosynthetic carbon acquisition by diatoms is still poorly understood.

It has generally been held that diatoms have biophysical CCMs, based on transport of inorganic carbon across cellular membranes (Giordano et al., 2005; Roberts et al., 2007). However, evidence has recently emerged of C₄ photosynthesis, a biochemical CCM, in the marine diatom Thalassiosira weissflogii (Reinfelder et al., 2000, 2004; Morel et al., 2002; compare with Johnston et al., 2001; Granum et al., 2005), emphasizing the need for additional photosynthetic labeling experiments. The case for C₄ photosynthesis has been further strengthened by the occurrence of relevant genes in recently sequenced marine phytoplankton genomes, including the diatoms Thalassiosira pseudonana (Armbrust et al., 2004) and Phaeodactylum tricornutum (Montsant et al., 2005) and the green alga Ostreococcus tauri (Derelle et al., 2006). Of particular relevance to this study is the finding that T. pseudonana possesses the enzymatic apparatus to operate C₄ photosynthesis of the kind suggested for T. weissflogii (Reinfelder et al., 2000, 2004; Morel et al., 2002), including phosphoenolpyruvate carboxylase (PEPC) and phosphoenolpyruvate carboxykinase (PEPCK). The hypothetical mechanism of unicellular C₄ photosynthesis is a compartmentalized carboxylation-decarboxylation cycle analogous to terrestrial C₄ plants, albeit utilizing different intracellular compartments rather than different specialized cells (Edwards et al., 2004). In the proposed model, PEPC functions as primary carboxylase in the cytoplasm, forming oxaloacetate (C₄) from phosphoenolpyruvate (C₃) and HCO₃⁻. C₄ acids are then transported into the chloroplast (possibly the pyrenoid) and decarboxylated by PEPCK, releasing CO₂ that is refixed by Rubisco. To complete the cycle, C₃ acids are transported back to the cytoplasm.
Important components of most biophysical CCMs are carbonic anhydrases (CAs), which catalyze the reversible hydration of CO₂ and usually depend on the trace metal zinc for activity (Giordano et al., 2005). A potential advantage of a biochemical versus a biophysical CCM in *T. weissflogii* is that of economizing on zinc (Reinfelder et al., 2000), which may be limiting or co-limiting in parts of the ocean (Crawford et al., 2003; Franck et al., 2003). However, the presence of cadmium-specific CAs in *T. weissflogii* and other diatoms (Lane et al., 2005; Park et al., 2007) further complicates consideration of how zinc influences their CCM.

The work described here includes short-term photosynthetic labeling studies on the marine diatoms *T. weissflogii* and *T. pseudonana*, and measurements in the latter of relevant gene transcripts and proteins (specific primers and antisera were only obtained for *T. pseudonana*). This study compared cells grown at near-ambient (380 μL L⁻¹; similar to the present atmospheric level) and low (100 μL L⁻¹; lower than last glacial maximum) air-equilibrium CO₂ concentrations, since earlier work suggested that C₄ photosynthesis is induced by low CO₂ in *T. weissflogii* (Reinfelder et al., 2000, 2004; Morel et al., 2002). It is known that growth in media equilibrated with CO₂ at below the present atmospheric level increases the photosynthetic affinity for inorganic carbon in, for example, *T. pseudonana* (Fielding et al., 1998) and the freshwater green alga *Chlamydomonas reinhardtii* (Vance and Spalding, 2005).

### RESULTS

Short-term photosynthetic ¹⁴C labeling was studied in *T. weissflogii* and *T. pseudonana* grown at near-ambient (380 μL L⁻¹) or low (100 μL L⁻¹) CO₂ concentrations. In *T. weissflogii*, both C₃ (glycerate-P and triose-P) and C₄ (mainly malate) compounds were major initial products, with respectively approximately 45% and 30% of label after 2 s (Fig. 1). The fraction of these early products then decreased rapidly, while that of C₆ (hexose-P) compounds increased reciprocally (from 15% to 60% within 30 s). The sigmoid shape of the C₆ labeling curve is consistent with transient C₄ labeling. Growth of the diatom at different CO₂ concentrations (380 or 100 μL L⁻¹) had no significant effect on the short-term labeling pattern in *T. weissflogii* (Fig. 2). The results indicate that a combination of glycerate 3-P (formed by Rubisco) and malate (derived from oxaloacetate formed by PEPC) are formed as primary products and sugar-P as secondary products by C₃-C₄ intermediate photosynthesis in *T. weissflogii*.

In *T. pseudonana*, the fraction of ¹⁴C-labeled C₃ compounds was similar to that in *T. weissflogii* (40% after 2 s, and then rapidly decreasing), but there was very little label in malate (C₄) and no label detected in Asp (C₄) at any time studied (Fig. 1). Most of the remaining label was incorporated into C₆ compounds, which increased from 40% (after 2 s) to 65% within 30 s. The hyperbolic shape of the C₆ labeling curve is consistent with the lack of C₄ labeling. As with *T. weissflogii*, there was no significant effect of the growth CO₂ concentration (380 or 100 μL L⁻¹) on the short-term labeling pattern in *T. pseudonana* (Fig. 2). The results indicate that glycerate 3-P is formed (by Rubisco) and malate (derived from oxaloacetate formed by PEPC) are formed as primary products and sugar-P as secondary products by C₃-C₄ intermediate photosynthesis in *T. pseudonana*.

In both diatoms there was significant ¹⁴C labeling of glycolate 2-P (C₂), the immediate product of Rubisco oxygenase activity, and early intermediates in the photosynthetic carbon oxidation cycle (PCOC), which contributed a higher fraction in *T. pseudonana* (10%–14%) than in *T. weissflogii* (5%–8%; Fig. 1).

---

Figure 1. Distribution of ¹⁴C-labeled products in *T. weissflogii* and *T. pseudonana* as a function of time after the addition of 1.2 mM NaH¹⁴CO₃ (0.85 MBq mL⁻¹) to the medium (total ¹⁴C incorporation in Supplemental Fig. S1; HPLC chromatograms in Supplemental Fig. S2). C₂, Combined glycolate 2-P, glycolate, and Gly; C₃, combined glycera-P (including glycerate 3-P) and triose-P; C₆, combined Glc-P and Fru-P; Mal, malate. Data are means ± SE of three separate determinations.

---

Plant Physiol. Vol. 145, 2007
Growth CO₂ concentration (380 or 100 μL L⁻¹) had small or negligible effects on transcripts of the C₄-metabolic genes PEPC₁, PEPC₂, and PEPCK, or Rubisco large subunit (RBCL), in T. pseudonana (Fig. 3). Transcription of RBCL, but neither PEPCs nor PEPCK, was strongly enhanced (approximately 30-fold) at the start of the light period compared to the start of the dark period. In accordance with the gene transcripts, CO₂ concentration had no significant effect on protein abundances of PEPC₁, PEPC₂, PEPCK, and RBCL (Fig. 4), nor did the transcripts or proteins respond to transient changes in CO₂ concentration (data not shown). In contrast, transcription of the PCOC gene for the P-subunit of Gly decarboxylase (GDCP) was highly up-regulated (3- to 6-fold) by low CO₂ concentration (Fig. 3). The results indicate that the putative C₄-photosynthetic carboxylases and decarboxylase in T. pseudonana are not influenced by inorganic carbon.

**DISCUSSION**

In T. weissflogii, the results of metabolic ¹⁴C labeling are consistent with a combination of glycerate 3-P (formed by Rubisco) and malate (derived from oxaloacetate formed by PEPC) as primary products of photosynthesis and sugar-P as secondary products (Fig. 1). This labeling pattern resembles that of a C₃-C₄ intermediate flowering plant such as Flaveria linearis (Monson et al., 1986), rather than a pure C₄ plant, in which malate is the dominant initial product and C₃ compounds appear as intermediates (Hatch and Slack, 1966). Interestingly, there was little label in Asp (C₄), which is a significant or dominant initial product in C₄ flowering plants with PEPCK as their decarboxylase (Wingler et al., 1999). Although C₄-metabolic gene transcripts or proteins in T. weissflogii were not analyzed in this study (due to the lack of specific primers and antisera), significant PEPC and PEPCK activities have previously been measured in this diatom (Reinfelder et al., 2000).

While the labeling pattern in T. weissflogii could be explained by C₃-C₄ intermediate photosynthesis, another explanation to consider is nonphotosynthetic C₄ metabolism. An alternative function of PEPC in diatoms is anaplerotic production of C₄ skeletons for nitrogen assimilation; the rate of light-independent carbon fixation in Skeletonema costatum, a close relative of Thalassiosira spp. (Kaczmarska et al., 2006), agrees well with the computed anaplerotic requirement (Granum and Myklestad, 1999). Differences in the diel pattern of NO₃⁻ assimilation between T. pseudonana and T. weissflogii (Needoba and Harrison, 2004) suggest that the anaplerotic rate is higher in the latter during the photoperiod, resulting in higher fixation of inorganic carbon into C₄ compounds. However, the extensive labeling of malate compared to anaplerotic requirements and negligible labeling of other organic and amino acids suggest that the C₄ fixation is mainly photosynthetic. The nonphotosynthetic roles of PEPCK are yet to be fully resolved in plants and are made more enigmatic in diatoms by its apparent chloroplastic location (Cabello-Pasini et al., 2001; Granum et al., 2005), but it too may be involved in nitrogen metabolism (Delgado-Alvarado et al., 2007). A recent study of PEPC and PEPCK activities in P. tricornutum indicated that both enzymes are strictly anaplerotic (Cassar and Laws, 2007).

In C₄ photosynthesis with HCO₃⁻ entering the cells, HCO₃⁻ fixed by PEPC in the cytoplasm, and CO₂ released by PEPCK in the chloroplast, there is no overt role for CAs (Reinfelder et al., 2000, 2004; Morel et al., 2002; Granum et al., 2005). However, the partial C₃ photosynthesis demonstrated in this study suggests the involvement of a parallel biophysical mechanism in T. weissflogii utilizing CAs. Facilitated or energized
uptake of inorganic carbon would require both a CA converting HCO$_3^-$ to CO$_2$ in the pyrenoid and a CA converting CO$_2$ to HCO$_3^-$ in some more peripheral compartment (Giordano et al., 2005; Roberts et al., 2007). Such involvement of CAs in the biophysical component of a combined CCM means less economy in the use of zinc than is the case with exclusive C$_4$ photosynthesis (Reinfelder et al., 2000).

While *T. pseudonana* possesses an enzymic complement that could permit C$_4$ photosynthesis, the combined metabolic $^{14}$C labeling, gene transcript, and protein measurements (Figs. 1–4) indicate solely C$_3$ photosynthetic biochemistry. Although posttranscriptional regulation of PEPC and PEPCK cannot be ruled out, regulatory phosphorylation domains characteristic for flowering plant enzymes are absent in the diatom enzymes (Granum et al., 2005). In neither diatom is the pathway(s) of inorganic carbon assimilation significantly altered by the CO$_2$ concentration used for growth, although there are clearly other acclimatory responses of both species (Fielding et al., 1998; Reinfelder et al., 2000, 2004; Burkhardt et al., 2001; Morel et al., 2002; Wilhelm et al., 2006; Roberts et al., 2007). Taken together, the evidence suggests that *T. pseudonana* acclimates to low inorganic carbon concentration by increasing its affinity for inorganic carbon using a biophysical CCM. Inorganic carbon depletion experiments with *T. pseudonana* and *T. weissflogii* showed similar growth rates as functions of inorganic carbon concentration (Clark and Flynn, 2000). Hence, any differences in the CCMs of these diatoms are not reflected in their growth rates.

Our results indicate significant rates of photorespiration in both *T. weissflogii* and *T. pseudonana*, and suggest that glycolate 2-P is metabolized by some PCOC, which is still not completely resolved in diatoms (Wilhelm et al., 2006). Transcription of *GDCP* was highly upregulated by low CO$_2$ concentration in *T. pseudonana* (Fig. 3), in accordance with previous evidence of high light induction of the Gly decarboxylase T-subunit in *T. pseudonana* (Parker and Armbrust, 2005) and *T. weissflogii* (Parker et al., 2004). Early PCOC intermediates were more extensively labeled in *T. pseudonana* than in *T. weissflogii* (Fig. 1). These data suggest that the combined biochemical and biophysical CCM in *T. weissflogii* is more effective in suppressing Rubisco oxygenase activity than is the exclusively biophysical CCM of *T. pseudonana*.

The work reported here highlights the hazards of assuming that the pathway(s) of photosynthetic carbon assimilation is consistent throughout a genus of diatoms. Interestingly, a recent molecular phylogenetic study showed that the genus *Thalassiosira* is paraphyletic, with *T. weissflogii* and *T. pseudonana* rather distantly related within the *Thalassiosira* plus *Skeletonema* clade (Kaczmarska et al., 2006). Variation in photosynthetic pathways is already recognized in many genera of flowering plants (Monson et al., 1986), and therefore caution should be exercised when inferring metabolic

---

**Figure 3.** Gene transcripts (mRNA) of PEPC1, PEPC2, PEPCK, RBCL, and GDCP (normalized to Act1) at the start of the light period (9 AM; 09:00) and dark period (9 PM; 21:00) in *T. pseudonana* grown at near-ambient (380 μL L$^{-1}$) or low (100 μL L$^{-1}$) CO$_2$. Data are means ± se of three separate determinations.

**Figure 4.** Immunoblots with antisera against PEPC1, PEPC2, PEPCK, and RBCL proteins from *T. pseudonana* grown at near-ambient (380 μL L$^{-1}$) or low (100 μL L$^{-1}$) CO$_2$ (full-length blots in Supplemental Fig. S3).
function in vivo from genomic data (Armbrust et al., 2004; Montsant et al., 2005; Derelle et al., 2006). This study suggests that diatoms utilize a combination of complementary CCMs, which may confer plasticity in acclimating to the changing atmospheric (and sea surface) CO₂ concentrations and variations in the availability of other resources (Giordano et al., 2005).

**MATERIALS AND METHODS**

**Growth of Diatoms**

*Thalassiosira pseudonana* (Hustedt) Hasle et Heimdal (clone CCMP 1335) and *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle (clone ACTIN, CCMP 1336) were batch cultured in artificial seawater, Aquil (Price et al., 1988), containing either 2.4 mM (near-ambient) or 0.6 mM (low) NaHCO₃. Inorganic carbon-free Aquil had a pH of 7.8, and Aquil with 2.4 mM NaHCO₃ had a pH of 8.1. Aquil with 0.6 mM NaHCO₃ presumably had an intermediate pH value. Cultures were grown with a 12-h photoperiod (200 μmol quanta m⁻² s⁻¹) at 15°C and air-equilibrated at either 380 μL L⁻¹ (near-ambient) or 100 μL L⁻¹ (low) CO₂. Cells were acclimated for at least 7 days, and were harvested during exponential growth (μ ≥ 1 d⁻¹). Cell numbers and sizes (Supplemental Table S1) were measured by microscopy (using hemocytometer and micrometer, respectively).

**Metabolic ¹⁴C Labeling**

To optimize photosynthetic ¹⁴C fixation, metabolic labeling experiments were conducted at mid-light period when circadian-regulated photosynthetic capacity is at a maximum (Putt and Prézelin, 1988). Triplicate cultures were harvested on membrane filters (0.45-µm pore), resuspended in 600 μL NaHCO₃-free Aquil (rather than the less ecologically relevant sorbitol buffer [Reinfelder et al., 2000, 2004]) to give densities of 2.5 to 5.0 × 10⁷ cells mL⁻¹ for *T. pseudonana* and 2.5 to 5.0 × 10⁶ cells mL⁻¹ for *T. weissflogii*, and incubated at 200 μmol quanta m⁻² s⁻¹ at 22°C. Metabolic labeling was initiated by adding 600 μL Aquil containing 2.4 mM NaH¹⁴CO₃ (1.0 MBq), and terminated after 2 to 30 s by adding 2.4 mL of 100°C Milli-Q water. Hence, during labeling, the cells were exposed to an inorganic carbon concentration (1.2 mM) and pH intermediate between those of the two growth media. The inorganic carbon system in the labeling solution was at, or very close to, chemical and isotopic equilibrium. Cells were extracted at 100°C for 30 min, acidified (pH ≤ 2.0) for 24 h to remove inorganic ¹⁴C, freeze-dried, and resuspended in 2.0 mL of Milli-Q water after centrifugation, the supernatant was freeze-dried to a final volume of 300 μL, and a 30-μL portion was assayed for radioactivity using a TriCarb 1100TR liquid scintillation analyzer with QuantraSmart Version 1.31 software (Packard). The ¹⁴C signal retained in the acid-stable products constituted <0.5% of the total ¹⁴C available from NaH¹⁴CO₃. The efficiency of the termination method was examined in experiments with *T. pseudonana*. When the labeling reaction was terminated immediately after the addition of NaH¹⁴CO₃, the total acid-stable ¹⁴C signal was only 1% of that after 5-s reaction, and the samples showed no discernable peaks in HPLC analysis. After cell extraction and centrifugation, 91% ± 5% (n = 47) of all acid-stable radioactivity was recovered in the supernatant. An ANOVA test showed that neither labeling time nor cell species had any effect on the proportion of radioactivity recovered, indicating that the ¹⁴C signal retained in the cell debris came from residual extract.

**Gene Transcript Analysis**

Gene-specific primers for PEP1C, PEP2C, PEPC, RBCL, GDCP, and the housekeeping gene actin 1 (Act1) were designed (Supplemental Table S2) using genomic sequence data for *T. pseudonana* (http://genome.jgi-psf.org/Thaps3/). Triplicate cultures of *T. pseudonana* were harvested at the start of the light period (9 a.m.) and dark period (9 p.m.) by centrifugation or gentle filtration and flash frozen. Cellular genomic DNA and total RNA were isolated with the DNeasy and the RNeasy plant mini kit (Qiagen), respectively. RNA was treated with RNase-free DNase (Qiagen) and tested for DNA contamination by PCR. One to 10 μg of RNA was reverse transcribed to cDNA using 150 units of BioScript RTase H Minus (Bioline) with 1 μg oligo(dT)₄ primers (Promega), 0.1 μmol dNTP mix (Bioline), and 40 units of RNAsin RNase inhibitor (Promega). The cDNA was purified with a QIAquick PCR purification kit (Qiagen). cDNA and genomic DNA standards were amplified by real-time PCR using gene-specific primers and PowerSYBR Green PCR master mix (Applied Biosystems) with an ABI Prism 7700 detection system (Applied Biosystems) run at 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. cDNA was quantified by DNA standard curves, and normalized to Act1. PCR products were resolved by PAGE and stained with ethidium bromide.

**Protein Analysis**

Polycystin custom peptide antisera were raised in rabbits against *T. pseudonana* PEP1C, PEP2C, and PEPC based on predicted protein sequences (Grunam et al., 2005). Custom peptides were used were PEP1C, KRLRESEGS-SEEEEC; PEP2C, KYLRSMPEPDDSPLTEPC; and PEPC, CIENTTWKEDE. The PEP1C and PEP2C conjugates were conjugated to keyhole limpet hemocyanin via the N-terminal Lys (K) primary amino groups, whereas the PEPC peptide was conjugated to keyhole limpet hemocyanin via the N-terminal Cys (C) sulfydryl group. RBCL antiserum was raised in rabbit against Rubisco purified from *Brassica napus* leaves. For protein extraction, cells were harvested as before at mid-light period (3 p.m.) and homogenized in six volumes of extraction buffer (50 mM MOPS [pH 7.2], 10 mM MgCl₂, 5 mM MnCl₂, 5 mM EDTA, 2 mM dithiothreitol, 0.5 mM 4-[2-aminoethyl]-benzenesulfonyl fluoride, and 0.05% v/v Triton X-100) at 4°C. The protein was quantified by the BioRad assay (Bradford, 1976), and denatured in 0.125 μL Tris-HCl (pH 6.8), 20% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol, 4% (w/v) SDS, and 0.025% (w/v) bromphenol blue at 95°C for 3 min. SDS-PAGE and immunoblotting were performed as described previously (Walker and Leegood, 1996).

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Total cellular ¹⁴C incorporations.

**Supplemental Figure S2.** HPLC chromatograms.

**Supplemental Figure S3.** Full-length immunoblots.

**Supplemental Table S1.** Cell size measurements.

**Supplemental Table S2.** PCR primer sequences.

**ACKNOWLEDGMENTS**

We thank Dr. Rob Hancock and Paul Walker (Scottish Crop Research Institute) for assistance with HPLC methodology, Drs. Arthur J.G. Moir (Department of Molecular Biology and Biotechnology, University of Sheffield) and David Parkinson (Biomedical Research Centre, Sheffield Hallam University) for producing custom peptides, and Dr. Simon C. Smith (Antibody Resource Centre, University of Sheffield) for producing antisera.

**LITERATURE CITED**

Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Nicholas H, Putnam NH, Zhou S, Allen AE, Apt KE, et al (2004) The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. Science 306: 79–86.
Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 28: 248–254
Burkhardt S, Amoroso G, Riebesell U, Sültemeyer D (2001) CO₂ and HCO₃⁻ uptake in marine diatoms acclimated to different CO₂ concentrations. Limnol Oceanogr 46: 1378–1391
Cabello-Pasini A, Swift H, Smith GJ, Alberte RS (2003) Iron. In: Edwards GE, Franceschi VR, Voznesenskaya EV. Single-cell C₄ photosynthesis in a marine diatom. Nature 421: 99–103
Cassar N, Laws EA (2007) Potential contribution of β-carboxylases to photosynthetic carbon iso fractionation in a marine diatom. Phycologia 46: 307–314
Clark DR, Flynn KJ (2007) Phosphoenolpyruvate carboxykinase from the marine diatom Skeletonema costatum and the phaeophyte Laminaria setchellii. II. Immunological characterization and subcellular localization. Bot Mar 44: 199–207
Casser DR, Flynn KJ (2000) The relationship between the dissolved inorganic carbon concentration and growth rate in marine phytoplankton. Proc R Soc Lond B Biol Sci 267: 953–959
Crawford DW, Lipsen MS, Purdie DA, Lohan MC, Statham PJ, Whitney FA, Putland JN, Johnson WK, Sutherland N, Peterson TD, et al (2003) Influence of zinc and iron enrichments on phytoplankton growth in the northeastern subarctic Pacific. Limnol Oceanogr 48: 1583–1600
Delgado-Alvarado A, Walker RP, Leegood RC (2007) Phosphoenolpyruvate carboxykinase in developing pea seeds is associated with tissues involved in solute transport and is nitrogen-responsive. Plant Cell Environ 30: 225–235
Derelle E, Ferraz C, Rombauts S, Rouzé P, Worden AZ, Robbens S, Derelle E, Ferraz C, Rombauts S, Rouzé P, Worden AZ, Robbens S, Partensky F, Degroeve S, Echeynie S, Cooke R, et al (2006) Genome analysis of the smallest free-living eukaryote Ostreococcus tauri unveils many unique features. Proc Natl Acad Sci USA 103: 11647–11652
Edwards GE, Franceschi VR, Voznesenskaya EV (2004) Single-cell C₄ photosynthesis versus the dual-cell (Kranz) paradigm. Annu Rev Plant Biol 55: 173–196
Falkowski PG, Raven JA (2007) Aquatic Photosynthesis, Ed 2. Princeton University Press, Princeton, NJ
Fielding AS, Turpin DH, Guy RD, Calvert SE, Crawford DW, Harrison PJ (1998) Influence of the carbon concentrating mechanism on carbon stable isotope discrimination by the marine diatom Thalassiosira pseudonana. Can J Bot 76: 1098–1103
Franck VM, Bruland KW, Hutchins DA, Brzezinski MA (2003) Iron and zinc effects on silicic acid and nitrate uptake kinetics in three high-nutrient, low-chlorophyll (HNLC) regions. Mar Ecol Prog Ser 252: 1–33
Giordano M, Beardall J, Raven JA (2005) CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annu Rev Plant Biol 56: 99–131
Granum E, Myklestad SM (1999) Effects of NH₄⁺ assimilation on dark carbon fixation and β-1,3-glucan metabolism in the marine diatom Skeletonema costatum (Bacillariophyceae). J Phycol 35: 1191–1199
Granum E, Raven JA, Leegood RC (2005) How do marine diatoms fix 10 billion tonnes of inorganic carbon per year? Can J Bot 83: 898–908
Hatch MD, Slack CR (1966) Photosynthesis by sugar-cane leaves. A new carboxylation reaction and the pathway of sugar formation. Biochem J 101: 103–111
Johnston AM, Raven JA, Beardall J, Leegood RC (2001) Photosynthesis in a marine diatom. Nature 412: 40–41
Kaczmarcka I, Beaton M, Benoit AC, Medlin LK (2006) Molecular phylogeny of selected members of the order Thalassiosirales (Bacillariophyta) and evolution of the fucoxanthin. J Phycol 42: 121–138
Lane TW, Saito MA, George GN, Pickering IJ, Prince RC, Morel FMM (2005) A cadmium enzyme from a marine diatom. Nature 435: 42
Monson RK, Moore BD, Ku MSB, Edwards GE (1986) Co-function of C₃ and C₄-photosynthetic pathways in C₃, C₄ and C₃-C₄ intermediate Flaviera species. Planta 168: 493–502
Montant A, Jabbari K, Maheswari U, Bowler C (2005) Comparative genomics of the pernnate diatom Phaeodactylum tricornutum. Plant Physiol 137: 500–510
Morel FMM, Cox EH, Kraepiel AML, Lane TW, Milligan AJ, Schaperdoth I, Rein fellowship for light in the centric diatom Thalassiosira weissflogii. Funct Plant Biol 29: 301–308
Needoba JA, Harrison PJ (2004) Influence of low light and a light:dark cycle on NO₃⁻ uptake, intracellular NO₃⁻ concentration and nitrogen isotope fractionation by marine phytoplankton. J Phycol 40: 505–516
Park H, Song B, Morel FMM (2007) Diversity of the cadmium-containing carbonic anhydrase in marine diatoms and natural waters. Environ Microb8iol 9: 403–413
Parker MS, Armbrust EV (2005) Synergetic effects of light, temperature, and nitrogen source on transcription of genes for carbon and nitrogen metabolism in the centric diatom Thalassiosira pseudonana (Bacillariophyceae). J Phycol 41: 1142–1153
Parker MS, Armbrust EV, Piovia-Scott J, Keil RG (2004) Induction of photosynthesis by light in the centric diatom Thalassiosira weissflogii (Bacillariophyceae): molecular characterization and physiological consequences. J Phycol 40: 557–567
Price NM, Harrison GI, Hering JG, Hudson RF, Nirel PMV, Palenik B, Morel FMM (1988) Preparation and chemistry of the algal carbonic anhydrase medium Aquil. Biol Oceanogr 6: 443–461
Püt M, Prželinić BB (1988) Diel periodicities of photosynthesis and cell division compared in Thalassiosira weissflogii (Bacillariophyceae). J Phycol 24: 315–324
Reinfeld JR, Kraepiel AML, Morel FMM (2000) Unicellular C₄ photosynthesis in a marine diatom. Nature 407: 996–999
Reinfeld JR, Milligan AJ, Morel FMM (2004) The role of the C₄ pathway in carbon accumulation and fixation in a marine diatom. Plant Physiol 135: 2106–2111
Roberts K, Granum E, Leegood RC, Raven JA (2007) Carbon acquisition by diatoms. Photosynth Res doi/10.1007/s11120-007-9172–2
Vance P, Spalding MH (2005) Growth, photosynthesis, and gene expression in Chlamydomonas varius. Environ Microb 1120: 500–513
Walker RP, Leegood RC (1996) Phosphorylation of phosphoenolpyruvate carboxykinase in plants. Studies in plants with C₄ photosynthesis and Crassulacean acid metabolism and in germinating seeds. Biochem J 317: 653–658
Wilhelm C, Büchel C, Fisahn J, Goss R, Jakob T, LaRoche J, Lavaud J, Wilhelm C, Büchel C, Fisahn J, Goss R, Jakob T, LaRoche J, Lavaud J, Lavo J, Loehr M, Riebesell U, Stehfest K, et al (2006) The regulation of carbon and nutrient assimilation in diatoms is significantly different from green algae. Protist 157: 91–124
Wingler A, Walker RP, Chen ZH, Leegood RC (1999) Phosphoenolpyruvate carboxykinase is involved in the decarboxylation of aspartate in the bundle sheath of maize. Plant Physiol 120: 539–545