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The *N*-Acetylmuramic Acid 6-Phosphate Etherase Gene Promotes Growth and Cell Differentiation of Cyanobacteria under Light-Limiting Conditions▽†

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Inactivation of *sll0861* in *Synechocystis* sp. strain PCC 6803 or the homologous gene *alr2432* in *Anabaena* sp. strain PCC 7120 had no effect on the growth of these organisms at a light intensity of 30 μmol photons m^{-2} s^{-1} but reduced their growth at a light intensity of 5 or 10 μmol photons m^{-2} s^{-1}. In *Anabaena*, inactivation of the gene also significantly reduced the rate of heterocyst differentiation under low-light conditions. The predicted products of *sll0861* and *alr2432* and homologs of these genes showed similarity to *N*-acetylmuramic acid 6-phosphate etherase (MurQ), an enzyme involved in peptidoglycan recycling, in *Escherichia coli*. *E. coli murQ* and the cyanobacterial homologs could functionally substitute for each other. We hypothesize that *murQ* in cyanobacteria promotes low-light adaptation through reutilization of peptidoglycan degradation products.

Cyanobacteria are procaryotes that perform oxygenic photosynthesis and have a Gram-negative cell wall structure (7). They are found in oceans, bodies of freshwater, and the soil surface and contribute significantly to global primary productivity (33). In many environments, light often is a limiting factor for their growth.

The efficiency of light harvesting and the distribution of excitation energy in photosystems are important in low-light adaptation. In *Prochlorococcus marinus*, high- and low-light-adapted ecotypes differ in the number of *pcb* genes that encode light-harvesting antenna proteins (3, 11). In *Synechocystis* sp. PCC 6803, *rpaC*, a gene required for the transition state, can promote growth in white light at an intensity of 2 μmol photons m^{-2} s^{-1} (10, 22). On the other hand, reutilization of secreted substances or degradation products may promote growth under light-limiting conditions. For example, low-light conditions can stimulate the uptake of amino acids in the cyanobacterium *Planktothrix rubescens* (31).

Bacteria can break down peptidoglycan (PG) and reutilize the degradation products to synthesize new PG. This process is called PG recycling. In cyanobacteria and other Gram-negative bacteria, PG forms a continuous layer completely surrounding the cell between the cytoplasmic membrane and the outer membrane (12). The net-like layer consists of glycan strands cross-linked by short peptides with GlcNAc–anhydro-*N*-acetylmuramic acid (anhMurNAc)–L-Ala–D-Glu–*meso*-diaminopimelic acid–D-Ala as the repeating unit (23). In *Escherichia coli*, PG is degraded to GlcNAc-anhMurNAc-peptides or GlcNAc-anhMurNAc and peptides in the periplasmic space, and the GlcNAc-anhMurNAc-peptides and GlcNAc-anhMurNAc are then imported into the cytoplasm by the permease AmpG (13). GlcNAc-anhMurNAc-peptides are processed into GlcNAc-anhMurNAc and tripeptides by AmpD (anhydro-*N*-acetylmuramyl-L-Ala amidase) and LdcA (LD-carboxypeptidase) in the cytoplasm and reutilized (13, 26). PG accounts for about 2% of the cell mass in Gram-negative bacteria. The reutilization of PG degradation products may promote growth under nutrient-limiting conditions. However, so far, no experimental evidence directly supports this hypothesis. For example, inactivation of *ampG* or other genes involved in PG recycling apparently does not affect the normal growth rate of *E. coli* (8, 13, 14, 27, 30), except that it results in autolysis during the stationary growth phase in an *ldcA* mutant (26).

Cyanobacteria have a PG structure similar to that of Gram-negative bacteria, except for small differences, such as the thickness, degree of cross-linking, and covalent linkage of the polysaccharide (15, 16). In the present study, we found that a gene that is highly conserved in cyanobacteria has a function similar to that of *murQ*, a gene involved in reutilization of GlcNAc-anhMurNAc in *E. coli*. As shown in Fig. 1, GlcNAc-anhMurNAc is processed into GlcNAc and anhMurNAc by NagZ (β-*N*-acetylglucosaminidase) (8), and then GlcNAc is phosphorylated by NagK (GlcNAc kinase), producing GlcNAc-6-P (24), while anhMurNAc is phosphorylated by AnmK (anhMurNAc kinase), producing MurNAc-6-P (28), and is converted by MurQ (MurNAc-6-P etherase) into GlcNAc-6-P (14, 29). GlcNAc-6-P deacetylase (NagA) further converts GlcNAc-6-P to GlcN-6-P, which can be used in synthesis of new PG or enter carbohydrate metabolism (24). We show here that *murQ* and its homologs in cyanobacteria can promote growth under light-limiting conditions. Also, in a filamentous N_2-fixing cyanobacterium,

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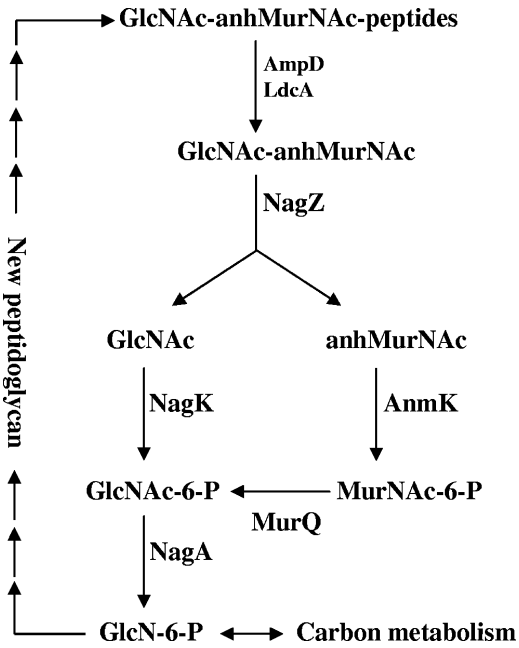

FIG. 1. Schematic diagram showing the PG recycling pathway described by Uehara et al. (29). anhMurNAC, anhydro-*N*-acetylmuramic acid; GlcN-6-P, glucosamine 6-phosphate; GlcNAc, *N*-acetylglucosamine; GlcNAc-6-P, *N*-acetylglucosamine 6-phosphate; MurNAC-6-P, *N*-acetylmuramic acid 6-phosphate.

Anabaena sp. strain PCC 7120, the *murQ* homolog enhances heterocyst differentiation at a low light intensity.

MATERIALS AND METHODS

Strains and culture conditions. *Synechocystis* sp. strain PCC 6803 was obtained from J. Zhao, Beijing University. *Anabaena* sp. strain PCC 7120 and *Microcystis aeruginosa* PCC 7806 were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences.

The cyanobacterial strains were grown as previously described (37, 38) using light intensities of 5, 10, and 30 μmol photons m^{-2} s^{-1}. For light-activated heterotrophic growth (LAHG), *Synechocystis* strains were cultured in BG11 medium with 5 mM glucose and exposed to light at an intensity of 5 μmol photons m^{-2} s^{-1} for 5 min per day but otherwise were kept in the dark. Specific growth rates (day^{-1}) were calculated as follows: $[(\log OD_{t2} - \log OD_{t1})/\log 2]/(t_2 - t_1)$, where OD_{t1} is the turbidity (optical density at 730 nm) after t_1 days and OD_{t2} is the turbidity (optical density at 730 nm) after t_2 days. For induction of heterocyst formation, *Anabaena* PCC 7120 grown in BG11 medium was collected, washed twice in $BG11_0$ medium (BG11 medium without $NaNO_3$), and incubated in the same medium with different light intensities for 24, 48, and 72 h. Heterocyst frequencies were calculated by determining the number of heterocysts in 1,000 cells. The turbidity of cyanobacteria and heterocyst frequencies were calculated from the results for 3 to 6 parallel cultures.

E. coli murQ mutant strain TJ2 (MC4100 *murQ*::Km^r) was a kind gift from Christoph Mayer, University of Konstanz (14). Mutant TJ2 and the complemented strains were cultivated in shaking flasks at 37°C in minimal medium A [10.5 g $liter^{-1}$ K_2HPO_4, 4.5 g $liter^{-1}$ KH_2PO_4, 1 g $liter^{-1}$ $(NH_4)_2SO_4$, 0.5 g $liter^{-1}$ sodium citrate, 0.1 g $liter^{-1}$ Mg_2SO_4] supplemented with 0.2% *N*-acetylmuramic acid (Sigma) as described by Jaeger et al. (14).

Plasmid construction. Molecular manipulations were performed by using standard methods. Tool enzymes were used according to the manufacturers' instructions. PCR fragments were cloned in the T-vector pMD18-T (Takara) and confirmed by sequencing. The primers used are listed in Table 1.

(i) Plasmids used for gene disruption. To inactivate *sll0861* or *sll0862* in *Synechocystis* PCC 6803, pHB187 carrying *sll0861*::C.K and pHB200 carrying *sll0862*::C.K were constructed. To inactivate *alr2432* in *Anabaena* PCC 7120, pHB322-2 carrying *alr2432*::C.K and *sacB* was constructed. C.K is a kanamycin resistance cassette (GenBank accession no. EU346690.1) (9).

(ii) Plasmids used for complementation. For *sll0861*, pHB2982 carrying P_{lacZ}-*sll0861* and pHB3908 carrying P_{lacZ}-*sll0861′* were constructed to complement *E. coli* TJ2, and pHB3055 carrying *sll0860-sll0861*-C.CE2 was constructed to complement the *Synechocystis sll0861*::C.K mutant. *sll0861′* was *sll0861* fused with an *E. coli* ribosome-binding site. C.CE2 is a chloramphenicol-erythromycin resistance cassette (9).

For *mlla-1*, the *sll0861* homolog in *Microcystis* PCC 7806 (NCBI GenBank accession no. AM778954.1), pHB2909b carrying P_{lacZ}-*mlla-1* was constructed to complement *E. coli* TJ2, pHB2949 carrying *omega*-$P_{7120rbcL}$-*mlla-1* was constructed to complement the *Anabaena alr2432*::C.K mutant, and pHB3054 carrying *omega*-$P_{6803rbcL}$-*mlla-1* was constructed to complement the *Synechocystis sll0861*::C.K mutant.

For *E. coli murQ*, pHB3157 carrying *omega*-$P_{6803rbcL}$-*murQ* was constructed to complement the *Synechocystis sll0861*::C.K mutant, and pHB3158 carrying *omega*-$P_{7120rbcL}$-*murQ* was constructed to complement the *Anabaena alr2432*::C.K mutant.

Details concerning construction of these plasmids are shown in Table 1.

Generation of cyanobacterial mutants. Transformation of *Synechocystis* PCC 6803 was performed as described by Williams (34). Conjugative transfer of plasmids into *Anabaena* PCC 7120 was performed as described by Elhai and Wolk (9). *Synechocystis* mutants were generated by transformation with corresponding plasmids. *Anabaena* mutants were generated using a two-step protocol involving *sacB*-based positive selection of double-crossover mutants (6). Complete segregation of mutants was confirmed by PCR. Details concerning mutant generation are shown in Table 1.

Western blot detection. *Synechocystis* PCC 6803 grown with different light intensities was collected by centrifugation at 6,000 rpm, resuspended in 40 mM Tris-Cl (pH 8.0) with 1 mM phenylmethylsulfonyl fluoride (PMSF), and ruptured by ultrasonication on ice. The cell debris and unbroken cells were removed by centrifugation at 6,000 rpm and 4°C for 10 min. The supernatant was then centrifuged at 30,000 rpm at 4°C for 30 min to separate membrane proteins from soluble proteins. Equal amounts of membrane and soluble proteins were loaded, separated by 12% SDS-PAGE, transferred to nitrocellulose filters (Millipore), detected with anti-Sll0861 rabbit antiserum, and visualized with goat anti-rabbit alkaline phosphatase antibody (Invitrogen) with nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolylphosphate (BCIP) (Amresco, United States) as the substrates. SDS-PAGE and Western blotting were performed using standard methods.

RESULTS AND DISCUSSION

Role of *sll0861* in low-light adaptation in *Synechocystis* PCC 6803. *Synechocystis* PCC 6803 can grow heterotrophically in the dark with daily brief exposure to weak light (1). This phenomenon is called light-activated heterotrophic growth (LAHG). In an effort to identify genes required for LAHG, we obtained *Synechocystis* mutants whose growth rates were significantly reduced under such conditions (18). In one of the mutants, *sll0861* was inactivated. We then generated mutants with a kanamycin resistance cassette (C.K) inserted into *sll0861* or the downstream gene *sll0862* (Table 1). The *sll0861*::C.K mutant showed greatly reduced growth under LAHG conditions, while the *sll0862*::C.K mutant had the wild-type phenotype (Table 2). The LAHG phenotype of the *sll0861* mutant was fully complemented by a DNA fragment containing the *sll0860-sll0861* sequence (Table 2). Because *sll0861* may be cotranscribed with *sll0860*, *sll0860* and the upstream sequence were included to promote expression of *sll0861* in the complementation experiment.

We wondered if *sll0861* affects photoautotrophic growth under low-light conditions. The *sll0861*::C.K mutant had a growth rate almost identical to that of the wild type at a light intensity of 30 μmol photons m^{-2} s^{-1} (Fig. 2A). However, when cultured with 5 μmol photons m^{-2} s^{-1}, the mutant showed slower growth than the wild type (Fig. 2B). When *sll0860-sll0861* was added to the mutant, the level of growth was restored to the wild-type level (Fig. 2B). These results suggest that *sll0861*

TABLE 1. Strains, plasmids, and primers

Strain, plasmid, or primer	Derivation and/or relevant characteristics[a]	Source or reference(s)
Synechocystis sp. strains		
PCC 6803	Wild type	J. Zhao
wt-1188	Km^r, Km^r cassette integrated into the neutral platform in *slr0168*	19, 34
DRHB199[b]	Km^r, *sll0861*::C.K, generated by transformation with pHB1099	This study
DRHB200	Km^r, *sll0862*::C.K, generated by transformation with pHB200	This study
DRHB199 DRHB3054	Km^r Sp^r, *omega*-$P_{6803rbcL}$-*mlla-1* integrated into the neutral platform in the genome of the *sll0861*::C.K mutant, generated by transformation of DRHB199 with pHB3054	
DRHB199 DRHB3055	Km^r Em^r, *sll0860-sll0861*-C.CE2 integrated into the neutral platform in the genome of the *sll0861*::C.K mutant, generated by transformation of DRHB199 with pHB3055	This study
DRHB199 DRHB3157	Km^r Sp^r, *omega*-$P_{6803rbcL}$-*murQ* integrated into the neutral platform in the genome of the *sll0861*::C.K mutant, generated by transformation of DRHB199 with pHB3157	This study
Anabaena sp. strains		
PCC 7120	Wild type	FACHB[c]
DRHB322-2	Nm^r, *alr2432*::C.K, generated by homologous double crossover of pHB322-2 with *Anabaena* chromosome	This study
DRHB322-2(pHB2949)	Nm^r Sp^r, pHB2949 carrying *omega*-$P_{7120rbcL}$-*mlla-1* introduced into the *alr2432*::C.K mutant	This study
DRHB322-2(pHB3158)	Nm^r Sp^r, pHB3158 carrying *omega*-$P_{7120rbcL}$-*murQ* introduced into the *alr2432*::C.K mutant	This study
Microcystis aeruginosa PCC 7806	Wild type	FACHB[c]
Escherichia coli TJ2	*murQ*::Km^r	14
Plasmids[d]		
pET21b	Ap^r, expression vector	Novagen
pHB187	Ap^r, PCR fragment bearing *sll0861-sll0862* (*Synechocystis* PCC 6803 chromosomal bp 1161737 to 1165229), generated with primers sll0861-F and sll0861-R, cloned into the EcoRI site of pRL500	This study
pHB199	Ap^r, C.K cloned into KpnI-cut and T4 DNA polymerase-blunted pHB187, with *sll0861* disrupted	This study
pHB200	Ap^r, C.K cloned into NheI-cut and T4 DNA polymerase-blunted pHB187, with *sll0862* disrupted	This study
pHB313	Ap^r, PCR fragment bearing *alr2432* (*Anabaena* PCC 7120 chromosomal bp 2923983 to 2925710), generated with primers alr2432-F and alr2432-R, cloned into pMD18-T	This study
pHB322-1	Km^r Ap^r, C.K inserted into HpaI-cut and T4 DNA polymerase-blunted pHB313, with *alr2432* disrupted	This study
pHB322-2	Cm^r Km^r, *alr2432*::C.K fragment excised with SphI and SalI from pHB322-1, cloned into SphI/SalI-cut pRL271	This study
pHB1347	Km^r Sp^r, plasmid containing *omega*-$P_{7120rbcL}$ in pHB912	32
pHB2739	Ap^r, PCR fragment containing $P_{6803rbcL}$ (*Synechocystis* PCC 6803 chromosomal bp 2477976 to 2478410), generated with primers 6803rbcL-F and 6803rbcL-R, cloned into pMD18-T	This study
pHB2759	Ap^r Sp^r, *omega* cassette excised with BamHI from pRL57, blunted with T4 DNA polymerase, cloned into SalI-cut and T4 DNA polymerase-blunted pHB2739	This study
pHB2909a	Ap^r, PCR fragment containing *mlla-1* (*Microcystis* PCC 7806 chromosomal bp 65241 to 66280), generated with primers 7806-F and 7806-R, cloned into pMD18-T, oriented against P_{lacZ}	This study
pHB2909b	Ap^r, PCR fragment containing *mlla-1* (*Microcystis* PCC 7806 chromosomal bp 65241 to 66280), generated with primers 7806-F and 7806-R, cloned into pMD18-T, oriented against like P_{lacZ}	This study
pHB2912	Ap^r Sp^r, *omega*-$P_{6803rbcL}$ excised with PstI/XbaI from pHB2759, blunted with T4 DNA polymerase, inserted into SalI-cut and T4 DNA polymerase-blunted pHB2909a, oriented like *mlla-1*	This study
pHB2913	Ap^r Sp^r, *omega*-$P_{7120rbcL}$ excised with NheI from pHB1347, blunted with T4 DNA polymerase, inserted into SalI-cut and T4 DNA polymerase-blunted pHB2909a, oriented like *mlla-1*	This study
pHB2949	Ap^r Sp^r Km^r, *omega*-$P_{7120rbcL}$-*mlla-1* excised with PvuII from pHB2913, inserted into EcoRI-cut and T4 DNA polymerase-blunted pRL25C	This study
pHB2982	Ap^r, PCR fragment containing *sll0861* (*Synechocystis* PCC 6803 chromosomal bp 1163322 to 1164363), generated with primers 6803sll0861-F and 6803sll0861-R, cloned into pMD18-T, oriented like P_{lacZ}	This study

Continued on following page

TABLE 1—*Continued*

Strain, plasmid, or primer	Derivation and/or relevant characteristics[a]	Source or reference(s)
pHB3012	Ap^r, PCR fragment bearing *sll0860-sll0861* (*Synechocystis* PCC 6803 chromosomal bp 1163322 to 1165090), generated with primers 6803sll0860-F and 6803sll0861-R, cloned into pMD18-T	This study
pHB3032	Ap^r Cm^r, C.CE2 excised with SalI from pRL598, blunted with T4 DNA polymerase, inserted into BamHI-cut and T4 DNA polymerase-blunted pHB3012	This study
pHB3054	Ap^r Sp^r, *omega*-$P_{6803rbcL}$-*mlla-1* excised with PvuII from pHB2912, cloned into EcoRI-cut and T4 DNA polymerase-blunted pKW1188, replacing the Km^r fragment	This study
pHB3055	Ap^r Cm^r, *sll0860-sll0861*-C.CE2 excised with SacI/PstI from pHB3032, cloned into EcoRI-cut and T4 DNA polymerase-blunted pKW1188, replacing the Km^r fragment	This study
pHB3146	Ap^r, PCR fragment bearing *E. coli murQ*, generated by PCR with primers murQ-F and murQ-R, cloned into pMD18-T	This study
pHB3147	Ap^r Sp^r, *omega*-$P_{6803rbcL}$ excised with PstI/XbaI from pHB2759, blunted with T4 DNA polymerase, inserted into SalI-cut and T4 DNA polymerase-blunted pHB3146, oriented like *murQ*	This study
pHB3148	Ap^r Sp^r, *omega*-$P_{7120rbcL}$ excised with NheI from pHB1347, blunted with T4 DNA polymerase, inserted into SalI-cut and T4 DNA polymerase-blunted pHB3146, oriented like *murQ*	This study
pHB3157	Ap^r Sp^r, *omega*-$P_{6803rbcL}$-*murQ* excised with PvuII from pHB3147, cloned into EcoRI-cut and T4 DNA polymerase-blunted pKW1188, replacing the Km^r fragment	This study
pHB3158	Ap^r Sp^r Km^r, *omega*-$P_{7120rbcL}$-*murQ* excised with PvuII from pHB3148, inserted into EcoRI-cut and T4 DNA polymerase-blunted pRL25C	This study
pHB3908	Ap^r, fragment bearing an *E. coli* ribosome-binding site and partial *sll0861* sequence was excised from pHB1330 with BglII/BalI and used to replace the BalI/BamHI fragment in pHB2982, resulting in a clone with *sll0861* expressed based on P_{lacZ} and *E. coli* ribosome-binding site (designated *sll0861′*)	This study
pKW1188	Ap^r Km^r, plasmid bearing a neutral integrative platform for *Synechocystis* PCC 6803	34
pMD18-T	Ap^r, T-cloning vector	Takara, Dalian, People's Republic of China
pRL25C	Km^r, pDU1-based *E. coli-Anabaena* shuttle vector	36
pRL57	Sp^r, cloning vector with the spectinomycin resistance cassette *omega*	4
pRL271	Cm^r, *sacB*-bearing positive selection vector	4
pRL446	Km^r, cloning vector with the C.K cassette	9; GenBank accession no EU346690.1
pRL500	Ap^r, positive-selection vector	9
pRL598	Cm^r Em^r, cloning vector with the C.CE2 cassette	9
Primers (5′–3′)		
6803rbcL-F	CCGATGAAGTGGTGGAGCA	
6803rbcL-R	GGTCAGTCCTCCATAAACATTG	
6803sll0860-F	CTAGTTCATTTCTCCACCGG	
6803sll0861-F	CCCGATCATCTTTCTCCCATCC	
6803sll0861-R	TGGCGAAGACAATGGGGACT	
7806-F	TCTTACGAAAAGCTCAATTAAACCG	
7806-R	AAGATAGCACCAGCGACGAGG	
alr2432-F	TCCCAAGATGATGCTGTCCGT	
alr2432-R	CCGACAACCGTAGGAGGTAA	
murQ-F	CGTAAATAGTAAGGTCACCACCG	
murQ-R	CGACACGGGTAAGAATGGTG	
sll0861-F	TTGAATTCCACTGTCCAACGACCATAGAC	
sll0861-R	ACGAATTCAAGATACGGAAGTAGTGCTG	

[a] Ap, ampicillin; Cm, chloramphenicol; Em, erythromycin; Km, kanamycin; Nm, neomycin; Sp, spectinomycin.
[b] DRHB indicates a product of double homologous recombination between a pHB plasmid and the *Synechocystis* sp. or *Anabaena* sp. genome.
[c] FACHB, Freshwater Algal Culture Collection of the Institute of Hydrobiology.
[d] The templates used for PCRs were genomic DNA of *Anabaena* PCC 7120, *E. coli* DH5α, and *Synechocystis* PCC 6803; PCR clones were confirmed by sequencing.

probably plays a role in low-light adaptation in *Synechocystis* PCC 6803.

Using Western blot analysis, we found similar levels of Sll0861 in *Synechocystis* PCC 6803 cultures exposed to light at intensities of 30, 5, and 0 μmol photons m^{-2} s^{-1} (see Fig. S1 in the supplemental material). Unlike Sll0886 (21), another protein involved in LAHG, Sll0861 was not associated with membranes.

***sll0861* homologs in *Anabaena* and *Microcystis*.** Homologs of *sll0861* have been found in most cyanobacterial species (see

TABLE 2. Specific growth rates of *Synechocystis* PCC 6803 and mutants of this strain cultured under LAHG conditions

Strain	Specific growth rate (day^{-1})[a]
Wild type	0.435 ± 0.014
sll0861::C.K mutant	0.166 ± 0.007
sll0862::C.K mutant	0.441 ± 0.024
sll0861::C.K mutant complemented with:	
sll0860-sll0861	0.458 ± 0.023
mlla-1	0.447 ± 0.028
E. coli murQ	0.415 ± 0.006

[a] The values are means ± standard deviations and were calculated using the results for three parallel cultures.

TABLE 3. Heterocyst frequencies of *Anabaena* PCC 7120 and mutants of this strain with 5 μmol photons m^{-2} s^{-1}

Time after nitrogen stepdown (h)	Heterocyst frequency (‰)[a]			
	Wild type	*alr2432*::C.K mutant	*alr2432*::C.K mutant complemented with:	
			mlla-1	*E. coli murQ*
24	47.7 ± 11.3	6.0 ± 0.7	54.3 ± 2.8	35.9 ± 6.2
48	65.2 ± 10.0	25.9 ± 6.7	69.0 ± 4.8	52.1 ± 5.2
72	74.5 ± 15.9	46.0 ± 17.2	74.0 ± 6.4	62.2 ± 4.9

[a] The heterocyst frequency was calculated by determining the number of heterocysts in 1,000 cells. The values (means ± standard deviations) were calculated by using the results for 6 cultures (2 × 3) of the wild-type or *alr2432*::C.K strain or for 3 cultures of each complemented strain.

Fig. S2 in the supplemental material). We used the homologous genes of the filamentous N_2-fixing cyanobacterium *Anabaena* PCC 7120 and the bloom-forming cyanobacterium *Microcystis* PCC 7806 to investigate the roles of these genes in low-light adaptation. Unlike *Synechocystis* PCC 6803, *Anabaena* PCC 7120 and *Microcystis* PCC 7806 are not able to grow heterotrophically. *Anabaena* PCC 7120 can produce specialized cells, which are called heterocysts, upon nitrogen stepdown to fix dinitrogen (35).

We inactivated the homologous *alr2432* gene in *Anabaena* PCC 7120 by inserting the C.K cassette. The *alr2432*::C.K mutant grew like the wild type when it was cultured with 30 μmol photons m^{-2} s^{-1} (Fig. 2C), but with 10 μmol photons m^{-2} s^{-} its growth was slower than that of the wild type (Fig. 2D). Because illumination is required for initiation of heterocyst differentiation in *Anabaena* (5), we used induction of heterocyst differentiation as an alternative criterion to evaluate the role of *alr2432* in low-light adaptation. With 5 μmol photons m^{-2} s^{-1}, heterocyst differentiation was significantly slower in the mutant (Table 3). Apparently, *alr2432* plays a role in low-light adaptation similar to that of *sll0861*.

We attempted to inactivate the homologous gene in *Microcystis* PCC 7806 but obtained no transformant. We then used the homologous gene from *Microcystis* PCC 7806, *mlla-1* (NCBI GenBank accession no. AM778954.1), to complement the *Synechocystis* PCC 6803 *sll0861*::C.K and *Anabaena* PCC 7120 *alr2432*::C.K mutants. *mlla-1* was expressed from P_{rbcL} (the promoter of the ribulose 1,5-bisphosphate carboxylase/oxygenase [Rubisco] large subunit-encoding gene) of *Synechocystis* PCC 6803 or *Anabaena* PCC 7120. As shown in Fig. 2B, expression of *mlla-1* in the *Synechocystis* mutant fully restored growth with 5 μmol photons m^{-2} s^{-1}. Also, the levels of growth and heterocyst differentiation of the *Anabaena* mutant

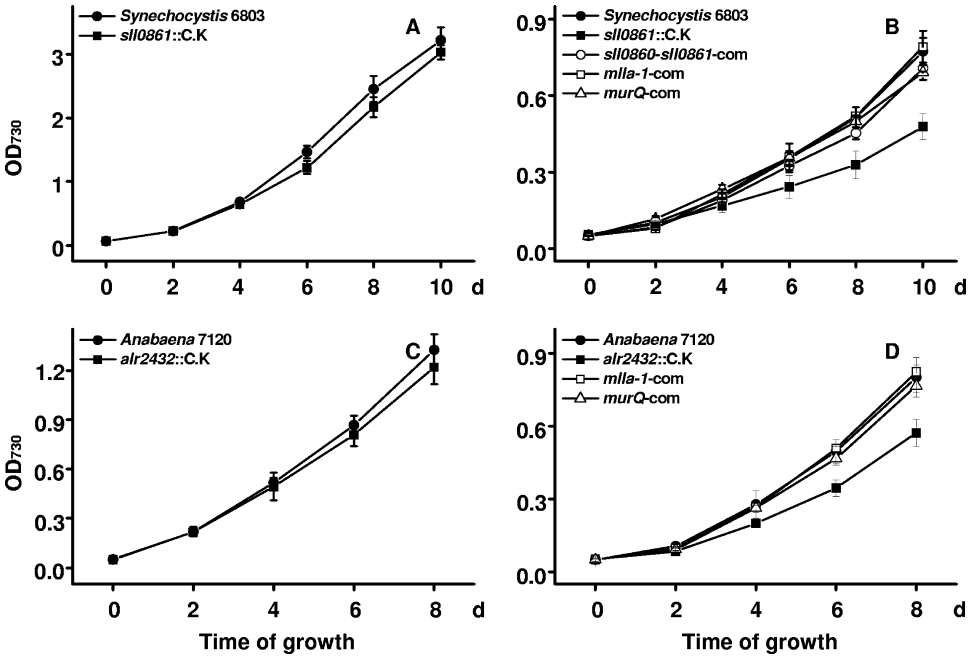

FIG. 2. Autotrophic growth of the *Synechocystis* PCC 6803 (A and B) and *Anabaena* PCC 7120 (C and D) strains with light intensities of 30 μmol photons m^{-2} s^{-1} (A and C), 5 μmol photons m^{-2} s^{-1} (B), and 10 μmol photons m^{-2} s^{-1} (D). Cells were cultured in BG11 medium at 30°C, and the growth was determined by monitoring the optical density at 730 nm (OD_{730}). *sll0860-sll0861*-com, complemented with *sll0860-sll0861*; *mlla-1*-com, complemented with *omega*-$P_{6803rbcL}$-*mlla-1* or *omega*-$P_{7120rbcL}$-*mlla-1*; *murQ*-com, complemented with *omega*-$P_{6803rbcL}$-*murQ* or *omega*-$P_{7120rbcL}$-*murQ*.

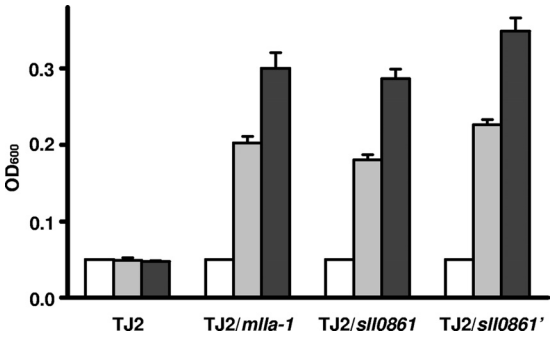

FIG. 3. Growth of *E. coli murQ* mutant TJ2 and this mutant containing pHB2982 (TJ2/*sll0861*), pHB2993 (TJ2/*mlla-1*), or pHB3908 (TJ2/*sll0861'*) in minimal medium A supplemented with MurNAc. Growth in liquid medium at 37°C was monitored by determining the turbidity (optical density at 600 nm [OD_{600}]) at zero time (open bars), 48 h (light gray bars), and 96 h (dark gray bars).

under low-light conditions were restored to the wild-type levels by complementation with *mlla-1* (Fig. 2D and Table 3). Based on these results, we concluded that *sll0861* in *Synechocystis* and homologs of this gene in *Anabaena* and *Microcystis* should have the same function in low-light adaptation.

***sll0861* and homologs of this gene function like *murQ* (*N*-acetylmuramic acid 6-phosphate etherase) genes.** *sll0861* is predicted to encode a sugar isomerase (SIS) domain (2) protein with similarity to the glucokinase regulatory protein (GKRP) in humans (E value, 4×10^{-10}). Also, this protein is similar to the *N*-acetylmuramic acid 6-phosphate etherase (MurQ) in *E. coli* (E value, 3×10^{-65}). We first compared the glucose kinase activities in the mutant and wild type and found no difference. Thus, we tested if *sll0861* and its homologs can complement *E. coli murQ*::Km^r mutant TJ2. Unlike the wild type, the TJ2 mutant could not grow on *N*-acetylmuramic acid (MurNAc) as the sole source of carbon and energy (14). We expressed *sll0861* and *mlla-1* in *E. coli* TJ2 from the *lacZ* promoter. In one of the constructs, pHB3908 (Table 1), a typical *E. coli* ribosome-binding site (RBS) was used to promote translation of *sll0861* (designated *sll0861'*). *sll0861*, *sll0861'*, and *mlla-1* all enabled *E. coli* TJ2 to grow in liquid medium with MurNAc as the sole carbon source (Fig. 3). The use of an *E. coli* ribosome-binding site did not have an apparent effect on growth. Tests on plates also clearly showed that there was complementation of TJ2 by the cyanobacterial genes (data not shown).

On the other hand, we complemented the cyanobacterial mutants with *murQ* from *E. coli*. *murQ* was expressed from the *rbcL* promoter of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120 in the *sll0861*::C.K and *alr2432*::C.K mutants, respectively. The *E. coli murQ* gene restored the autotrophic growth of both mutants to wild-type levels (Fig. 2B and D) under low-light conditions. Also, it restored heterocyst differentiation (Table 3) in the *Anabaena* PCC 7120 *alr2432*::C.K mutant at an intensity of 5 μmol photons m^{-2} s^{-1} and LAHG (Table 2) of *Synechocystis* PCC 6803 *sll0861*::C.K. From the results described above, we concluded that *sll0861* and its homologs have a function similar to that of *murQ* in *E. coli* and that the encoded *N*-acetylmuramic acid 6-phosphate etherase is involved in PG recycling in cyanobacteria.

Predicted MurQ and other enzymes involved in PG recycling in *Prochlorococcus* ecotypes. Kettler et al. (17) classified 12 strains of *P. marinus* in six high-light-adapted ecotypes (MED4, MIT 9215, MIT 9301, MIT 9312, MIT 9515, and AS9601) and six low-light-adapted ecotypes (MIT 9303, MIT 9313, MIT 9211, SS120, NATL2A, and NATL1A). Homologs of *murQ* were found in the six low-light-adapted ecotypes but in none of the high-light-adapted ecotypes (Table 4).

Consistently, there are no homologs of *nagZ*, *nagK*, *anmK*, and *nagA*, the other four genes involved in reutilization of PG amino sugars, in the six high-light-adapted ecotypes (Table 4). There are homologs of *nagZ*, *anmK*, and *nagA* in all six low-light-adapted ecotypes, while there are *nagK* homologs only in two of the low-light-adapted ecotypes, MIT 9303 and MIT 9313 (Table 4). The other four low-light-adapted ecotypes may reutilize MurNAc but not GlcNAc.

Hypothesis that MurQ in cyanobacteria may promote low-light adaptation through peptidoglycan recycling. Based on our experimental investigation of *murQ* from three cyanobacterial species and the bioinformatics analysis of light-adapted ecotypes of *P. marinus*, we propose that MurQ promotes low-light adaptation in cyanobacteria. However, we showed that the *sll0861*::C.K mutant of *Synechocystis* PCC 6803 did not differ in oxygen evolution or the transition state from the wild type (unpublished data); therefore, the role of *murQ* in low-light adaptation is different from that of *rpaC* (10, 22).

We hypothesize that the effect of MurQ on low-light adaptation is based on its role in PG recycling. PG is the major material in the Gram-negative cell wall, and it accounts for about 2% of the cell mass. The reutilization of PG degradation products should reduce the loss of fixed carbon in cyanobacteria. When there is sufficient light, the contribution of PG recycling to the increase in biomass can be neglected. However, at a low light intensity, this energy-saving strategy could have an effect on promoting cell propagation. In *Anabaena*, illumination in first several hours after nitrogen stepdown is required for initiation of heterocyst differentiation (5). No molecular mechanism for the role of illumination in heterocyst differentiation has been reported yet, but it is conceivable that photosynthesis can promote cell division and accumulation of

TABLE 4. Predicted genes involved in recycling of PG amino sugars in light-adapted ecotypes of *P. marinus*[a]

Strain	Ecotype[b]	Homolog of[c]:				
		nagZ	*nagK*	*anmK*	*murQ*	*nagA*
AS9601	HL	−	−	−	−	−
MED4	HL	−	−	−	−	−
MIT9215	HL	−	−	−	−	−
MIT9301	HL	−	−	−	−	−
MIT9312	HL	−	−	−	−	−
MIT9515	HL	−	−	−	−	−
MIT9211	LL	+	−	+	+	+
MIT9303	LL	+	+	+	+	+
MIT9313	LL	+	+	+	+	+
NATL1A	LL	+	−	+	+	+
NATL2A	LL	+	−	+	+	+
SS120	LL	+	−	+	+	+

[a] The genome information was obtained from the study of Kettler et al. (17).
[b] HL, high-light-adapted ecotype; LL, low-light-adapted ecotype.
[c] +, yes; −, no.

2-oxoglutarate, both of which are required for initiation of heterocyst differentiation (20, 25). Under light-limiting conditions, PG recycling may affect these processes by increasing the pool of carbon metabolites. Alternatively, MurQ may exert its effects on growth or cell differentiation by reducing the accumulation of MurNAc-6-P or upstream metabolites (Fig. 1). In the future, inactivation of other genes involved in PG recycling and analyses of metabolites in *Synechocystis* PCC 6803 and *Anabaena* PCC 7120 should provide further evidence to clarify these possibilities.

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