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mgpS, a Complex Regulatory Locus Involved in the Transcriptional Control of the *puc* and *puf* Operons in *Rhodobacter sphaeroides* 2.4.1

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A new method has been developed in order to select mutants showing decreased *puc* operon transcription in *Rhodobacter sphaeroides* 2.4.1. A transcriptional fusion of a promoterless fragment derived from the *sacB* gene, encoding the levansucrase from *Bacillus subtilis*, to the upstream regulatory region of the *puc* operon has been constructed. With appropriate levels of exogenous sucrose, survivors of a sucrose killing challenge have been isolated. Subsequent analysis revealed the presence of both *cis*- and *trans*-acting “down” mutations in relation to *puc* operon expression. One of the *trans*-acting regulatory mutations was chosen for further study. The original mutation showed less than 2% of the level of *puc* operon transcription compared with the wild type under aerobic conditions and an 86% reduction under dark dimethyl sulfoxide conditions. This mutation can be complemented by a 3.9-kb *Bam*HI DNA fragment derived from a cosmid contained within a genomic cosmid bank. DNA sequence analysis of this fragment revealed the presence of a 2.8-kb open reading frame, designated *mgpS*, which would encode a 930-amino-acid protein. The N-terminal portion of the putative protein product presents homologies to proteins of the RNA helicase family. Disruption of the chromosomal *mgpS* resulted in decreased transcription of both *puc* and *puf*, while the presence of *mgpS* in multicopy in the wild type, 2.4.1, increased *puc* expression by a factor of 2 under aerobic conditions. Structural analysis of the *mgpS* locus revealed that expression of *mgpS* was likely to be complex. A smaller protein containing the 472 C-terminal amino acids of *MgpS* is able to act by itself as an activator of *puc* transcription and is expressed independently of the large open reading frame in which it is contained.

Rhodobacter sphaeroides is a purple, nonsulfur bacterium able to grow under photoheterotrophic or chemoheterotrophic conditions. The pigment-protein complexes which carry out light harvesting and primary photochemistry are the antenna complexes, designated B875 and B800-850, and the reaction center, respectively (6, 7, 31). They are localized to the intracytoplasmic membrane. The intracytoplasmic membrane, formed by invagination of the cytoplasmic membrane, is induced when the partial pressure of oxygen is lowered below 2.5% relative to that of air (5, 20), and its abundance is inversely related to the incident light intensity (24). The *puc* operon of *R. sphaeroides* comprises the *pucBA* structural genes encoding the B800-850 β and α polypeptides as well as an additional gene(s) downstream of *pucA* which encodes a function essential for posttranscriptional expression of the B800-850 complex (24, 27). Two of the three polypeptides of the reaction center (L and M) as well as the α and β polypeptides of the light-harvesting complex B875 are encoded by the *puf* operon (22, 42).

It has been shown that transcription of *puc* is highly regulated by light as well as oxygen (23). A two-component regulatory system, PrrA/PrrB, sensitive to oxygen partial pressure, has been described. PrrB is the histidine kinase responsive to anaerobiosis. PrrA, the response regulator, positively regulates the transcription of the *puc* and *puf* operons (11, 12). The descriptions of the PrrA/PrrB system as well as those of other

trans-acting effectors of photosynthesis gene expression have been facilitated by the isolation of mutants which display increased expression of photosynthesis gene expression, e.g., App and PpsR (16, 17).

Here, we describe an alternative method to the isolation of mutants (those displaying decreased transcription of the *puc* operon). The promoterless *sacB* gene of *Bacillus subtilis* has been transcriptionally fused to the upstream regulatory region of the *puc* operon. *sacB* encodes a levansucrase, which when expressed in the presence of sucrose at the appropriate concentration, leads to cell death. SacB hydrolyzes sucrose and synthesizes branched polymers of fructosyl residues which have been shown to be lethal for a variety of gram-negative bacteria (15). This construction has been moved into *R. sphaeroides* 2.4.1, and when grown on plates containing carefully titrated levels of sucrose, only those cells with reduced synthesis of the *sacB* product or a defective product can survive. Using this selection, we have isolated and subsequently characterized several mutants with decreased *puc* operon transcription. By complementation analyses, we have localized and described a new activator of *puc* and *puf* operon expression. We have designated this new locus, *mgpS*, for modulator of genes for photosynthesis.

(A preliminary report of these results was presented at the 95th General Meeting of the American Society for Microbiology, Washington, D.C., 21 to 25 May 1995.)

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. The *R. sphaeroides* wild type, 2.4.1, and its derivatives were grown at 30°C in Sistrom minimal medium supplemented with succinate as the carbon source (8). *Escherichia coli* strains were

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TABLE 1. Bacteria and plasmids used in this study

Strain or plasmid	Genotype or phenotype	Source or reference
Strains		
<i>E. coli</i> DH5 α phe	F ⁻ ϕ 80dlacZ Δ M15 Δ (lacZYA-argF)U169 <i>recA1 endA1 hsd</i> R17(τ_K^- m $_K^+$) <i>supE44</i> λ^- <i>thi-1 gyrA relA1 phe::Tn10dCm</i>	11
<i>R. sphaeroides</i>		
2.4.1	Wild type	W. R. Siström
Ω E	2.4.1 <i>mgpS</i> :: Ω Sm ^r Sp ^r at <i>EspI</i> ^a site	This study
Ω P	2.4.1 <i>mgpS</i> :: Ω Sm ^r Sp ^r at <i>PstI</i> site	This study
MGP 1	2.4.1 <i>mgpS</i> Δ <i>EcoRI</i> ^b - <i>PstI</i> ^b :: Ω Sm ^r Sp ^r	This study
MGP 2	2.4.1 <i>mgpS</i> Δ <i>EcoRI</i> ^a - <i>XmaI</i> ^a :: Ω Sm ^r Sp ^r	This study
MGP 3	2.4.1 <i>mgpS</i> Δ <i>PstI</i> ^b - <i>XmaI</i> ^b :: Ω Sm ^r Sp ^r	This study
MGP 4	2.4.1 <i>mgpS</i> Δ <i>AccI</i> ^a - <i>XmaI</i> ^a :: Ω Sm ^r Sp ^r	This study
MGP 5	2.4.1 <i>mgpS</i> Δ <i>EspI</i> ^a - <i>EcoRV</i> ^a :: Ω Sm ^r Sp ^r	This study
M7	Mutant derived from 2.4.1, lower <i>puc</i> and <i>puf</i> transcription levels, reduced coloration	This study
Plasmids		
pRK415	Tc ^r	21
pBluescript II SK	Ap ^r with T3 and T7 promoters	Stratagene
pUC18	Ap ^r	44
pPDK1	Sm ^r /Sp ^r Tc ^r <i>puc::aph</i>	25
pHP45W	Source of the Ω Sm ^r /Sp ^r cassette	33
pSUP202Km	pSUP 202 + Ω Km ^r in the <i>HindIII</i> site of <i>tet</i> from pSUP202	Reference 36 and S. Dryden
pCF200Km	Km ^r Sm ^r /Sp ^r IncQ <i>puc::lacZYA</i> ^c	25
pUI1830	Sm ^r /Sp ^r Tc ^r <i>pufB::lacZYA</i> ; pCF1010 derivative + 1.3-kb <i>RsaI</i> fragment from <i>puf</i> operon in the <i>XbaI</i> ^a site of pCF1010	L. Gong
pUI1851	pUI523A derivative + 1.3-kb <i>RsaI</i> fragment from <i>puf</i> operon in the <i>StuI</i> site of pUI523A	Reference 40 and L. Gong
pPS400	pRK415 derivative + 0.7-kb <i>PstI</i> - <i>DraII</i> <i>puc</i> DNA between Ω Sm ^r /Sp ^r and 1.6-kb <i>AccI</i> - <i>EcoRV</i> <i>sacB</i> DNA; Tc ^r and Sm ^r /Sp ^r	This study
pUI8256	pLA2917-derived cosmid from <i>R. sphaeroides</i> 2.4.1 cosmid library, Tc ^r	10
pUI3102	pRK415 derivative + 3,900-bp <i>BamHI</i> fragment from pUI8256 containing <i>mgpS</i> , in the <i>BamHI</i> site of pRK415(-) ^c ; Tc ^r	This study
pUI3103	pUI3102 derivative with the 0.8-kb <i>PstI</i> fragment containing the 3' end of <i>mgpS</i> (-) deleted; Tc ^r	This study
pUI3104	pBS derivative + 3.9-kb <i>BamHI</i> fragment from pUI8256 containing <i>mgpS</i> in the <i>BamHI</i> site of pBS(+) ^d ; Ap ^r	This study
pUI3105	pUI3104 derivative with the first 2,033 nucleotides of the 3.9-kb <i>BamHI</i> fragment deleted and containing the last 1,828 bp of <i>mgpS</i> (+); Ap ^r	This study
pUI3106	pUI3104 derivative with the first 1,070 nucleotides of the 3.9-kb <i>BamHI</i> fragment(+) deleted; Ap ^r	This study
pUI3107	pUI3104 derivative with the first 2,381 nucleotides of the 3.9-kb <i>BamHI</i> fragment deleted and containing the last 1,493 bp of <i>mgpS</i> (+); Ap ^r	This study
pUI3108	pRK415 derivative + 1.5-kb <i>SstI</i> - <i>HindIII</i> ^a fragment from pUI3107 inserted into <i>SstI</i> - <i>HindIII</i> of pRK415(-); Tc ^r	This study
pUI3109	pRK415 derivative + 1.5-kb <i>SstI</i> - <i>HindIII</i> ^a fragment from pUI3107 inserted into <i>SstI</i> - <i>EcoRI</i> ^a of pRK415(+); Tc ^r	This study
pUI3110	pRK415 derivative + 1.9-kb <i>SstI</i> - <i>HindIII</i> ^a fragment from pUI3105 inserted into <i>SstI</i> - <i>HindIII</i> of pRK415(-); Tc ^r	This study
pUI3111	pRK415 derivative + 1.9-kb <i>SstI</i> - <i>HindIII</i> ^a fragment from pUI3105 inserted into <i>SstI</i> - <i>EcoRI</i> ^a of pRK415(+); Tc ^r	This study

^a The overhangs were made blunt with the Klenow fragment of DNA polymerase I before cloning.

^b The overhangs were made blunt with T4 DNA polymerase before cloning.

^c (-), insert DNA fragment having an orientation in the opposite direction of the *tet* and *lac* promoters.

^d (+), insert DNA fragment having an orientation in the same direction as the *tet* and *lac* promoters.

grown at 37°C in Luria-Bertani medium (28). When appropriate, tetracycline, spectinomycin, streptomycin, and kanamycin were added at 1, 50, 50, and 25 μ g/ml, respectively, for *R. sphaeroides* and 20, 50, 50, and 25 μ g/ml for *E. coli*.

DNA manipulation and sequence analyses. Plasmid DNA was isolated according to standard protocols or manufacturers' instructions, as were restriction endonuclease and other enzymatic treatments of DNA. Deletion clones for sequencing were prepared with the exonuclease III-mung bean nuclease deletion kit from Stratagene (La Jolla, Calif.). A series of deletions in both orientations differing by approximately 300 nucleotides were made from pUI3104 according to the instructions of the manufacturer. These clones were used to determine the DNA sequence of the insert DNA of pUI3104 with the pBST3 primer (5'-CCCTCACTAAAGGGAACAAAAGCT-3') and the EXT7-1 primer (5'-AATACGACTCACTATAGGGC-3'). Part of the sequence was confirmed with the primers 5'-GCCCTGAAGGAAGCCGATG-3' (-534), 5'-CACCTCGAAC

GAATGGCTCT-3' (888), 5'-ACCGTCACTTCACCCCTTGTC-3' (1409), and 5'-CGAAGGATTTGGGTTGGTTG-3' (2713). The numbers in parentheses represent the nucleotide coordinates to which the primers hybridize (see discussion pertaining to Fig. 3). Sequence determination was performed with an ABI 373A automatic DNA sequencer and with a *Taq* dye deoxy terminator sequencing kit (Perkin-Elmer, Applied Biosystems Division, Foster City, Calif.) at the DNA Core Facility of the Department of Microbiology and Molecular Genetics. Sequence analyses were performed with the computer program DNA Strider (Institut de Recherche Fondamentale, Commissariat à l'Énergie Atomique, Paris, France) and the program manual for the Wisconsin Package (Genetics Computer Group [GCG software], Madison, Wis.).

Construction of MGP 1 to 5. The 3.9-kb blunt-ended *BamHI* fragment was cloned into pBluescript II SK, which had been previously digested with *Ecl* 136 and *HincII*. Portions of the insert were removed with the appropriate restriction

enzymes, and an Ω cartridge encoding resistance to spectinomycin and streptomycin (Sm^r/Sp^r) was cloned in their place. *Pvu*II fragments were excised from these constructs and cloned into pSUP202Km, which had been previously treated with *Sca*I. The resulting plasmids, unable to replicate into *R. sphaeroides*, were moved from *E. coli* to *R. sphaeroides* by standard procedures (9). Presumptive even-numbered crossovers were selected from the exconjugants on Sm/Sp -containing plates, and these were tested for kanamycin resistance. It was assumed that $Sm^r Sp^r Km^r$ cells would have undergone a single crossover, while $Sm^r Sp^r Km^s$ cells would have undergone a double crossover. For several candidates chosen at random, the double crossover event was confirmed by Southern hybridization analysis.

Pulsed-field agarose gel electrophoresis. Genomic DNA was prepared and analysed by pulsed-field agarose gel electrophoresis according to previously described protocols (38) with a transverse alternating field electrophoresis gel apparatus (Beckman Instruments, Inc., Fullerton, Calif.).

Southern DNA analysis. DNA was transferred by capillary action to nylon membranes (Micron Separations, Inc., Westboro, Mass.), and the nucleic acids were bound by UV cross-linking with a UVC1000 (Hoefer Scientific Instruments, San Francisco, Calif.). Hybridizations were performed at 68°C, and the final wash was at 68°C in $0.1 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS). Labeling of DNA probes with biotin and detection of hybridizing sequences by chemiluminescence were performed with the NEBlot Phototope kit (New England Biolabs, Inc., Beverly, Mass.).

Preparation of cell extracts and assay of β -galactosidase. *R. sphaeroides* cultures used for the measurement of β -galactosidase activities were grown aerobically by sparging with gas mixtures or anaerobically in the presence of 70 mM dimethyl sulfoxide (DMSO) as described previously (9). Cells were harvested at an optical density at 660 nm of approximately 0.2. Cell breakage with a French press, preparation of crude extracts, and β -galactosidase assays (at 30°C for 5 min) with *O*-nitrophenyl- β -galactoside hydrolysis were performed as described previously (40). All experiments were done at least three times. Protein determinations were done with the bicinchoninic acid protein assay (Pierce, Rockford, Ill.).

Spectrophotometric assay. Cells were washed and resuspended in 10 mM phosphate buffer–1 mM EDTA (pH 7.2). After a 2-min sonication (50% duty, intensity level 5) (Sonifier cell disruptor; Branson Sonic Power Co.), extracts were centrifuged for 5 min at top speed in a microcentrifuge. Absorption spectra of the supernatants were analyzed with a Perkin-Elmer Corp. (Norwalk, Conn.) Lambda 4C spectrophotometer.

Protein electrophoresis. *E. coli* cells grown overnight in Luria-Bertani medium were diluted fourfold with Luria-Bertani medium supplemented with 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG) and grown for 4 h. The cells were harvested, washed with Tris–50 mM HCl (pH 7.0), and then resuspended in the same buffer. After a 2-min sonication (Sonifier cell disruptor), the cells were centrifuged for 2 min at top speed in a microcentrifuge, and the supernatant was then centrifuged for 1 h at 200,000 \times g. The supernatant (soluble fraction) was removed, and the pellet (membrane fraction) was resuspended in the same buffer. The proteins were separated on an 8% polyacrylamide gel as previously described (35).

Chemicals and materials. Restriction endonucleases and other nucleic acid-modifying enzymes were purchased from New England Biolabs, Inc. (Beverly, Mass.), Boehringer Mannheim Biochemicals (Indianapolis, Ind.), Bethesda Research Laboratory Life Technologies, Inc. (Gaithersburg, Md.), and Promega Corp. (Madison, Wis.). 5-Bromo-4-chloro-3-indolyl β -D-galactopyranoside (*X*-Gal) was obtained from Boehringer Mannheim Biochemicals. Antibiotics, *O*-nitrophenyl- β -D-galactopyranoside, and other reagents were reagent grade and were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Accession number. The nucleotide sequence of the *mgpS* region has been assigned EMBL accession number Z50182.

RESULTS

Isolation of *cis*- and *trans*-acting mutations involved in *puc* operon expression. To provide positive selection for the isolation of down regulatory mutations involved in the control of *puc* operon transcription, we transcriptionally fused a promoterless fragment of the *sacB* gene to the *puc* upstream regulatory DNA sequence. We isolated a 1,572-bp *Acc*I-*Eco*RV fragment containing the *sacB* gene from plasmid pRL271 (4) and filled the unpaired bases with the Klenow fragment of *E. coli* DNA polymerase I. This fragment was then inserted into the *Sma*I site of pUC18. The fragment was reisolated with the *Xba*I and *Sst*I restriction enzymes. The plasmid pPDK1 (26), a pRK415 derivative containing the 699-bp *Pst*I-*Dra*II fragment of the *puc* upstream regulatory region cloned between the transcription translation stop cartridge $\Omega Sm^r/Sp^r$ and the *aph* gene was treated with *Xba*I and *Sst*I to remove the *aph*-containing DNA fragment. The *Xba*I-*Sst*I fragment containing the

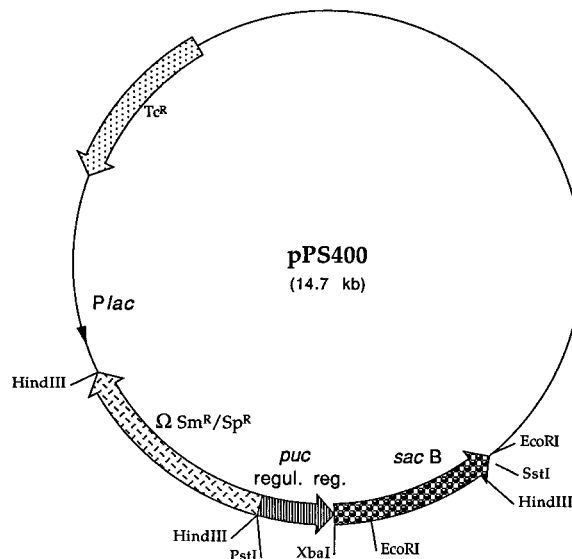


FIG. 1. Map of pPS400. The 1.6-kb *Acc*I-*Eco*RV promoterless fragment of the *sacB* gene of *Bacillus subtilis* has been cloned downstream of the *Pst*I-*Dra*II fragment of the upstream regulatory region (regul. reg.) of the *puc* operon in pRK415. An Ω cartridge encoding resistance to streptomycin and spectinomycin is present upstream of the *puc* regulatory region.

promoterless *sacB* gene was then cloned into the subsequently linearized plasmid. Figure 1 shows a map of the resulting plasmid, pPS400.

When this plasmid was moved into *R. sphaeroides*, *sacB* was expressed under control of the *puc* promoter and levansucrase was synthesized. In the presence of sucrose, this enzyme forms polymers of fructosyl residues lethal for the bacteria. We determined that the minimal lethal concentration of sucrose under aerobic growth conditions on the surface of a petri dish was 0.05%. Under these conditions, only mutants with a reduced level of the *sacB* product or a defective *sacB* product can survive. Mutations can occur either within the *puc* upstream regulatory region or within the *sacB* gene itself; these are “*cis*-mutations.” Mutations can also occur within the genomic DNA of the host, and these are “*trans*-mutations.” To localize each mutational type, plasmid DNA was isolated from sucrose-resistant colonies, mobilized back into the *R. sphaeroides* wild type, 2.4.1, from *E. coli* without a sucrose challenge, and the sensitivity of the exconjugants to sucrose was tested under the original conditions of isolation. When the mutation appeared not to be on the plasmid (exconjugants were sensitive to sucrose), we took the original mutant strain, cured it of its resident plasmid, reintroduced pPS400, and tested it for sucrose sensitivity. The insensitivity of the exconjugants to sucrose confirmed that the mutation was localized on the chromosomal DNA of the original isolate. Using 0.05% sucrose, we obtained both *cis*- and *trans*-mutations. At higher concentrations of sucrose (0.1 to 5%), the only surviving cells were those presenting deletions into the *puc-sacB* region of the plasmid. We measured β -galactosidase activity of the first eight *trans*-mutations isolated, with a *puc::lacZ* transcriptional fusion (pCF200 Km) placed in *trans* in each of these mutants as well as in the wild type, and grown under aerobic conditions. All of the isolates showed decreased *puc* expression ranging from 2 to 14% of the value obtained for the wild type (data not shown). We chose one of these mutants, designated M7, for further studies. This mutant presents a pale pink coloration but is still able to grow slowly under photoheterotrophic conditions.

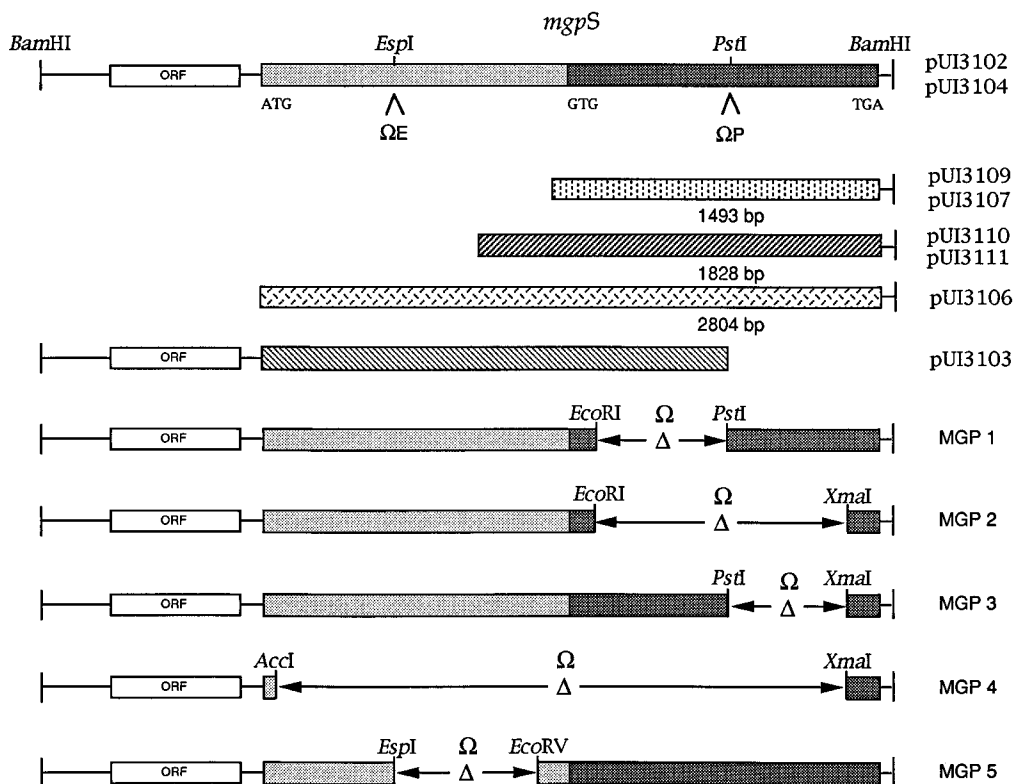


FIG. 2. Structure of the 3.9-kb *Bam*HI fragment which complements the mutation in M7. The most upstream region designated ORF corresponds to the 594-bp ORF immediately upstream of the *mgpS* locus. An Ω cartridge encoding resistance to spectinomycin and streptomycin has been inserted into the two unique *Esp*I and *Pst*I sites of pUI3104, to create the strains Ω E and Ω P. The strains MGP 1 to 5 were constructed by deletion (extent indicated by the arrows) of part of *mgpS* and insertion of an Ω cartridge. Portions of the 3.9-kb fragment contained in some of the plasmids constructed for this study are shown. The numbers indicate the length of the shaded area.

Cloning and sequencing of *mgpS*. A cosmid bank containing *R. sphaeroides* 2.4.1 genomic DNA (10) was mobilized from *E. coli* into M7. The initial phenotype used to score for complementation was the change from a decreased pigmentation to the pigmentation of the wild type. Several cosmids were able to restore the wild-type coloration. Among these, some are known to contain regulatory genes capable of affecting photosynthesis gene expression as characterized by *puc* operon activation, such as *PrrA* or *App* (11, 16). However, one cosmid, pUI8256 had not been previously observed in any of our studies. pUI8256 was able to partially restore to the mutant both coloration and β -galactosidase activity with pCF200Km in *trans*. Cosmid pUI8256 was subcloned into pRK415, and all subclones were mobilized into M7 by conjugation. One of the plasmids (pUI3102) containing a 3.9-kb *Bam*HI fragment was able to partially restore wild-type coloration to mutant M7. This fragment was cloned in pBluescript II SK (pUI3104), and deletion clones were obtained in both orientations. These deletion clones were used to sequence the entire 3.9-kb *Bam*HI fragment. The GCG software package was used in conjunction with a *R. sphaeroides* codon usage table (13) to identify two open reading frames (ORFs) with sizes of 594 and 2,793 bp, respectively, apparently transcribed in the same orientation and separated by 86 bp. pUI3102 was treated with *Pst*I and religated, removing the terminal 78 bp of the 2,793-bp ORF. The resulting plasmid (pUI3103), which contains a 3.1-kb *Bam*HI-*Pst*I fragment from cosmid pUI8256 including the whole 594-bp ORF but only part of the 2,793-bp ORF (Fig. 2), was unable to restore coloration to mutant M7. We therefore

concluded that the 594-bp ORF itself was not responsible for restoration of the phenotype to mutant M7.

The 2,793-bp ORF could encode a putative protein with a size of 930 amino acids. Both the DNA sequence and deduced amino acid sequences are shown in Fig. 3. We designated this locus *mgpS*, for modulator of genes of photosynthesis. When the DNA sequence was examined for the presence of a Shine-Dalgarno sequence, no obvious match was found. A portion of the sequence downstream of the *Bam*HI site was obtained (Fig. 3) with the cosmid pUI8256 as a template and the oligonucleotide 5'-CAACCAACCCAAATCCTTCG-3' (2694). Two GC-rich stems at the end of *mgpS* followed by a series of four T's (Fig. 3) could act as a rho-independent terminator.

Alignment of *mgpS*. The DNA and deduced protein sequences of *mgpS* were compared against sequences in the GenBank database. For the first half of the deduced protein sequence, homologies were found to proteins from the RNA helicase family, i.e., proteins involved in folding, splicing, and translation of RNA. The best matches (Fig. 4) were to the product of the yeast nuclear gene *snv3*, a putative ATP-dependent RNA helicase which affects mitochondrial posttranscriptional processes (37), and the SKI protein of *Saccharomyces cerevisiae*, which blocks the expression of viral mRNA (41). Similarities were found in a number of regions of conserved amino acid motifs, like GRAGR, a putative RNA binding domain (37) (Fig. 4). The C-proximal portion of the putative protein presents a region rich in alanine and proline residues in a repeated motif similar to those found in the H8 antigen of *Neisseria gonorrhoeae* (43) and the dihydrolipoamide acetyl-

GGATCCAGTCCGCATGCGCTTGGCCGGTCCCGGGGAGGGCGGGCTG
*Bam*HI

AAAAGTGCGCCGCTCAACTCAGCGCTGCCCCACCTTCATCGCCATCGGGATCGAGCCCTCGACGGCGAGGGCCGGACATGAAGCCATCGTGGGA
 TTGAGTGCGCCCTCGAGGATTCGCCGAACACGTCGGCCGAGGGCGTACATGTCAGCTCGCCCTCGCCATCGCCCTCGCCGACGCCCTGTTCGTCCATC
 ATGATCAGCGCCCTCGTGGCAATGACGAACCTTCGGCTGCATCGAAAGTCGAAACCTTCGGCTCAGGGCTCCACCGCGCGCTCGATCACCTTCGTC
 ATCCCGGCTGTCTCAATCCCGTTTCTTTCAGCTTCGCCACTGGCGTTGGCCGTGGCAGCTTACATTTCGGACTATGAAGCGGGCGACCGTTTC
 ACAACGCTATCGTGGGGCAATCGTCCGATTGCTCGGTCTCGCACCGTGGCGCGAGAGCGCCGGCGGCCCGGGCGCATGGACGAGCTTTCG
 CGCGCTGAAGGAAGCCGATGCGGCCGGCGGGGATCGAGCGGCAGATCTTACCAGATGGTGAATCCGGCTTCGCTCGCCGATCTCTCTGC
 TCGAGAGGGGGCGAAGGCGTGGCCGGCGGACCCGGAGCGCCATCGACCACTGACGGCGCTCATGATCACCGCCCGATTTCGCCGAAGGCT
 GGAACGGCGGGCCACGGCCATTTTCAAGCGGGCATGTTCCGCCCTCGATGGCCGACATCGCCCATGTGTGACGCTCAACCCCGCCATTTCCGGCG
 CTCGCGCGGCTCGGCACCATGCTGGAGAGATGGACCGGCCAAGCCGGCACTCGAGGCTATCGGGCGCGCTCGGTATACATCGCCACTCGACG
 GGTAGAGCAGGGCGTGAACGGCTGAAGCGAAGGCTGCCGGTCAGGAACCTTAAAGGCGAGCCATCGCCGGACCGCGAGGGTACCGCGGTTCTCG
 GGCGACCAACCGGCCAAGACCCTATTTCCATCGAACGGATCTGGGCCATCGGACGGCGTATCGGCTCGCCCTCGCGCTTCGGCGCGGAG 57
 M L G H R T G V I G L P L R L L A R E
 1
 GTCCTAGACCGGATCGTGGCCAGCGCCGCCCTCGTGGCGCTCGTACCAGGGAGCGGATCGTCCCAGCGGACGCAATCTGGTCTGC 156
 V^{ACT}Y D R I V A Q R G P S V V A L V T G E E R I V P E R T Q Y W V C
 20
 ACCGTGAGGCCATCGGATGGAGATCGGCGGGATTCGTGGCGTGGACGAGATCCAGCTTCGGCGCACCCCGAGCGCGCATGTCTTACCAGC
 53
 T V E A M P M E I G A D F V A V D E I Q L C G D P E R G H V F T D
 CGGTGCTGGGGCGGGGCTCGTGCAGACATGTTCTCGGTCTGGAGTGATGGGGCGCCATTCGCGCGTGTGGCGCATGTGACTTCTCG 354
 R L L R A R G L V E T M F L G S D V M R G A I A A L V P H V T F L
 86
 CGCGCGAGCGCTTCGACCTCTCTATGCGGATCGAAGAAGTACCGCGGATGCCCGCGCACCATCGTGGGCTTCGCGTGCATAACGTC
 119
 R R E R F S T L S Y A G S K K I S R M P P R S A I V G F S V D N V
 TATGCCATCGCGAGCTCATCCCGCTCAGAAGGGGGCTCGCCGCTGGTATGGGGCGCTTACCGCCCGCACCGGAACCGCGAGTGGCCCTTAC 552
 Y A I A E L I R R Q K G G C A V V M G A L S P R T R N A Q V A P Y
 152
 CAGACGGCGCGGATGATATCTCTCGCCACCGATCGATCGGGATGGGGTGAACCTCGACATCGCCCATGTGGCTTTTTCGACGTAATTC 651
 Q N G D V D Y L V A T D A I G M G L N L D I R H V A F S S T V K F
 185
 GAGCGCGGGGATCGGGCGCTGTTCCCGACGAGTGGGGAGATCGCGGTTCGGCGGGCGCCACACCGAGCGGGCACCTTGGCGTACCGGC
 218
 D G R R M R P L F P H E L G Q I A G R A G R H T E A G T F G V T G
 GAGCGCGCCCTCGACGAGGGCGTGGTCGATCGATCGAGAACCACCTTTTCGCCCGATCGAGCGGTGCATCGCGCAACGACCGCGTGAATTC 849
 251
 E A S P L D E G V V D A I E N H R F A P I Q R L H W R N D R L E F
 GGCACGGTGCAGCGGTGATCGCTCGCTCGAGGAGCCACCTCGAAGCAATGGCTTCGCGGGCGGGGAGTCGGACATCTCATCGCTGAAGCGG 948
 284
 G T V E R L I A S L E E P T S N E W L S R A R E S D D L H A L K A
 CTGTCCGAGGCCCGAGGTGCGTGGCGGCTGCATCGCGCACAGAGTGGCGTCTCTGGGACGCTCGCCGATTCGCGGATTCGCGAGCATCTCG 1047
 317
 L S E E P E V R A R L H S P H E V R L L W D V C R I P D P R S I S
 ACCACCGAGCATGTGACTTCTTCCCGGATTTTCGGCTTCTGTGCGAAGGAAAGTGGCAACGACTGGTTCGAGCGCCATTCGCCGGATCGAC 1146
 350
 T E H V T L L A R I F P F L C E G K V P N D W L A G A I A R I D
 CGCCCGCGGAGATATCGACCGCTTTCGAAGCGCTGGCTATATTCGGACTGGACTATGCGCGCAGAGACCGCGGTGGGTGCGAGCAGAAAT 1245
 383
 R P S G D I D T L S K R L A Y I R T W T Y V A Q R R G W V Q D E S
 CATTTGGCGGAGAAACCGCGGCTAGAAGACCGCTGTCGGATCGCTCCACCGCGGCTGACTCAGAGATTTCGACCGCGCACCTCTGCTC 1344
 416
 H W R E E T R A V E D R L S D A L H G A L T Q R F V D R R T S V L
 ATGCGCGGTGTAACAGAGGAGCCTTCTGGCTGAGTCAATGACAAGGTGAAGTACGCTGGAGGGCGAATTCGCGCGCTCGAAGGGTTC 1443
 449
 M R R L K Q K E S L V A E V N D K G E V T V E G E F V G R L E G F
 CGTTCGCGAGGATCGTGAACTCGCCGAGGAGCGACCCCTCGCGGAGCGCGCTTCAGCGCGTTCGCGCGCGAGTTCACCTCAGGGCGGAC 1542
 482
 R F R Q D A S N S P D E A R T L R Q A A V Q A L R P E F H L R A D
 CGTCTTACAATTCGCCGACACCGAGATGGATTTACCAGAGCGGGCGCTCATGTGGGCGAGCAGCGGTGGGCAAGCTGATCGCGGCCAGAG 1641
 515
 R F Y N S P D T E M D F T E Q G G L M W G E Q A V G K L I A G P E
 CCGATCGCCCGAGGTCGAGGCTTCGTCGAGGAGTGCAGCGCCCGAGGTCGCGGAGAGGTGCGCGCGAGGCTCAGCATTTTCATCGACCGCAAG 1740
 548
 P M R P Q V E A F V D E D A G P E V A E K V R R R R L Q H F I D R K
 GTCGCGCCAGTTCGAGCGCTTCTGGCCATGAGCCGGACGAGAGCTGACGGCGCTTCCCGCGGCTTCGCGCTTCGGTGGTAAAGCTCGGG 1839
 581
 V A A Q F E P L L A M S R D E T L T G L A R G F A F R L V E S L G
 CTGATCCCGCGAGCAGGTGGCCGAAGGTCAGTTCGCTGATCAGGAGCGCGCTCGGCATCGCGAAGCATGGCTCGCGTTCGGCGAGTTCAGG 1938
 614
 L I P R E Q V A E E V K S L D Q E A R S A L R K H G V R F G Q F T
 ATCTTCTCGCGCTCTGCTGAAACCGCGCGACCGGCTGGCTGCTGCTTGGTTCGGAACCGCTCGAGGATTTCGCGAAAGCCCGCG 2037
 647
 I F L P L L L K P A P T R L R L V L W S L A N G L Q E F P E S P P
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 680
 P G L V T I P H I A D V P K M H Y T L A G Y H P A G Q R A I R I D
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 M L E R L A D L L R A K D S R A G F E A T P D M L S I T G M T L E
 CAGTTCGCGGCTCATGACGAGGCTGGGTTACCGGGCGAGCCCGCGAGCGGGCGAAGTGAAGCCCTCCGCGCGCTGAGGCGCTCCCGCC 2334
 746
 Q F A G L M Q G L G Y R A E P G E R P K V K A A P A A E A A P A
 GAGGGCGAGCGCCGAGAGTGCAGCGGAGGAGCGCGCGCGGCGAAGTCCGTCGCGGAGCGCGAGGCGAGCCCGGAGCGCGCGCGGAGAG 2433
 779
 E G E A P A E V P A E E A P A A E A P S A D A E A A P D A P A E E
 CCGCGCGGCTGAGGTCGAGGCTTACACCTTCACTGGCGCGCGGCTCGGATCGCGGTCCGCGCGGCGAGCGCGGGCGAGGGCGAGGGG 2532
 812
 P A A V E V E S F Y T F T W A P R R P D R G P R R H D R G Q G E G
 CGCAACCGCGCGCGCGCAACCGGGCGAGCGCCCGCGGAAGCGCTGCGCGAGGCGCTCGCGCGGCGAGCGCGCGCGGAGCGTCAGGGC 2631
 845
 R N R G P R A T P G D A P A E G A A E A R P P R G D R P E R Q G
 CAGCGCATGGGGCAAGCCAAAGGGCAAGGGCGCAAGCGGACCGCAAGGGCGCGAGCGCAACCAACCAATCTTCGAATCGCGCGCGCGGG 2730
 878
 H G H G K P K G K G K P D R K G A D R N Q P K S F E S R P A R
 AGCGACGAGATCGACCGGCAATCGTTCGCGCTCTCGCGCGCTCGCGCGGAGCTGATCGGTTTCGCTGCGCGGAGCGGATTCGCGCTGACA
 911
 S D R I D P D N P F A V L A A L R D R S stop
 AATGGTGGTGCAGCGCGCTTCTTCAAGACGCGCGCTTCGCCACGGCCCTCGTTCGGAAGCGGGCGGATCCGGCTCAACGGCGAGCATGTCGCCAAT

FIG. 3. Nucleotide sequence and predicted amino acid sequence of *mgpS*. The putative start (ATG) and stop codons for the 594-bp ORF and for *mgpS* are underlined. Sequences underlined by asterisks correspond to a potential Shine-Dalgarno sequence for a putative smaller ORF starting with a GTG (underlined). Two stem loops followed by a series of T's which could act as a rho-independent terminator are double underlined.

A

MGPS	MLGHRTGVIG	L	PLRLLA REY	YDR IVAQ RG P	30			
SUV3	QK LKSVDRGYA	G	PLRLLA REY	YDR FHA EK IR	282			
MGPS	S	VVA LV T GEE	..	R IV P ERT Q	...Y W V CT V E	AMP ME IG AD F	V AM DE IQ LC G	75	
SUV3	C	..N LT T GEE	V	IR DL DR GN	S AG L T S G T V E	M V P IN Q R F D V	V VI DE IQ M S	330	
MGPS	DPER GH V FT D	R LL R AR GL VE	T M FL S	D V M	...R GA I AL	112		
SUV3	DGR GW A WT N	A LL G V V S RE V	H LC GE K	S V L	PL V K S I V R M T	369		
MGPS	V PH V T LR RE	R F ST LS ..YA	G...	S KK IS R	M.....	P P	RS AI V G F S V D	150	
SUV3	G DK L T INE Y E	R L G KL S.. VE	E K P IK D G IK G	L.....	R K	G DC V V A F S KK	410		
MGPS	N V Y AI AE L IR	R Q K G .G CA V V	M GA L S P TR N	A Q V AP Y Q NG D	V D Y I V AT D AI	199			
SUV3	K IL DL K L K IE	K D N L K V AV I	Y G S L P P ET R V	Q QA L F N NG E	Y D IM V AS DA I	910			
SKI2	R DL P Q IL K TR	S L LER GI AV I	H G L L P I V KE	L IE IL F S K G F	I K V I E AT ET F	725			
DBPA	K DC Q AV CD AL	N EV G S AL S L	H G DE Q RD R D	Q T L V R F ANG S	A R V I V AT D V A	301			
MGPS	G M G L N L D IR H	V A F S S I V K F D	G R R M R P L F P H	E L G Q I G R AG	R H TE AC T FG V	249			
SUV3	G M G L N L S IR	V V F T N M K Y N	G E EL M E M T S S	Q IK Q I G R AG	R F K S R S A S G G	960			
SKI2	A M G L N L P TR	V I F S S I R K H D	G N L R E L T P G	E F T L A G R AG	R R GL D S T C T V	774			
DBPA	A R G ..	L D I K S	L E L ..	V V N F E	L A W D E V H V E	R I G R ..	A R A G	N S G L A I S F CA	346

B

MGPS	MTLEQ F AG L M Q GL G Y R A E P GER P K V K A AP A AA E AP A E G E A P ..	A EV P A E E	790
H8	M K K S L F A AL L S L A L A CC G GE K A E A P A E A S T E A P A E A P A A E P A A E		50
MGPS	A P A E A P S A D A E A AP D AP A E P A A V E S F	820	
H8	A A A E A P A E A ..	P A E A P A A E A A T E P A A	79
MGPS	I D M L ER L A D L L R A K D S R A G F E A T P D M L S I T G M L E Q F A G L M Q GL G Y R A E P	760	
AZO2	I E L V K T G D L I E V E Q L V L E S A R A S M E V P S K A G V V S V S K L G D L K E	65	
MGPS	G E R P K V K A A P A A E A P A E GE A P A V P A E R A P A E A P S A D A E AP D AP A E	810	
AZO2	G D A I I E L P A AG A AA A P A P A AA V P A P T Q A V D E A E A P S P G A S A T P ..	A P A A	114

FIG. 4. Amino acid alignment of domains of the deduced protein product of *mgpS*. (A) The upstream region of the ORF showing homologies with RNA helicase is compared with those of SUV3 (*S. cerevisiae*) (37), SKI2 (*S. cerevisiae*) (41), and DBPA (*E. coli*) (14). Shaded boxes represent conserved domains in the RNA helicase family. Amino acids which are conserved are in boldface. (B) The downstream region is compared with the H8 antigen of *N. gonorrhoeae* and the dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase of *A. vinelandii* (AZO2). Alanine and proline residues are in boldface. For each protein, the lysine residue to which the lipoic acid is attached is outlined.

transferase component of the pyruvate dehydrogenase of *Azotobacter vinelandii* (1). Those two proteins bind a lipoic acid to a lysine residue proximal to this region. In the putative protein encoded by the 2,793-bp ORF, there are two lysine residues (Lys-765 and Lys-767) just upstream of the alanine- and proline-rich region. For the dihydrolipoamide acetyltransferase component, there are several lipoic acid domains separated by interdomain linker sequences rich in alanine and proline. These interdomain sequences have a high degree of conformational flexibility and provide the lipoyl domains with the mobility required for interaction with the catalytic centers of the molecule (29). No matches were found for the region of the protein between amino acids 250 and 750 of MgpS.

Disruption of *mgpS* with an Ω Sm^r Sp^r cartridge: effects on *puc* and *puf* transcription and spectral complex levels. An Ω Sm^r Sp^r cartridge (33) was cloned into either the *EspI* or the *PstI* site in the 3.9-kb *Bam*HI fragment of pUI302 (Fig. 2). The *Bam*HI fragment was then cloned into pSUP202Km, which is incapable of replicating in *R. sphaeroides*. Ultimately these plasmids were moved into *R. sphaeroides* by conjugation, and even-numbered crossover events were selected among the ex-conjugants as described in Materials and Methods. The structure of each recombinant clone was confirmed by Southern analysis. Two of these were chosen for further study and designated Ω E for the insertion of the Ω cartridge in the *EspI* site and Ω P for the insertion in the *PstI* site. The Ω P strain showed a decreased coloration, while the Ω E strain presented a coloration only slightly lighter than that of the wild type. Both of the mutants were able to grow under phototrophic conditions (3 or 10 W/m²) with a rate identical to that of the wild type.

Spectral analyses of membrane extracts of these mutants as well as of those of the original M7 and 2.4.1 were performed,

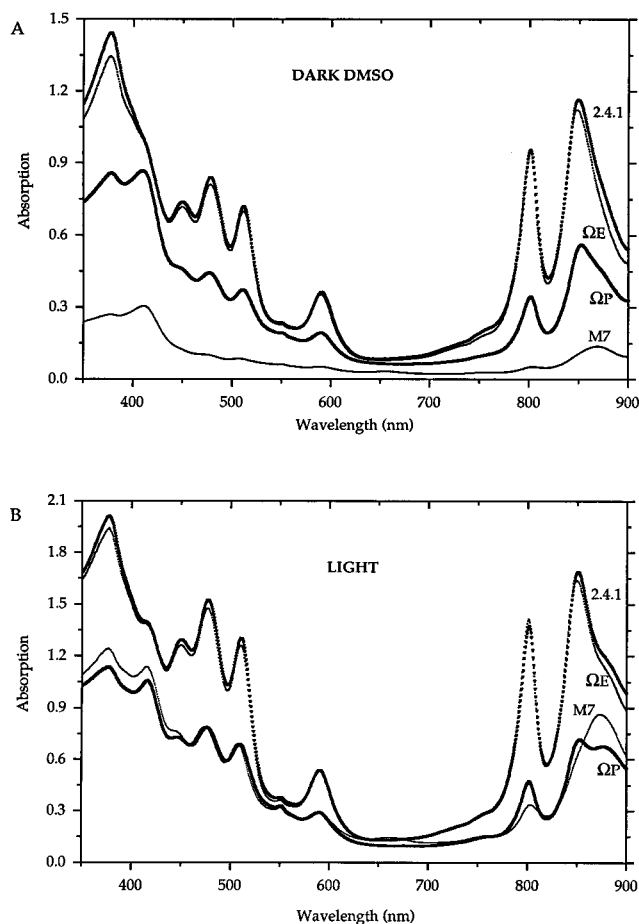


FIG. 5. Absorption spectra of membranes of *R. sphaeroides* 2.4.1, M7, Ω E, and Ω P. Cells were grown anaerobically in the dark with DMSO (A) or photosynthetically (10 W/m²) (B). All samples contain 1 mg of protein per ml. The levels of the B800-850 and B875 complexes were determined by the equation of Meinhardt et al. (30).

and the results are presented in Fig. 5. The original mutant, M7, contained less than 2% of the B800-850 complex and less than 25% of B875 complex compared with the wild type, while the Ω P strain revealed ~33% of the B800-850 complex and ~50% of the B875 wild-type levels in cells grown under dark DMSO conditions (Fig. 5A). Under phototrophic conditions (Fig. 5B), mutant M7 contained levels of B875 identical to those of the wild type, but the quantity of the B800-850 complex was very low. Under the same conditions, Ω P revealed ~25% of the B800-850 levels compared with 2.4.1 but only slightly lower levels of B875. Surprisingly, the Ω E strain showed no significant difference from the wild type, 2.4.1, when grown under both photosynthetic and dark DMSO conditions.

The final cellular level of antenna complexes contained within the intracytoplasmic membrane is the result of a series of events including transcription, translation, insertion, and ultimately assembly of the proteins and the pigments in the membrane. To determine the effect of disruption of *mgpS* at the transcriptional level, we measured β -galactosidase activity with two plasmids, pCF200Km and pUI1830, bearing *puc::lacZ* and *puf::lacZ* transcriptional fusions, respectively, and one plasmid, pUI1851, bearing a *puf::lacZ* translational fusion. Under both aerobic and dark DMSO growth conditions, insertion of an Ω cartridge into the *mgpS* locus resulted in a 75% de-

TABLE 2. β -Galactosidase activities from cell extracts of *R. sphaeroides* strains bearing a *puf::lacZ* transcriptional fusion (pUI1830) or *puf::lacZ* translational fusion (pUI1851)

Cell extract	β -Galactosidase activity ^a			
	<i>puf::lacZ</i> transcriptional fusion		<i>puf::lacZ</i> translational fusion	
	+O ₂	-O ₂	+O ₂	-O ₂
2.4.1	530 ± 32	4,876 ± 279	671 ± 25	3,067 ± 126
M7	264 ± 65	3,193 ± 804	84 ± 4	2,256 ± 375
ΩP	121 ± 25	1,188 ± 179	137 ± 26	1,591 ± 80
ΩE	170 ± 8	2,155 ± 264	300 ± 26	2,949 ± 107

^a β -Galactosidase activity is expressed as micromoles of *O*-nitrophenyl- β -galactopyranoside hydrolyzed per minute per milligram of protein. +O₂, aerobic growth. Cells were sparged with 30% O₂, 69% N₂, and 1% CO₂. -O₂, anaerobic growth in the dark in the presence of 70 mM DMSO.

crease in *puf* operon transcription when the insertion was in the *Pst*I site (ΩP) and a 56 to 68% decrease when insertion was in the *Esp*I site (ΩE) (Table 2). These effects were even more severe than those in the original mutant isolate, M7. These results suggest that *mgpS* may act in a positive manner in its effect on the *puf* operon expression under aerobic and anaerobic conditions. When β -galactosidase activity was measured with a translational *puf::lacZ* fusion (Table 2), under aerobic conditions, the results were similar to those obtained with the transcriptional fusion, showing β -galactosidase activities approximately 80% lower for ΩP and 55% lower for ΩE than those for the wild type. Under anaerobic conditions, ΩP showed only a 52% decrease and ΩE was virtually identical to the wild type. Importantly, under aerobic conditions, the original mutant, M7, showed only a 50% decrease in *puf* expression with the transcriptional fusion but a >85% decrease with the translational fusion. Thus, the upstream mutation showed no effect on *puf* operon expression as measured from a *puf::lacZ* translational fusion under anaerobic conditions, whereas with a transcriptional fusion, the effect was considerable. The nature of and reason for this difference are unknown at the present time.

β -Galactosidase activity was also measured with a *puc::lacZ* transcriptional fusion (Table 3). Under anaerobic conditions, the level of *puc* transcription in the two disrupted strains was approximately 65% lower than that in 2.4.1, showing that under those conditions, *mgpS* is also a positive effector of *puc* transcription. Under aerobic conditions, the disruption of the gene at the *Pst*I site resulted in a 90% decrease in *puc* transcription, while the disruption at the *Esp*I site had no effect. In M7, *puc* expression is severely depressed in the presence of

TABLE 3. β -Galactosidase activities from cell extracts of *R. sphaeroides* strains bearing a *puc::lacZ* (pCF200Km) transcriptional fusion

Cell extract	β -Galactosidase activity with transcriptional fusion <i>puc::lacZ</i> ^a	
	+O ₂	-O ₂
2.4.1	423 ± 23	4,519 ± 136
M7	7 ± 1	631 ± 110
ΩP	38 ± 3	1,684 ± 40
ΩE	452 ± 18	1,448 ± 270

^a β -Galactosidase activity is expressed as micromoles of *O*-nitrophenyl- β -galactopyranoside hydrolyzed per minute per milligram of protein. +O₂, aerobic growth. Cells were sparged with 30% O₂, 69% N₂, and 1% CO₂. -O₂, anaerobic growth in the dark in the presence of 70 mM DMSO.

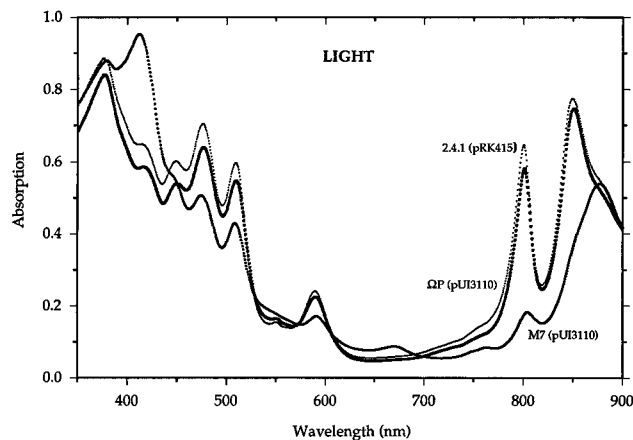


FIG. 6. Absorption spectra of membranes of *R. sphaeroides* 2.4.1, M7, and ΩP containing pUI3110 or pRK415 in *trans*. Cells were grown under photosynthetic conditions (10 W/m²). All samples contain 1 mg of protein per ml.

oxygen. However, the fold induction in both M7 and ΩP when *puc* expression is measured anaerobically versus aerobically remains substantial in both mutant strains. On the basis of other studies, given the levels of expression aerobically, we would have predicted an approximately 10-fold induction.

Does *mgpS* encode one or two proteins of different sizes?

The data so far present something of a paradox. On the one hand, analysis of the spectral complex data suggests that the *mgpS* locus is in reality two separate loci as defined by the ΩE and ΩP strains. Similarly, measurements of β -galactosidase activity with pCF200Km in each of these strains, under aerobic conditions, reinforces this contention. However, the β -galactosidase results determined under anaerobic conditions, as well as the DNA sequence, suggest that *mgpS* is a single locus. To extend this analysis in more detail, we elected to study the role of the C-terminal portion of the *mgpS* locus alone on photosynthesis gene expression. From the clones obtained after exonuclease III deletion of pUI3104, we obtained two DNA fragments encompassing the terminal 1,493 bp and the terminal 1,828 bp of *mgpS* and cloned each in both orientations into pRK415 (Fig. 2). These plasmids were moved into ΩP, and we examined the restoration of pigmentation in the exconjugants. When plasmid pUI3109 containing the terminal 1,493-bp fragment of *mgpS* was cloned in the same orientation as the vector *tet* and *lac* promoters, the presence of the plasmid was able to restore wild-type pigmentation to ΩP, while in the opposite orientation (pUI3108), there was no effect on ΩP coloration. However, the 1,828-bp fragment was able to restore wild-type coloration in either orientation relative to the *tet* and *lac* promoters (plasmids pUI3110 and pUI3111). Spectral analysis of membranes of ΩP containing pUI3110 (fragment in opposite orientation relative to *tet* and *lac* promoters) in *trans* showed that it was able to restore the levels of antenna complexes in the membranes to values similar to those of the wild type, 2.4.1 (Fig. 6). This was not the case with the original mutant, M7. Spectra obtained with M7 bearing plasmid pUI3110 or pUI3102 were identical, showing only partial restoration of the B800-850 complex.

β -Galactosidase activities with pCF200Km, which contains a transcriptional *puc::lacZ* fusion in *trans*, were also measured in ΩP, also containing various subfragments of *mgpS* in *trans*, in order to gain insight into the effect of the distal portion of the *mgpS* locus on photosynthesis gene expression (Table 4). The presence of pUI3110 increased *puc* expression in ΩP by a

TABLE 4. β -Galactosidase activities from cell extracts of *R. sphaeroides* strains bearing a *puc::lacZ* transcriptional fusion (pCF200Km) and containing pRK415, pUI3110, or pUI3102 plasmids in *trans*

Cell extract (transcriptional fusion plasmid)	β -Galactosidase activity with transcriptional fusion <i>puc::lacZ</i> ^a	
	+O ₂	-O ₂
2.4.1(pRK415)	634 \pm 33	3,399 \pm 441
2.4.1(pUI3110)	1,661 \pm 76	3,454 \pm 316
2.4.1(pUI3102)	1,149 \pm 200	ND
Ω P(pRK415)	25 \pm 5	1,411 \pm 162
Ω P(pUI3110)	1,717 \pm 266	3,603 \pm 232
Ω P(pUI3102)	1,621 \pm 301	ND
M7(pRK415)	7 \pm 1	ND
M7(pUI3110)	61 \pm 10	ND
M7(pUI3102)	653 \pm 28	ND

^a β -Galactosidase activity is expressed as micromoles of *O*-nitrophenyl- β -D-galactopyranoside hydrolyzed per minute per milligram of protein. + O₂, aerobic growth. Cells were sparged with 30% O₂, 69% N₂, and 1% CO₂. -O₂, anaerobic growth in the dark in the presence of 70 mM DMSO. ND, not determined.

factor of 14 under aerobic conditions. In the wild type, 2.4.1, in the presence of this 1,828-bp fragment in multicopy, *puc* transcription was increased 2.6-fold relative to that of the control. The presence of the whole 3.9-kb fragment in *trans* (pUI3102) increased *puc* transcription by a factor of 2 in the wild type, 2.4.1. Under anaerobic conditions, the presence of plasmid pUI3110 in Ω P restored *puc* transcription to levels identical to those of the wild type. Unlike under aerobic conditions, pUI3110 does not increase *puc* transcription when present in the wild type, 2.4.1, most probably because expression levels are already high. The fact that the 1,828-bp fragment when cloned in either orientation in pRK415 is able to complement the insertion mutation in Ω P, whereas the 1,493-bp fragment can only complement Ω P when it is under control of the *tet* and *lac* promoters, suggests that the biological activity associated with *mgpS* is contained within the 1,493-bp fragment but can be expressed from promoter sequences present within the 1,828-bp fragment. The presence of a putative Shine-Dalgarno sequence just prior to the GTG codon localized 74 bp downstream of the beginning of the 1,493-bp fragment makes it a potential candidate for a start codon (Fig. 3). These findings support the contention that *mgpS* is actually composed of two loci. However, DNA sequencing of both DNA strands on the exonuclease III deletion clones has been performed several times and has been reconfirmed with internal primers with the intact gene as the template (see Materials and Methods), and no evidence has been obtained for the existence of a stop codon by which the end of a putative upstream ORF could be identified.

To further analyze the structure of *mgpS*, a series of chromosomal deletions were constructed, as described in Materials and Methods and depicted in Fig. 2. The strains MGP 1, MGP 2, MGP 3, and MGP 4, which have had various portions of the 1,493-bp sequence deleted, revealed a decreased coloration identical to that of the Ω P strain. Mobilization of pUI3110 into each of these strains was sufficient to restore the normal red pigmentation. Strain MGP 5, which has a deletion in the proximal portion of *mgpS* but still contains the distal 1,632 bp of the locus, revealed the same phenotype as Ω E; i.e., a red coloration slightly less intense than that observed for 2.4.1.

Expression of the *mgpS* product in *E. coli*. As already described, in order to sequence the 3.9-kb *Bam*HI fragment, we cloned this fragment into pBluescript II SK and performed an exonuclease III deletion of the insert DNA. *E. coli* DH5 α ph was subsequently transformed with the altered plasmids.

Among the clones obtained, one bears a plasmid (pUI3106) in which the proximal 1,070 nucleotides of the 3.9-kb *Bam*HI fragment have been deleted (Fig. 2). The remaining fragment starts 11 nucleotides upstream of the putative ATG start codon of *mgpS*. In this plasmid, the translation initiation site of *mgpS* is in frame with the translation initiation site of the α peptide of β -galactosidase, present in pBluescript II SK. Another derivative was used in the construction of pUI3109. Plasmid pUI3107 contains the distal 1,493-bp fragment, and in this case too, the sequence derived from *mgpS* is in frame with *lacZ*. Soluble and membrane protein extracts of the strains containing the plasmids pUI3106 and pUI3107, as well as that containing the original pBluescript II SK plasmid, grown in the presence of IPTG, were prepared and separated by SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 7). The membrane fraction derived from the strain bearing the pUI3107 plasmid (1,493-bp fragment) contained two proteins with apparent sizes of 65 and 60 kDa not present in the strain bearing pBluescript II SK. On the basis of the nucleotide sequence, presuming there is one translation product from each of the α translation initiation sites and the translation initiation site identified for the 1,493-bp fragment, two proteins with sizes of 54 and 51 kDa are expected. The presence of the induced proteins in the membrane fraction rather than in the soluble fraction suggests that the protein encoded by the 1,493-bp fragment is a membrane-bound protein.

From the strain bearing pUI3106, we would expect the synthesis of a 105-kDa protein. Neither a protein of this size nor one of a smaller size was observed in either the membrane fraction or the soluble fraction of this strain. The 45-kDa protein, which seems to be present in a larger amount in the soluble fraction (Fig. 7A, lane 3) appears to be the protein which is found in the membrane fraction in extracts of cells bearing pBluescript II SK or pUI3107 (Fig. 7B, lanes 1 and 2). This has been observed several times in separate experiments. This does not seem to be the result of contamination of the soluble extract by membranes, since other proteins present in large amounts in the membrane fraction are not visible in the soluble fraction. We have no explanation for this phenomenon, although it may represent the ability of the protein to partition itself to different cellular compartments.

Mapping of *mgpS* in the *R. sphaeroides* 2.4.1 genome. The *R. sphaeroides* 2.4.1 genome has been physically mapped, and it

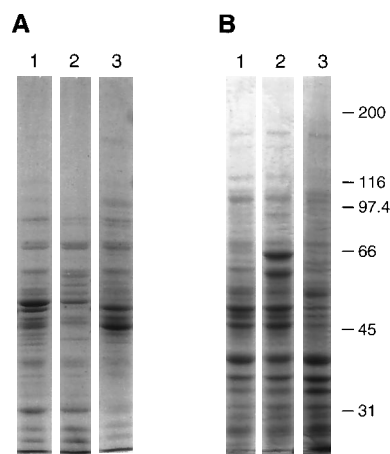


FIG. 7. SDS-PAGE of soluble (A) and membrane (B) extracts of *E. coli* cells containing the plasmid pBluescript II SK (lane 1), pUI3107 (lane 2), or pUI3106 (lane 3) (25 μ g of protein per lane). Molecular masses (in kilodaltons) are given to the right.

has been shown that this bacterium possesses two chromosomes and five endogenous plasmids (38, 39). All of the known photosynthesis structural genes have been mapped to a region of the large chromosome designated the photosynthesis gene cluster. The localization of *mgpS* was accomplished by restriction analysis of Ω P with rare cutting enzymes defined for *R. sphaeroides* DNA (e.g., *AseI*, *DraI*, and *SnaBI*). The Ω Sm^r Sp^r cartridge introduced new *AseI* and *DraI* sites into the genome. After digestion with *AseI*, it was observed that the original 1,105-kb *AseI* A fragment of the large chromosome (39) disappeared and two new bands with sizes of approximately 500 and 600 kb appeared. When a double digestion was performed with *SnaBI* and *AseI*, the 370-kb *SnaBI* fragment gave rise to two new fragments with sizes of 60 and 310 kb. This means that *mgpS* is localized 60 kb from one of the ends of the 370-kb *SnaBI* fragment. Because the digestion of Ω P genomic DNA with *DraI* gave an electrophoresis pattern similar to the one from the wild type (no significant difference in size for the 660-kb fragment), we concluded that *mgpS* maps 2,110 kb clockwise from the *puf* operon in chromosome I of *R. sphaeroides* 2.4.1, far from the photosynthesis gene cluster and other known regulatory elements.

DISCUSSION

The expression of *sacB* encoding the levansucrase of *B. subtilis* is lethal for gram-negative bacteria in the presence of sucrose. A plasmid containing a fusion of the promoterless *sacB* gene to the upstream regulatory region of the *puc* operon was constructed and transferred into *R. sphaeroides* 2.4.1. The exconjugants able to grow on plates containing a previously defined concentration of sucrose showed mutations or deletions in the *puc-sacB* DNA fragment on the plasmid (*cis*-mutations) or mutations in the genomic DNA of the host (*trans*-mutations). One of the *trans*-mutants, M7, contained very small amounts of both types of antennae but was still able to grow photosynthetically, although slowly. The residual level of *puc* transcription, measured with a *puc::lacZ* transcriptional fusion, was less than 2% of that observed for the wild type in cells grown in the presence of oxygen and only 14% of that of the wild type in dark DMSO cultures (Table 3). A 3.9-kb *BamHI* fragment derived from cosmid pUI8256 (10) was able to restore partial pigmentation and full *puc* expression to mutant M7. A 2,793-bp ORF designated *mgpS* was identified within this fragment.

When considered together, the data indicate that the original M7 mutant may contain more than a single mutation. On the one hand, mutations in *mgpS* and M7 have similar, although not identical, effects on *puf* and *puc* transcription, and in both, *puc* expression is more severely compromised. Likewise, either *mgpS* or subclones derived therefrom in *trans* can have a substantial effect on *puc* operon transcription. However, none of these DNA fragments in *trans* can fully restore to M7 spectral complex levels identical to those of the wild type, although mutants derived from mutations in *mgpS* can be fully complemented. M7 is also unusual in that of the numerous photosynthesis mutants we have isolated, this is the first in which we have observed such a dramatic difference between the spectrum obtained under dark DMSO conditions and that at 10 W/m². This may also speak to the presence of more than a single mutation in M7. However it is possible, because of the unusual structure of the *mgpS* locus, that a complex mutational event entirely within that locus is responsible for the unusual phenotype of M7.

mgpS has been interrupted by insertion of an Ω cartridge at two different sites, namely *EspI* and *PstI*, localized in the N-

proximal and C-proximal parts of the predicted protein product, respectively (Fig. 2). Both of the resulting strains, Ω E and Ω P, showed decreased *puf* transcription under aerobic and anaerobic conditions and decreased *puc* transcription under anaerobic conditions (Tables 2 and 3). These results demonstrate that the *mgpS* locus is probably an activator of both the *puc* and *puf* operons. However, under aerobic conditions, Ω P showed less than 10% of the wild-type level of *puc* transcription, while Ω E showed no difference from the wild type, 2.4.1 (Table 3). Moreover, the amount of antennae present in the membrane of Ω E was identical to that in the wild type, while Ω P showed two-thirds less B800-850 complex and ~75% of the B875 complex under dark DMSO conditions (Fig. 5A). One salient feature of these spectral data is that the B800-850 complex is more severely restricted than the B875 complex in both the original mutant and Ω P. At the transcriptional level, Ω P is more highly compromised than M7 for *puf* operon expression, while the reverse is true for *puc* operon expression, although both *puf* expression and *puc* expression are substantially reduced in Ω P.

To further assess the hypothesis that *mgpS* comprises two loci, we decided to further study the corresponding C-proximal portion of *mgpS* alone. The results convincingly demonstrated that the C-terminal region of *mgpS* encodes a protein which has a significant effect upon *puc* and *puf* operon expression and that this portion of the locus is driven by its own promoter. The GTG codon localized 74 bp downstream of the beginning of the 1,493-bp fragment is a good candidate for a start codon, since it is preceded by a good Shine-Dalgarno sequence. Even in the wild type, 2.4.1, this region in multicopy gave rise to a 2.6-fold increase in *puc* transcription. The existence of two loci seems to be confirmed by the fact that strains which had various portions of the 1,493-bp fragment deleted (strains MGP 1, MGP 2, and MGP 3 [Fig. 2]) always resulted in a decreased coloration identical to that of the Ω P strain, which was restored to normal in the presence of pUI3110, while strain MGP5, which had a deletion in the N-proximal part but still contained the terminal 1,632 bp of *mgpS*, had a nearly red coloration only slightly less intense than that observed for 2.4.1.

However, DNA sequencing analyses, repeated several times and confirmed with internal primers, did not reveal the existence of a stop codon which could terminate the first ORF. It has been shown that ribosomes can "slip" during translation (2) and possibly generate a stop codon out of frame (32) and thereby terminate the translation process. It is also possible that if the N portion of the mRNA encodes an RNA helicase, it can affect translation of the mRNA corresponding to this complex locus.

To gain further insight into the coding capacity of *mgpS*, we cloned the fragment containing the last 1,493 bp of *mgpS* and a fragment starting 11 bp upstream of the putative ATG start codon for *mgpS* into pBluescript II SK and expressed these constructions in *E. coli*. In both cases, the DNA sequences were in frame with the sequence of the α peptide. With the plasmid containing the 1,493-bp fragment, we obtained the synthesis of two membrane-bound proteins with sizes of 65 and 60 kDa. These sizes are higher than those expected for proteins expressed from the α peptide translation initiation site and the translation initiation site identified for the 1,493-bp fragment (54 and 51 kDa, respectively). However, it has been shown that the presence of the highly rich alanine-proline repeat regions is responsible for anomalous electrophoretic mobility in other proteins like lipoylacyltransferase or the bovine β -crystallin B1 chains (3, 18). When the plasmid containing the entire sequence of *mgpS* was expressed in *E. coli*, no protein products were observed. The band with a size of approximately 45 kDa,

which seems to be present in a larger amount in the soluble fraction (Fig. 7, lane 3A) appears to be a protein which is present in the membrane fraction in the control and which can partition to two cellular compartments.

If *mgpS* is composed of two separate loci and the distal locus is expressed from its own promoter sequence contained within the 1,828-bp fragment, we would have to conclude that both of these loci encode different proteins, each able to serve as *puc* and *puf* effectors, since disruption of the proximal *mgpS* region at the *EspI* site also resulted in altered *puc* and *puf* transcription (Tables 2 and 3). A more likely hypothesis is that *mgpS* is a single locus encoding two proteins with sizes of 930 and 472 amino acids derived from two different mRNAs, with the sequence of one (distal) contained entirely within the sequence of the other and with both terminated by the same UGA stop codon. A similar but not identical phenomenon has been observed with transposon Tn5, in which two proteins, one a transposase and the other an inhibitor of transposition, are synthesized from the same ORF (same promoter but two different translation initiation sites). The two proteins share the same amino acid sequence, except that the first one has an additional 40 residues at its N terminus (19, 34). If this is the case, both protein products might be expected to activate transcription of the *puc* and *puf* operons, since we saw a decrease in transcription with both ΩP and ΩE disrupted strains. It is also possible that the RNA helicase activity can affect translation of one or both of the mRNA species, such that the protein products are altered and therefore modulate any effect it might have on transcription.

The homology found with RNA helicase proteins which are involved in modifications of mRNA suggests that MgpS could also be involved in posttranscriptional control of photosynthesis gene expression or, in fact, control of other nonphotosynthesis genes. To further examine this hypothesis, we compared the β -galactosidase activities obtained with a *puf::lacZ* transcriptional fusion and a *puf::lacZ* translational fusion present in the disrupted strains ΩP and ΩE (Table 2). The results suggest that the disruption of *mgpS* had a positive effect on posttranscriptional events involved in *puf* operon expression. MgpS could therefore act as an activator of photosynthesis genes at the transcriptional level but could also, under some conditions, negatively regulate their expression at the posttranscriptional level. Whatever the case, disruption of the RNA helicase domain of the *mgpS* locus had no discernible effect on cell growth under the conditions employed here. Additional studies may reveal a unique role for this activity. The finding and initial characterization of the *mgpS* locus raise some interesting possibilities regarding photosynthesis gene expression. Both the behavior of the original mutant and that of the derived mutants reveal the lack of an absolute linkage between transcriptional and posttranscriptional expression of the photosynthetic apparatus. These observations are the subject of ongoing studies.

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