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**EXPERIMENTAL ARTICLES**

# The *gap3* Gene of *Synechococcus* PCC 7942 Is Induced during Adaptation to Low $CO_2$ Concentrations

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**Abstract**—The *gap3* gene of the *Synechococcus* and *Anabaena* cyanobacteria fulfills a thus far unknown function. A homolog of this gene has recently been found in the nuclear genomes of diplonemids, which are heterotrophic flagellates closely related to kinetoplastids and euglenoids. To understand the function of the *gap3* gene in the cyanobacteria, we performed Northern blotting experiments with *gap3* probes under different growth conditions. Under the standard photosynthetic growth conditions (high illumination and 1% $CO_2$ in the gas phase), the expression of the *gap3* gene was very low, but it significantly increased during cell adaptation to the low $CO_2$ concentration (0.03%). The *gap3* operon was expressed as a polycistronic transcript of about 7 kb in size, which included ORF2 (1259 bp) immediately downstream of *gap3*. ORF2 probably encodes a putative transporter of $HCO_3$. The nucleotide sequence of ORF2 has been submitted to GenBank under accession number AF428100.

*Key words*: cyanobacteria, glyceraldehyde-3-phosphate dehydrogenase, *gap3*, gene expression, $CO_2$-concentrating mechanism, membrane protein, transporter, functional pleiotropism.

The cytoplasmic and chloroplast glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) are the key enzymes of, respectively, glycolysis and the Calvin cycle in plants and cyanobacteria (the latter are evolutionarily related to the chloroplasts of higher plants). The *gap* genes, which encode these enzymes, serve as molecular probes in studies of the evolutionary relations between various organisms [1].

Some cyanobacteria (such as *Synechocystis* sp. PCC 6803 [2]) have two *gap* genes (*gap1* and *gap2*), whereas other cyanobacteria (including *Anabaena variabilis* [3] and *Synechococcus* PCC 7942 [4]) have three divergent *gap* genes (*gap1, gap2*, and *gap3*). The *gap3* gene has recently been found in the diplonemid genome [5]. The function of this gene is as yet unknown, although there is phylogenetic evidence that *gap3* is genetically related to the *gapB* gene of *Escherichia coli* [6], which actually codes for erythrose 4-phosphate dehydrogenase [7] and not for glyceraldehyde-3-phosphate dehydrogenase.

The aim of this work was to find conditions that promote the expression of the *gap3* gene in the cyanobacterium *Synechococcus* PCC 7942. This knowledge can provide insight into the possible physiological role of the product of this gene in cyanobacteria.

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## MATERIALS AND METHODS

**Bacterial strains, plasmids, and cultivation conditions.** The unicellular cyanobacterium *Synechococcus* PCC 7942 was obtained from S.V. Shestakov, Department of Genetics, Faculty of Biology, Moscow State University. This cyanobacterium was grown at 28–30°C under either low (1500 lx) or high (3000 lx) illumination in BG11 medium [8], which was sparged with air containing the normal (about 0.03%) or an increased (1%) concentration of $CO_2$. In the latter case, the cultivation medium was supplemented with 10 mM HEPES–NaOH buffer (pH 8.0). The filamentous nitrogen-fixing wild-type cyanobacterium *Anabaena variabilis* ATCC 29413 was kindly provided by Prof. Bothe of the Institute of Botany, Köln University, Germany. This cyanobacterium was grown as described by Hu *et al.* [9]. *Escherichia coli* strain XL-1 Blue was grown in LB broth supplemented with the appropriate antibiotics. The cloning vector was pBluescript SK(+) (Stratagene). *E. coli* cells were transformed and the transformants were tested by the standard procedures [10].

**The cloning, sequencing, and sequence analysis of the ORF2 of *Synechococcus* PCC 7942.** The genomic DNA of *Synechococcus* PCC 7942 was digested with *Hin*dIII restriction endonuclease, and a *gap3*-containing DNA fragment was subcloned on the pBluescript-SK(+) vector and sequenced with T7 polymerase by using an automatic DNA Sequenator

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(Pharmacia) according to the manufacturer's instructions. The data obtained were analyzed with the aid of the Blast Search program [11] available on the NCBI GenBank BLAST e-mail server and by using the genomic sequence information in CyanoBase (http://www.kazusa.or.jp/cyanobase/).

**Nucleotide sequence accession number.** The nucleotide sequence of the gene encoding a transporter protein was deposited in GenBank under accession number AF428100.

**The isolation of RNA and Northern blotting analysis.** The total RNA was extracted from *Synechococcus* PCC 7942 cells with TRIzol reagent (Life Technologies Division, Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA samples were denatured and subjected to denaturing electrophoresis in 1.3% formaldehyde gel (at a load of 30 μg per lane) in 40 mM MOPS buffer (pH 7.0) containing 10 mM sodium acetate and 1 mM EDTA. Then the RNA was transferred onto Hybond-$N^+$ nylon membranes (Amersham Pharmacia) by the routine procedure [10]. The concentration of RNA was measured spectrophotometrically at 260 nm before the RNA was applied onto the gel. In addition, the RNA was quantified by the intensity of the luminescence of the ethidium bromide–stained rRNA bands under UV light. The size of transcripts was determined by using RNA markers in the interval of 0.28–6.58 kb (Promega). RNA was fixed on nylon membranes at 80°C for 2 h. Then, the fixed RNA was hybridized with α-$^{32}$P-labeled DNA probes by using a Multiprime DNA labeling system (Amersham Pharmacia). The hybridization and washing procedures were performed as described in [10]. The DNA probes were prepared by PCR with the following specific primers: 5'-GAA CCC TAC GGT GAG GC-3' and 5'- ATC ACG CGT GTG GAC AC-3' for the *gap3* gene of *Synechococcus* PCC 7942 and 5'-GCA GGA ACA GTA TTT TCT G-3' and 5'-CGT TAA TAC CTG ATA CCC A-3' for the *gap3* gene of *A. variabilis* ATCC 29413.

## RESULTS AND DISCUSSION

Some cyanobacteria of the genera *Synechococcus* and *Anabaena* have the *gap3* gene, which is a third divergent gene of the *gap* family with an unknown function [3, 4, 12]. To understand the function of the *gap3* gene in the cyanobacteria, we attempted to reveal cultivation conditions that favor the expression of this gene. For this purpose, we employed the Northern blotting approach. The total RNA was isolated from cyanobacterial cells grown under different cultivation conditions.

In cells grown under high illumination, the expression of the *gap3* gene was very low, if expression occurred at all, irrespective of whether the air was enriched in $CO_2$. Unexpectedly, the expression of this gene significantly increased when the cyanobacterial cells grown under high illumination in the presence of 1% $CO_2$ were incubated under the same illumination in the presence of 0.03% $CO_2$ (the normal concentration of carbon dioxide in air). This was evident from the appearance of a long mRNA molecule on the respective lane (Fig. 1) when cells were incubated for 30 or more hours in the presence of 0.03% $CO_2$. The shorter RNA transcripts might result from the degradation of the long transcript.

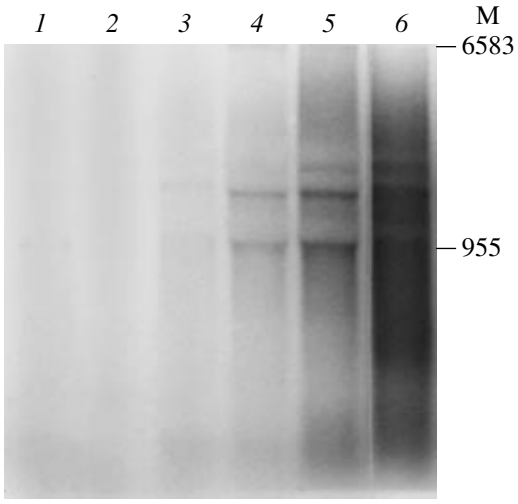


**Fig. 1.** The Northern hybridization of the total *Synechococcus* PCC 7942 RNA with the *gap3* gene as a hybridization probe. The total RNA was isolated from wild-type *Synechococcus* PCC 7942 cells grown for 3 days under high illumination in an atmosphere containing 1% $CO_2$ (lane *1*) and then incubated for 24, 30, 36, 48, and 72 h (lanes *2–6*, respectively) in an atmosphere containing 0.03% $CO_2$ and having the same illumination. The solid lines show the positions of molecular weight markers containing 6583 and 955 nucleotides.

Northern hybridization experiments with another cyanobacterium, *A. variabilis* ATCC 29413, showed that the *gap3* gene was expressed in this cyanobacterium in a similar way. Namely, this gene was expressed as a monocistronic mRNA when *A. variabilis* cells were cultivated under high illumination in the presence of 1% $CO_2$ and as a long mRNA molecule when they were transferred and incubated under the same illumination in the presence of 0.03% $CO_2$ (Fig. 2). The long transcript of *A. variabilis* ATCC 29413 appeared earlier than in the case with *Synechococcus* PCC 7942 and did not degrade under the experimental conditions used. The intensity of nitrogen fixation did not influence the expression of the *gap3* gene in the nitrogen-fixing cyanobacterium *A. variabilis* ATCC 29413. The long transcript was observed when the cyanobacterial cells grown at the high $CO_2$ concentration (1%) were transferred and incubated at the low $CO_2$ concentration (0.03%) irrespective of the presence of nitrate in the medium (data not presented).

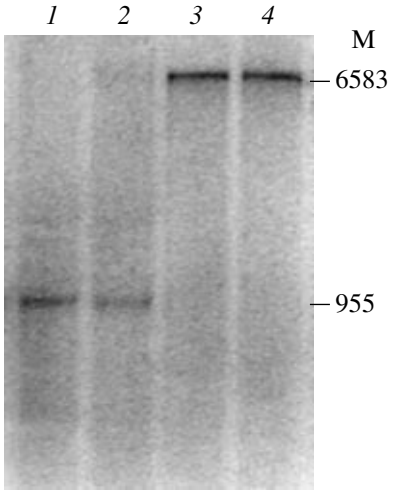


**Fig. 2.** The Northern hybridization of the total *A. variabilis* ATCC 29413 with the *gap3* gene as a hybridization probe. The total RNA was isolated from wild-type *A. variabilis* ATCC 29413 cells grown for 3 days under high illumination in an atmosphere containing 1% $CO_2$ (lane *1*) and then incubated for 8, 24, and 48 h (lanes *2–4*, respectively) in an atmosphere containing 0.03% $CO_2$ and having the same illumination. The solid lines show the positions of molecular weight markers containing 6583 and 955 nucleotides.

The sequence analysis of the DNA region containing the *gap3* gene of *Synechococcus* PCC 7942 revealed the presence of a 1259-bp ORF2, which encodes a hydrophobic protein with a molecular mass of 44584 Da. Analysis with the Blast Search program showed that ORF2 is homologous to the 53.1-kDa protein of *E. coli*, which is a putative membrane protein of the PTR2 transporter family. ORF2 also showed a homology to the transporter ORF (*alr1096*) of *Anabaena* sp. PCC 7120 [13] and the cephamycin export protein of *Rhodobacter capsulatus* SB 1003 [14]. Like the *gap* gene and the cephamycin export protein gene of *Rh. capsulatus* SB 1003, the *gap3* gene and the ORF2 of *Synechococcus* PCC 7942 overlap by 4 bp. In *Anabaena* sp. PCC 7120, the *gap3, alr1095* (NP_485138.1), and *alr1096* (NP_485139.1) genes are adjacent and probably belong to one operon [13]. The sequencing of the cyanobacterium *Thermosynechococcus elongatus* BP-1 genome (http://www.kazusa.or.jp/cyanobase/) showed that the homologous *gap3* gene (*tll1941*) is adjacent to the orthological ORF2 gene (*tll1940*) of the same operon. The sequence of the *gap3* operon of *T. elongatus* BP-1 showed that it has a length of 7239 bp. The sequence of the entire genome of *Synechococcus* sp. PCC 7942 is to be accomplished in the nearest future (http://genome.ornl.gov/microbial/syn_PCC7942), which will provide information on the nucleotide sequence of the whole region of the *gap3* gene of this cyanobacterium.

The ability to rapidly adapt to variable environmental conditions is very important for bacteria to survive and grow in nature. Of great interest in this respect are cyanobacteria, which consume carbon dioxide and evolve oxygen, thereby contributing to the maintenance of life on the earth. Cyanobacteria may be useful in the study of the evolution of plants and their plastids since cyanobacteria and plants have a common ancestor [15]. Cyanobacteria are more convenient genetic objects for research than are many other oxygenic photoautotrophs [16]. For this reason, they are widely used to study the regulatory mechanisms that are involved in the adaptation of photosynthesizing bacterial cells to the environment. To optimize carbon fixation at low ambient concentrations of carbon dioxide, cyanobacteria use the so-called carbon-concentrating mechanism, a light-driven mechanism for concentrating $CO_2$ near the major carboxylating enzyme, ribulose bisphosphate carboxylase [17]. The molecular and biochemical mechanisms of this process are not understood in depth, although there is evidence that light and synthesis of new proteins are necessary for the carbon-concentrating mechanism to be completely induced [17]. According to the hypothesis of Sueltemeyer *et al.* [18], the full induction of this mechanism occurs in two steps. At the first step, the post-translational modification of the constitutive $HCO_3$ transporter protein provides for a fast adaptive cell response to limiting concentrations of inorganic carbon. At the second step, which is not as fast as the first, the biosynthesis of new proteins fully optimizes the adaptive cell response. The homology of the ORF2 product to some transport proteins suggests that this product is also a transport protein. The putative $HCO_3$ transporter that was identified with the aid of the CCM mutant IL-2 of *Synechococcus* PCC 7942 [19] did not show a homology to the ORF2 product. This suggests that *Synechococcus* PCC 7942 may have different $HCO_3$ transporters that provide the cyanobacterial cell with carbon under different ambient conditions.

Thus, the *gap3* genes of *Synechococcus* PCC 7942 and *Anabaena variabilis* ATCC 29413 are expressed as long mRNA molecules during the adaptation of these cyanobacteria to low $CO_2$ concentrations. The *gap3* gene of *Synechococcus* PCC 7942 overlaps the gene that codes for a membrane transporter protein. The *gap3* gene may be involved in the functioning of the carbon-concentrating mechanism of *Synechococcus* PCC 7942 and *Anabaena variabilis* ATCC 29413. Further studies are necessary to understand the role of the Gap3 and transporter proteins.

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## REFERENCES


1. Cerff, R., The Chimeric Nature of Nuclear Genomes and the Antiquity of Introns as Demonstrated by the GAPDH Gene System, *Tracing Biological Evolution in Protein and Gene Structures*, Gö, M. and Schimmel, P., Eds., Amsterdam: Elsevier, 1995, pp. 205–227.
2. Koksharova, O., Schubert, M., Shestakov, S., and Cerff, R., Genetic and Biochemical Evidence for Distinct Key Functions of Two Highly Divergent GAPDH Genes in Catabolic and Anabolic Carbon Flow of the Cyanobacterium *Synechocystis* sp. PCC 6803, *Plant Mol. Biol.*, 1998, vol. 36, pp. 183–194.
3. Martin, W., Brinkmann, H., Savona, C., and Cerff, R., Evidence for a Chimeric Nature of Nuclear Genomes: Eubacterial Origin of Eukaryotic Glyceraldehyde-3-Phosphate Dehydrogenase Genes, *Proc. Natl. Acad. Sci. USA*, 1993, vol. 90, pp. 8692–8696.
4. Figge, R., Schubert, M., Brinkmann, H., and Cerff, R., Glyceraldehyde-3-Phosphate Dehydrogenase Gene Diversity in Eubacteria and Eukaryotes: Evidence for Intra- and Inter-Kingdom Gene Transfer, *J. Mol. Biol. Evol.*, 1999, vol. 16, pp. 429–440.
5. Qian, Q. and Keeling, P.J., Diplonemid Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and Prokaryote-to-Eukaryote Lateral Gene Transfer, *Protist*, 2001, vol. 152, pp. 193–201.
6. Alefounder, P. and Perham, R., Identification, Molecular Cloning and Sequence Analysis of a Gene Cluster Encoding the Class II Fructose 1.6-Bisphosphate Aldolase, 3-Phosphoglycerate Kinase and a Putative Second Glyceraldehyde 3-Phosphate Dehydrogenase of *Escherichia coli, Mol. Microbiol.*, 1989, vol. 3, pp. 723–732.
7. Zhao, G., Pease, A., Bharani, N., and Winkler, M., Biochemical Characterization of *gapB*-Encoded Erythrose 4-Phosphate Dehydrogenase of *Escherichia coli* K-12 and Its Possible Role in Pyridoxal 5'-Phosphate Biosynthesis, *J. Bacteriol.*, 1995, vol. 177, pp. 2804–2812.
8. Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., and Stanier, R.Y., Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria, *J. Gen. Microbiol.*, 1979, vol. 111, pp. 1–61.
9. Hu, N.-T., Thiel, T., Giddings, T.H., and Wolk, C.P., *Anabaena* and *Nostoc* Cyanophages from Sewage Settling Ponds, *Virology*, 1982, vol. 114, pp. 236–246.
10. Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor: Cold Spring Harbor Lab., 1989.
11. Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J., Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs, *Nucleic Acids Res.*, 1997, vol. 25, pp. 3389–3402.
12. Valverde, F., Peleato, M.L., Fillat, M.F., Gomez-Moreno, C., Losada, M., and Serrano, A., Simultaneous Occurrence of Two Different Glyceraldehyde-3-Phosphate Dehydrogenases in Heterocystous $N_2$-Fixing Cyanobacteria, *Biochem. Biophys. Res. Commun.*, 2001, vol. 283, pp. 356–363.
13. Kaneko, T., Nakamura, Y., Wolk, C.P., Kuritz, T., Sasamoto, S., Watanabe, A., Iriguchi, M., Ishikawa, A., Kawashima, K., Kimura, T., Kishida, Y., Kohara, M., Matsumoto, M., Matsuno, A., Muraki, A., Nakazaki, N., Shimpo, S., Sugimoto, M., Takazawa, M., Yamada, M., Yasuda, M., and Tabata, S., Complete Genomic Sequence of the Filamentous Nitrogen-Fixing Cyanobacterium *Anabaena* sp. Strain PCC 7120, *DNA Res.*, 2001, vol. 8, pp. 205–213.
14. Vlcek, C., Paces, V., Maltsev, N., Paces, J., Haselkorn, R., and Fonstein, M., Sequence of a 189-kb Segment of the Chromosome of *Rhodobacter capsulatus* SB1003, *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, pp. 9384–9388.
15. Gray, M.W., Origin and Evolution of Organelle Genomes, *Curr. Opin. Genet. Develop.*, 1993, vol. 3, pp. 884–890.
16. Koksharova, O.A. and Wolk, C.P., Genetic Tools for Cyanobacteria, *Appl. Microbiol. Biotechnol.,* 2002, vol. 58, pp. 123–137.
17. Kaplan, A., Schwarz, R., Lieman-Hurwitz, J., Ronen-Tarazi, M., and Reinhold, L., Physiological and Molecular Studies on the Response of Cyanobacteria to Changes in the Ambient Inorganic Carbon Concentration, *The Molecular Biology of Cyanobacteria*, Bryant, D.A., Ed., Amsterdam: Kluwer, 1994, pp. 469–485.
18. Sueltemeyer, D., Klughammer, B., Badger, M., and Price, G., Fast Induction of High-Affinity $HCO_3$ Transport in Cyanobacteria, *Plant. Physiol.*, 1998, vol. 116, pp. 183–192.
19. Bonfil, D.J., Ronen-Tarazi, M., Sultemeyer, D., Lieman-Hurwitz, J., Schatz, D., and Kaplan, A., A Putative $HCO_3$ Transporter in the Cyanobacterium *Synechococcus* sp. Strain PCC 7942, *FEBS Lett.*, 1998, vol. 430, pp. 236–240.