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MapA, an Iron-Regulated, Cytoplasmic Membrane Protein in the Cyanobacterium *Synechococcus* sp. Strain PCC7942

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Growth of *Synechococcus* sp. strain PCC 7942 in iron-deficient media leads to the accumulation of an ~34-kDa protein. The gene encoding this protein, *mapA* (membrane-associated protein A), has been cloned and sequenced (GenBank accession number, L01621). The *mapA* transcript is not detectable in normally grown cultures but is stably accumulated by cells grown in iron-deficient media. However, the promoter sequence for this gene does not resemble other bacterial iron-regulated promoters described to date. The carboxyl-terminal region of the derived amino acid sequence of MapA resembles bacterial proteins involved in iron acquisition, whereas the amino-terminal end of MapA has a high degree of amino acid identity with the abundant, chloroplast envelope protein E37. An approach employing improved cellular fractionation techniques as well as electron microscopy and immunocytochemistry was essential in localizing MapA protein to the cytoplasmic membrane of *Synechococcus* sp. strain PCC 7942. When these cells were grown under iron-deficient conditions, a significant fraction of MapA could also be localized to the thylakoid membranes.

Iron is an essential micronutrient for most organisms. In the cyanobacteria, this element is involved in chlorophyll biosynthesis and nitrate reduction and serves as a cofactor in a variety of other redox reactions (24, 29). The concentration of free iron in most microbial environments is generally lower than that required to support microbial growth (about 10^{-6} M) (20); this is due to the fact that, in the presence of oxygen, the ferrous iron is converted to the ferric form which precipitates and is then essentially unavailable to cells. In response, organisms have evolved various mechanisms for iron solubilization and uptake. The well-studied siderophore-mediated iron uptake systems of *Escherichia coli* are known to utilize outer membrane, periplasmic, and inner membrane proteins for iron assimilation (reviewed in references 11 and 22). Mutations in genes essential for iron acquisition, such as siderophores, reveal obvious phenotypes. However, mutations at other loci in the 3 min of the *E. coli* chromosome dedicated to iron metabolism may be silent because of the multiplicity of high-affinity iron transport systems that are available to *E. coli* (5).

By contrast, comparatively little is known about the iron metabolism of the cyanobacteria. One feature of the cyanobacterial response to iron limitation is the replacement of iron-containing cofactors with other cofactors. For example, iron-limited cultures of *Synechococcus* sp. strain PCC 7942 replace the iron-containing redox protein, ferredoxin, with the flavoprotein flavodoxin (19). Changes of this type result in negligible alterations of growth rates. A few species of cyanobacteria are apparently capable of producing siderophores and expressing proteins involved in iron uptake (8). *Anabaena* spp. produce a high-affinity iron transport compound called schizokinen (32), iron-deficient *Synechococcus* sp. strain PCC 7002 produces a hydroxamate-type siderophore (3), and *Synechococcus* sp. strain PCC 7942 expresses a cytoplasmic membrane protein of 38.6 kDa, IrpA, which participates in iron acquisition and storage (25).

Synechococcus sp. strain PCC 7942 is a unicellular, photosynthetic prokaryote capable of oxygenic photosynthesis. This organism demonstrates dramatic changes in cellular ultrastructure and physiology in response to iron limitation (30). We have determined that the photosynthetic apparatus is significantly altered under conditions of iron stress, including decreased cellular levels of the light-harvesting phycobilisomes and assembly of fewer thylakoid membranes per cell (16). In these iron-deficient cells, the cell envelope represents a larger proportion of the total cell membrane fraction and may have enhanced functional significance. We describe here the isolation of an iron-repressed gene (membrane-associated protein, *mapA*) of *Synechococcus* sp. strain PCC 7942. The MapA protein, with a molecular mass of ~ 35 kDa, has been localized to the cytoplasmic membrane of iron-sufficient cells by using immunocytochemistry and cellular subfractionation. We demonstrate that this protein serves as a reliable marker for the cell envelope during membrane preparation but that thylakoid fractions apparently contain this protein under certain environmental conditions such as iron deficiency. We have constructed deletion and insertion mutants of *mapA* that result in no obvious phenotype in *Synechococcus* sp. strain PCC 7942. However, a search of the Protein Information Resource database revealed similarities of MapA to two protein classes. The first class consists of bacterial envelope proteins involved in iron acquisition, whereas the second is a protein of unknown function that is one of the most abundant proteins in the chloroplast envelope inner membrane.

MATERIALS AND METHODS

Organisms and culture conditions. *Synechococcus* sp. strain PCC 7942 was grown with aeration in liquid BG-11 medium (1) at 30°C under a light intensity of 70 to 100 microeinsteins m^{-2} s^{-1}. Growth under iron-deficient conditions was in BG-11 prepared without added iron (30). When appropriate, the iron concentration of BG-11 was adjusted to 40 nM by addition of

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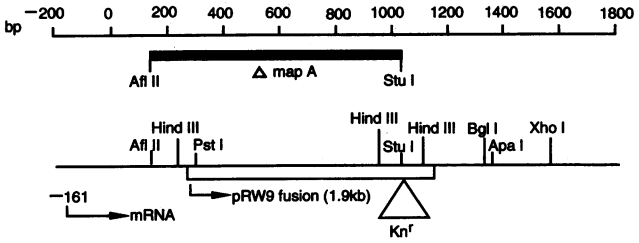

FIG. 1. Restriction map of the *Synechococcus* strain PCC 7942 *mapA* gene and description of the mutant constructs. Plasmid pRW9J includes the entire *mapA* gene and flanking sequences. The position of the *lacZ-mapA* fusion in pRW9 is noted below, and the *mapA* interruption construct is indicated by a triangle to show where a kanamycin-resistance cassette was ligated into the unique *Stu*I site. The Δ*mapA* strains were constructed by deletion of the region between the *Afl*II and *Stu*I sites (solid bar) and replaced with the same kanamycin-resistance cassette. The start of transcription, as determined by primer extension analysis, is indicated at the bottom left.

ferric ammonium citrate solution to iron-deficient BG-11. This yields an iron concentration 1/1,000th of the normal 40 μM level. Cell densities were determined with a Petroff-Hausser cell counting chamber.

Isolation of the *mapA* gene. A *Synechococcus* sp. strain PCC 7942 λZAP (Stratagene, LaJolla, Calif.) genomic expression library (36) was screened with antibodies produced against proteins which are expressed by this organism under iron stress conditions (28). Excision in vitro resulted in the isolation of pRW9. The overlapping clone pRW9J, necessary for the sequencing of the *mapA* 5′ region, was isolated from the λZAP library by use of ^{32}P-labeled randomly primed oligonucleotide probes (Pharmacia, Piscataway, N.J.) with the *Pst*I-*Hin*dIII fragment from pRW9 as the template.

Recombinant DNA techniques. Restriction enzymes were obtained from Promega (Madison, Wis.), Bethesda Research Laboratories (Bethesda, Md.), and New England Biolabs (Beverly, Mass.). DNA was isolated from *Synechococcus* sp. strain PCC 7942 as described by Golden et al. (15) and blotted to nylon membranes (Nytran, Schleicher and Schuell, Keene, N.H.) as described by the manufacturer. Southern analysis was performed as described in reference 4. The *mapA* gene was sequenced from double-stranded plasmid clones by use of Sequenase (U.S. Biochemicals, Cleveland, Ohio) and synthetic DNA-sequencing primers.

The *Synechococcus* sp. strain PCC 7942 *mapA* gene was interrupted by use of the kanamycin-resistance cassette CK.3 constructed by Elhai (14) by inserting *Sma*I-restricted CK.3 into the unique *Stu*I site of pRW9. The *mapA* gene was deleted by replacement of the *Afl*II-*Stu*I fragment of pRW9J with the same *Sma*I fragment of CK.3. These constructs were used to transform *Synechococcus* sp. strain PCC 7942 (15), and complete segregation of the mutant alleles was demonstrated by Southern analysis.

Cyanobacterial RNA was isolated, and Northern (RNA) blots were performed as described in reference 27. Primer extension analysis was performed as described in reference 37 by use of synthetic oligonucleotide primers.

Computer analysis. DNA and protein sequence analysis was performed with the MacVector software package (IBI/Kodak, Rochester, N.Y.). Hydropathy plots were prepared with DNA Strider software (Christian Marck, Département de Biologie, Institut de Recherche Fondamentale, Commissariat a l'Énergie Atomique, Gif-sur-Yvette, France). Sequence database searches were performed through the Purdue Agriculture Center Laboratory for Computational Biochemistry.

Protein methods. *Synechococcus* sp. strain PCC 7942 cells were harvested, resuspended to approximately 3×10^8 cells per ml (iron sufficient) or 3×10^9 cells per ml (iron deficient) and broken by three passages through a French pressure cell at 16,000 lb/in^2 in 50 mM MES [2-(*N*-morpholino)ethanesulfonic acid; pH 6.5]–1 mM Benzamidine–1 mM caproic acid–1 mM phenylmethylsulfonyl fluoride. DNase I and $MgCl_2$ were added to the lysates to final concentrations of 50 μg/ml and 6 mM, respectively, and the lysates were incubated on ice (4°C) for 1 h. EDTA was added to a final concentration of 10 mM, and unbroken cells and large pieces of cell debris were cleared from the lysate by centrifugation in a Beckman J2-21 centrifuge at 5,500 × *g* for 10 min. The cleared lysate was pelleted at 150,000 × *g* for 2 h (4°C) in a Beckman L8-70M ultracentrifuge. Cell membrane components were prepared by a modification (35) of the sucrose gradient purification previously described (23). The pelleted membranes were homogenized into 50 mM MES buffer and loaded directly onto sucrose gradients. Cell walls were harvested from the 2.2 to 2.6 M sucrose layer interface, and thylakoids were harvested from the center of the green band within the 1.5 M sucrose layer. Membrane fractions were washed with 50 mM MES buffer several times. Membrane proteins were precipitated by several acetone extractions of membrane fractions with 5 volumes of cold acetone at 20°C for 2 h followed by centrifugation at 12,000 × *g* for 10 min in a Beckman J2-21 centrifuge. Protein concentration was determined by the Bradford assay (9). Electrophoresis of protein was performed according to the methods of Delepelaire and Chua (12) by use of the buffer system of Laemmli (18). Polyacrylamide gradient gels (10 to 20% resolving gel with 5% stacking gel) were run at constant power (1.5 W) in the cold (4°C). Samples were solubilized with 1% lithium dodecyl sulfate–1% β-mercaptoethanol at 25°C for 10 min and cleared by centrifuging at 14,000 rpm for 5 min in an Eppendorf Microfuge just prior to loading. Protein blotting was performed as described previously (21), and immunostaining was based on the methods of Towbin et al. (34).

MapA antiserum preparation. The *E. coli* strain BB4 (Stratagene), containing the plasmid pRW9, was used to overexpress the LacZ-MapA fusion protein. The LacZ-MapA fusion protein was purified by denaturing acrylamide gel electrophoresis; the protein band then was excised from Coomassie-stained preparative polyacrylamide gels, homogenized into 5 ml of 0.1% sodium dodecyl sulfate, frozen in 1-ml syringes (for storage), and injected into New Zealand White rabbits. The initial immunization used 1.5 ml of this slurry (approximately 60 to 75 μg of protein) injected subcutaneously into 15 spots on the rabbit's back (approximately 100 to 150 μl per spot). Booster immunizations of the same or similarly prepared slurries (approximately 30 to 50 μg of protein) were given every 3 to 4 weeks, and serum was collected the week following each booster injection. The serum was collected and frozen (−80°C) in small aliquots. A 1:2,000 dilution of the antiserum was used for specific staining of the MapA protein on Western blots (immunoblots) by use of alkaline phosphatase-conjugated secondary antibody (Promega).

Freeze substitution preparation and immunocytochemistry for electron microscopy. The complete freeze substitution procedure is described by Reddy et al. (26). Briefly, cells were concentrated, resuspended in agarose, and spread thinly onto glass slides. Small squares of the cell-agarose sheet were quick-frozen by plunging them into liquid propane which is cooled to −190°C by liquid nitrogen. During the next few days, the cellular water was gradually replaced with an organic

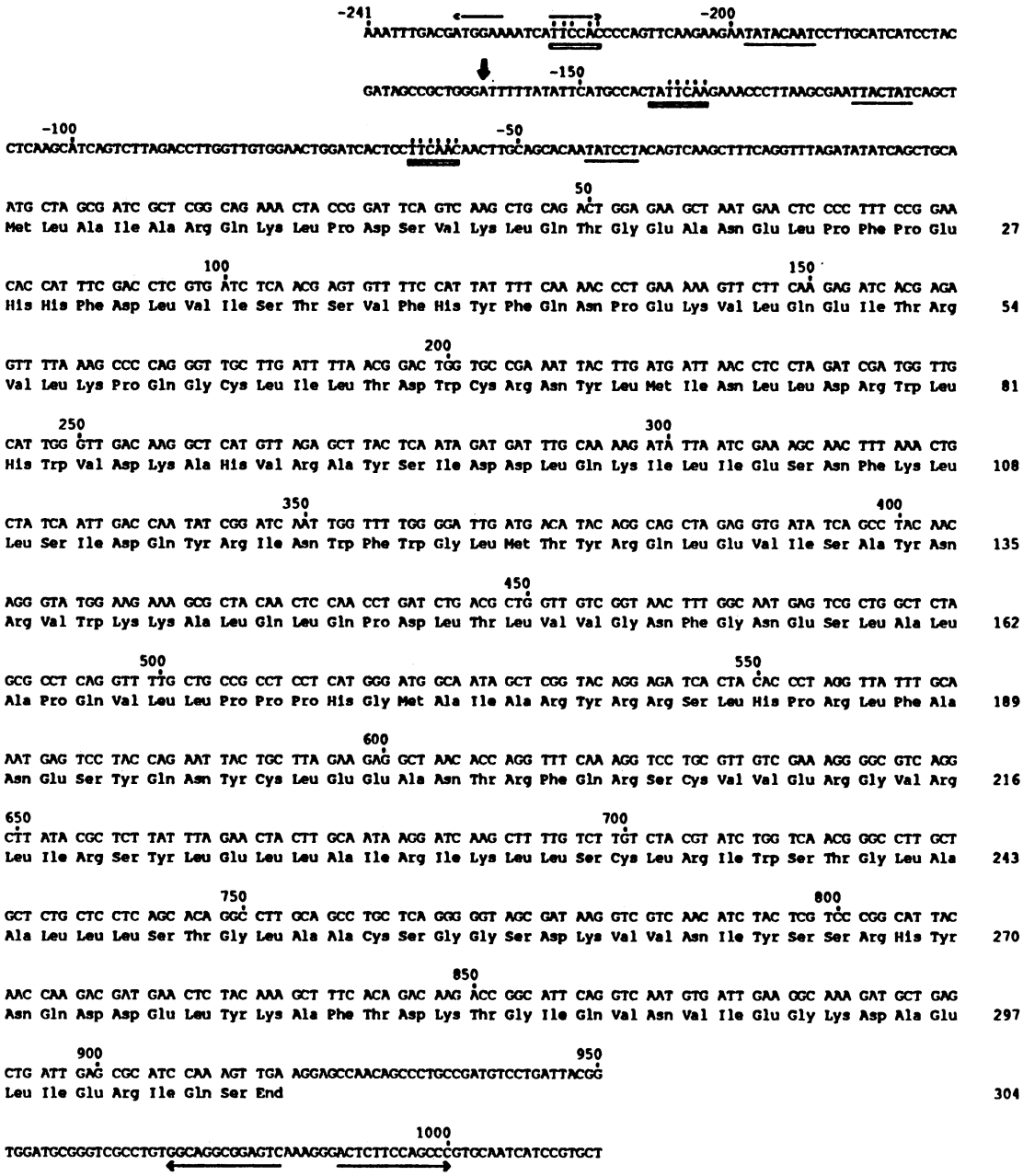

FIG. 2. DNA base and derived amino acid sequences of the *mapA* gene. The nucleic acid residues are numbered above their sequence, and amino acid residues are numbered at the end of each line. The down arrow at base number −161 points to the first base of the transcript as determined by primer extension analysis. Potential regulatory features are highlighted. The double-underlined regions denote repeated sequences. The quality of the repeat is shown by the number of dots above each sequence. Potential cyanobacterial −10 sequences are underlined. The 5′ inverted repeat thought to be involved in transcriptional regulation is highlighted with arrows (centered at base number −223), as is the 3′ inverted repeat capable of forming a hairpin structure.

solvent. For immunocytochemistry, we determined that ethanol was the best freeze substitution solvent. The cells were then embedded in Lowicryl HM20 and photopolymerized by UV light for 48 h at 4°C and then for an additional 48 h at room temperature.

Immunocytochemical localization of antigens in cyanobacteria was performed by preparing cyanobacteria for freeze substitution with ethanol as described above. The solutions contained Tris-buffered saline (TBS; 20 mM Tris HCl [pH 7.4] plus 150 mM NaCl). To this buffer was added 1% bovine serum albumin (BSA) (TBS-B), 0.3% Tween 20 (TBS-T), or both 0.3% Tween 20 and 1% BSA (TBS-TB). Thin sections (~80 to 100 nm) on carbon-coated formvar grids were incubated in TBS-TB for 20 min to block nonspecific immunoglobulin sites. Grids were then placed on 50-μl drops of primary antibody which had been diluted in TBS-B to the appropriate concentration as determined by trial; this procedure was performed in a moist chamber for ~15 h at 4°C. Between the changes of

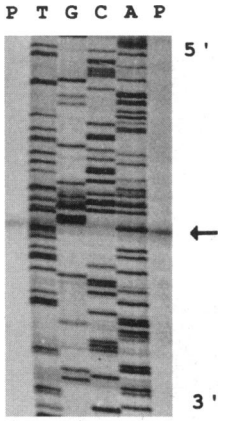

FIG. 3. Primer extension analysis of the *mapA* gene. The products of DNA sequencing reactions are presented in the four lanes labeled T, G, C, and A. The primer-extended products from isolated RNA shown in lanes 1 and 5 are labeled P.

solution, excess liquid was drawn off the grids with filter paper, with care taken not to let the grids dry. The grids were removed from the antibody and washed at room temperature by flotation on TBS-T placed in the wells of spot plates. They were briefly incubated on TBS-TB for ~5 min prior to a final 1-h incubation on a dilute solution of 10-nm colloidal gold conjugated to goat anti-rabbit immunoglobulin (Sigma, St. Louis, Mo.) in TBS-TB. The grids were floated on TBS-T, jet washed with double-distilled H_2O, and dried. They were stained with uranyl acetate and lead citrate prior to examination in a Philips Em-400 transmission electron microscope at an accelerating voltage of 80 keV.

Nucleotide sequence accession number. The DNA base sequence of the *mapA* gene has been assigned the GenBank accession number L01621.

RESULTS

Cloning, sequencing and expression of *mapA*. Our goal was to isolate iron-regulated genes from *Synechococcus* sp. strain PCC 7942 that coded for membrane proteins. We used a previously characterized (28) polyclonal antibody preparation that was directed against iron stress-induced membrane proteins. Immunoscreening of the λZAP expression library of *Synechococcus* sp. strain PCC 7942 with this antibody resulted in the identification of 10 immunopositive clones. The clones were assigned to six groups by restriction analysis and then analyzed on Northern blots to identify genes that demonstrated iron-regulated transcription. The colony containing plasmid pRW9 met these criteria and was used for further characterization.

The overlapping clone pRW9J was isolated and analyzed to construct the restriction map shown in Fig. 1, and the *Afl*II-*Bgl*I fragment was sequenced. Inspection revealed a nearly perfect (10 of 13 residues) inverted repeat centered 70 bp from the termination codon (Fig. 2). Primer extension analysis identified adenosine residue number −160 as the first base of the mRNA transcript (Fig. 3). The 5′ region of this gene possesses a number of interesting features which may be involved in the increased expression of this transcript by iron-limited cells. The arrows above the DNA sequence between bases −214 and −232 (Fig. 2) highlight a nearly perfect inverted repeat (6 of 7 bases). The double underlined sequence under the right arrow (TTCCAC) is repeated nearly perfectly twice in this 5′ region. These sequences are double underlined, and residues common to all three iterations are indicated with dots above them. Each of these sequences is followed by a promising candidate for cyanobacterial −10 sequences (single underline).

The open reading frame encodes a protein of 304 amino acids with a derived molecular mass of 35 kDa (Fig. 2). The hydrophobicity plot predicts a single hydrophobic domain of approximately 37 amino acids between amino acid residues 217 and 254. This protein partitioned with the hydrophobic material upon Triton X-114 phase partitioning (10), suggesting that MapA is an intrinsic membrane protein (data not shown).

The pattern of transcription of *mapA* in cells grown under iron-sufficient and iron-deficient conditions indicated that the gene was iron repressible. The *Pst*I-*Hin*dIII fragment from pRW9 identified an iron-repressible, 1.2-kb transcript. This mRNA was barely detectable in RNA isolated from normally grown cells but was stably accumulated in RNA prepared from iron-deficient cells (data not shown). We conclude that the transcription of *mapA* is regulated by iron concentrations in the culture.

Localization of the MapA protein in *Synechococcus* sp. strain PCC 7942. The localization of MapA was determined by immunocytochemistry and Western blot analysis of cellular fractions with an antiserum prepared as described in Materials and Methods. Our original Western blots indicated that MapA was associated with the thylakoids of iron-deficient cells (35). Immunocytochemistry, however, clearly demonstrated that MapA was associated with the cytoplasmic membranes (Fig. 4) of iron-sufficient as well as iron-deficient cells. In Fig. 4a to d, virtually all of the gold is associated with the envelope and especially with the cytoplasmic membrane (innermost envelope membrane). Nonetheless, about 5% of the gold was found on thylakoids, with most of this label on the thylakoid proximal to the cytoplasmic membrane. This feature is accentuated in iron-deficient cells (Fig. 4e to g), which only have one thylakoid leaflet per cell. The freeze substitution procedure is important for these studies in that it maintains cellular morphology, especially the relationship of the thylakoids to the cell wall. Thus, in iron-deficient cells, MapA is localized in both the cytoplasmic membrane and the thylakoid, which corresponds very closely to the Western blot results described below. It is noteworthy that the immunogold was usually not randomly deployed along the membranes but was arrayed in small patches as seen in each of the cells in Fig. 4. This feature was not considered an artifact based on the gold because it was not typically seen with other antibodies. However, the functional significance of this patchiness is unknown.

These results were corroborated and amplified by Western blot analysis of cellular proteins. Cell lysates from iron-deficient, wild-type cells yield a band at 34 kDa (Fig. 5, lane 2). This immunoreactive band is barely discernible in lysates from iron-sufficient, wild-type cells (Fig. 5, lane 1) and is absent from lysates of the *mapA* insertion mutant (Fig. 5, lane 3). The analysis of the thylakoid and envelope fractions from iron-deficient cells generated the most interesting results. MapA antibody reacted very strongly against a 34-kDa protein in thylakoids from wild-type cells (Fig. 5, lane 4) and not at all against thylakoids from the *mapA* mutant (Fig. 5, lane 5). The antibody also reacted with a 34-kDa protein in the cytoplasmic membrane fraction (Fig. 5, lane 6) and the envelope fraction from iron-deficient wild-type cells (Fig. 5, lane 7). The protein band in the thylakoid fraction appeared to migrate faster (a 0.5- to 1-kDa size difference) than the band in the envelope or lysate fraction (Fig. 5). The difference in MapA migration between fractions is likely an electrophoretic aberration due to the large quantity of protein found in that particular size range

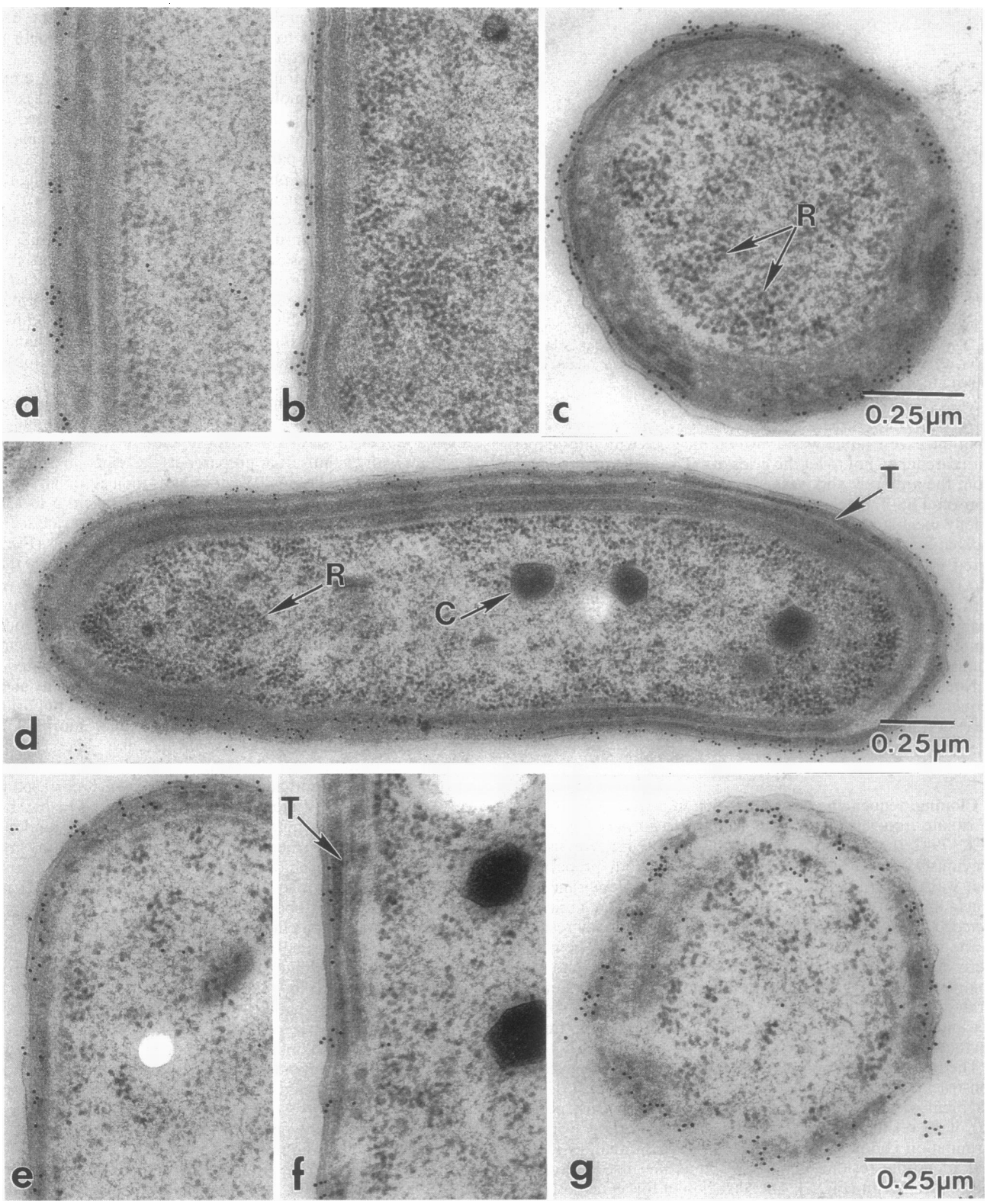

FIG. 4. Immunocytochemical localization of MapA in freeze substituted cells of *Synechococcus* sp. strain PCC 7942 grown under iron-sufficient conditions (a to d) and iron-deficient conditions (e to g). The antibody was prepared against MapA protein from *Synechococcus* strain PCC 7942 and used at a dilution of 1:5,000. The electron micrographs indicate that the vast majority of the gold particles associated with cytoplasmic membrane in iron-sufficient cells (a to d). However, a higher percentage of the gold was localized over the thylakoids in iron-deficient cells (e to g), although much of the MapA was still over the cytoplasmic membrane. T, thylakoid; C, carboxysome; R, ribosomes.

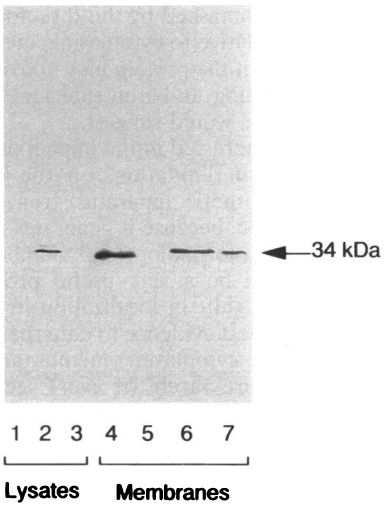

FIG. 5. Western blot of *Synechococcus* sp. strain PCC 7942 cell lysates and purified membranes (30 μg of total protein per lane) probed with a MapA antiserum produced against a LacZ-MapA fusion protein. Lanes 1 to 3 represent cell lysates from iron-sufficient wild-type (lane 1), iron-deficient wild-type (lane 2), or iron-deficient MapA insertion mutant (lane 3) cells. Lanes 4 to 7 represent acetone-extracted membrane fractions from iron-deficient cells. Lane 4, wild-type thylakoids; lane 5, MapA insertion mutant thylakoids; lane 6, wild-type cytoplasmic membranes; lane 7, wild-type envelope fraction.

in iron-deficient thylakoids (28). We believe that this strongly immunoreactive band represents the same protein in all fractions despite the fact that the protein from thylakoid fractions runs anomalously on gels.

Construction of insertion and deletion mutants in *mapA*. Mutations in the *mapA* gene were constructed as described in Materials and Methods and shown in Fig. 1. We have carefully studied over 15 such mutants and have demonstrated by Southern blot analysis that the insertions or deletions are as stated and that each chromosome in a cell has the altered gene (38). The Western blot of a cell lysate from one such deletion strain is shown in Fig. 5. The lysate of Δ*mapA* shows no band that cross-reacts with the MapA antisera (Fig. 5, lane 3), demonstrating that the gene product has not been produced. Nonetheless, neither this mutant nor any of the other mutant strains that we analyzed demonstrated an observable phenotype. The strains grew well in iron-deficient media and were unchanged according to numerous other criteria. Electron microscopy of such mutants indicated a tendency for the iron-deficient cells to contain more area of thylakoid membrane than similarly grown cells of the wild type. However, it is impossible to assess the significance of this finding given our current understanding of cyanobacterial physiology. We conclude that MapA is a nonessential protein and that it is not required for growth under iron-deficient conditions.

MapA is related to two types of proteins. The location of MapA and similarities to amino acid sequences of proteins with known functions may provide important clues as to the possible function of MapA. Comparison of our derived amino acid sequence (Fig. 2) with the sequences present in the Protein Information Resource database led to the similarities reported in Fig. 6. In Fig. 6A, we demonstrate that the C terminus of MapA (residues 233 to 304) displays 36% amino acid identity with the N-terminal 75 residues of SfuA from *Serratia marcescens* (2) and 29% identity with the N terminus of Fbp from *Neisseria gonorrhoeae* (6). This is significant, since both SfuA (36 kDa) and Fbp (37 kDa) are postulated to be involved in iron acquisition. A pairwise comparison of SfuA and Fbp in this region yields a score of 36% identity. These sequences were also compared with an amino-terminal sequence from a periplasmically located 40-kDa iron-repressed gene product from *Haemophilus influenzae* (17). The comparison of this sequence with *Serratia marcescens* Sfu reveals 82% identity; furthermore, this sequence is 32% identical to both *N. gonorrhoeae* Fbp and *Synechococcus* sp. strain PCC 7942 MapA. Hydropathy plots of the three sequences compared in Fig. 6A are remarkably similar, in that the region encompassing the first 20 amino acids was predicted to be hydrophobic, the next region of 20 amino acids was predicted to be hydrophilic, and the last region of 20 amino acids received a neutral score (data not shown).

A.

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MKLRISSLGPVALLASSMMLAFGAQAASADQGIVIYNAQHENLVKSWVDGFTK 1-53 S.m.SfuA
**TS*RY----****AALTA*TP*L---**--*TV**G**KEAAQAVA*A**R 1-44 N.m. Fbp
**TS*RY----****AALTA*TP*L---**--*TV**G**KEAAQAVA*A**R 1-44 N.g. Fbp
SC***W*T*LA***L*T-G**--*CSGGS*KVVN**SSR*Y*QDDELYK+**D 233-282  MapA
                          *--*+++*+**++++T***K+*EQ 1-40  H.i. 40

              DTGIKVTLRNGGDSELGNQL 54-75 S.m.SfuA
              A*****K*NSAKGDQ*AG*I 45-66 N.m. Fbp
              A*****K*NSAKGDQ*AG*I 45-66 N.g. Fbp
              K***Q*NVIE*+*A**IER+ 283-304   MapA
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B.

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QLAKAKAKKPLKECRIIEGDAEDLPFPTDYADRYVSAGSIEYWPDPQRGIREAYRVLKLGGKA 153-215 E37
MLAIARQKLP-DSVKLQTGEANELPFPEHHFDLVISTSVFHYFQNPEKVLQEITRVLK--PQG  1-60  MAPA
 ** *+ * *    ++  *+* +****    *  +*     *   * + ++*  ****

CLIGPVYPTFWLSRFFADVWMLFPKEEE----Y-IEWFQK----AGFKDVQLKRIGPKWYRGV 216-269 E37
CLILTDWCRNYLMINLLDRW-LHWVDKAHVRAYSIDDLQKILIESNFKLLSIDQYRINWFWGL 61-122 MAPA
***        *     * * *   +      * *+  **      ** + + +    *  *+
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FIG. 6. Alignment of the MapA sequence with potential homologs. (A) Alignment with proteins involved in cellular iron acquisition. The amino acid sequences are identified and numbered on the right. Abbreviations: S.m. SfuA, SfuA from *Serratia marcescens*; N.m. Fbp, Fbp from *Neisseria meningitidis*; N.g. Fbp, Fbp from *N. gonorrhoeae*; MapA, *Synechococcus* sp. strain PCC 7942 MapA; H.i. 40, amino-terminal sequence of the 40-kDa periplasmic protein of *H. influenzae*. An asterisk indicates identity with the *Serratia marcescens* sequence. A plus indicates identity with the *Neisseria* spp. sequences. The hyphens indicate gaps introduced to optimize alignment. (B) Alignment with the chloroplast envelope protein E37. The E37 sequence is presented above the sequence of MapA, and the amino acid residues are numbered to the right. An asterisk indicates amino acid identity, a plus indicates a conservative replacement, and the hyphens indicate gaps introduced to optimize the alignment.

In addition to the relationship of the C terminus of MapA to bacterial iron-acquisition proteins, the N terminus of MapA demonstrated a strong similarity to a major chloroplast envelope protein (Fig. 6B). In this figure, the N-terminal 122 residues of MapA are compared with the central one-third of the sequence of the chloroplast envelope inner membrane protein E37 (7, 13). The pairwise comparison of these sequences reveals a 30% identity. This score increases to 43% if conservative amino acid replacements are considered. The function of E37 has not yet been elucidated, but it is an abundant protein that comprises 5 to 10% of the total chloroplast envelope protein. An E37 antiserum obtained from M. A. Block (see reference 7) showed cross-reaction with several proteins in the mid-30-kDa size range in both the thylakoids and envelopes of iron-deficient *Synechococcus* sp. strain PCC 7942 cells. We were not able to determine conclusively if one of these cross-reactive proteins was MapA. The MapA antibody, however, showed no cross-reactivity against proteins from well-purified chloroplast envelopes (35).

DISCUSSION

We have identified *mapA*, a *Synechococcus* sp. strain PCC 7942 gene with enhanced expression during iron-deficient growth but which codes for a protein that is not essential for growth in iron-deficient media. At the transcriptional level, *mapA* shows fairly strong repression by available iron. It is tempting to speculate that the inverted repeat centered at residue number −223 of the DNA sequence, as well as the two other iterations of part of this sequence, is involved in this type of regulation. This sequence shows a low level of similarity to the iron-repressible promoters identified in *Synechococcus* sp. strain PCC 7942 and *E. coli*, in which the transcriptional control elements contain the DNA sequence TCATT as part of the right repeat.

The region of MapA that is similar to SfuA from *Serratia marcescens* (2) and Fbp from *N. gonorrhoeae* (6) includes the last two-thirds of the predicted hydrophobic domain of MapA and extends to the carboxy terminus. Although the precise function of SfuA from *Serratia marcescens* is unknown, this *sfuABC* operon can restore the ability of *E. coli* strains unable to produce siderophores to grow in iron-limited media (2). Iron has been shown to copurify with Fbp at a molar ratio of 1:1. All of these proteins have been shown to be localized to the periplasm, although they may be anchored to the cytoplasmic membrane through the hydrophobic domains described here. The common domain identified may also provide part of a channel for the transfer of iron through hydrophobic membranes. Thus, MapA could be part of an as yet unrecognized iron acquisition system, which would be consistent with the transcriptional response of this gene to iron limitation.

Growth in iron-limiting conditions increases the transcription of the message for MapA, and the protein becomes relatively abundant. Localization of MapA by immunocytochemistry clearly indicates a cytoplasmic membrane association in iron-sufficient cells. However, there is only about a twofold increase in MapA in cells grown under iron limitation, as determined from a count of gold particles on electron micrographs (as in Fig. 4) of 25 images each of iron-deficient and iron-sufficient cells (data not shown). As the number of thylakoid membranes per cell decreases from three to one under iron limitation (30), the cytoplasmic membrane would be present in a 1:1 ratio with the thylakoids and its proteins would become easier to detect and therefore would appear to increase in number. It is possible that the MapA protein does not so much accumulate heavily under iron-limiting conditions as reveal its presence, unmasked by the threefold decrease in thylakoid membranes relative to cytoplasmic membranes (30). The absolute quantities of the protein may not vary as dramatically between iron-limiting and iron-sufficient conditions as the Western blot analysis would suggest.

We are particularly interested in the impact of environmental stresses, such as iron limitation, on the structure and function of the photosynthetic apparatus. Iron deficiency has been particularly valuable, because it generates massive structural changes in cellular morphology and membrane structure (16, 28, 30). MapA will be a very useful probe for future research because of the shift in localization in iron-deficient cells. This is among the best evidence to date that there may be connections between the cytoplasmic membrane and the thylakoids. Such contacts are rarely (if ever) seen in electron micrographs of chemically fixed cells; however, freeze substituted, iron-deficient cells indicate a very close association between these two membranes (Fig. 4). Evidence from Western blots (31, 33) and electron microscopy (31) has recently shown that some photosynthesis proteins may be localized in both the thylakoids and the cytoplasmic membrane. The patchiness in the distribution of MapA, as evidenced by immunogold distribution, may be a reflection of contact zones where these two membrane systems intersect. This hypothesis may help explain the extreme difficulty in separating thylakoids and envelopes in cyanobacteria. It may be that these contact zones prevent complete separation of the membrane systems during mechanical breakage, and a proportion of the contaminating envelopes in the thylakoid fraction as well as the contaminating thylakoids in the envelope fraction may represent functional contact zones. Of course, this may be the reason that E37 is a major protein in the chloroplast envelopes.

The MapA protein may be useful in future analysis of two important questions. First, the relationship of MapA with chloroplast E37 may be of value in determining the evolutionary relationship of cyanobacteria and chloroplasts. Further study of MapA also may be of use in assigning a role to E37. In addition, the shift of MapA from an exclusively cytoplasmic membrane localization to a substantial thylakoid distribution as environmental conditions change may provide a tool for understanding the relationship of these membranes. Most importantly, we are interested in determining if MapA can be used as a monitor of contact zones between the thylakoid and the cytoplasmic membrane. One future line of research will use the MapA antibody as a probe against isolated membranes from cells that are actively producing new photosynthetic membranes.

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