JOURNAL OF BACTERIOLOGY, Feb. 1997, p. 714–720 Vol. 179, No. 3
0021-9193/97/$04.00+0

The *ggtA* Gene Encodes a Subunit of the Transport System for the Osmoprotective Compound Glucosylglycerol in *Synechocystis* sp. Strain PCC 6803

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Received 12 July 1996/Accepted 2 October 1996

The *ggtA* gene was sequenced during the analysis of a mutant of *Synechocystis* sp. strain PCC 6803 with impaired salt tolerance. It showed striking sequence similarities to ATP-binding proteins of binding-protein-dependent transport systems (ABC transporters). Mutants of *ggtA* and three neighboring reading frames were constructed by inserting an *aphII* gene cassette and were physiologically and genetically characterized. The *ggtA* insertion mutant lost its glucosylglycerol (GG) uptake ability, but its salt tolerance did not change. Therefore, it was concluded that active transport of the osmoprotective compound GG in *Synechocystis* is mediated by an ABC transporter. The genes for the GG-specific ABC transporter are not organized in an operon as usually found for comparable transporters, since the other insertion mutants showed normal GG transport activity. After cultivation of the *ggtA* mutant at high salt concentrations, significant amounts of GG were found in the cultivation medium, indicating that GG transport is mainly necessary for recovery of GG leaked through the cytoplasmic membrane. The Northern blot technique revealed increased transcription of the *ggtA* gene in cells adapted to higher salt concentrations, whereas in cells from basal medium, its transcription was weak.

Salinity is one of the most important abiotic factors in aquatic biotopes. An increase in salt concentration represents a combination of two stress conditions for living cells. The reduction of the water potential of the surrounding medium causes the cells to lose water. In contrast to this purely osmotic stress, an increase in salinity also means a dramatic increase in inorganic ions (especially Na^+ and Cl^-) in the surrounding medium which enter the cells along chemical gradients (33). High intracellular concentrations of inorganic ions are toxic to most eubacteria. During adaptation to salt stress, these bacteria usually achieve a balanced osmotic potential by extruding excess inorganic ions and accumulating osmoprotective compounds (compatible solutes), which are low-molecular-weight hydrophilic compounds that do not interfere with cell metabolism because they are largely inert and carry no net charge (4, 10). Osmoprotective compounds reduce the osmotic potential of the cytoplasm to prevent further loss of water and apparently prevent denaturation by helping macromolecules to retain their natural configuration.

Two strategies are used to acquire high intracellular amounts of osmoprotective compounds: de novo synthesis and/or uptake from the surroundings. Salt-loaded cyanobacterial species accumulate sucrose, trehalose, glucosylglycerol [2-*O*-(α-D-glucopyranosyl)-glycerol (GG)], glycinebetaine, or glutamatebetaine, the principal osmoprotective compounds, by de novo synthesis (34). Only the biosynthetic pathway for GG synthesis has so far been elucidated. It is synthesized from ADP-glucose and glycerol-3-phosphate by a salt-activated enzyme system (16). A GG uptake system was recently found (25) in the cyanobacterium *Synechocystis* sp. strain PCC 6803, which accumulates GG and tolerates up to 1.2 M NaCl (32). This uptake system also showed affinity for the osmoprotective compounds sucrose and trehalose. The transporter allowed salt-sensitive mutants of *Synechocystis* lacking the ability to synthesize GG to grow in the presence of exogenous GG under high-salt conditions (25).

Unlike the cyanobacteria, many heterotrophic eubacteria prefer to acquire osmoprotective compounds by uptake from their surrounding environment. Several active transport systems for proline, glycinebetaine, and its precursor choline have been characterized in *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis* (5, 19, 30). The activities of these systems are directly stimulated by higher salt concentrations and are further increased by salt-regulated induction of their gene expression (22). *E. coli* is able to synthesize trehalose under osmotic stress, but if glycinebetaine is available in the medium, trehalose de novo synthesis is completely suppressed and adaptation to high osmotic pressure is achieved by accumulation of glycinebetaine alone (11, 21). In *E. coli*, glycinebetaine is transported mainly by the osmoregulated ProU system, a binding-protein-dependent transport system (5). A related system was recently characterized in *B. subtilis* (19). Binding-protein-dependent transport systems belong to the superfamily of ABC (ATP-binding cassette) transporters. They consist of at least three parts: a periplasmic binding protein with high affinity for the substrate, an integral cytoplasmic membrane protein acting as a permease, and a protein located at the cytosolic face of the membrane, which is responsible for transport energization by ATP splitting (ATP-binding protein). Usually these three subunits are encoded together in one operon (1).

Several salt-sensitive mutants of *Synechocystis* have been generated to isolate genes involved in salt adaptation (15). The mutated region from one of these mutants and the corresponding wild-type regions have been cloned and sequenced (17). In this study, we present results that led to the identification of a gene involved in GG transport. One of the sequenced open

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reading frames (ORFs) that was not impaired in the salt-sensitive mutant showed a high degree of similarity to ATP-binding subunits of the family of ABC transporters. An insertion mutant, in which this gene was interrupted by an *aphII* (aminoglycoside phosphotransferase II conferring kanamycin resistance) gene, showed defective GG transport. We therefore concluded that this gene encodes a protein involved in GG transport. The transport seems to be mediated by a binding-protein-dependent transport system. The expression of the gene was increased about threefold in cells adapted to high salinity. We propose that this gene be designated *ggtA* (glucosylglycerol transport A).

MATERIALS AND METHODS

Strains and culture conditions. A derivative of *Synechocystis* sp. strain PCC 6803 with enhanced transforming capacity obtained from S. Shestakov (Moscow State University, Moscow, Russia) was used in all experiments. Axenic cells were cultivated on agar plates at 30°C under constant illumination, using a mineral medium (15). Transformants were initially selected on medium containing kanamycin (Sigma) at 10 $\mu g\ ml^{-1}$, while the segregation of clones and cultivation of mutants were performed on medium containing kanamycin at 50 $\mu g\ ml^{-1}$. *E. coli* JM101 (35) was used for routine DNA manipulations. *E. coli* was cultivated in Luria broth (LB) medium at 37°C.

For physiological characterization, axenic cultures of the cyanobacteria were grown photoautotrophically in batch cultures at 29°C under continuous illumination of 20 W m^{-2} and bubbling with CO_2 (2.5%)-enriched air. To achieve the desired salt concentrations, NaCl was added to the basal growth medium containing 2 mM NaCl (15). To obtain salt-adapted cells, the cyanobacteria were precultured for 5 days in NaCl-enriched media. The cultures were checked for contamination by heterotrophic bacteria by microscopy or spreading of 0.2 ml of culture on LB plates.

DNA manipulations. Total DNA was isolated from *Synechocystis* by lysozyme treatment and phenol-chloroform purification (2). All other DNA techniques such as plasmid isolation, transformation of *E. coli*, ligations, restriction analysis (restriction enzymes from Life Technologies), Southern hybridization analysis, and labeling of DNA probes for hybridization experiments by random priming using [α-^{32}P]dATP (Amersham Buchler) were carried out by standard methods (35). Correct integration of the *aphII* gene into plasmids was checked by restriction analysis and DNA sequencing with the dideoxy-chain termination method, using [α-^{35}S]dATP (Amersham Buchler) and a Sequenase 2.0 kit (U.S. Biochemical). For sequencing, double-stranded plasmid DNA was isolated by means of a QIAprep plasmid kit (Qiagen). The following synthetic primers were specifically used for the *aphII* gene: kan 5′ (caggcctggtatgagtcagc) and kan 3′ (atttttatcttgt gcaatgt) (custom oligonucleotide synthesis; Pharmacia). Computer analysis of the DNA sequence was done with the DNASIS/PROSIS and the GCG (7) software packages.

RNA isolation and Northern blot experiments. Total RNA was isolated from *Synechocystis* by a modified method (31). Instead of ultracentrifugation, the RNA was treated with DNase (Boehringer Mannheim) to remove contaminating DNA. After denaturation, the RNA was separated in 1.3% agarose gels containing 7% formaldehyde in morpholine propanesulfonic acid (MOPS) buffer. It was then transferred overnight to nylon membranes (Hybond N; Amersham Buchler), using capillary transfer with 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (35), and fixed to the filters by UV illumination for 3 min. The filters were hybridized overnight with ^{32}P-labeled DNA probes at 53°C in high-sodium dodecyl sulfate (SDS) concentration buffer (7% SDS, 50% formamide, 5× SSC, 2% blocking reagent [Boehringer Mannheim], 50 mM sodium phosphate buffer [pH 7.0], 0.1% *N*-lauroylsarcosine [Boehringer Mannheim]). After washing (three times in 2× SSC–0.1% SDS at room temperature for 20 min and once in 0.1× SSC–0.1% SDS at 53°C for 30 min), the filters were incubated for autoradiography at −80°C. The amount of radioactivity in the single bands was determined with a PhosphorImager (Molecular Dynamics). The RNA concentration was determined by absorption measurements at 260 nm in a model U2000 double-beam UV/Vis spectrophotometer (Hitachi).

Generation of insertion mutants. For the generation of mutants affected in specific ORFs, the *aphII* gene was integrated into the sequences encoding the specific ORFs at selected single restriction sites. Where necessary, *aphII* gene integration was performed after appropriate DNA subfragments had been cloned into *E. coli* plasmids. The *aphII* gene was isolated from the *E. coli* plasmid pUC4K (39). DNA of these constructs was isolated from *E. coli* with a QIAprep spin plasmid kit (Qiagen). One microgram of DNA was used for *Synechocystis* transformation, and kanamycin-resistant (Km^r) clones were selected (15).

Transport assays. ^{14}C-GG with a specific activity of 0.659 dpm $pmol^{-1}$ was obtained by ^{14}C labeling of salt-loaded *Synechocystis* cells and purification (25). GG uptake was measured under the culture conditions described above without aeration. The cell suspension (0.5 ml) was taken directly from the cultures (total cell volume, 1.2 to 2.6 $mm^3\ ml^{-1}$) and incubated for 15 min with 0.2 mM ^{14}C-GG. The tests were stopped by addition of 3 ml of medium of the same salt concentration and immediate filtration through membrane filters (0.45-μm pore size, SM 11306; Sartorius). Subsequently the filters were washed three times with 3 ml of cultivation medium and solubilized in a scintillation cocktail as described in reference 25. Radioactivity was measured by liquid scintillation counting. Glucose uptake was measured as described in reference 18.

Other physiological parameters. The GG content of the cells was estimated by high-pressure liquid chromatography (HPLC) (36) after extraction in 80% ethanol. The amount of GG leaked into the medium was also determined by HPLC after desalting the sterile-filtered supernatants on ion-exchange columns. Cell number and diameter were determined by means of an electronic particle counter (8). The total cell volume was estimated as the product of average single-cell volume and cell number. Photosynthetic oxygen evolution was measured by a Clarke-type electrode (8).

Nucleotide sequence accession number. The nucleotide sequence reported has been deposited in GenBank under accession number U32936.

RESULTS AND DISCUSSION

Sequence analysis of the *ggtA* gene. Partial deletion of the *stpA* gene (28) and the gene upstream of it (*orfI*) was found to be responsible for the salt-sensitive phenotype of *Synechocystis* mutant 4. The salt sensitivity was caused by reduced synthesis of the osmoprotective compound GG (17). The downstream gene (*orfII*) of *stpA* remained intact in mutant 4 (Fig. 1). The deduced amino acid sequence of *orfII* was compared with amino acid sequences from databases by means of the TFASTA program (7). Significant similarities (about 35% identical amino acid residues) to several ATP-binding proteins from ABC transporters were found (Fig. 2). This homology was observed throughout the whole sequence but was most pronounced among the first 220 amino acids of the N-terminal part. The motifs for the ATP-binding sites (amino acid residues 37 to 45 and 154 to 159) could also be identified in the sequence of ORFII protein (Fig. 2). The size of the ORFII protein, 40.9 kDa, matches well this type of protein from several bacteria.

The most remarkable similarities were found to two proteins from *E. coli*: UgpC, which is involved in the uptake of glycerol-3-phosphate and glycerolphosphoryl diesters (29), and MalK, which is a component of the maltose uptake system (12). A high degree of homology to the CysA protein of the binding-protein-dependent sulfate uptake system of the cyanobacterium *Synechococcus* sp. strain PCC 6301 (14) was also observed. MbpX, a protein of unknown transport function, is encoded on the chloroplast genome of *Marchantia polymorpha* (38). Furthermore, we found significant sequence similarities to the ProV protein, the ATP-binding protein of the osmotically regulated ProU system for glycinebetaine uptake in *E. coli* (13).

Construction of mutations in *ggtA* and flanking genes. The sequence similarities suggested that *orfII* also encodes an ATP-binding protein of an unknown cyanobacterial ABC transporter. However, it was not possible to identify the transport process in which ORFII might be implicated from these sequence data alone, as the homologous proteins are involved in the transport of substrates with various structures. To identify the substrate transported by this system, an insertion mutant of *orfII* was constructed. After the sequence encoding *orfII* had been subcloned on a *Stu*I/*Bgl*II fragment, a single *Hpa*I site was present (Fig. 1). The *aphII* gene from pUC4K was inserted into this site. One clone harboring the *aphII* gene at the right position in a transcription direction opposite that of *orfII* was selected after restriction and sequence analysis (Fig. 1). This DNA construct was transformed into *Synechocystis*, and Km^r clones were selected.

The genes encoding the subunits of ABC transporters are often organized into operons (1). Therefore, insertion mutants specific to the reading frames upstream (*orfI* and *stpA*) and downstream (*orfIII*) of *orfII* were also constructed and used in this study (Fig. 1). All of these mutants were constructed after

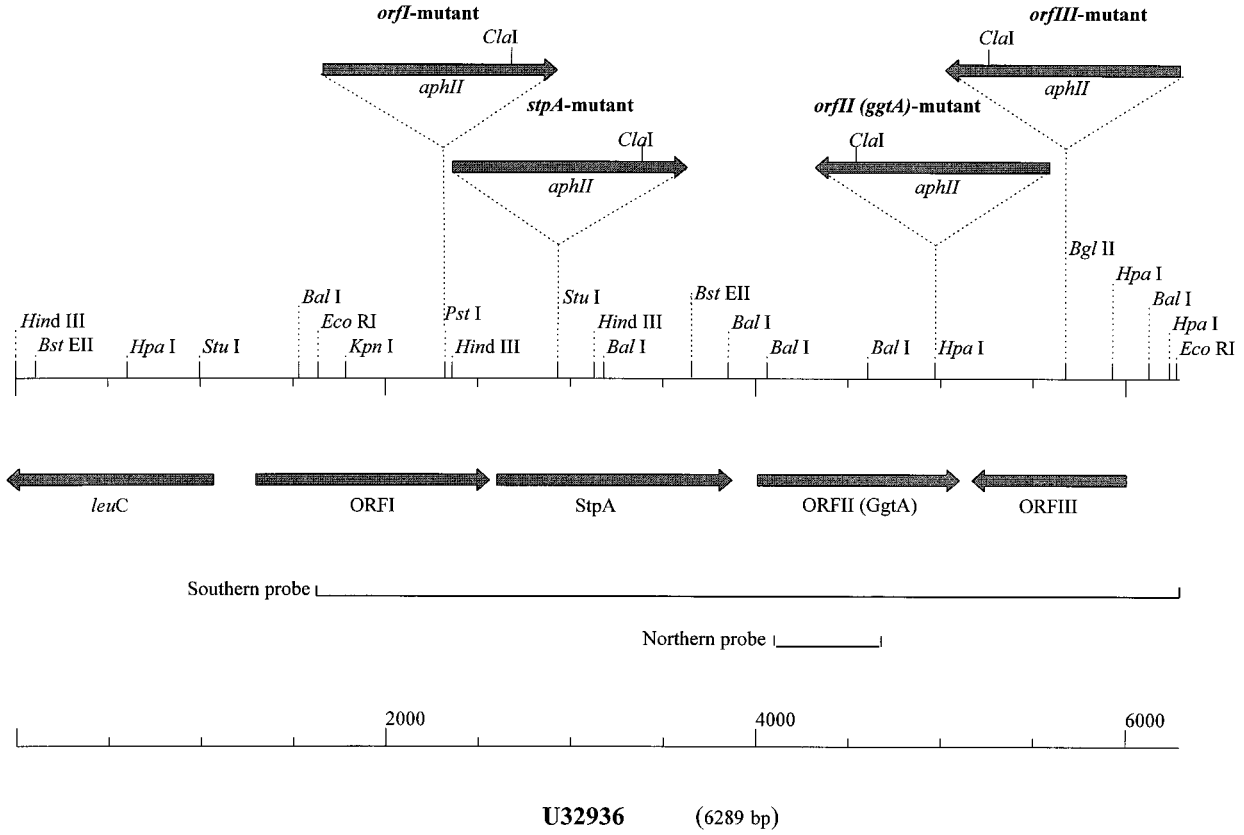

FIG. 1. Restriction map and protein-encoding regions (arrows below) of the sequence U32936 (17). Integration sites of the *aphII* gene in the mutants that are specific for single protein-encoding regions are shown above the restriction map. The arrows indicate the direction of transcription. Probes used in the Southern and Northern hybridization experiments are given under the protein-encoding regions.

insertion of an *aphII* gene into a single restriction site of each ORF, transformation of these constructs into *Synechocystis*, and selection of Km^r clones. Isolated chromosomal DNA from such clones was analyzed in Southern hybridization experiments. Probing with the *aphII* gene indicated that kanamycin resistance in all of these clones was based on the integration of this gene into the genome (not shown). Additionally, the *Eco*RI fragment of the wild-type DNA was used as a probe (Fig. 1). Chromosomal DNAs of the different mutants and the wild type were double digested by *Eco*RI to excise the wild-type fragment and by *Cla*I, which cuts once in the *aphII* gene. Only the 4.6-kb fragment corresponding to the probe was found in the wild-type DNA, while in mutant DNA, the probe recognized two fragments of the sizes expected after correct insertion in each case (Fig. 3). All mutants lacked the 4.6-kb wild-type fragment, indicating a double crossover event during recombination and complete segregation.

Analysis of GG transport activity in the mutants. All mutants grew at about the same rate as the wild type in low-salt media (Table 1). Only the *stpA* mutant produced a salt-sensitive phenotype (resistance limit at 550 mM NaCl), all others being able to adapt to high-salt conditions (normal growth at 684 mM NaCl). As expected in view of the salt tolerance limits, the accumulation of GG was not significantly changed except in the *stpA* mutant (Table 1). Because *orfII* was found downstream of the *stpA* gene suspected to be involved in GG synthesis, it seemed appropriate to check the *orfII* mutant for GG transport ability. Glucose uptake was measured at the same time to check for overall transport activity and energy supply. In wild-type cells, GG uptake was enhanced after salt adaptation, while in the *orfII* mutant, almost no GG uptake could be detected, either in cells grown in basal medium or in cells grown in high-salt media (Table 2). However, glucose uptake remained unchanged in the *orfII* mutant compared to the wild type (not shown). The lack of GG transport activity in the *orfII* mutant indicates that the *orfII* gene most probably encodes a protein involved in GG transport. It could also be concluded that GG uptake is mediated by a binding-protein-dependent transport system. Therefore, we propose the designation *ggtA* for *orfII*.

The genes upstream and downstream of *ggtA* do not encode other proteins involved in this transport process, since all other mutants were able to transport GG, and as in the wild type, GG uptake was enhanced in salt-adapted cells (Table 2). The rate of GG uptake was slightly lower in salt-treated cells of the *stpA* mutant than in wild-type cells. This could be explained by the salt sensitivity of the *stpA* mutant leading to reduced growth and metabolic activity at high salt concentrations, since photosynthetic oxygen evolution in this mutant was reduced to 40% of the value seen in wild-type cells after cultivation in 513 mM NaCl (not shown).

As yet, only a few binding-protein-dependent transport systems have been characterized in cyanobacteria, and these have been involved in sulfate, nitrate, and manganese uptake (3, 14, 23, 24, 27). In contrast to the GG transporter, all subunits of these ABC transporters have been clustered and organized like an operon. A polycistronic transcript was found for the genes encoding the nitrate transport system. It includes further genes

FIG. 2. Amino acid sequence comparison between ORFII (GgtA) and several ATP-binding subunits of binding-protein-dependent transport systems (UgpC [29], MalK [12], CysA [14], MbpX [38], and ProV [13]). The GCG program PILEUP was used for the alignment (7). Uppercase letters represent amino acids that are identical or strongly homologous in five or six of the aligned sequences (homology index of ≥1.0 in the Dayhoff table [6]). Shaded boxes indicate common conserved motifs of ATP-binding proteins. The first motif represents the ATP-binding site, G-X-X-G-X-G-K-T/S. The second ATP-binding motif has the following structure: hydrophobic stretch-D-E (9, 20).

involved in nitrate metabolism (23, 27). Genes encoding ABC transporters responsible for glycinebetaine uptake in *E. coli* and *B. subtilis* are also organized in operons and transcribed together in a single polycistronic mRNA (5, 19).

GG uptake does not seem to be essential for the salt adaptation of *Synechocystis*, since cells of the *ggtA* mutant had a growth rate similar to that of the wild type at high NaCl concentrations. Both wild-type *Synechocystis* and the *ggtA* mutant are able to synthesize sufficient GG de novo to adapt to high salt concentrations (Table 1). Moreover, it is unlikely that the natural habitats of planktic cyanobacteria contain sufficient GG to contribute significantly by their uptake for salt adaptation. The GG transport system seems to be mainly responsible for minimizing losses of intracellular GG. Evidence for the leakage of GG from the cytoplasmic to the periplasmic space has been reported (25). We therefore checked the medium used to cultivate the salt-adapted wild-type and *ggtA* mutant cells for dissolved GG. No dissolved GG was detected in the cultures of wild-type cells, whereas the cells of the *ggtA* mutant had lost significant amounts of GG (Fig. 4). In the latter case, the quantity of GG found in the medium after 48 h of cultivation amounted to 10% of the total GG content of the culture. Therefore, in salt-adapted *Synechocystis* cells, GG transport seems to be necessary for the recovery of GG lost by leakage through the cytoplasmic membrane. This strategy helps the cell to save carbon. A similar phenomenon has been reported for salt-grown *E. coli* cells, which accumulate trehalose in the absence of glycinebetaine. Trehalose leaking through the cytoplasmic membrane is cleaved by a trehalase specifically expressed in osmotically stressed cells, and the glu-

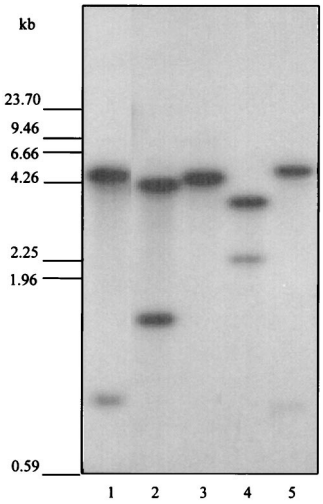

FIG. 3. Southern blot experiments for characterization of complete segregation of the mutants used the ^{32}P-labeled *Eco*RI fragment (Fig. 1) as a probe for the hybridization to *Eco*RI/*Cla*I-double-digested chromosomal DNA from the *orfI* mutant (lane 1), the *stpA* mutant (lane 2), the wild type (lane 3), the *orfII* (*ggtA*) mutant (lane 4), and the *orfIII* mutant (lane 5). The molecular masses of *Hin*dIII-digested λ DNA size standards were drawn by using the positions from the photo of the ethidium bromide-stained gel of the same magnitude.

cose reenters the cells through the phosphoenolpyruvate sugar phosphotransferase system (37). A glycinebetaine uptake system has also been found in cyanobacterial strains that are able to synthesize this compound (26). This glycinebetaine transporter should carry out the same function (32). In our experiments, CO_2-enriched medium was used to cultivate the wild type and the *ggtA* mutant, and cell growth was mainly limited by light. Under CO_2-limiting conditions, the lack of GG transport activity might lead to a lower salt resistance level or at least a reduced growth rate in the presence of high NaCl concentration.

Analysis of the salt-dependent expression of the *ggtA* gene. In the uptake experiments, only low GG transport activities were found in cells cultivated in the basal medium. Activities were clearly enhanced in salt-adapted cells. This increase could be caused by direct salt-induced activation of the transport system as has been found for GG-synthesizing enzymes (16) or by enhanced expression of the corresponding genes. The expression of *ggtA* was investigated by Northern blot experiments using RNA isolated from *Synechocystis* cells adapted to different salt concentrations and a probe specific to the *ggtA* gene (Fig. 1). In the Northern blots, a strong signal corresponding to a size of 1.2 kb was observed. This is about the size of the *ggtA* gene and indicates a monocistronic RNA, but the accumulation of a stable breakdown product of a larger mRNA cannot be ruled out (Fig. 5). A weaker signal of about 2.6 kb was sometimes visible and could also represent a transcript containing *stpA*. The possible occurrence of this bicistronic RNA might also be responsible for the slightly reduced GG transport activity in the *stpA* mutant, since the mutation might also reduce the amount of mRNA specific to *ggtA*. RNA from cells grown in basal medium gave only a weak signal, while the amount of *ggtA* transcript in salt-adapted cells increased about three- to fourfold (Fig. 5). This general increase in the amount of *ggtA* mRNA is in good agreement with the increase in GG transport activity and makes salt-regulated transcriptional control of the expression of this gene seem very likely. The amount of mRNA specific to *ggtA* was highest in cells adapted to 342 mM NaCl and lower in cells adapted to higher salt concentrations (Fig. 5C). In contrast to the bell-shaped curve describing mRNA synthesis, the increase in GG transport activity as the salt concentration increased was found to be linear (25). This discrepancy indicates posttranscriptional regulation of activity besides the general salt-induced activation of transcription for the GG transporter. Alternatively, expression of the currently unknown genes encoding the other subunits of the GG transporter might depend directly on the NaCl content and thus give rise to the observed linear increase of transporter activity.

Conclusions. *orfII* found downstream of *stpA* was designated *ggtA*. Three lines of evidence indicate that *ggtA* represents an ATP-binding protein involved in the GG transport system of *Synechocystis*: (i) the GgtA protein sequence shows striking similarities to sequences of other members of this protein

TABLE 1. Growth rates and GG accumulation in different insertion mutants of *Synechocystis* sp. strain PCC 6803 after cultivation in enhanced NaCl concentrations for 2 days

Strain	Growth rate (h^{-1})		GG content ($\mu g\ mm^{-3}$ [CV][a])	
	2 mM NaCl	684 mM NaCl	256 mM NaCl	513 mM NaCl
Wild type	0.0425	0.0309	33.3 (100)	100.4 (100)
orfI mutant	ND[b]	ND	23.6 (70.8)	90.7 (90.4)
stpA mutant	0.0405	No growth	4.6 (13.8)	14.8 (14.7)
ggtA mutant	0.0421	0.0328	35.6 (106.9)	89.9 (89.6)
orfIII mutant	ND	ND	38.0 (114.1)	100.5 (100.1)

[a] CV, total cell volume (product of average single-cell volume and cell number). Percentage of wild-type value is given in parentheses.
[b] ND, not determined.

TABLE 2. Uptake of ^{14}C-labeled GG by cells of the wild type and the *orfI*, *stpA*, *orfII* (*ggtA*), and *orfIII* mutants after cultivation in basal and NaCl-enriched media for 2 days

Strain	Mean GG uptake (pmol min^{-1} mm^{-3} [CV][a]) ± SD		
	2 mM NaCl	257 mM NaCl	513 mM NaCl
Wild type	42.8 ± 7.6	93.0 ± 3.3	137.3 ± 9.1
orfI mutant	40.8 ± 5.0	101.9 ± 3.6	130.7 ± 5.0
stpA mutant	50.3 ± 1.9	66.8 ± 1.8	90.5 ± 1.6
ggtA mutant	6.3 ± 2.0	1.3 ± 0.4	8.2 ± 1.9
orfIII mutant	59.5 ± 5.6	105.6 ± 11.1	134.5 ± 10.9

[a] CV, total cell volume.

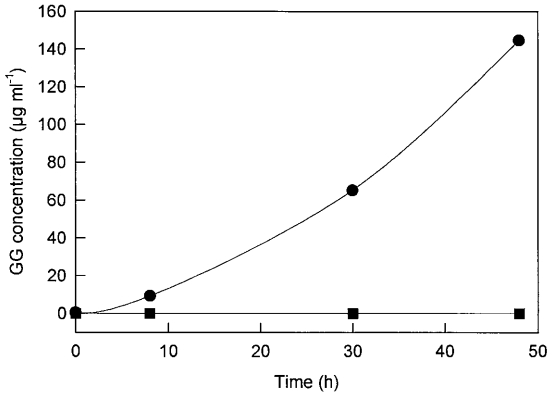

FIG. 4. Leakage of GG into the medium after cultivation of wild-type (■) and *orfII* (*ggtA*) mutant (●) cells adapted to 684 mM NaCl.

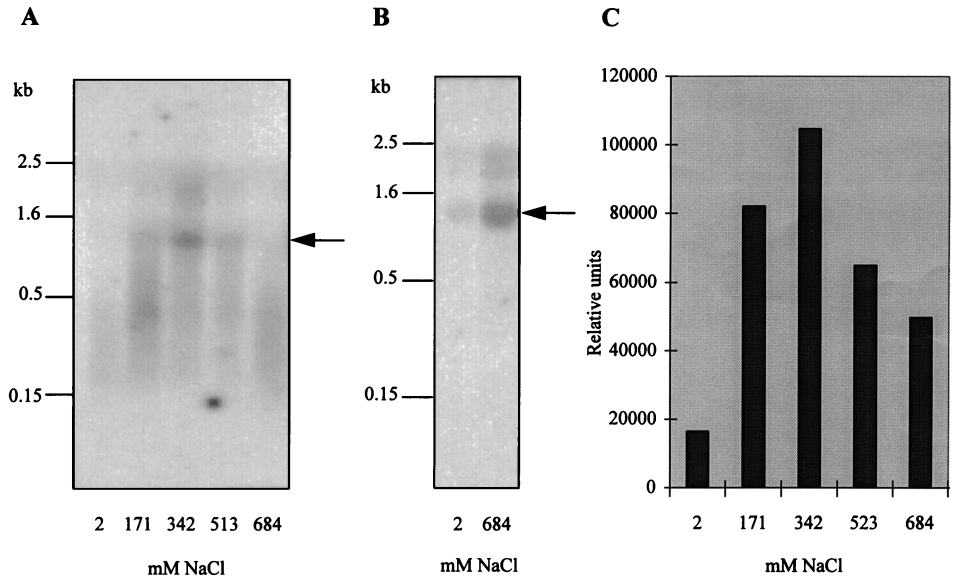

FIG. 5. Characterization of the salt-dependent expression of the *ggtA* gene in *Synechocystis*. RNA isolated from cells adapted to different NaCl concentrations was hybridized with the ^{32}P-labeled internal *Bal*I fragment of *orfII* (*ggtA*) (Fig. 1) as a probe. In panel A, 10 μg of total RNA was applied to each lane, and the filter was exposed for 4 days for autoradiography. In panel B, 20 μg of total RNA was applied to each lane, and the filter was exposed for 10 days for autoradiography. (C) Quantitative estimation of the radioactivity in the 1.2-kb transcript (arrows in panels A and B) by means of a PhosphorImager.

group; (ii) an insertion mutation of *ggtA* impaired GG uptake ability; and (iii) after cultivation of the *ggtA* mutant, significant amounts of GG were found in the medium. Furthermore, the physiological characteristics of the *ggtA* mutant indicate that the active transport of the osmoprotective compound GG is mediated by a binding-protein-dependent transport system in *Synechocystis*, the genes for which are not organized in an operon like those for other transporters, because mutation of the genes upstream and downstream of *ggtA* did not inhibit GG transport. The GG transporter is responsible for the recovery of GG leaking through the cytoplasmic membrane from high-salt-grown cells. Beside activation of transcription, other mechanisms seem to be involved in the increase of the GG transport activity in salt-adapted cells. Further experiments are planned to clone and characterize the other subunits (binding protein and permease) of the GG transport system of *Synechocystis*.

ACKNOWLEDGMENTS

We thank Arne Schoor for estimation of GG by HPLC. Excellent technical assistance by I. Dörr, B. Brzezinka, and A. Schmidt is thankfully acknowledged. The *stpA* mutant used in the experiments was kindly provided by F. Joset, CNRS, LCB, Marseille, France. Critical reading of the manuscript by E. Bremer, Phillips-Universität, Marburg, Germany, is acknowledged.

The work was supported by a grant of the Deutsche Forschungsgemeinschaft.

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