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EXPERIMENTAL ARTICLES

The *gap1* Operon of the Cyanobacterium *Synechococcus* PCC 7942 Carries a Gene Encoding Glycogen Phosphorylase and Is Induced under Anaerobic Conditions

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Abstract—The cloning and sequencing of the *gap1* operon, which encodes the glycolytic NAD-specific glyceraldehyde-3-phosphate dehydrogenase in the cyanobacterium *Synechococcus* PCC 7942, showed that the *gap1* gene is closely linked to the *glgP* gene encoding glycogen phosphorylase (an enzyme that catalyzes the first step of glycogen degradation). Northern blotting experiments showed that the *gap1* and *glgP* genes are coexpressed and organized in a bicistronic operon, whose expression is enhanced under anaerobic conditions. The nucleotide sequence of the operon has been submitted to GenBank under accession number AF428099.

Key words: anaerobic stress, glyceraldehyde-3-phosphate dehydrogenase, glycogen phosphorylase, glycolytic enzymes, operon, gene expression, cyanobacteria.

Oxygenic photosynthetic cyanobacteria, ancient relatives of chloroplasts, are widely used for the study of genetic mechanisms that control photosynthesis; nitrogen fixation; cell differentiation; and nitrogen, hydrogen, and carbon metabolisms [1]. In plants, the cytoplasmic and the chloroplast glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) are the key enzymes of glycolysis and the Calvin cycle, respectively. The cyanobacterium *Synechocystis* sp. PCC 6803, which is capable of heterotrophic growth in the dark, has two *gap* genes, *gap1* and *gap2* [2]. In spite of the absence of cell compartments in prokaryotic cyanobacteria, the *gap1* and *gap2* genes code for the glycolytic and the Calvin cycle enzymes, which are functionally distinct [2]. The cyanobacteria *Anabaena variabilis* and *Synechococcus* PCC 7942 have three divergent genes (*gap1, gap2*, and *gap3*) [3, 4], which have not yet been investigated in depth.

The aim of this work was to study the expression of the *gap1* gene in the obligately photoautotrophic cyanobacterium *Synechococcus* PCC 7942 by the RNA–DNA hybridization method.

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. The bacterium was grown at 28–30°C at a low illumination of 1500 lx or at a high illumination of 3000 lx in BG11 medium [5], which was sparged with air containing the normal amount of CO_2 (about 0.03%) or 1% CO_2. In the latter case, the cultivation medium was supplemented with 10 mM HEPES–NaOH buffer (pH 8.0). Anaerobic cultivation was performed by placing liquid culture flasks in a 2.5-l Anaerocult/EA container (Merck). *Escherichia coli* strain XL-1 Blue was grown in LB broth supplemented with the corresponding antibiotics. The cloning vector was pBluescript SK(+) (Stratagene). *E. coli* cells were transformed and the transformants obtained were tested by the standard procedures [6].

The cloning and sequencing of the *gap1–glg* operon of *Synechococcus* PCC 7942 and the sequence analysis. The *gap1* gene was cloned from the gene library of *Synechococcus* PCC 7942 as described previously [4]. The genomic fragment that carried the *gap1* gene was obtained with the aid of *Sal*I endonuclease. The fragment was subcloned on the pBluescript SK(+) vector and sequenced with T7 polymerase by using an automatic DNA Sequenator (Pharmacia) according to the manufacturer's instructions. The data obtained were analyzed with the aid of the BLAST search program available on the NCBI GenBank BLAST server [7] and with the use of information on the *Synechocystis* sp. PCC 6803 genome sequence,

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which is available in Cyanobase (http://www.kazusa.or.jp/cyanobase/).

Nucleotide sequence accession number. The nucleotide sequence of the *glgP* gene was deposited in GenBank under accession number AF428099.

The isolation of RNA and Northern blotting hybridization. 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 a Hybond-N$^+$ nylon membrane (Amersham Pharmacia) by the routine procedure [6]. The RNA concentration 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 under UV light. The size of the transcripts was determined by using RNA markers in the interval 0.28–6.58 kb (Promega). RNA was fixed on nylon membranes at 80°C for 2 h. Then the membranes with the fixed RNA were hybridized with α-32P-labeled DNA probes by using a Multiprime DNA labeling system (Amersham Pharmacia). The hybridization and washing procedures were performed as described by Sambrook *et al.* [6]. The DNA probes were prepared by PCR with the following specific primers: 5'-GAC CTC GTT CCG CCA AA-3' and 5'-CCG AGC ATC GCC AAT AAAG-3' for the *gap1* gene, and 5'(+)-CCA CCA CGA GCT ACC TCG-3' and 3'(-)-CCG TTG CTA GCC CGG G-5' for the *glgP* gene.

RESULTS AND DISCUSSION

The *gap1* and *glgP* genes of the photoautotrophic cyanobacterium *Synechococcus* PCC 7942 code for two key carbon metabolism enzymes, GAPDH and glycogen phosphorylase. RNA hybridization experiments showed that the *gap1* gene was expressed in *Synechococcus* PCC 7942 as a quite extended RNA molecule 3.8–4.0 kb in size (Fig. 1). The *Sal*I fragment of the genomic DNA with the *gap1* gene was subcloned on the pBluescript vector, and both strands were sequenced. As a result, we identified a long open reading frame (ORF) located 295 bp downstream of the 3'-end of the *gap1* gene. A comparison of the amino acid sequence encoded by this ORF with the data available in GenBank showed that this sequence is very similar to those of the known glycogen phosphorylases, especially with the two enzymes of *Synechococcus* PCC 6803 encoded by two different *glg* genes. Namely, the *glg* sequence of *Synechococcus* PCC 7942 was found to have 60% identical amino acids with the sequence encoded by the *glgP1* (*sll1356*) gene and 63% identical amino acids with the sequence encoded by the

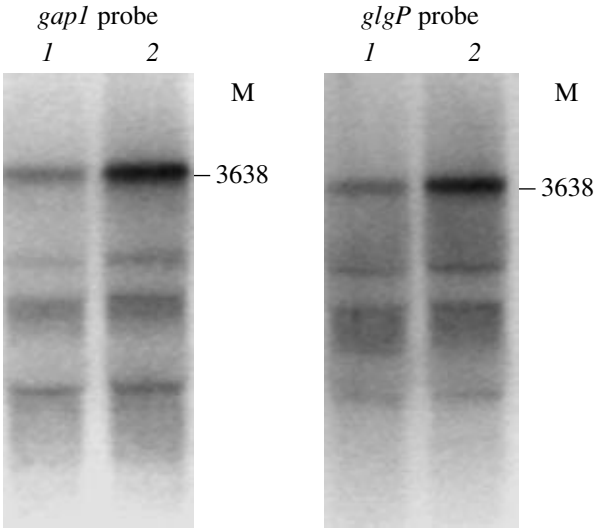

Fig. 1. The results of RNA hybridization performed by the Northern blotting technique. The total RNA was isolated from the wild-type *Synechococcus* PCC 7942 cells grown for 2 days under high illumination in air containing 1% CO_2 (lane *1*) and then incubated for 24 h under anaerobic conditions and the same illumination (lane *2*). The solid lines show the position of a marker containing 3638 nucleotides.

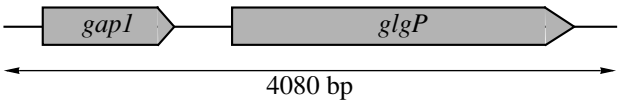

Fig. 2. The *gap1* operon with the *glgP* gene coding for glycogen phosphorylase.

glgP2 (*sll1367*) gene of *Synechocystis* PCC 6803 [8]. Thus, the genome of *Synechococcus* PCC 7942 has two genes (*gap1* and *glgP*), which are organized in a *gap1–glgP* operon (Fig. 2).

Northern hybridization experiments revealed a long transcript 3.8–4.0 kb in size and shorter RNA molecules (Fig. 1). The size of the long transcript corresponded to the sum of the sizes of the *gap1* and *glgP* transcripts. The shorter transcripts might represent RNA degradation products. The expression of the *gap1–glgP* operon of *Synechococcus* PCC 7942 increased during cultivation under anaerobic conditions (Fig. 3).

The gene clusters that code for carbon metabolism enzymes have been described for a number of prokaryotic organisms. For instance, the *E. coli* genes coding for fructose bisphosphate aldolase, phosphoglycerate kinase, and GAPDH form one gene cluster [9], whereas the genes coding for triosephosphate isomerase and phosphofructokinase form another cluster [10]. In *Bacillus stearothermophilus*, GAPDH and phosphoglycerate kinase are encoded by adjacent genes [11]. In *Bacillus megaterium*, the genes coding for GAPDH, phosphoglycerate kinase, and triosephosphate isomerase belong to one operon [12].

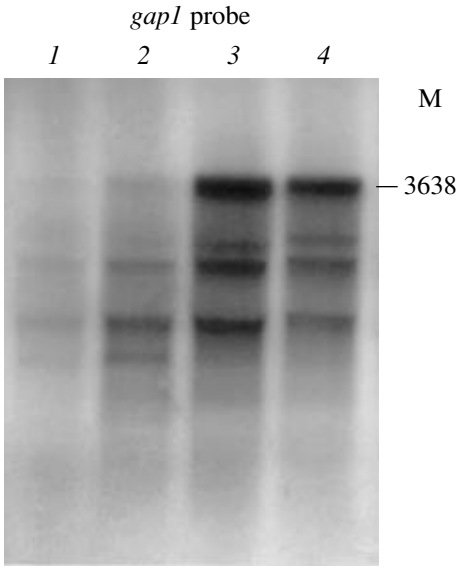

Fig. 3. The results of RNA hybridization performed by the Northern blotting technique. The total RNA was isolated from wild-type *Synechococcus* PCC 7942 cells grown for 5 days under low illumination (1500 lx) in the air (lane *1*) and then incubated for 28 h under high illumination (3000 lx) and aerobic conditions (lane *2*), under high illumination and anaerobic conditions (lane *3*), and under low illumination and anaerobic conditions (lane *4*). The solid line shows the position of a marker containing 3638 nucleotides.

The *gap* operon of *Synechococcus* PCC 7942 differs from the *gap* operons of other organisms in that it has the *gap1* gene and the gene coding for glycogen phosphorylase, which is an important allosteric enzyme involved in the metabolism of bacterial glycogen [13]. Glycogen has been found in many bacteria, however, only a few bacterial glycogen phosphorylases have been comprehensively studied at genetic and protein levels. The complete sequence of the *Synechocystis* PCC 6803 genome shows that it contains several genes (*sll0158, sll0945, sll1393, slr0237, slr1367*, and *sll1356*) of glycogen metabolism [8]. As for *Synechococcus* PCC 7942, only one gene of glycogen metabolism (*glgB*) of this cyanobacterium has been cloned and sequenced [14]. The ORF (the *gemE* gene) found near the 3'-terminus of the *glgB* gene of *Synechococcus* PCC 7942 codes for a protein that has a relatively high similarity (41% identical amino acids) to human and rat uroporphyrinogen decarboxylase (EC 4.1.1.37). The close arrangement of the *glgB* and *hemE* genes and the absence of a promoter sequence upstream of the latter gene allowed Kiel *et al.* to suggest that both genes are expressed coordinately in *Synechococcus* PCC 7942 [14]. However, this suggestion was not supported by RNA–DNA hybridization experiments.

Our studies of the *gap1–glgP* operon and the study of the *glgB* gene by Kiel *et al.* [14] showed that the structural organization of the glycogen metabolism genes of *Synechococcus* 7942 greatly differs from that found in other bacteria. For instance, the glycogen synthesis genes of *E. coli* are organized in two adjacent operons, *glgBX* and *glgCAY* [15]. The *glg* operon of *Agrobacterium tumefaciens* contains five ORFs (*glgP, glgB, glgC, glgA*, and the phosphoglucomutase gene *pmg*) [16]. The gene cluster responsible for glycogen biosynthesis in *Bacillus stearothermophilus* also contains five ORFs (*glgBCDAP*). Of interest is the fact that the ATPase operon of *Streptococcus* mutants is flanked by the genes that encode glycogen phosphorylase and NADP-dependent GAPDH [17].

Phosphorylases are likely to be involved in the regulation of glycogen metabolism over periods of cell response to stresses and of quick cell adaptation to varying environmental conditions [13]. Exposure to anaerobiosis is a stress for aerobic organisms. This explains why the expression of the plant gene coding for cytoplasmic GAPDH is controlled by anaerobic conditions [18]. The data presented in Fig. 3 show that the expression of the cyanobacterial *gap1–glgP* operon is also enhanced under anaerobic conditions. Therefore, primary carbon metabolism is very responsive to varying environmental conditions.

Thus, our experiments for the first time demonstrated that the two genes of the unicellular photoautotrophic cyanobacterium *Synechococcus* PCC 7942 that code for the carbon metabolism enzymes GAPDH and glycogen phosphorylase are expressed as one mRNA molecule and that the expression of these genes is enhanced under anaerobic conditions.

Northern blotting experiments revealed a long transcript and shorter mRNA molecules (Fig. 1), which might represent degradation products of the long transcript. The regulation of glycogen biosynthesis by control of the stability of mRNA has not yet been described for cyanobacteria, although it is known that the product of the *E. coli* gene *csrA* accelerates the degradation of the ADP-glucose pyrophosphorylase gene *glgC* by means of selective binding to mRNA [19, 20]. This type of regulation, which is as yet poorly studied, may play an important role in the expression of bacterial genes.

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