University of Nebraska - Lincoln Digital Commons@University of Nebraska - Lincoln

Papers in Biochemical Engineering

Chemical and Biomolecular Engineering Research and Publications

7-1-2005

The phosphoglycolate phosphatase gene and the mutation in the phosphoglycolate phosphatase-deficient mutant (*pgp1-1*) of *Chlamydomonas* reinhardtii

Kensaku Suzuki

National Agricultural Research Center for Tohoku Region, Morioka, Japan, suzuki@affrc.go.jp

Hidenobu Uchida

National Agricultural Research Center for Tohoku Region, Morioka, Japan

Tarlan G. Mamedov

University of Nebraska-Lincoln, tmammedov2@unl.edu

Follow this and additional works at: http://digitalcommons.unl.edu/chemengbiochemeng



Part of the Biochemical and Biomolecular Engineering Commons

Suzuki, Kensaku; Uchida, Hidenobu; and Mamedov, Tarlan G., "The phosphoglycolate phosphatase gene and the mutation in the phosphoglycolate phosphatase-deficient mutant (pgp1-1) of Chlamydomonas reinhardtii" (2005). Papers in Biochemical Engineering.

http://digitalcommons.unl.edu/chemengbiochemeng/8

This Article is brought to you for free and open access by the Chemical and Biomolecular Engineering Research and Publications at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Papers in Biochemical Engineering by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

The phosphoglycolate phosphatase gene and the mutation in the phosphoglycolate phosphatase-deficient mutant (pgp1-1) of Chlamydomonas reinhardtii¹

Kensaku Suzuki, Hidenobu Uchida, and Tarlan G. Mamedov

Abstract: The sequences of the phosphoglycolate phosphatase (PGPase) gene *Pgp1* and the 5'-upstream region from *Chlamydomonas reinhardtii* wildtype 2137 and the *pgp1-1* mutant N142 that lacks the activity of PGPase (PGP1) were determined. The comparison revealed the alteration of a G to A at position 98 relative to the start codon. This destroyed the "GT" splice donor site at the beginning of the first intron of this gene, resulting in an extension of the first exon to 49 translatable codons followed by a stop codon, containing the codons corresponding to whole transit peptide for the chloroplast stroma and the first four N-terminal amino-acid residues of the PGP1 subunit. The comparison of the upstream nucleotide sequence of *Pgp1* with those of 37 other genes including those involved in the CO₂-concentrating mechanism and (or) photorespiration showed the high similarity of *Pgp1* upstream to a periplasmic carbonic anhydrase gene *Cah1*; the motifs RAGGTCAGN₈₋₉CCR and TTGGCAG were found only within the low-CO₂ responsive genes, including *Pgp1* and *Cah1*. GAN₇CGNTTGGAAN₂AG, TTGGAAGGAG, and CAGAGGTCAGN₈CCG were found only with *Pgp1* and *Cah1*, and ACGCTTGGCAGT and CATTACCAT were found only with *Pgp1* and alanine aminotransferase gene *Aat1*. The possibility of functional PGPase isozyme(s) in *C. reinhardtii* is also discussed.

Key words: Chlamydomonas reinhardtii, CO₂-concentrating mechanism, low-CO₂ responsive gene, pgp1-1 mutation, phosphoglycolate phosphatase.

Résumé : Les auteurs ont déterminé les séquences du gène de la phosphatase du phosphoglycolate (PGPase) et de la région 5' en amont, chez la souche sauvage 2137 du *Chlamydomonas reinhardtii*, ainsi que du gène *pgpl-1* du mutant N142 qui est dépourvu de l'activité de la PGPase (PGP1). Une comparaison révèle l'altération d'un G vers un A à la position 98, par rapport au codon de départ, ce qui détruit le site du fournisseur de jonction « GT », au début du premier intron de ce gène. Ceci conduit à une extension du premier exon à 49 codons transposables, suivi d'un codon d'arrêt, et contenant les codons correspondants au peptide de transition entier du stroma chloroplastique ainsi qu'à la première sous unité N terminale à quatre résidus d'acides aminés du PGP1. La comparaison de la séquence des nucléotides en amont du gène *Pgp1* avec ceux de 37 autres gènes, incluant ceux impliqués dans le mécanisme de concentration du CO₂ et (ou) de la photorespiration, montre une forte similarité du *Pgp1* en amont, jusqu'au gène de l'anhydrase carbonique périplasmique *Cah1*; on ne retrouve les motifs RAGGTCAGN₈₋₉CCR et TTGGCAG uniquement avec les gènes de réaction au faible CO₂, incluant *Pgp1* et *Cah1*. Les séquences GAN₇CGNTTGGAAN₂AG, TTGGAAGGAG et CAGAGGTCAGN₈CCG ne se retrouvent qu'avec les gènes *Pgp1* et *Cah1*, et les séquences ACGCTTGGCAGT et CATTACCAT qu'avec le gène *Pgp1* ainsi que le gène *Aat1* de l'aminotransférase de l'alanine. Les auteurs discutent la possibilité de l'existence d'une ou plusieurs isoenzyme(s) fonctionnelle(s) PGPase, chez le *C. reinhardtii*.

Mots clés : Chlamydomonas reinhardtii, mécanisme de concentration du CO₂, gène de réaction au faible CO₂, mutation *pgp1-1*, phosphatase du phosphoglycolate.

[Traduit par la Rédaction]

Received 25 August 2004. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 29 August 2005.

K. Suzuki.² Department of Research Planning and Coordination, and Department of Biology and Environmental Sciences, National Agricultural Research Center for Tohoku Region, Shimo-Kuriyagawa, Morioka, 020-0198, Japan.

H. Uchida³ and T.G. Mamedov.⁴ Department of Biology and Environmental Sciences, National Agricultural Research Center for Tohoku Region, Shimo-Kuriyagawa, Morioka, 020-0198, Japan.

¹This article is one of a selection of papers published in the Special Issue on CO₂-Concentrating Mechanisms in Photoautotrophic Microorganisms and was presented at the Fifth International Symposium on Inorganic Carbon Utilization by Aquatic Photosynthetic Organisms.

²Corresponding author (e-mail: suzuki@affrc.go.jp).

³Present address: Research Institute of Agricultural Resources, Ishikawa Agricultural College, Nonoichi, Ishikawa, 921-8836, Japan.

doi: 10.1139/B05-071

⁴Present address: Department of Biochemistry, University of Nebraska, Lincoln, NE 68588-0664, USA.

Introduction

Phosphoglycolate phosphatase (PGPase), a photorespiratory enzyme that catalyzes the hydrolysis of phosphoglycolate produced by the ribulose-1,5-bisphosphate oxygenase activity of ribulose-1,5-bisphosphate carboxylase-oxygenase, is essential for the growth of photosynthetic organisms in the light. The PGPase-deficient mutant of Chlamydomonas reinhardtii pgp1-1 requires elevated levels of CO2 and cannot grow under air (Suzuki et al. 1990). This high-CO₂ requirement likely results because phosphoglycolate accumulates in the pgp1-1 cells under air during photosynthesis (Suzuki et al. 1999), as phosphoglycolate is a strong inhibitor of triosephosphate isomerase (Wolfenden 1970; Anderson 1971; Norman and Colman 1991). However, it is also possible that the mutation blocks the trigger for the induction of the CO₂concentrating mechanism (CCM). In the pgp1-1 mutant, the function of the CCM was considerably lower than that in the wild type, in terms of photosynthetic affinity for inorganic carbon and total carbonic anhydrase activity (Suzuki et al. 1990), and intracellular inorganic carbon accumulation (Suzuki and Spalding 1987). On the other hand, it has been reported that PGPase is quickly and transiently upregulated by transfer of high-CO₂-grown cells to air (Marek and Spalding 1991), although the mechanism is not clear. Thus, the regulation of PGPase activity seems to be closely related to that of the CCM when the cells adapt to different CO₂ conditions, and use of the PGPase mutants should provide valuable information to investigate both regulatory mechanisms. In this report, we compared DNA sequences of the Pgp1 gene and upstream regions of C. reinhardtii wild-type 2137 and pgp1-1 mutant N142 to determine the pgp1-1 mutation. Possible regulatory sites in the upstream region of *Pgp1* and the possibility of functional PGPase isozyme(s) are also discussed.

Materials and methods

Strains and growth conditions

Chlamydomonas reinhardtii mutant strain N142 mt-(pgp1-1, cw15) was isolated from the progenies of RPR-pgp1 mutant 7FR2N (Suzuki 1995; Suzuki et al. 1999) after three crossings with strains containing the CC125-background, such as 124⁸ mt- (wild type) and TW3 mt+(thi10, cw15), which were obtained from K. Shimogawara (Teikyo University, Japan). The pgp1-1 mutant N142 and wild-type 2137 (Suzuki et al. 1990) were grown photoauto-trophycally as described previously (Suzuki 1995).

Determination of DNA sequences

DNA sequences of the PCR products and (or) the subclones ligated into pGEM-T (Promega, Madison, Wisconsin) were determined with automated DNA-sequencing systems 310 and 373 (Applied Biosystems, Foster City, California) using both the Dye Terminator Cycle Sequencing Kit and the Dye Primer Cycle Sequencing Kit (Applied Biosystems). The PCR reactions were performed using TAKARA LA *Taq* with GC Buffer (TAKARA BIO INC., Otsu, Japan) with purified whole genomic DNA from 2137 and N142 cells, and combinations of the following primers. The primers for the forward direction were PGP1S1

(GCACGAGGGATTATCTGACG), PGP1iSi12 (GCGCAG-PGP1iS51 (CTCAAGAGC-TTGCCACCAACGAG), GGCGAGTTCAT). PGP1iS65 (TGTTCATCGCCA-CCAACCG), PGP1tS2 (AAGGTCCACCCCGACTTCGT), PGP1p41 (TGTGAACTCTCGTTTGGAAG), and PGP1p61 (TTGCTGATGGCCCGTCAGAG). The primers for the reverse direction were PGP1iA21 (GAAGATGAAGCACTC-CACCT), PGP1iA24 (TCCACCTTCTTCAGCAGCTC), PGP1iA62 (ACGTAGCGGTCAAAACCAAC), PGP1iA81 (CATGAAGTCCGAGGGCTTG), PGP1A1 (ACCAGG-CTCCCTTAGC), and PGP1A162 (AGCAACTACGTT-TACTGAGC). Most primers were designed based on the cDNA sequence of PGPase (AB052169). PGP1iSi12 was designed based on the sequence containing the first intron determined in the present study, and PGP1p41 and PGP1p61 were designed based on the 5'-upstream sequence found in the scaffold 1006 obtained from the JGI Chlamydomonas reinhardtii version 1.0 database, using the BLAST search (http://genome.jgi-psf.org/cgi-bin/runBlast?db = chlre1). The sequence determination was done at least twice for both forward and reverse directions. Sequences were analyzed with DNASIS software (Hitachi Software Engineering, Yokohama, Japan).

Sequence interpretation

To help find candidates for regulatory DNA elements, the sequences of a 1000-bp 5'-upstream region of Pgp1 were also analyzed with PLACE, the motif database for plant cisacting regulatory DNA elements (Higo et al. 1999; Prestridge 1991) via Internet (http://www.dna.affrc.go.jp/ PLACE/), and TESS, transcription element search software on the Internet (Schug and Overton 1997; http://www.cbil. upenn.edu/tess/). Because the DNA sequence for the Pgp1 gene we determined was from -634 to 5090 relative to the start codon, the complete 1000-bp 5'-upstream sequence of Pgp1 was obtained from JGI Chlamydomonas reinhardtii version 2.0 database (JGI Chlamy 2.0), which was found in scaffold 34 using the BLAST search (http://genome.jgipsf.org/cgi-bin/runBlast?db = chlre2), where there was no mismatch within the 634-bp upstream sequences, although the complete sequence of Pgp1 gene was not available in the database. The results obtained were compared with those of the following genes: Aatl (alanine aminotransferase, GenBank accession No. U31975,); Ald1 (fructose-1,6bisphosphate aldolase, GenBank accession No. S72951, upstream from JGI Chlamy 2.0); AOX1 (alternative oxidase, GenBank accession No. AF047832, upstream from JGI Chlamy 2.0); AOX2 (alternative oxidase, GenBank accession No. AF285187, upstream from JGI Chlamy 2.0); Cahl (periplasmic carbonic anhydrase, GenBank accession No. AB026126); Cah2 anhydrase, (periplasmic carbonic GenBank accession No. X54488, upstream from JGI Chlamy 2.0); Cah3 (chloroplast anhydrase, carbonic GenBank accession No. U73856, upstream from JGI Chlamy 2.0); Cah6 (chloroplast carbonic anhydrase, GenBank accession No. AY463238, upstream from JGI Chlamy 2.0); Ccp1 (chloroplast envelop protein LIP36G1, GenBank accession No.U75345, upstream from JGI Chlamy 2.0); Ccp2 (chloroplast envelop protein LIP36G2, U75346, upstream from JGI Chlamy 2.0); Ccm1 (a regulatory gene for CCM, Miura et al. 2002, GenBank accession No.

844 Can. J. Bot. Vol. 83, 2005

Fig. 1. The position of the *pgp1-1* mutation in the sequence of the *Pgp1* gene from the mutant N142. (A) PCR fragments that were used for nucleotide sequencing, directly or after the subcloning. Each PCR product was obtained from *Chlamydomonas reinhardtii* wild-type 2137 and *pgp1-1* mutant N142, and was sequenced at least twice for both directions. (B) Outline of the *Pgp1* gene, with the exons indicated by thick lines. (C) Comparison of the nucleotide and deduced amino acid (bold letters) sequences from wild-type 2137 and the mutant N142. The sequence of the whole mutant gene with the 634-bp 5'-upstream region is available in the DDBJ, EMBL, and GenBank databases (accession No. AB191485).

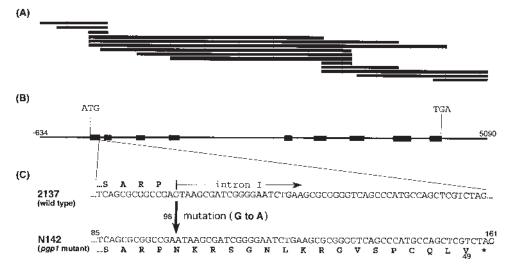


Fig. 2. Nucleotide sequence of the *Pgp1* gene and the 5'-upstream region that is expected to contain the promoter region, as compiled from the gene sequence with the 634-bp 5'-upstream region (GenBank accession No. AB091101, version AB091101.2) from *Chlamydomonas reinhardtii* wild-type 2137, and that obtained using the BLAST search from the JGI *Chlamydomonas reinhardtii* version 2.0 database (http://genome.jgi-psf.org/cgi-bin/runBlast?db = chlre2). Deduced amino acid sequence of the PGPase precursor protein (accession No. AB052169) is shown by one-letter codes under the nucleotide sequence. The nucleotide sequences found within the enhancer element EE-1 (Kucho et al. 2003) of *Cah1* upstream are boxed by regular lines. The sequences indicated by bold letters were found only in the low-CO₂ responsive genes, and those underlined by thick lines were found only with *Aat1* and *Pgp1* (Fig. 3). The CAAT and GATA motifs (putative promoter elements) are boxed by thick lines.

```
-991
-901
-811
\tt ATCCCACCTGCATCCT{\color{red}{C}}{\color{blue}{CAGCAGGGCCGGCCAAGCGCCAAGCGCCGGCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGGCCGGCCAAGCGGCCGGCCAAGCGGCCGGCCAAGCGGCCAGGCAAGCGCCGGCCAAGCGGCCGGCCAAGCGGCCGGCCAAGCGGCCGGCCAAGCGGCCGGCCAAGCGGCCGGCCAAGCGGCCGGCCAAGCGGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCGCCGGCCAAGCAAGCGCCGGCCGGCAAGCAAGCGCCGGCCAAGCGCCGGCAAGCAAGCAAGCAAGCGCCGGCCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAAGCAA
                                                                                                                                                                                                                     -721
 \underline{\texttt{CGCGCGCAGCACGCAGGCTG}}\underline{\texttt{CGGCGCC}}\underline{\texttt{GCGCCGCG}}\underline{\texttt{AGCT}}\underline{\texttt{GCCGGCAG}}\underline{\texttt{TAGATGCTGGAGCGCTTGCTGATGGCCCGT}}\underline{\texttt{CA}\underline{\textbf{GAGGTCA}}}
                                                                                                                                                                                                                     -631
-541
                                                                                                                                                                                                                     -451
CATGTGTGAACTCTCGTTTGGAAGGAGTCGGCGGCGGGGGATCTCCATAATACCTGCTGCCGGAGCAGGTCATCCTCGGAGCCAAAGTC
                                                                                                                                                                                                                     -361
                       agattttcac-cggttggaaggaggt (EE-1 in Cah1 upstream)
{\tt CAGGTCGCGGCGCTTGGGAAGCGCTTCCGGCAGTTCCTCTGATGCCTCCATCTTGA} \underline{{\tt ACCGGTT}} {\tt CGGCTCGGCTACCTAAGTGCCAGACTCCT} \\
                                                                                                                                                                                                                     -271
\texttt{GCGACGA} \underline{\texttt{TTGGAA}} \underline{\texttt{CAGGGT}} \\ \texttt{CAGCTATTTCCTTCCAAACCTAGTGACCATAGTATCTGTAATGCATACATTACGCTTCAACTTATCCTG} \\
                                                                                                                                                                                                                     -181
\underline{GATGTC} A GTTTGGC \overline{AG} TTCCTTAGTGACCGCTTO \underline{CAATTG} CAGCCCCATCCCGATGCTGCTCT\underline{GTGCAG} CTCGTTTGCTTGTTTGCCC\underline{CG}
                                                                                                                                                                                                                        -91
 <u>CCATGT|</u>ACGCTTGCAGTCGCATTACCATCTTGCACAGATCGTCGGACCAGGACGCTGCAGG<mark>CACTTG</mark>CATATTGTAGGATTATCTGACG
                                                                                                                                                                                                                          - 1
90
        L S L K Q L P S A R C A A R P V R P V R R M
180
270
              NEOKL
                                                                     K
                                                                            K
```

AB052694); *Csbp* (sedoheptulose-1,7-bisphosphatase, GenBank accession No. Y14608); *GapA* (chloroplastic glyceraldehydes 3-phosphate dehydrogenase, GenBank accession No. L27668, upstream from JGI Chlamy 2.0); *Gdh1* (putative glycolate dehydrogenase, Miura et al. 2004, upstream from JGI Chlamy 2.0); *GscP* (putative glycine cleavage system P-protein, Miura et al. 2004, upstream from JGI Chlamy 2.0); *H43* (high-CO₂-inducible periplasmic protein, GenBank accession No. AB042098, upstream from JGI Chlamy 2.0); *LciA* (putative inorganic carbon transporter,

GenBank accession No. AB168092, upstream from JGI Chlamy 2.0); *LciB* (putative membrane protein, GenBank accession No. AB168093, upstream from JGI Chlamy 2.0); *LciC* (putative chloroplast protein, GenBank accession No. AB168094, upstream from JGI Chlamy 2.0); *LciI* (putative membrane protein, GenBank accession No. U31976, upstream from JGI Chlamy 2.0); *LcrI* (a low-CO₂-inducible protein, AB168089); *McaI* (mitochondrial carbonic anhydrase, GenBank accession No. U80804); *Mca2* (mitochondrial carbonic anhydrase, GenBank accession No.

U80805); Mdh1 (NADP-malate dehydrogenase, GenBank accession No. AJ277281, upstream from JGI Chlamy 2.0); Nar1 (nitrate transporter, GenBank accession No. AF149737, upstream from JGI Chlamy 2.0); nad-MDH GenBank (NAD-malate dehydrogenase, accession No.AJ250844, upstream from JGI Chlamy 2.0); Pgk (phosphoglycerate kinase, GenBank accession U14912,); Pgp2 (C_15019, a putative PGPase gene proposed in JGI Chlamy 2.0); Pgp3 (C_270015, a putative PGPase gene proposed in JGI Chlamy 2.0); PrkA (phosphoribulokinase, GenBank accession No. AF228914, upstream from JGI Chlamy 2.0); RbcS1 (ribulose-1,5bisphosphate carboxylase-oxygenase small subunit, GenBank accession No. X04471, upstream from JGI Chlamy 2.0); RbcS2 (ribulose-1,5-bisphosphate carboxylase-oxygenase small subunit, GenBank accession No. X04472); Rh1 (a CO₂-channel protein, GenBank accession No. AY013258, upstream from JGI Chlamy 2.0); Sgat (putative serine glyoxylate aminotransferase, upstream from JGI Chlamy 2.0); Shmt (serine hydroxymethyltransferase, GenBank accession No. AF442558, upstream from JGI Chlamy 2.0); Sta2 (granule-bound starch synthase, GenBank accession No. AF026420, upstream from JGI Chlamy 2.0); and Sta3 (soluble starch synthase, GenBank accession No. AF026422, upstream from JGI Chlamy 2.0). To determine whether the genes were low-CO2 responsive or not, we referred to Miura et al. (2004) and the supplemental data of Yoshioka et al. (2004), which is available on the Internet (http://www. plantcell.org/cgi/content/full/tpc.021162/DC1).

Results and discussion

pgp1-1 mutation

The cDNA sequence for the Chlamydomonas PGPase, which we have reported previously (Mamedov et al. 2001), allowed us to determine the pgp1-1 mutation. The sequences of the Pgp1 gene and the upstream region were determined for Chlamydomonas reinhardtii wild type 2137 and PGPasedeficient mutant pgp1-1-N142, from the position -634 to 5112 relative to the start codon (Figs. 1 and 2, entire sequences not shown). The comparison of these two sequences revealed the alteration of a G to A at position 98 (Fig. 1), a result consistent with the recent quantitative finding that more than 99% of ethylmethane sulfonate-induced mutations were G/C-to-A/T transitions (Greene et al. 2003). This change destroyed the "GT" splice donor site at the beginning of the first intron of this gene and resulted in an extension of the first exon to 49 translatable codons, followed by a stop codon (Fig 1). Because the expected peptide contained the complete signal peptide for stroma and four N-terminalamino-acid residues of the PGPase subunit, it is quite likely that the gene product is digested soon after being translated, even if the mRNA is fully functional and is not digested soon. As we have not been able to detect both the small peptide (Mamedov et al. 2001) and the responsible mRNA (data not shown) in either high-CO2-grown or air-adapted cells of the pgp1 mutant, it is necessary to determine if it was because of a quick digestion of the mutant peptide or mRNA, or some other reason. Because of such characteristics of the mutation, the suppressor double mutants of pgp1-1 mutant in which PGPase activity is recovered fully or partially to allow the growth under air (Suzuki 1995) should help define the regulatory mechanism of PGPase.

Thus, the deficiency of PGPase activity in the *pgp1-1* mutant was caused by the lack of the functional enzyme, and further investigation is required to make clear why the mutant shows quite low CCM activity even in air-adapted cells.

The upstream sequence of Pgp1 gene

The regulation of PGPase activity seems to be closely related to that of the CCM when the cells adapt to different CO₂ conditions. PGPase is quickly and transiently upregulated after transfer of high-CO2-grown cells to air (Marek and Spalding 1991). Although the mechanism is not clear, such a response is expected to be a result of a different gene regulation from that proposed for the periplasmic carbonic anhydrase gene Cahl (Kucho et al. 2003). In addition, phosphoglycolate, the substrate of PGPase, has been thought to be a candidate for the trigger of low-CO₂ adaptation (e.g., Suzuki et al. 1990; Kaplan and Reinhold 1999). To help define the regulatory mechanism of the Pgp1 gene, the DNA sequence of the 1000-bp upstream region was compared with that of 37 other genes, including the genes and putative genes involved in the CCM and photorespiration, most of which are low-CO₂ responsive (Fig. 3).

The upstream region of Pgp1 gene seems to have the highest similarity to that of the Cahl gene in Fig. 3. Although the CAAT motif was found with all genes, except Aat1 and Aox2 (data not shown), which are listed in Fig. 3, two motifs containing CAAT were observed at similar positions in Pgp1 and Cah1. The first one, CAATCA, was found at -454 in Pgp1 and -473 in Cah1, and the second one, CAATTG, was found at -146 in Pgp1 and at -150 in Cah1. Such similarity was not observed with any other genes that we compared. The region between the two CAAT motifs of Pgp1 upstream has a sequence similar to the EE-1 motif (Kucho et al. 2003) of *Cah1* upstream (Fig. 2). Although the EEC motif (GANTTNC, Kucho et al. 2003) was not found, two regions have the consensus sequence GAN₇CGN-TTGGAAN₂AG (-436 to -424 and -266 to -254), which was also found in EE-1 of Cah1 upstream (-283 to -271). The sequence TTGGAAGGAG, which contained the TGGAAGG motif (R02828 in the TRANSFAC database), was found in Pgp1 upstream and the EE-1 motif in Cah1 upstream (Fig. 3). Such motifs are not found in any other genes, and likely involved in the common regulatory mechanism for Pgp1 and Cah1.

It has been proposed that the EE-1 and EE-2 motifs, which contain the EEC motif as the consensus sequence, are essential for binding the transcriptional regulator CCM1 (CIA1) (Fukuzawa et al. 2001; Xiang et al. 2001). Recently it has been proposed that the CCM1 is a master regulatory factor of low-CO₂ inducible genes (Fukuzawa et al. 2001; Xiang et al. 2001; Miura et al. 2004). Although *Pgp1* gene may be one of the CCM1-regulated genes (Miura et al. 2004), it is not likely that this gene is regulated directly by CCM1 because of the lack of the EEC motif, but it is likely that this gene has another regulatory mechanism that the *Cah1* gene also has. It is also possible that CCM1 is able to bind to another site in the upstream region or that it requires other factor(s) for proper operation because the EEC motif was not found in the 1000-bp 5'-upstream region of 11 low-

846 Can. J. Bot. Vol. 83, 2005

Fig. 3. Comparison of the 5'-upstream 1000-base-nucleotide sequence of the Pgp1 gene with those of 37 other genes mostly involved in carbon and nitrogen metabolism. The upstream sequences were obtained from the JGI Chlamydomonas reinhardtii version 2.0 database (JIG Chlamy 2.0, http://genome.jgi-psf.org/chlre2/chlre2.home.html), after searching corresponding genes with BLAST or a key word search with the gene or cDNA sequences available in the DDBJ, EMBL, GenBank, and Kazusa Chlamydomonas reinhardtii EST (Kazusa Chlamy EST, Asamizu et al. 2000, http://www.kazusa.or.jp/en/plant/chlamy/EST/index.html) databases or published papers. Numbers starting with "C" are the gene model names in JGI Chlamy 2.0, and others are DDBJ, EMBL, or GenBank accession numbers. The accession numbers in parentheses indicate sequences that were used only for the BLAST searches. CGCGCC, GGGTTGAANTCCC, and AACCCCNGNTGCA are the sequences reported to be conserved in some CO₂-responsive genes, including Cah1 (Kucho et al. 1999). AACCAA is the sequence annotated as "REalpha" in the Lemna gibba Lhcb21 gene promoter (Degenhardt and Tobin 1996). TTGGAAGGAG is the sequence observed within the EE-1 region (Kucho et al. 2003) of the Cah1 gene upstream. AGGTCA is the sequence annotated as "Q (quantitative)-element" in the maize Zm13 gene promoter in the PLACE database (http://www.dna.affrc.go.jp/PLACE/). ACGCTTGGCAGT and CATTACCAT are sequences observed only in the Aat1 and Pgp1 upstreams in this report. Pgp2 and Pgp3 are putative genes, based on sequence similarity with the Pgp1 gene, as annotated in JGI Chlamy 2.0. +, positive low-CO₂ response; (+), probable positive low-CO₂ response; -, negative low-CO₂ response (or positive high-CO₂ response); (blank), no apparent CO₂ response. The EST fragments corresponding to Pgp2 and Pgp3, identified by using the BLAST search in Kazusa Chlamy EST, did not have low- or high-CO₂ responsive characteristics in the web-only supplemental table (Yoshioka et al. 2004, http://www.plantcell.org/cgi/content/full/tpc.021162/DC1). The position from the start codon is indicated for each motif found in the upstream region. The motifs found only in low-CO₂ responsive genes are indicated by bold letters, and the motifs found only in Pgp1 and other genes involved in CCM and photorespiration are boxed by thick lines.

Fig. 4. Comparison of the amino acid sequences of *Pgp2* and *Pgp3* gene products (CrPGP2 and CrPGP3, respectively) with PGPase precursor (CrPGP1). The alignment was calculated using ClustalW version 1.8 (http://clustalw.genome.jp/), and the similarity is indicated below the amino acid sequences with the following characters: *, positions that have a single, fully conserved residue; :, one of the following "strong" groups is fully conserved: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, or FYW; ., one of the following "weaker" groups is fully conserved: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, or HFY. These are all the positively scoring groups that occur in the Gonnet Pam250 matrix. Strong and weak groups had scores >0.5 and 0.5, respectively. Core motifs for the families of *p*-nitrophenyl phosphatases and bacterial PGPases within the haloacid dehalogenase superfamily (Selengut 2001) are indicated below the similarity indicators, using the following groupings: a, aromatic (FHWY); c, charged (DEHKR); f, small polar (CDNST); h, hydrophobic (ACFGHIKLMPRTVWY); l, aliphatic (ILV); o, hydroxyllic (ST); p, polar (CDEHKNQRST); X, any; and Z, E or Q.

```
1 --MLSLKQLPSARCAARPVR-----
CrPGP1
                                                     ---PVRRMVAAOASARPTAT
                    ------MKKAT
CrPGP2
          1 MSNLALQARRMALGATRQARGCHAVLAGGAPFTARAATAATAVDVPSTSATSVTPLTVLD
CrPGP3
CrPGP1
         36 NEQKLELLKKVECFIFDCDGVIWLGDKVIEGVPETLDMLRGMGKKVFFVTNNSTKSRAGY
CrPGP2
          6 DRDKLEAFQQYDAWVFDLDGTLWKGSTLIPGAKEFIELLRYYNKKVFFVTNNATKSRATN
         61 ERTAPERLRETSTLIFDCDGVLWRGSEIIHNAPEALKEFRRQGKRLLFVTNNSSKSRAGY
CrPGP3
                 * ::: .
                                    *. :* .. * :. :*
                                                        *:::*****::***
                        hhlhDhDGz1h
(NPPase)
                                                        hXhhhloNN
                        hlhFDhDGTLh
(bact.PGP)
                                                        hhhzlhoNN
                         (Motif I)
                                                        (Motif II)
CrPGP1
         96 MSKFOSI,GI,NVKAEETYSSSYAAAAYI,ESTNFN-----KKVYVIGETGII,EELDI,KGI
         66 AAKLTAMGINATQAEMYTSSFAAAAYLKAISFN-----KKAYVIGEEGLVEELTAVGV
CrPGP2
CrPGP3
        121 VAKFSSLGLEVAAEEIVSSSYCAAAYLTSQGFGPGGSRPCSKVLLLGWSGVEQELEQAGI
                          *::**:.**** : .*.
             :*: ::*::.
                                                    .*. ::*
CrPGP1
        149 RHVGGPGDADKKVTLKSGE-FMEHDHDVGAVVVGFDRYVNYYKIOYATLCIRENPGCMFI
CrPGP2
        119 QCVGGPAHRGVEVDWSQAEPHVEVDPEVGAVVVGLDRYISYYKLQYATLCLANNDSCMFL
        181 PYVGGRALKVPPMDDLDAMKALKVDPDVGAVVVGWDPNFSYSRLVYASIHLRELPGCLLV
CrPGP3
                                 :: * :*****
                                                      .. **.. . .
                        :
        208 ATNRDAVTHLTDAOEWAGNGSMVGAIVGSTKREPIVVGKPSDFMLKNISASLGLRPDOIA
CrPGP1
CrPGP2
        179 ACNTDARGHFSOAOEWAGAGTMVAALIGSSEREPMLLGKPASFILDHLCATHOVARDKTI
CrPGP3
        241 ATNMDCADHIGGGRMMPGTGGLVKAVETASGVSAVNVA\underline{\kappa}GGE\underline{w}LLPYLCRTYGLEPAH\underline{\tau}A
                        .: .* * :* *: :: ..: :.*
(NPPase)
                                                  KPphh
(bact.PGP)
                                                  KPpzX
                                                                       z1
                                                (Motif IIIa)
CrPGP1
        268 MVGDRLDTDIMFGKNGGLATALVLSGVTTPEVLNSPDNKVHPDFVLNSLPDLLSVKEKAM
        239 VVGDRLDTDILWGIQNGAGTCCVLSGVTSEAQLLAESNKVHPKLYMSDIGDFLTIKNQIP
CrPGP2
CrPGP3
        301 IVGDRMDTDIHLGRQGGLFTCLPLTGVTTLKRLEGLPASEHPDVVVRSVAQLAGLPA---
             ****
                               *. *:***:
(NPPase)
            hlGDphXoD1Xhz
(bact.PGP)
            <u>hlGD-fXXDlZzz</u>
            (Motif IIb)
CrPGP1
        328 VAA-
CrPGP2
        299 SNCAIM
CrPGP3
```

										Motif								
	Gene	Response to low CO ₂	cececc	GANTTNC	GGGTT <u>GAANTCC</u> C	AACCCCNGNTGCA	AACCAA	СААТСА	СААТТG	ТТGGAA	<u>TTGGAA</u> GGAG	АВВТСА	R <u>AGGTCA</u> GN ₈ °,CCR	TTGGCAG	ACGC <u>TTGGCAG</u> T	ТАССАТ	CAT <u>TACCAT</u>	Corresponding gene model in JGI Chlamy v2.0 and/or DDBJ/EMBL/GenBank accesion number
Photrespiration	Aat1	+	-914 -791 -97	-	-	1	-	1	-	-	-	1	-	-46	-50	-129	-132	C_320082, (U31975)
	GcsP	(+)	-547	-64	-	-	-752	-	-	-	-	-	-	-	-	-	-	C_910041, putative gene
	Gdh1	+	-	-	-	-	-	-	-210	-	-	-	-	-	-	-	- 70	C_290029, putative gene
	Pgp1	+	-690	-	-	-	-	-454	-146	-433 -263	-433	-636 -167	-637	-171 -80	-84	-67	-70	C_340088, AB091101.2
	Sgat	+	-562 -475	-15	-	-	-	-	-540	-	-	-	-	-	-	-	-	C_900004, putative gene
	Shmt	+	-	-604		-	-938	-	-	-	-		-	-	-	-	-	C_970018, (AF442558)
	Cah1	+	-890 -360	-656 -591 -334 -282	-596	-200	-218	-473	-150	-322	-322	-696	-697	-824 -348	-	-	-	AB026126, C_40150
	Cah3	+	-	-	-	-	-	-	-	-	-	- '	-	-	-	-	-	C_710031, U73856
	Cah6	+	-68	-	-	-	-	-954	-	-975	-	-	-	-	-	-	-	C_1150014, AY463238
	Ccm1		-563	-135	-	-	-	-	-	-	-	-873	-	-	-	-	-	AB052694, C_2060016
	Ccp1	+	-367	-288	-	-	-494	-	-	-	-	-	-	-	-	-	-	C_40052, (U75345)
_	Ccp2	+	-	-	-	-	-	-	-	-	-	-323	-324	-	-	-	-	C_40147, (U75346)
CCM	Lci1	+	-	-639	-	-	-	-228	-	-	-	-	-	-	-	-	-	C_1350038?, (U31976)
0	Lci5	+	-	-	-	-	-	-	-	-	-		-	-725	-	-	-	C_150094, AF394230
	LciA	+	-	-	-	-	-	-	-	-	-	-360	-361	-	-	-	-	C_90197, (AB168092)
	LciB	+	-329	-953 -373	-	-	-554	-	-484	-628	-	-	-	-	-	-	-	C_8740001, (AB168093)
	LciC	+	-822	-	-	-	-365	-	-143	-	-	-	-	-	-	-	-	C_90183, (AB168094)
	Lcr1	+	-733	-523	-	-	-173	-385	-	-	-	-	-	-820 -446	-	-	-	AB168089, C_1530002
	Mca1	+	-261	-165	-299	-284	-696	-	-	-	-	-	-	-	-	-949	-	U80804
	Mca2	+	-261	-165		-284	-696	-	-	-	-	-725	-726	-	-	-	-	U80805, C_2030011
	Ald1		-101	-653		-	-	-	-	-	-	-	-	-	-	-	-	C 4220001, (S72951)
	Aox1		-565	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C_330029, (AF047832)
	Aox2		-947	-996	-	-	-	-	-	-	-	-	-	-	-	-	-	C_340013, AF314255.2
	Cah2	-	-	-693	-	-	-		-20 -12	-	-	-	-	-	-	-	-	C_40149, X54488
	Mdh1	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-	-805	-	C_520009, (AJ277281)
	Csbp		-	-	-	-	-		-141	-	-	-	-	-	-	-	-	Y14608, C_30202
	GapA		-529	-173	-	-	-	-	-	-	-	-737	-	-	-	-	-	C_280107, L27668
	H43	-	-742	-297		-	-	-	-989	-	-	-	-	-	-	-	-	C_70187, AB042099
	nad-MDH		-230			-	-671	-707		-	-	-	-	-	-		-	C_550067, (AJ250844)
	Nar1			-740		-	-	-	-	-	-	-	-	-	-	-	-	AF149738, C_520040
	Pgk	-	-164 -138	-	-	-	-	-5		-	-	-	-	-	-		-	C_140184, (U14912)
	PrkA		-379	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C_950008, (AF228914)
	RbcS1		- 270	-422		-	-	-	-	-	-	-	-	-	-	-	-	C_660013, X04471
	RbcS2 Rh1	_	-378	-	-	-	-	-	- -842	- -297	-	-	-	-	-	-	-	X04472, C_660012 C 1460033, (AY013258)
	Sta2	+	-539	-	-	-	-	-	-842	-379		- -315	-	-	-	-	-	C_10205, (AF026420)
	Sta3	+	-25 -	-157	-	-	-	-	-284	-	-	-	-	-	-	-	-	C 970042, (AF026422)
	Pgp2 *		-576	-667		-	-163	-	-	-	-	-	-	-	-	-	-	C_150191, putative gene
<i>gp1</i>	Pgp3 *		-658			-	-	-5		-967		-723		-	-	-	-	C_270015, putative gene
್ರ.≔	<i>.</i>		-273															

CO₂ responsive genes such as *Aat1*, *LciA*, and *Sta2*, and was also found with eight genes that were not low-CO₂ responsive, such as *GapA*, *H43*, and *Cah2* (Fig. 3).

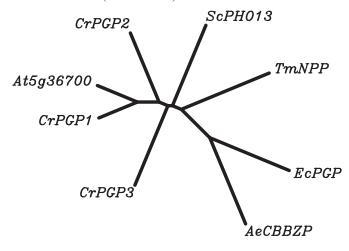
The motif CAGAGGTCAGN₈CCG, which contains the AGGTCA motif annotated as the "Q (quantitative)-element" in the maize Zm13 gene promoter (Hamilton et al. 1998) in the PLACE database, is a good candidate for the region involved in the common regulatory mechanism of Pgp1 and Cah1. This motif was found only with these two genes at the

similar positions (-639 to -619 and -699 to -679, respectively).

Although CAGAGGTCAGN₈CCG was not found with any other genes in Fig. 3 (data not shown), a more leaky motif, RAGGTCAGN₈₋₉CCR, was also found with *Ccm1*, *Ccp2*, *LciA*, and *Mca2* (Fig. 3). TTGGCAG, which contains the TTGGCA motif (Paonessa et al. 1988), was also found with *Pgp1* and *Cah1*, as well as with *Aat1*, *Lci5*, and *Lcr1* (Fig. 3). Thus, although there was no motif that was com-

848 Can. J. Bot. Vol. 83, 2005

Fig. 5. A phylogenetic tree calculated from an alignment of the PGPase subunit (CrPGP1), Pgp2 and Pgp3 gene products (CrPGP2 and CrPGP3, respectively), and NPPases and bacterial PGPases. The output tree is displayed as an unrooted N-J tree after the alignment using ClustalW version 1.8 (http://clustalw. genome.jp/). CrPGP1, Chlamydomonas reinhardtii PGPase (PGP1) subunit; CrPGP2, C. reinhardtii Pgp2 gene product (C_150191 in JGI Chlamy 2.0); CrPGP3, C. reinhardtii Pgp3 gene product (C_270015 in JGI Chlamy 2.0); At5g36700, Arabidopsis thaliana putative PGPase gene product (AT5G36700.1 in the Arabidopsis Information Resource, http:// www.arabidopsis.org/index.jsp); AeCBBZP, Alcaligenes eutrophus PGPase (AAA20195.1); EcPGP, Escherichia coli PGPase (AAC76410.1); ScPHO13, Saccharomyces cerevisiae pnitrophenylphosphatase (CAB56540.1); TmNPP, Thermotoga maritima PGPase (AAD36807.1).



mon among the low-CO₂ responsive genes shown in Fig. 3, the two motifs, RAGGTCAGN₈₋₉CCR and TTGGCAG, were found only within the low-CO₂ responsive genes involved in the CCM and photorespiration, suggesting that these two motifs are involved in the signal transduction for low-CO₂ adaptation in *C. reinhardtii*.

The *Pgp1* upstream region also has some motifs quite similar to those in *Aat1* upstream (Figs. 2 and 3). The following sequences were found only in the similar regions of *Pgp1* and *Aat1* upstreams (Fig. 3): ACGCTTGGCAGT was found at -84 to -73 in *Pgp1* and 50 to -39 *Aat1*, and CATTACCAT was found at -50 to -39 in *Pgp1* and -132 to -124 in *Aat1*. Thus, *Pgp1* and *Aat1* genes are quite likely to have common regulatory mechanism(s) in which these two sequences are involved together with the above-mentioned sequence TTGGCAG, although these motifs were not found in the upstreams of the other photorespiratory genes *GcsP*, *Gdh1*, *Shmt*, and *Sgat*.

Further investigations are necessary to clarify the regulatory mechanism of the Pgp1 gene to help understand the mechanism that optimizes photosynthesis when organisms adapt to different concentrations of inorganic carbon.

Possible PGPase isozymes

The deduced sequence of mutant PGP1 contains the complete stroma-targeting signal peptide, followed by 21 amino acid residues, but contains only four amino acid residues of the wild-type PGPase mature subunit (Fig. 1). It is, there-

fore, quite unlikely that the mutant PGP1 is functional and responsible for the residual PGPase activity observed in the *pgp1-1* mutants (Suzuki 1995; Suzuki et al. 1990, 1999). The observed residual activity in the mutants could be caused by nonspecific phosphatase(s) and (or) isozyme(s) of PGPase.

Two putative PGPase genes, Pgp2 and Pgp3, have been proposed in JGI Chlamy 2.0 based on similarity with the Pgp1 cDNA sequence. Comparison of the deduced amino acid sequences of putative gene products with that of PGP1 showed that all three gene products had all consensus motifs of p-nitrophenyl phosphatases (Selengut 2001), except motif IIIa (Fig. 4). PGP2 was very likely to lack any transit peptide, but PGP3 seems to have a transit peptide region quite similar to those of thylakoid lumen-targeted peptides (Fig. 4). On the other hand, a phylogenetic tree produced after the ClustalW alignment with the related gene products showed that the sequences of putative PGP2 and PGP3 subunits were rather similar to that of p-nitrophenyl phosphatases rather than that of PGP1 subunit, and were less similar to those of bacterial PGPases (Fig. 5). Based on a comparison of these sequence characteristics, PGP2 and PGP3 are likely to have PGPase activity, although they are less similar to PGP1 than the Arabidopsis putative PGPase, and it is not clear if Pgp2 and Pgp3 are really functional or not. As the expression of Pgp2 and Pgp3 did not change after transfer from high- to low-CO2 levels (Fig. 3), based on the supplemental data of Yoshioka et al. (2004), the physiological role(s) is likely not in photorespiration if they are functional PGPases but rather in nonphotosynthetic reactions such as DNA repair (Pellicer et al. 2003; Kim et al. 2004). However, some of the suppressor double mutants of pgp1-1-18-7F with a partly recovered PGPase activity (Suzuki 1995) may have enhanced Pgp2 and (or) Pgp3 expression. It is, on the other hand, still possible that the low rate of glycolate production observed in pgp1 mutants (Suzuki et al. 1999) are catalyzed by other phosphatase(s) such as galactose-1phosphatase that has been reported in Arabidopsis thaliana to have some PGPase activity (Laing et al. 2004).

Acknowledgments

We thank Tomoya Yamaguchi and Katsuhiro Nakayama for helpful assistance and useful discussions. This work was supported by a grant-in-aid from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Bio-Design Project).

References

Anderson, L. 1971. Chloroplast and cytoplasmic enzymes II. Pea leaf triosephosphate isomerases. Biochim. Biophys. Acta, **235**: 237–244.

Asamizu, E., Miura, K., Kucho, K., Inoue, Y., Fukuzawa, H., Ohyama, K., Nakamura, Y., and Tabata, S. 2000. Generation of expressed sequence tags from low-CO₂ and high-CO₂ adapted cells of *Chlamydomonas reinhardtii*. DNA Res. 7: 305–307.

Degenhardt, J., and Tobin, E.M. 1996. A DNA binding activity for one of two closely defined phytochrome regulatory elements in an *Lhcb* promoter is more abundant in etiolated than in green plants. Plant Cell, **8**: 31–41.

Fukuzawa, H., Miura, K., Ishizaki, K., Kucho, K., Saito, T., Kohinata, T., and Ohyama, K. 2001. *Ccm1*, a regulatory gene

controlling the induction of a carbon-concentrating mechanism in *Chlamydomonas reinhardtii* by sensing CO₂ availability. Proc. Natl. Acad. Sci. U.S.A. **98**: 5347–5352.

- Greene, E.A., Codomo, C.A., Taylor, N.E., Henikoff, J.G., Till, B.J., Reynolds, S.H., Enns, L.C., Burtner, C., Johnson, J.E., Odden, A.R., Comai, L., and Henikoff, S. 2003. Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in *Arabidopsis*. Genetics, 164: 731–740.
- Hamilton, D.A., Schwarz, Y.H., and Mascarenhas, J.P. 1998. A monocot pollen-specific promoter contains separable pollenspecific and quantitative elements. Plant Mol. Biol. 38: 663– 669.
- Higo, K., Ugawa, Y., Iwamoto, M., and Korenaga, T. 1999. Plant cis-acting regulatory DNA elements (PLACE) database:1999. Nucleic Acids Res. 27: 297–300.
- Kaplan, A., and Reinhold, K. 1999. CO₂ concentrating mechanisms in photosynthetic microorganisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50: 539–570.
- Kim, Y., Yakunin, AF., Kuznetsova, E., Xu, X., Pennycooke, M., Gu, J., Cheung, F., Proudfoot, M., Arrowsmith, C.H., Joachimiak, A., Edwards, A., and Christendat, D. 2004. Structure and function-based characterization of a new phosphoglycolate phosphatase from *Thermoplasma acidophilum*. J. Biol. Chem. 279: 517–526.
- Kucho, K., Ohyama, K., and Fukuzawa, H. 1999. CO₂-responsive transcriptional regulation of CAH1 encoding carbonic anhydrase is mediated by enhancer and silencer regions in *Chlamydomonas* reinhardtii. Plant Physiol. 121: 1329–1337.
- Kucho, K., Yoshioka, S., Taniguchi, F., Ohyama, K., and Fukuzawa, H. 2003. Cis-acting elements and DNA-binding proteins involved in CO₂-responsive transcriptional activation of *Cah1* encoding a periplasmic carbonic anhydrase in *Chlamydomonas reinhardtii*. Plant Physiol. 133: 783–793.
- Laing, W.A., Bulley, S., Wright, M., Cooney, J., Jensen, D., Barraclough, D, and MacRae, E. 2004. A highly specific Lgalactose-1-phosphate phosphatase on the path to ascorbate biosynthesis. Proc. Natl. Acad. Sci. U.S.A. 101: 16976–16981.
- Mamedov, T.G., Suzuki, K., Miura, K., Kucho, K., and Fukuzawa, H. 2001. Characteristics and sequence of phosphoglycolate phosphatase from a eukaryotic green alga *Chlamydomonas* reinhardtii. J. Biol. Chem. 276: 45573–45579.
- Marek, L.F., and Spalding, M.H. 1991. Changes in photorespiratory enzyme activity in response to limiting to CO₂ in *Chlamydomonas reinhardtii*. Plant Physiol. **97**: 420–425.
- Miura, K., Kohinata, T., Yoshioka, S., Ohyama, K., and Fukuzawa, H. 2002. Regulation of a carbon concentrating mechanism through CCM1 in *Chlamydomonas reinhardtii*. Funct. Plant Biol. 29: 211–219.
- Miura, K., Yamano, T., Yoshioka, S, Kohinata, T., Inoue, Y., Taniguchi, F., Asamizu, E., Nakamura, Y., Tabata, S., Yamato, K.T., Ohyama, K., and Fukuzawa, H. 2004. Expression profil-

- ing-based identification of CO₂-responsive genes regulated by CCM1 controlling a carbon-concentrating mechanism in *Chlamydomonas reinhardtii*. Plant Physiol. **135**: 1595–1607.
- Norman, E.G., and Colman, B. 1991. Purification and characterization of phosphoglycolate phosphatase from the cyanobacterium *Coccochloris peniocystis*. Plant Physiol. **95**: 693–698.
- Paonessa, G., Gounari, F., Frank, R., and Cortese, R. 1988. Purification of a NF1-like DNA-binding protein from rat liver and cloning of the corresponding cDNA. EMBO J. 7: 3115–3123.
- Pellicer, M.T., Nuñez, M.F., Aguilar, J., Badia, J., and Baldoma, L. 2003. Role of 2-phosphoglycolate phosphatase of *Escherichia coli* in metabolism of the 2-phosphoglycolate formed in DNA repair. J. Bacteriol. 185: 5815–5821.
- Prestridge, D.S. 1991. SIGNAL SCAN: A computer program that scans DNA sequences for eukaryotic transcriptional elements. CABIOS, 7: 203–206.
- Schug, J., and Overton, G.C. 1997. TESS: Transcription Element Search Software on the WWW. Technical Report CBIL-TR-1997-1001-v0.0. Computational Biology and Informatics Laboratory, School of Medicine, University of Pennsylvania, Philadelphia, Penn.
- Selengut, J.D. 2001. MDP-1 is a new and distinct member of the haloacid dehalogenase family of aspartate-dependent phosphohydrolases. Biochemistry, **40**: 12704–12711.
- Suzuki, K. 1995. Phosphoglycolate phosphatase-deficient mutants of *Chlamydomonas reinhardtii* capable of growth under air. Plant Cell Physiol. **36**: 95–100.
- Suzuki, K., and Spalding, M.H. 1987. Phothosynthetic characteristics of several high-CO₂-requiring mutants of *Chlamydomonas*. *In* Progress in Photosynthesis Research. Vol. 4. Proceedings of the VII the International Congress on Photosynthesis, Providence, R.I., 10–15 August 1986. *Edited by J. Biggins. Martinus Nijhoff*, Dordrecht. pp. 329–332.
- Suzuki, K., Marek, L.F., and Spalding, M.H. 1990. A photorespiratory mutant of *Chlamydomonas reinhardtii*. Plant Physiol. 93: 231–237.
- Suzuki, K., Mamedov, T.G., and Ikawa, T. 1999. A mutant of Chlamydomonas reinhardtii with reduced rate of photorespiration. Plant Cell Physiol. 40: 792–799.
- Wolfenden, R. 1970. Binding of substrate and transition state analogs to triosephosphate isomerase. Biochemistry, 9: 3404–3407.
- Xiang, Y., Zhang, J., and Weeks, D.P. 2001. The Cia5 gene controls formation of the carbon concentrating mechanism in Chlamydomonas reinhardtii. Proc. Natl. Acad. Sci. U.S.A. 98: 5341–5346.
- Yoshioka, S., Taniguchi, F., Miura, K., Inoue, T., Yamano, T., and Fukuzawa, H. 2004. The novel Myb transcription factor LCR1 regulates the CO₂-responsive gene *Cah1*, encoding a periplasmic carbonic anhydrase in *Chlamydomonas reinhardtii*. Plant Cell, **16**: 1466–1477.