

A Two-component Signal Transduction Pathway Regulates Manganese Homeostasis in *Synechocystis* 6803, a Photosynthetic Organism*

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Elemental manganese is essential for the production of molecular oxygen by cyanobacteria, plants, and algae. In the cyanobacterium *Synechocystis* sp. PCC 6803, transcription of the *mntCAB* operon, encoding a high affinity Mn transporter, occurs under Mn starvation (nM Mn) conditions but not in Mn-sufficient (μ M Mn) growth medium. Using a strain in which the promoter of this operon directs the transcription of the *luxAB* reporter genes, we determined that inactivation of the *slr0640* gene, which encodes a histidine kinase sensor protein component of a two-component signal transduction system, resulted in constitutive high levels of *lux* luminescence. Systematic targeted inactivation mutagenesis also identified *slr1837* as the gene encoding the corresponding response regulator protein. We have named these two genes *manS* (manganese-sensor) and *manR* (manganese-regulator), respectively. A polyhistidine-tagged form of the ManS protein was localized in the *Synechocystis* 6803 cell membrane. Directed replacement of the conserved catalytic His-205 residue of this protein by Leu abolished its activity, although the mutated protein was present in cyanobacterial membrane. This mutant also showed suboptimal rates of Mn uptake under either Mn-starved or Mn-sufficient growth condition. These data suggest that the ManS/ManR two-component system plays a central role in the homeostasis of manganese in *Synechocystis* 6803 cells.

Manganese is an essential transition metal in almost all organisms. It plays a critical role for the photoautotrophic life style of cyanobacteria, algae, and plants. During oxygenic photosynthesis, a cluster of four Mn atoms in the photosystem II complex in thylakoid membranes catalyzes photolysis of water to produce molecular oxygen (1). Despite this importance of Mn in the biosphere, the regulatory details of cellular Mn homeostasis remain poorly understood (2, 3).

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We have identified previously MntABC, an ABC-type permease that mediates high affinity Mn transport in the cyanobacterium *Synechocystis* sp. PCC 6803 (4, 5). The protein components of this transporter are encoded by three neighboring genes, *mntA*, *mntB*, and *mntC*, organized in an operon *mntCAB*. This transporter functions when *Synechocystis* 6803 cells are grown under Mn starvation conditions. A second high affinity Mn transporter functions in *Synechocystis* 6803 cells grown under Mn-sufficient conditions (5). Furthermore, a low affinity Mn transporter also operates in these cells. It is evident that Mn uptake in these cyanobacterial cells is controlled via a carefully modulated regulatory process.

The maintenance of homeostasis in a cell often requires complex sets of components that regulate the balance of metabolism. Such a regulatory cascade of events is initiated with the perception of the status of the environment with regard to specific metabolites or nutrients. Almost all cells utilize signaling cascades to respond to both positive and negative environmental stimuli. During recent years, two-component signal transduction has been recognized as a widely used strategy by which cells adapt and respond to their environment (6–10). This means of sensing the environment is utilized by bacteria, as well as plants, and involves at least two separate protein components. At the beginning of the signal transduction chain is a protein containing a sensor domain that is typically a histidine kinase with a His residue that is essential to a phosphorylation cascade. The second component is the response regulator that contains a receiver regulatory domain with a critical aspartic acid residue, an acceptor of the phosphate group from the His group in the histidine kinase. Often, a second domain of the response regulator protein binds directly to DNA and interacts with the transcription machinery to regulate the expression of a set of genes (7–9). Recent analysis has shown that among the various bacterial species with completely sequenced genomes, cyanobacteria have the largest numbers of two-component sensor regulator pairs (10, 11). In *Synechocystis* 6803 there are 43 proteins containing the canonical histidine kinase sensor domains and 40 proteins containing the response regulator signature (11). In the recently sequenced genome of the filamentous N_2 -fixing cyanobacterium *Anabaena* sp. PCC 7120, 195 genes encode components of such two-component signal transduction systems (12). To date, functional roles have been determined for only a limited number of such proteins in cyanobacteria. These include two-component systems for responses to extreme environmental conditions such as general nutrient limitation and high light stress (13), phosphate limitation (14), and cold stress (15, 16). One of the first two-component sensor-regulator pairs to be identified in *Synechocystis* 6803 was the Cph1/Rcp1 proteins that are in-

volved in light-regulation (17, 18). The Cph1 protein was identified originally as a homolog of phytochrome (19). Recently, a two-component pair has been described that is involved in regulating the stoichiometry between photosystem I and photosystem II complexes by sensing changes in the redox state of the plastoquinone pool in *Synechocystis* 6803 membranes (20).

To understand the mechanism by which cyanobacteria sense the status of the available pool of manganese, we have engineered a reporter strain in which the expression of bacterial luciferase genes is controlled by Mn concentration in the growth medium. Using this strain, we have discovered a two-component sensor protein, ManS, which is involved in the sensing of Mn in *Synechocystis* 6803. We have also identified ManR, the cognate response regulator protein. We discuss the critically important role of the ManS/ManR system for the regulation of the overall Mn homeostasis in these cyanobacterial cells.

MATERIALS AND METHODS

Bacterial Growth Conditions—Wild-type and mutant cells of *Synechocystis* 6803 were grown at 30 °C in BG11 medium (21), buffered at pH 8.0, and bubbled with air. Solid medium for cyanobacterial growth was BG11, supplemented with 1.5% agar and 5 mM sodium thiosulfate. Continuous illumination was provided by fluorescent lamps at 50 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. For Mn-uptake assays, *Synechocystis* 6803 cells were conditioned in Mn-free liquid BG11 medium, in which ferric ammonium citrate was replaced by ferric nitrate, and Mn was omitted from the trace element components. To starve *Synechocystis* 6803 for Mn, cells were grown in liquid BG11, washed, and then grown in Mn-free BG11 for 36 h prior to the uptake assays.

Escherichia coli cells for the propagation of plasmids and manipulation of DNA were grown in Luria Broth medium. Plasmids were propagated in *E. coli* strain DH5 α . Procedures for the growth of *E. coli* strains and for the manipulation of DNA were as described (22).

Construction of a pmntlux Reporter Strain and Isolation of Mutants—The construction and chromosomal insertion of the *luxAB* reporter fusion gene were as described previously (23). Specifically, a DNA fragment corresponding to the -6 to -139 nucleotides upstream of the translation initiation codon of the *mntC* gene (4), representing the promoter region (*pmnt*) of the *mntCAB* operon of *Synechocystis* 6803, was fused to the promoterless bacterial *luxAB* genes that encode the Lux reporter protein (see Fig. 1). This reporter construct was inserted at a site downstream of the *ndhB* gene in *Synechocystis* 6803, and the resulting strain (*pmntlux*) was used in the screening procedure outlined below.

A genomic priming system (New England Biolabs) was used to mobilize a transposon containing a chloramphenicol-resistance (Cm^{R}) gene for random insertion into the DNA of 110 different cosmids, which contained chromosomal fragments of *Synechocystis* 6803 used previously for genome sequencing (24). The *pmntlux* strain of *Synechocystis* 6803 was transformed with this transposon inactivation library, and Cm^{R} mutants that constitutively expressed the *pmnt::luxAB* reporter gene were isolated. For this purpose, the *lux* luminescence from Cm^{R} colonies was assayed using a VIM camera system (model C-1400-47; Hamamatsu Photonics Co., Hamamatsu, Japan) and processed on an Argus 50 image analyzer (Hamamatsu Photonics Co.). Genomic DNA isolated from each mutant strain was digested with *Hha*I, and after self-ligation it was used as the template for inverse PCR with outward primers corresponding to the 5'- and 3'-terminal regions of the Cm^{R} cassette. The exact positions of the cassette in the mutant genomes were determined by sequencing the respective PCR products (see Fig. 2). To identify the corresponding regulator protein, four response regulator genes that are not localized in the 110 cosmids mentioned above were inactivated individually in the *pmntlux* reporter strain by inserting a kanamycin-resistance (Km^{R}) cassette in the respective coding regions, essentially as described previously (24).

Modification of the Histidine Kinase Gene *manS*—The coding region of the *manS* gene was fused translationally to a DNA segment encoding a hexahistidyl domain followed by a c-Myc epitope (see Fig. 3) in the pTYE007 plasmid (25). In addition, the Km^{R} cassette and a 500-bp DNA fragment downstream of the *manS* gene were inserted distal to the *c-myc* gene. This construct (pTYE007-*manS*) was used to modify the *manS* gene. First, a previously described PCR-based method (26) was used to change the nucleotide A604 in the *manS* gene to a T, resulting in the replacement of His-205 in the ManS protein to a leucine residue.

Second, a periplasmic loop region (amino acids 60 to 132) of the ManS protein was removed by deleting a *Stu*I/*Eco*RI DNA fragment from pTYE007-*manS*. These constructs were then used to transform the *pmntlux* reporter strain to produce two Km^{R} mutant strains, *pmntlux-manS* Δ 60-132 and *pmntlux-manSH205L*, respectively.

SDS-PAGE and Western Blot Analysis of ManS Protein—Membranes from *Synechocystis* 6803 cells were prepared as described earlier (27). SDS-PAGE was performed as described in Ref. 28. Polypeptides were electrotransferred to nitrocellulose membranes, which were then incubated with anti c-Myc antibodies (Santa Cruz Biotechnology). Goat anti-rabbit IgG conjugated to peroxidase was used to amplify the signals, and the reacting bands were visualized using enhanced chemiluminescence reagents (Amersham Biosciences).

Kinetics of Luminescence from Cells Containing the *pmnt::luxAB* Reporter Gene—The strains containing *pmnt::luxAB* as a reporter gene were grown in BG11. Before and after exposure to Mn-deficient medium the cells were adjusted to a density of $\text{OD}_{750} = 0.1$ or 0.01. A 300- μl aliquot of the cell suspension was placed in the reaction tube in a Lumi-counter (model 2500; Microtech-Nichion, Chiba, Japan). The luminescence intensity was recorded in the absence of decanal for 1 min after which 10 μl of 5% decanal was added. The luminescence intensity rose rapidly to attain the maximum within 10 s and then declined gradually. The maximum luminescence intensity was taken as a measure of the expression level of the *pmnt::luxAB* reporter gene.

Manganese Uptake Assays— Mn^{2+} uptake assays were performed essentially as described earlier (5). *Synechocystis* 6803 cells were washed and resuspended in Mn-free BG11. After the addition of radioactive ^{54}Mn , 100- μl samples were collected at specific time points and quickly dispersed in 5 ml of BG11 containing 10 mM cold Mn. The samples were collected on nitrocellulose membrane filters (BA83; Schleicher & Schuell) by vacuum filtration, and the filters were immersed in scintillation fluid and counted on an LS 5000 TD scintillation counter (Beckman Instruments).

RT-PCR Analysis of Expression of Metal Transporter Genes—The relative amounts of transcripts from various genes were evaluated by the RT-PCR method (29). Total RNA from *Synechocystis* 6803 cells cultured in normal or Mn-free BG11 medium was extracted according to Ref. 30, treated with RNase-free DNase I (Roche Molecular Biochemicals), and then purified by phenol-chloroform extraction and ethanol precipitation. A reverse transcription reaction was performed using Superscript II enzyme (Invitrogen). The products were amplified by PCR and then analyzed by electrophoresis on 0.8% agarose gels. Primers were designed so that the amplified products would be internal to the coding region of each gene. All of the forward primers were designed for sequences downstream of the translation initiation codon, and the reverse primers were designed to obtain ~350-bp-long PCR products from each gene. The RNaseP gene was used as a control template with constitutive expression levels (31).

RESULTS

Isolation of Mutants with Unregulated Expression of the *pmnt::luxAB* Reporter Gene—The *mntCAB* operon encodes a high affinity ABC transporter protein complex found in *Synechocystis* 6803 cells grown under Mn starvation conditions (4, 5). Expression of this transporter is controlled by the concentration of available Mn. RT-PCR experiments have shown that when grown in the BG11 medium (containing 9 μM Mn), *Synechocystis* 6803 cells do not have any detectable level of the *mntCAB* transcript (data not shown). The presence of this transcript was, however, detected within 15 min of incubation of the same cells in Mn-deficient BG11 medium. The expression of these genes is evidently under tight transcriptional control.

To identify factors that mediate such Mn-mediated regulation, a *pmntlux* reporter strain was constructed (Fig. 1A; also see "Materials and Methods"). In this strain, the promoter of the *mntCAB* operon directs the transcription of the *luxAB* reporter genes. It is noteworthy that in this strain the endogenous *mntCAB* operon has not been modified. In these cells, the expression of the reporter gene and that of the *mntCAB* operon were similarly regulated by Mn (data not shown). This reporter strain was mutagenized randomly by transformation with a transposon inactivation library (see "Materials and Methods"), and the resultant Cm^{R} colonies were screened for mutants that exhibited expression of the reporter gene under Mn-sufficient

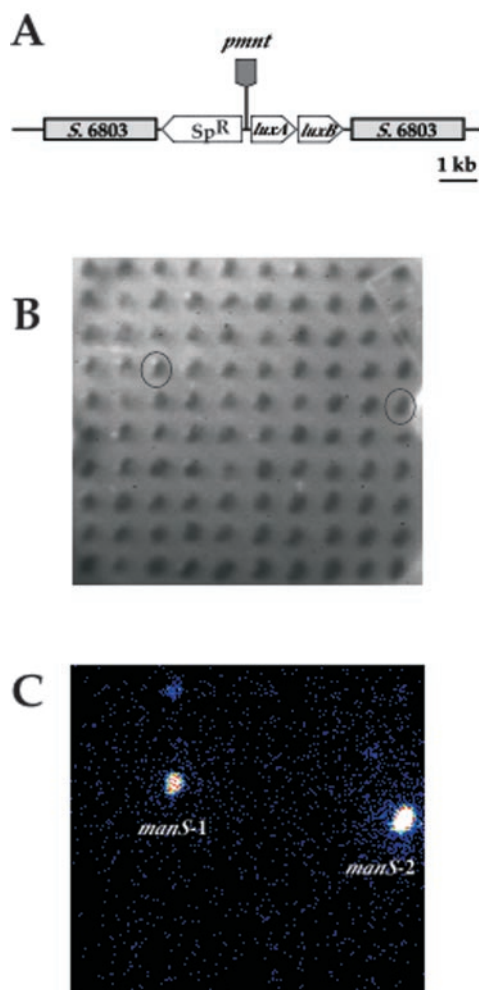


FIG. 1. A, a schematic map of the *pmnt* reporter construct. The shaded boxes represent flanking *Synechocystis* 6803 DNA sequences. *Sp^R*, spectinomycin/streptomycin resistance gene; *luxA* and *luxB*, promoterless *luxAB* reporter genes; *pmnt*, promoter region of the *pmntCAB* operon of *Synechocystis* 6803. See “Materials and Methods” for further details. B, a grid of Cm-resistant derivative clones of the *pmntlux* strain grown on solid BG11 medium. C, *lux* luminescence from the colonies shown in B. *manS-1* and *manS-2* are two independent clones (circled in B) exhibiting high levels of *lux* expression. See “Materials and Methods” for further details.

conditions (Fig. 1, B and C). Of nearly 20,000 Cm^R colonies, we identified two such colonies. They were called *manS-1* and *manS-2*, respectively.

Identification of a Genetic Locus That Regulates Expression of the *pmnt* Promoter—Analysis of the *manS-1* and *manS-2* strains showed that in both mutants, the Cm^R gene was inserted in the same open reading frame, *slr0640* (also termed *hik27*) (Cyanobase; www.kazusa.or.jp/cyano/cyano.html), although at two different positions (Fig. 2A). This gene encodes a histidine kinase sensor protein that belongs to a two-component signal transduction system in *Synechocystis* 6803 cells (11). Because this protein is involved in manganese sensing (see below), we have named it ManS, and we have named the corresponding gene *manS*. The ManS protein has 441 residues with a predicted molecular mass of 49.2 kDa. COG (www.ncbi.nlm.nih.gov/COG/) and SMART (smart.embl-heidelberg.de/) analysis indicated that ManS is a histidine kinase sensor protein (10), with two transmembrane domains, a HisKA domain that includes the conserved and catalytically important His-205 residue, a HAMP dimerization domain, and a HAT-Pase domain (Fig. 2B).

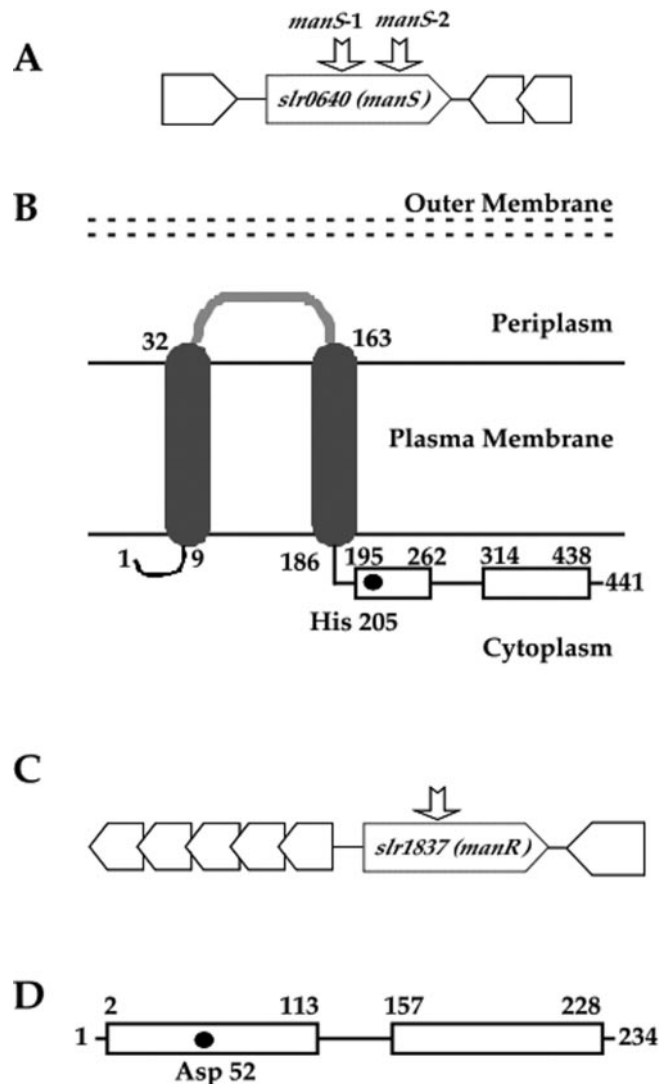


FIG. 2. A, a schematic map of the *slr0640* (*manS*) region of *Synechocystis* 6803 chromosome. The arrowheads indicate the sites of insertion of the Cm^R cassette in the *manS-1* and *manS-2* strains, respectively. B, a schematic depiction of various domains in the ManS protein in the plasma membrane of *Synechocystis* 6803 cells. The numbers correspond to amino acid residues in ManS. The open rectangles represent a HisKA domain (195 to 262) and a HATPase C domain (314 to 438), respectively. The black oval corresponds to the His-205 residue. See “Results” for further details. C, a schematic map of the *slr1837* (*manR*) region of *Synechocystis* 6803 chromosome. The arrowhead indicates the site of insertion of the Km^R cassette in the *manR* gene. D, a schematic depiction of various domains in the ManR protein in *Synechocystis* 6803 cells. The numbers correspond to amino acid residues in ManR. The open rectangles represent a CheY-like receiver domain (2 to 113) and a DNA binding domain (157 to 228), respectively. The black oval corresponds to the conserved Asp-52 residue. See “Results” for further details.

Detection of ManS and Analysis of Expression of the *pmnt::luxAB* Reporter Gene—To detect the ManS protein, we added both a polyhistidine tag and a c-Myc epitope tag at the C-terminal end of this protein (Fig. 3A). Using monospecific antibodies against the c-Myc protein, we were able to detect a 54-kDa protein (Fig. 3B) in the *pmntlux* reporter cells that have been transformed with the WT¹/His/Myc version of the *manS* gene. The predicted molecular mass of this tagged protein is 52.9 kDa. To determine the role of His-205 in ManS in the predicted autophosphorylation and phosphotransfer events in such a two-component signal transduction system, this res-

¹ The abbreviation used is: WT, wild-type.

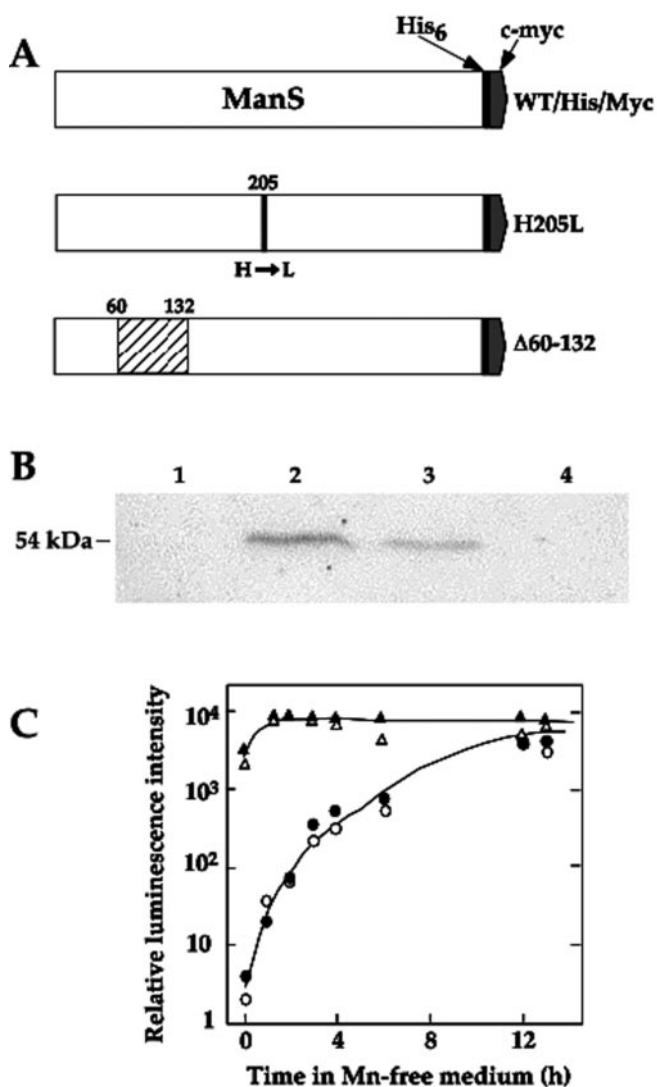


FIG. 3. Modification of the *manS* gene in *Synechocystis* 6803. *A*, a sequence encoding a hexahistidyl (*His*₆) tag and a c-Myc epitope tag was added to the 3'-end of the coding region of *mntS*. *H205L*, site-specific mutation resulting in the replacement of His-205 by Leu in *MntS*. The *bottom panel* represents a deletion mutation in which the sequence encoding residues 60 to 132 (hatched box) was deleted. *B*, Western immunoblot analysis of membrane proteins from the *pmntlux* reporter strain transformed with wild-type (lane 1), WT/His/Myc (lane 2), *H205L* (lane 3), and $\Delta 60-132$ (lane 4) constructs, using anti c-Myc antisera. 20 μ g of protein-containing sample was loaded in each lane. *C*, time course of luminescence from the *mntC::luxAB* reporter gene in *Synechocystis* 6803 cells. ●, wild-type; ○, WT/His/Myc; △, $\Delta 60-132$; and ▲, *H205L*.

idue was changed to Leu. Such a modification still allowed the accumulation of the ManS protein in cell membrane, although at a reduced level (Fig. 3B). Finally, to evaluate the potential role of the periplasmic domain of this protein in binding Mn, a conserved loop (residues 60 to 132) was deleted from the protein. However, such a mutation resulted in the absence of the protein in the membrane (Fig. 3B).

Fig. 3C shows the time-dependent changes in the lux luminescence intensity from the *pmntlux* cells, harboring various forms of the ManS protein. When grown in Mn-supplemented medium, luminescence from the WT, as well as the WT/His/Myc strains, were extremely low. Incubation in Mn-depleted medium increased the level of luminescence from these cells by nearly 10,000-fold. In contrast, both the $\Delta 60-132$ (with no *MntS* protein) and the *H205L* strains exhibited very high levels of luminescence even when grown in the presence of Mn. These

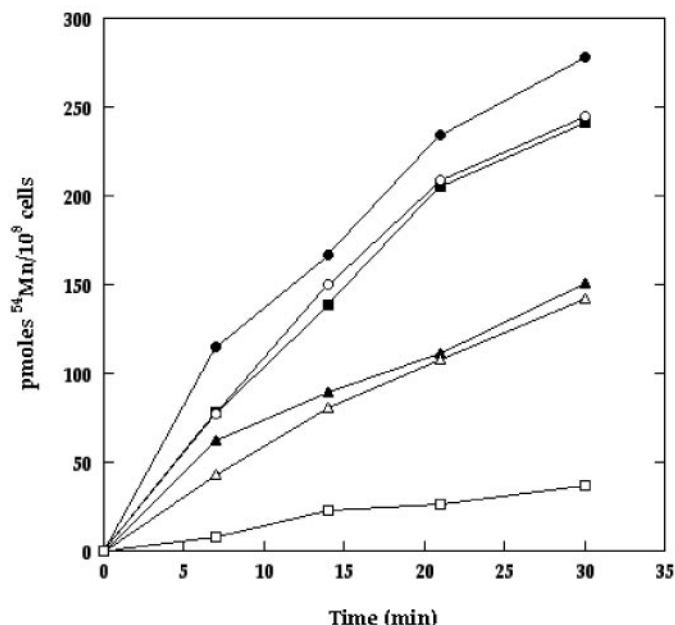


FIG. 4. Kinetics of ⁵⁴Mn²⁺ uptake by wild-type (circles), $\Delta mntC$ (rectangles), and *H205L* (triangles) cells by manganese in the growth media. Cells were grown in Mn-deficient (empty symbols) or Mn-supplemented (filled symbols) BG11 medium. Data are from one representative experiment.

data demonstrated that the ManS protein is a strong determinant in Mn-mediated regulation of transcription of the *pmnt* promoter, and the His-205 residue plays an important role in this process.

Identification of the Cognate Response Regulator ManR—As described above, extensive mutagenesis of the *pmntlux* reporter strain using the cosmid inactivation library identified the ManS sensor but not the corresponding response regulator protein. The known response regulator genes in *Synechocystis* 6803 (11) that are not represented in this cosmid library were inactivated systematically in the reporter strain. Among such inactivation mutants, only the *slr1837* (Fig. 2C) mutant cells exhibited high levels of lux luminescence, grown under either Mn-sufficient or Mn-depleted conditions (data not shown), similar to the observations with the *mntS* mutant cells described above. We concluded that the *slr1837* gene encodes the response regulator that interacts with ManS and named it ManR. The ManR protein is 234 residues long with a predicted molecular mass of 25.6 kDa. COG and SMART analysis (see above) indicated that ManR is a member of the OmpR subfamily of response regulators and has an N-terminal CheY-like receiver domain (Fig. 2D) that includes the conserved phosphate acceptor Asp-52 residue (10). ManR also has a C-terminal winged-helix-type DNA binding domain, suggesting that it interacts directly with the *pmnt* promoter.

Manganese Uptake Activities in the *manS* Mutant Cells—*Synechocystis* 6803 cells have at least two high affinity Mn-uptake systems (3, 5). Among them, the *MntABC* transporter is present and active when cells are grown under Mn starvation conditions. As a consequence, wild-type cells had high Mn-uptake activities when grown under both Mn-sufficient and Mn-deficient conditions (Fig. 4). As reported previously (5), the $\Delta mntC$ mutant lacking any functional *MntABC* transporter showed poor uptake activity under Mn-depleted conditions. However, it had normal Mn-uptake activity when grown in Mn-sufficient condition. In contrast, the Mn-uptake activity of the *H205L* mutant, as well as in the original *manS-1* mutant (data not shown), was unaffected by the presence or absence of

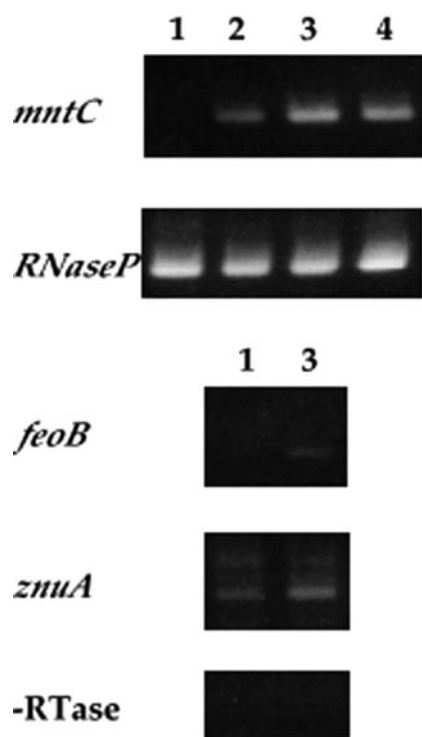


FIG. 5. The transcript levels of *mntC*, *feoB*, and *znuA* genes in the wild-type (1, 2) and H205L (3, 4) strains. Transcript abundance in cells grown in the presence (1, 3) or absence (2, 4) of added Mn in the BG11 medium was determined by the RT-PCR method. The transcript level of RNaseP in each sample is shown as a control. The absence of contamination of DNA was confirmed by PCR without reverse transcriptase (–RTase) reaction. See “Results” for further details.

the Mn in the growth medium. Notably, this activity was significantly less than that in wild-type cells, as well as in the Δ *mntC* cells grown in the presence of Mn. Evidently, the MntS sensor protein controls not only the expression of the *mntCAB* operon but also the cellular Mn status in *Synechocystis* 6803.

Transcription of Other Metal Transporter Genes in the *manS* Mutant Cells—As mentioned earlier, the level of the *mntC* transcript was undetectable in wild-type cells grown in BG11 medium and increased significantly under Mn-starvation conditions (Fig. 5). In contrast, *mntC* was expressed at a high level in the H205L mutant cells, both in the presence and absence of Mn in the growth medium. Interestingly, transcript levels of *feoB* (*slr1392*) and *znuA* (*slr2043*), two genes whose expression is repressed in complete BG11 medium, were increased slightly in the H205L mutant strain. It is noteworthy that *feoB* encodes an iron transporter (32), and *znuA* (formerly called *zntC*) encodes a component of an ABC transporter for zinc (3).

DISCUSSION

The data presented in this paper demonstrate that the two-component sensor protein ManS and the response regulator protein ManR are required for the regulation of transcription of the *mntCAB* operon encoding an uptake Mn permease in *Synechocystis* 6803 cells. In a canonical bacterial two-component system, the sensor His kinase protein is often localized in the cell membrane. As shown in Fig. 2B, the ManS protein is predicted to have two transmembrane domains. Cyanobacterial cells have two internal membrane systems, the plasma membrane and the thylakoid membrane (33). Because the ManS sensor is presumably involved in the perception of extracellular Mn concentration, we have reasoned that this integral membrane protein is localized in the plasma membrane (Fig. 2B). It is tempting to speculate that the periplasmic

domain of ManS is involved in the physical interaction with the Mn^{2+} cation.

To date, the ManS/ManR pair constitutes the only known two-component signal transduction system for manganese. Among various transition metals, such two-component systems have been identified for copper and silver. Two different copper-responsive two-component systems are present in *E. coli*, namely, CusS/CusR (34), and PcoS/PcoR (35). In *Pseudomonas syringae*, a plant pathogen, the CopS/CopR two-component system provides copper resistance (36), whereas in *Salmonella*, the SilS/SilR system provides resistance to silver ions (37). During the preparation of this manuscript, Reyes and co-workers (38) reported that in *Synechocystis* 6803 cells, the RppA/RppB two-component system identified originally by Li and Sherman (20) as a redox regulation system also has a role in nickel sensing. In all of these examples, the genes encoding the sensor His kinase and the response regulator are organized in operons. In contrast, the *manS* gene is located far away from the *manR* gene in the chromosome of *Synechocystis* 6803 (www.kazusa.or.jp/cyano/cyano.html).

During recent years, homologs of the MntABC transporter have been implicated to have significant roles in various bacterial infectious processes (39, 40). A number of regulator proteins for bacterial Mn homeostasis have also been identified. Notably, Que and Helmann (41) have studied MntR, a member of the DtxR diphtheria toxin repressor protein family, in *Bacillus subtilis* cells (41). *B. subtilis* cells have two distinctly different Mn transporter systems. Among them, MntABCD is an ABC-type transporter, similar to the MntABC transporter in *Synechocystis* 6803. A second Mn transporter is MntH, a member of the Nramp family of transporters (42). MntR is a bifunctional protein. In Mn-starved conditions, it activates transcription of the *mntABCD* operon, whereas in Mn-sufficient conditions, MntR represses expression of the *mntH* gene. In the presence of manganese, ScaR, a homolog of MntR in *Streptococcus gordonii*, acts as a repressor for the *sca* operon that encodes a Mn permease similar to the MntABC transporter (43). Interestingly, in *E. coli* cells, both Fur, an iron-dependent regulator, and MntR, a manganese-dependent regulator, control the expression of the *mntH* gene (44). It is noteworthy that none of these organisms has any known two-component signal transduction system for Mn.

As shown in Fig. 5, the ManS His kinase sensor protein appears to have some regulatory effect on the expression of the *feoB* and *znuA* genes. In other bacterial systems, expression of the iron transporter FeoB is known to be regulated by the well known regulator Fur. In *Synechocystis* 6803 cells, expression of the *znuA* gene is transcriptionally regulated by the Zur repressor protein, encoded by the *sll1938* gene.² It is known that the Mn^{2+} cation can bind to Fur (and presumably Zur) (45). It is possible that in the absence of ManS activity, an unregulated supply of Mn inside the cyanobacterial cells may lead to binding of this metal to Fur and Zur, with consequent transcriptional repression of the *feoB* and *znuA* genes. However, the dominant effect of ManS is on the *pmnt* promoter (Fig. 3C), indicating that the primary function of this protein is in sensing Mn.

Because of their oxygenic photosynthetic lifestyle, cyanobacterial cells must monitor carefully the available levels of Mn. The data presented in this manuscript demonstrate that the ManS/ManR two-component system in *Synechocystis* 6803 cells is an important determinant in the sensing of external Mn concentration. An additional interesting finding during this

² M. Bhattacharyya-Pakrasi, M. Shibata, T. Ogawa, and H. B. Pakrasi, unpublished data.

study is that in the H205L mutant strain, Mn-uptake activity is suboptimal under both Mn-sufficient and Mn-deficient conditions (Fig. 4), raising the question whether *pmnt* is the only promoter in *Synechocystis* 6803 cells that is regulated by ManS. It is possible that the Mn-responsive signal transduction pathway initiating with the ManS His kinase has more than one cognate response regulator, one of which (ManR) acts on *pmnt*, whereas the other(s) may control the expression of the second high affinity Mn transporter, as well as that of the Mn-efflux system(s) in these cyanobacterial cells. Which other promoters are regulated by the ManS sensor, as well as how and where Mn binds to this protein, are being investigated currently.

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