

# Nucleotide Sequence and Characterization of the *Rhodobacter capsulatus hvrB* Gene: HvrB Is an Activator of *S*-Adenosyl-L-Homocysteine Hydrolase Expression and Is a Member of the LysR Family

JOSEPH J. BUGGY, MICHAEL W. SGANGA,† AND CARL E. BAUER\*

Department of Biology, Indiana University, Bloomington, Indiana 47405

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Here we present the nucleotide sequence and characterization of two genes, *hvrB* and *orf5*, that are located in the regulatory gene cluster from *Rhodobacter capsulatus*. The *hvrB* gene, which encodes a protein with a predicted molecular mass of 32 kDa, is shown to be highly homologous to genes encoding members of the LysR family of bacterial transcriptional regulators. A chromosomal disruption of *hvrB* is shown to result in the failure to regulate expression from the nearby *ahcY* and *orf5* genes in response to alterations in light intensity. We show by primer extension mapping that the 5' end of *ahcY*-specific mRNA defines a promoter region exhibiting sequence similarity to known *R. capsulatus* promoter elements. Our mutational analysis further demonstrates that *hvrB* autoregulates its own expression in vivo.

*S*-Adenosyl-L-homocysteine hydrolase (AdoHcyase) is an enzyme responsible for the reversible hydrolysis of *S*-adenosyl-L-homocysteine (AdoHcy) to adenosine and homocysteine (8). AdoHcy is formed as a direct product of transmethylation reactions involving *S*-adenosyl-L-methionine (AdoMet) (25) and is known to be a potent inhibitor of most AdoMet-mediated methyl group transfer reactions. Previously, Sganga et al. (29) cloned and sequenced the gene encoding the AdoHcyase enzyme, *ahcY*, from the purple, nonsulfur photosynthetic bacterium *Rhodobacter capsulatus*. The results of their analysis demonstrated that the AdoHcyase primary structure was highly conserved, exhibiting >60% sequence identity between the eubacterial enzyme and its homologs from such evolutionarily divergent species as *Caenorhabditis elegans*, *Dicystostelium discoideum*, rat, and human (7, 19, 24). A mutant strain of *R. capsulatus* containing a disruption of the *ahcY* gene (SLB1) was shown to be incapable of growth on minimal medium but viable on complex medium or on minimal medium containing exogenously added methionine or homocysteine (29). The viability of this strain on complex medium was somewhat surprising in light of the greatly elevated intracellular AdoHcy level that the cells exhibited as a consequence of the absence of AdoHcyase activity (29). The elevated AdoHcy/AdoMet ratio should be toxic to many methyl group transfer reactions that occur within the cell.

In photosynthetic bacteria, methylation of the sixth propyl group of Mg-protoporphyrin IX during the synthesis of bacteriochlorophyll is catalyzed by the enzyme *S*-adenosyl-L-methionine:magnesium protoporphyrin methyltransferase (MPMT) (18). The reaction is accompanied by a methyl group transfer from AdoMet to Mg-protoporphyrin IX, resulting in the formation of Mg-protoporphyrin monomethylester. Mg-protoporphyrin monomethylester is the first stable intermediate which is unique to the bacteriochlorophyll biosynthetic path-

way (15). Previously, it was shown that a strain lacking AdoHcyase activity synthesizes only low levels of bacteriochlorophyll, indicating that alterations in the intracellular AdoHcy/AdoMet ratio can affect bacteriochlorophyll biosynthesis (29). The inhibitory effect of AdoHcy on bacteriochlorophyll synthesis presumably occurs at the level of the MPMT-catalyzed methyl group transfer, which is known to be inhibited by AdoHcy (11, 18). In this study, we present evidence that *ahcY* expression is transcriptionally repressed by a high light intensity in a manner that mimics the two- to threefold high-light repression of bacteriochlorophyll synthesis. We also provide evidence of the existence of a second gene, *orf5*, which is transcribed divergently and regulated inversely from *ahcY*. Finally, we also demonstrate that light-mediated regulation of *ahcY* and *orf5* is controlled by a nearby open reading frame (ORF) termed *hvrB*. Sequence analysis of *hvrB* indicates that the predicted protein product is very similar to a conserved family of *trans*-acting bacterial regulatory proteins known as the LysR family (17).

## MATERIALS AND METHODS

**Strains, culture, and lighting conditions.** Bacterial strains used in this study are listed in Table 1. *R. capsulatus* St. Louis and SLH5 were grown aerobically by culturing cells in a 250-ml flask that contained a 20-ml volume of PYS broth and that was shaken at 300 rpm and 34°C (33, 35). Photosynthetic cells were grown in 18-ml screw-cap tubes that were illuminated at 34°C with a bank of 60-W incandescent Lumiline lamps. Light intensity was controlled by shading cultures with neutral-density filters, light intensity being measured with a Weston Instruments Inc. model 755 illumination meter. Cultures were monitored for growth rate by measuring turbidity at various times with a Klett-Summerson spectrophotometer (red filter).

**Sequence analysis.** Plasmid pMW3.1, which encodes the regulatory gene cluster from which *hvrB* and *orf5* were sequenced, has been described elsewhere (29). Both strands of the *hvrB* and *orf5* genes were sequenced in an M13 subclone with Sequenase and Taqenase sequencing kits in accordance with the manufacturer's directions (United States Biochemical Corp.). DNA sequence data were analyzed on a  $\mu$ Vax com-

\* Corresponding author. Mailing address: Department of Biology, Indiana University, Jordan Hall, Bloomington, IN 47405. Phone: (812) 855-6595. Fax: (812) 855-6705.

† Present address: San Francisco General Hospital, San Francisco, CA 94110.

TABLE 1. Bacterial strains, bacteriophages, and plasmids

Strain, bacteriophage, or plasmid	Relevant characteristics	Source or reference
<b>Strains</b>		
<i>E. coli</i>		
NM522	<i>supE thi Δ (lac proAB) Δhsd-5 (F' proAB lacI<sup>q</sup>ZΔM15)</i>	Gaugh and Murray (12)
Tec5	C600(pDPT51) (mobilizing vector)	Taylor et al. (31)
<i>R. capsulatus</i>		
St. Louis		
SLH5	<i>rif-10</i>	Sganga et al. (29)
SLB1	<i>hvrB::Km<sup>r</sup> rif-10</i>	This study
CB1127	<i>ahcY::Km<sup>r</sup> rif-10</i>	Sganga et al. (29)
	<i>crnG121 rif-10</i> ; gene transfer agent overproducer	Young et al. (35)
<b>Bacteriophages</b>		
M13mp10		Messing and Vieira (22)
M13mp11		Messing and Vieira (22)
<b>Plasmids</b>		
pBR322Ω	Ω Sp <sup>r</sup> cassette	Prentki and Krisch (27)
pCB800	<i>XhoI-EcoRI</i> fragment from pMS3.1 cloned into <i>SalI-EcoRI</i> sites of pNM481	This study
pCB800Ω	Ω Sp <sup>r</sup> cassette cloned into <i>EcoRI</i> site of pCB800	This study
pCB801	<i>SmaI-BglIII</i> fragment from pMS3.1 cloned into <i>SmaI-BamHI</i> sites of pNM481	This study
pCB801Ω	Ω Sp <sup>r</sup> cassette cloned into <i>XhoI</i> site of pCB801	This study
pJB802	<i>BamHI-HindIII</i> fragment from pJB114 cloned into <i>BamHI-HindIII</i> sites of pNM481	This study
pJB802Ω	Ω Sp <sup>r</sup> cassette cloned into <i>SmaI</i> site of pJB802	This study
pJB114	2.8-kb <i>SmaI</i> fragment from pMS3.1 cloned into <i>HincII</i> site of pTZ19r	This study
pMS3.1	Cosmid clone of regulatory gene cluster	Sganga and Bauer (30)
pNM481	Translational <i>lacZ</i> fusion vector	Minton (23)
pTZ19r	Multifunctional cloning vector; Ap <sup>r</sup> <i>lacZ'</i>	United States Biochemical Corp.
pTZ18u	Multifunctional cloning vector; Ap <sup>r</sup> <i>lacZ'</i>	United States Biochemical Corp.
pUC4-KIXX	Km <sup>r</sup> cassette	Barany (1)

puter with programs from the Sequence Analysis Package of the University of Wisconsin Genetics Computer Group and submitted to GenBank under accession number L23836.

**Interposon mutagenesis.** Site-directed mutagenesis of *hvrB* was accomplished by recombining a kanamycin resistance (Km<sup>r</sup>) cassette into the chromosomal copy of *hvrB*. A plasmid-encoded disruption of *hvrB* was constructed by first subcloning a *BglIII-XhoI* restriction fragment from pMW3.1 (30) into *SalI-BamHI* sites of pTZ18 (United States Biochemical Corp.) and then cloning a *SmaI* restriction-digested Km<sup>r</sup> cassette obtained from pUC4-KIXX (1) into the *SmaI* site present in the subcloned *hvrB* fragment. The *hvrB::Km<sup>r</sup>* plasmid construct was then transformed into the *Escherichia coli* plasmid-mobilizing strain Tec5 and then mobilized by conjugation into the gene transfer agent-overproducing strain CB1127. The gene transfer agent was collected from the culture supernatant of the CB1127 exconjugant and subsequently used to recombine the interposon into the chromosome of strain St. Louis according to the method of Scolnik and Haselkorn (28). The resulting strain, SLH5, contains the Tn5 Km<sup>r</sup> gene recombined into the 157th codon of *hvrB*.

**Reporter plasmid construction.** pJB802 was constructed by a two-step cloning procedure (Table 1). First, a 2.8-kb *SmaI* restriction fragment which cuts within *hvrB* and which contains several hundred bases of upstream DNA was isolated from digested pMW3.1 DNA (29) and subsequently ligated into *HincII*-digested cloning vector pTZ19r, creating plasmid pJB114. Flanking *BamHI* and *HindIII* restriction sequences in the pTZ19r polylinker region enabled the reexcision of the 2.8-kb *hvrB*-containing fragment with *BamHI* and *HindIII*; this fragment was then cloned into similar sites present in *lacZ*

fusion vector pNM481 (23). Finally, a *SmaI* restriction fragment from pBR322Ω, containing the Ω spectinomycin resistance transcription-translation termination fragment, was subcloned into a unique *SmaI* site, creating plasmid pJB802Ω. Plasmid pCB800Ω, which contains an in-frame fusion of the 141st codon of the *orf5* gene product with β-galactosidase, was constructed by subcloning a 650-bp *XhoI-EcoRI* restriction fragment from pMS3.1 (28) into *EcoRI-SalI* sites of pNM481 (22). The Ω site was subsequently cloned as an *EcoRI* restriction fragment from pBR322Ω (27) into the *EcoRI* site of pCB800, creating construct pCB800Ω. Plasmid pCB801 was constructed by cloning a 1.7-kbp *SmaI-BglIII* restriction fragment from pMW3.1 into *SmaI-BamHI* restriction sites in pNM481, creating an in-frame fusion of the 35th codon of *ahcY* to the *lacZ* gene. A *SmaI* restriction fragment containing the Ω site was then cloned into a unique *XhoI* site in pCB801 by digesting pCB801 with *XhoI* and then filling in the 5'-overhanging ends with deoxynucleoside triphosphates and the Klenow fragment of DNA polymerase, resulting in plasmid construct pCB801Ω. Each of the reporter plasmids was transformed into strain Tec5 and then mobilized into relevant *R. capsulatus* strains by previously described conjugation techniques (35).

**β-Galactosidase activity.** Cultures were grown either photo-synthetically in 18-ml screw-cap tubes or aerobically in 250-ml Erlenmeyer flasks, as indicated above. When appropriate, overnight cultures were subcultured to 5 Klett units in RCV (or RCV supplemented with 150 μg of various metabolites per ml). Cultures were subsequently harvested at a cell density of 1.5 × 10<sup>8</sup> cells per ml, disrupted by sonication, and assayed for β-galactosidase activity as described previously (35). Units of

activity represent micromoles of *o*-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) hydrolyzed per minute per milligram of protein.

**RNA isolation.** The following RNA isolation procedure was adapted from the method of Chomczynski and Sacchi (5). A 500-ml culture of *R. capsulatus* St. Louis cells was grown anaerobically under high-light (6,000 lx) conditions to late log phase. Cells were harvested by centrifugation for 10 min at  $10,000 \times g$ , and the pellets were resuspended in a 250- $\mu$ l solution containing 5 mg of lysozyme per ml. After incubation at 4°C for 20 min, the cells were diluted with 15 ml of a denaturing solution consisting of 4.23 M guanidinium thiocyanate, 42 mM citrate, 0.83% sarcosine, and 0.2 mM  $\beta$ -mercaptoethanol buffer. To extract RNA, 1.2 ml of 2 M sodium acetate (pH 4.0) was added, and the mixture was vortexed with a 12-ml solution of phenol-chloroform-isoamyl alcohol (24:24:1). This mixture was then centrifuged at  $10,000 \times g$  for 20 min at 4°C. The top aqueous phase was removed, and the RNA was precipitated from this phase by the addition of an equal volume of isopropanol; incubation was done for 30 min at -20°C. The RNA was pelleted by centrifugation at  $10,000 \times g$  and resuspended in 2 ml of denaturing solution, from which the RNA was reprecipitated as described above. Finally, the RNA was pelleted, washed with 75% ethanol, and dried in a vacuum desiccator. The RNA was suspended in distilled H<sub>2</sub>O and stored at -70°C.

**Primer extension analysis.** An *ahcY*-specific synthetic oligonucleotide primer (5'-TTGCGGCCGAAGCTCGGCCAGTTT-3') was 5' end labeled by mixing 90 ng of oligonucleotide with 100  $\mu$ Ci of [ $\gamma$ -<sup>32</sup>P]ATP and 5 U of T4 polynucleotide kinase in a 50- $\mu$ l kinase buffer reaction mixture (kinase buffer contains 50 mM Tris-HCl [pH 9.0], 10 mM MgCl<sub>2</sub>, 5 mM dithiothreitol, and 5% glycerol) for 1 h at 37°C. The labeled primer was added to 75  $\mu$ g of RNA, and to this mixture was added a 1/4 volume of 2 M ammonium acetate and 3 volumes of ethanol. Following 10 min on dry ice, the mixture was centrifuged for 10 min and dried in a vacuum desiccator. The pellet was dissolved in a hybridization buffer consisting of 0.4 M NaCl, 400 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES) (pH 6.4), 1 mM EDTA, and 80% formamide. The mixture was heated to 80°C and slowly cooled to the hybridization temperatures of 55°C for the *orf5* primer and 54°C for the *ahcY* primer {hybridization temperature = 29.3 + [0.41(%G+C content of the primer)]} over a period of 2 h. The mixture was precipitated by adding 45  $\mu$ l of 2 M ammonium acetate, 145  $\mu$ l of distilled H<sub>2</sub>O, and 800  $\mu$ l of ethanol, chilled for 10 min on dry ice, and then pelleted by microcentrifugation for 10 min. The pellet was dissolved in 10  $\mu$ l of 10 mM PIPES-400 mM NaCl, and then S1 buffer was added (S1 buffer contains 500  $\mu$ M each deoxynucleoside triphosphate, 10 mM dithiothreitol, 8 mM MgCl<sub>2</sub>, 50 mM Tris-HCl [pH 8.2], and 25  $\mu$ g of actinomycin D per ml). Five units of avian myeloblastosis virus reverse transcriptase was added, and the mixture was incubated for 1 h at 42°C. The nucleic acid was phenol extracted, ethanol precipitated, resuspended in denaturing solution, heated to 90°C, and run on a 6% Long-Ranger polyacrylamide gel (from AT Biochem Corp.) alongside a sequencing ladder, which was created by use of the same oligonucleotide primers and chain termination sequencing reactions (Sequenase sequencing kit).

## RESULTS

**Sequence analysis of *hvrB*.** In a previous study, we demonstrated that *ahcY* was located within a cluster of regulatory genes known to be involved in controlling synthesis of the *R.*

*capsulatus* photosystem (29). In an effort to further characterize the regulatory gene cluster, we subcloned and sequenced from cosmid pMS3.1 a 1.9-kb *EcoRI*-*Bgl*II restriction fragment that contained a segment of DNA upstream of *ahcY* (Fig. 1). Inspection of the sequence in this region revealed the presence of two ORFs, called *orf5* and *hvrB*, of 615 and 878 nucleotides, respectively, that are divergently transcribed from *ahcY*. Figure 2 shows a codon preference plot (16) generated from the *hvrB* and *orf5* sequences by use of a codon preference table generated from known transcribed *R. capsulatus* genes (35). The plot indicates that this sequence indeed forms two adjacent ORFs containing a biased codon usage that is typical of transcribed *R. capsulatus* genes (35).

A computer-assisted search of the GenBank data base revealed no entries with significant similarities to the *orf5* sequence. However, a search of the GenBank data base with *hvrB* did reveal that the predicted amino acid sequence of HvrB shares a high degree of sequence similarity with several members of a well-characterized family of bacterial transcriptional regulators known as the LysR family (17). Proteins of the LysR family share several key distinguishing features: (i) they tend to be approximately 300 amino acids long; (ii) they contain a consensus helix-turn-helix DNA binding motif within a conserved amino terminus; (iii) they generally regulate the transcription of nearby divergently transcribed promoters; and (iv) their intracellular levels tend to be regulated autogenously (10, 17, 32). As diagrammed in Fig. 1 and 3, HvrB is typical in many respects of members of this family of regulatory proteins. It is a 292-amino-acid polypeptide that exhibits a high degree of sequence identity in its amino-terminal region to other members of this family (HvrB exhibits >50% sequence identity to the amino-terminal region of AmpR and TrpI, as shown in Fig. 3). HvrB also contains a putative helix-turn-helix DNA binding motif in its amino-terminal region (boxed region in Fig. 1), as predicted by the method of Dodd and Egan (9). Also presented below is evidence that HvrB is responsible for controlling expression of the nearby *orf5* and *ahcY* genes, as well as evidence that HvrB autogenously regulates its own expression.

***ahcY* and *orf5* expression is light regulated.** Members of the LysR family typically regulate tightly linked divergently transcribed genes in response to a metabolic compound that is generated or used by the regulated operon (4, 10, 26). We therefore set up assays to determine whether the nearby divergently transcribed *orf5* and *ahcY* genes are similarly regulated by HvrB in response to metabolites of the activated methyl cycle. To this end, we created translational reporter plasmid pCB801 $\Omega$ , which contains an in-frame fusion of the 35th codon of *ahcY* to the *E. coli lacZ* gene. We first postulated that *ahcY* expression could be regulated in response to the levels of methyl cycle-associated metabolites, such as AdoHcy, which is the toxic metabolite that is cleaved by the *ahcY*-encoded AdoHcyase enzyme. To address this possibility, we assayed *lacZ* expression from pCB801 $\Omega$  when this plasmid was present in both the wild-type parent strain, St. Louis, and the *ahcY*-disrupted strain, SLB1. A previous analysis indicated that strain SLB1 contains a 10-fold elevation in the intracellular level of AdoHcy (29). Thus, if *ahcY* expression were regulated in response to the intracellular AdoHcy/AdoMet ratio, then its expression should be altered in SLB1. Surprisingly, the results of  $\beta$ -galactosidase assays (Table 2) indicated that *ahcY::lacZ* expression from pCB801 $\Omega$  was the same in both strains. This result would indicate that *ahcY* expression is not significantly regulated by the intracellular level of AdoHcy. We next assayed *ahcY::lacZ* expression when cells were grown in media supplemented with 150  $\mu$ g of various methyl cycle metabolites

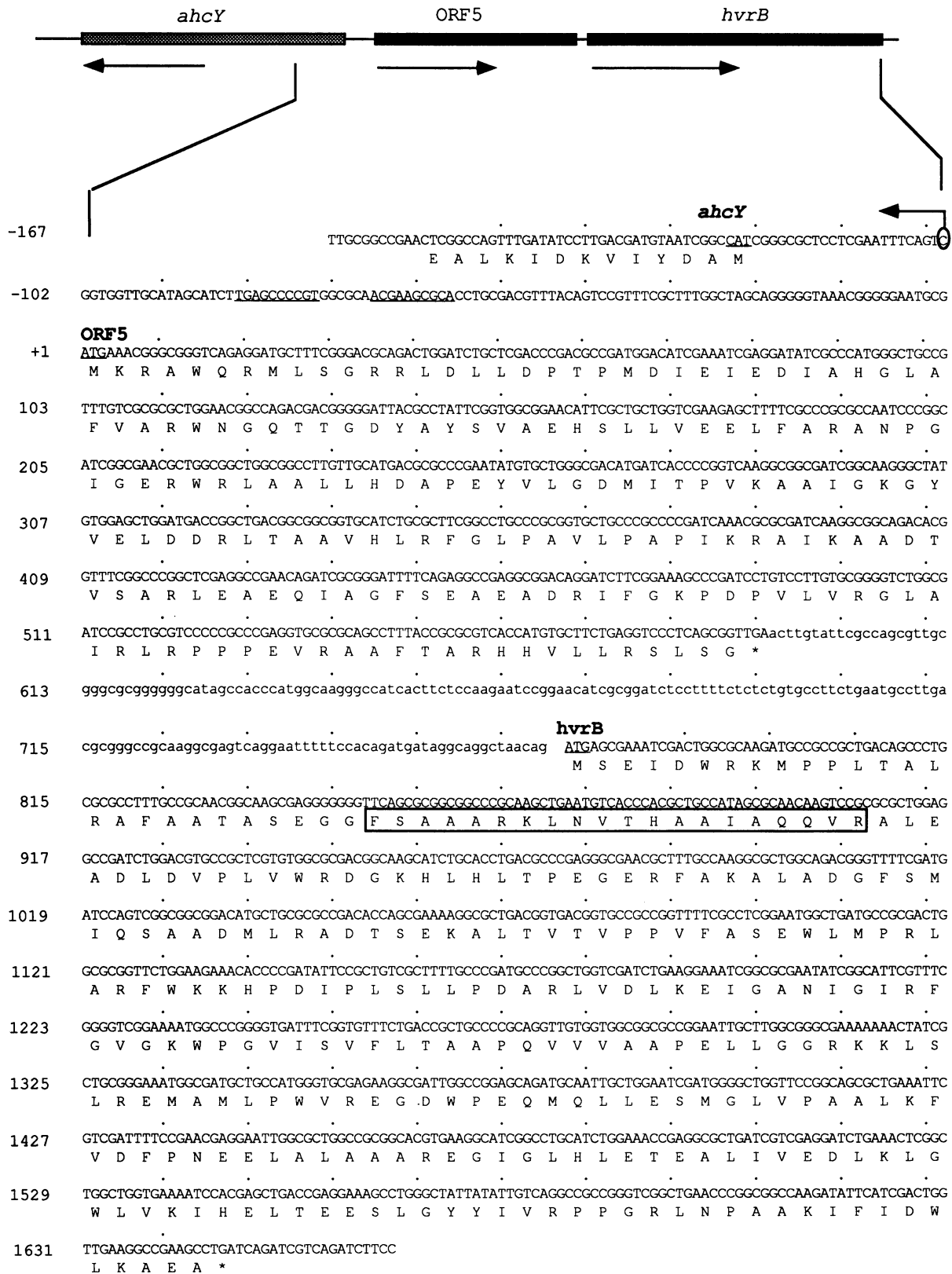


FIG. 1. Nucleotide sequence and organization of the *orf5* and *hvrB* loci. The sequence starts with the 5' end of the adjacent *ahcY* gene, which has been described elsewhere (29). The deduced amino acid sequence is shown below the nucleotide sequence, in the one-letter code. The ATG initiation codons (as predicted by codon preference analysis) are underlined. The transcription start site for *ahcY* is indicated by an arrow. A 26-bp inverted repeat just upstream from the *orf5* coding sequence is also underlined. The predicted helix-turn-helix DNA binding motif within HvrB is boxed.

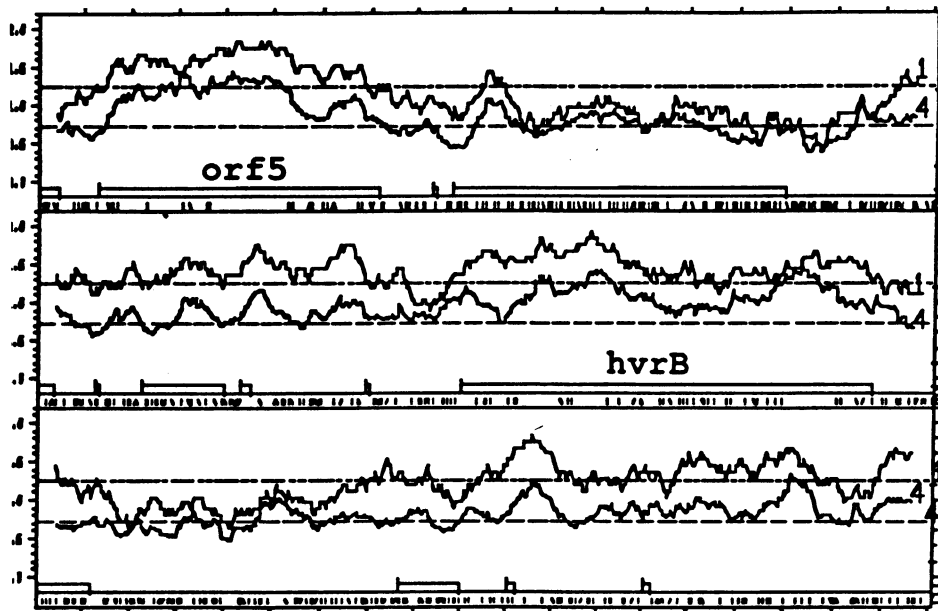


FIG. 2. Codon preference plot indicating translated regions of the *R. capsulatus* sequence. A statistical plot of the codon utilization (lower line) and third-position G · C bias (upper line) for each reading frame is shown in the three graphs, with each graph representing a different reading frame. The program identifies efficiently translated genes by virtue of ORFs (open boxes) which contain few rare codons (vertical dashes below the ORFs) and exhibits a rise in the graph above random utilization, which is denoted as a broken line. The *orf5* and *hvrB* ORFs are indicated. A codon utilization data base obtained from known *R. capsulatus* genes has been described elsewhere (35). This plot was generated by use of the statistical program of Gribskov et al. (16).

per ml as well as with analogs of metabolites in the methyl cycle to address whether any of these compounds could affect *ahcY* expression. The results of this analysis (Table 2) also indicated that *ahcY* expression is not significantly affected by the exogenous addition of these metabolites, at least at the concentrations that we tested. Thus, *ahcY* expression does not appear to be regulated either in response to the intracellular AdoMet/AdoHcy ratio or by a number of additional metabolites in the activated methyl cycle.

As shown in the study of Sganga et al. (29), one physiological consequence of disrupting *ahcY* in *R. capsulatus* is that it leads to a severe reduction of bacteriochlorophyll biosynthesis. This

reduction is presumed to be a consequence of an increase in the intracellular level of AdoHcy, which is known to be a potent inhibitor of a methylation reaction that occurs at the first committed step of the Mg-tetrapyrrole biosynthetic pathway (18). Since the intracellular level of bacteriochlorophyll is known to be repressed two- to threefold in response to an increase in environmental light conditions (6), it is possible that the regulation of intracellular AdoHcyase represents a contributing factor by which *R. capsulatus* regulates photopigment biosynthesis in response to various light conditions. As shown in the bar graphs in Fig. 4, *ahcY::lacZ* expression from wild-type cells harboring pCB801Ω is indeed repressed twofold

E. coli	LysR	5	N	L	R	H	I	E	I	F	H	A	V	M	T	A	G	S	L	T	E	A	A	H	L	L
E. coli	MetR	3	E	I	K	H	L	K	T	L	Q	A	L	R	N	S	G	S	L	A	A	A	A	A	L	L
E. coli	IlvY	2	D	L	R	D	L	K	T	F	L	H	L	A	E	E	R	H	F	G	R	S	A	R	A	M
R. meliloti	NodD	10	D	L	N	L	L	V	A	L	D	A	L	M	T	E	R	K	L	T	A	A	A	R	R	I
E. cloacae	AmpR	7	P	L	N	S	L	R	A	F	E	A	A	A	R	H	L	S	F	T	H	A	A	I	E	L
P. aeruginosa	TrpI	7	S	L	N	A	L	R	A	F	E	A	A	A	R	L	H	S	I	S	L	A	A	E	E	L
R. capsulatus	HvrB	11	P	L	T	A	L	R	A	F	A	A	T	A	S	E	G	G	F	S	A	A	A	R	K	L

H	T	S	Q	P	T	V	S	R	E	L	A	R	F	E	K	V	I	G	L	K	L	F	E	R	V	R	G	R	L	H	P	T	V	Q	G
H	Q	T	Q	S	A	L	S	H	Q	F	S	D	L	E	Q	R	L	G	F	R	L	F	V	R	K	S	Q	P	L	R	F	T	P	Q	G
H	V	S	P	S	T	L	S	R	Q	I	Q	R	L	E	E	D	L	G	Q	P	L	F	V	R	D	N	R	T	V	T	L	T	D	A	G
N	L	S	Q	P	A	M	S	A	A	I	A	R	L	R	T	Y	F	G	D	E	L	F	S	M	Q	G	R	E	L	I	P	T	P	R	A
N	V	T	H	S	A	I	S	Q	H	V	K	T	L	E	Q	H	L	N	C	Q	L	F	V	R	V	S	R	G	L	M	L	T	T	E	G
H	V	T	H	G	A	V	S	R	Q	V	R	L	L	E	E	D	I	G	V	A	L	F	G	R	D	G	R	G	V	K	L	T	D	S	G
N	V	T	H	A	A	I	A	Q	Q	V	R	A	L	E	A	D	L	D	V	P	L	V	R	D	G	K	H	L	H	L	T	P	E	G	

FIG. 3. Alignment of the N-terminal portions of the HvrB amino acid sequence and sequences of members of the LysR family. HvrB is aligned to six other peptides of the LysR family from four different species of bacteria. Residue numbers are given at the beginning of each sequence. Residues in black boxes indicate sequences in HvrB which are identical to at least four of the six residues within each group of proteins. Similar residues or conservative substitutions are shaded.

TABLE 2. Activity of *ahcY* in various media

Medium supplementation <sup>a</sup>	$\beta$ -Galactosidase activity <sup>b</sup> of:	
	St. Louis(pCB801 $\Omega$ )	SLB1(pCB801 $\Omega$ )
None	492	497
D-Methionine	429	527
L-Methionine	619	481
D-Homocysteine thiolactone	533	575
AdoHcy	482	521
DL-Homocysteine	501	501

<sup>a</sup> *R. capsulatus* cultures were grown overnight in RCV minimal media, subcultured into RCV supplemented with 150  $\mu$ g of each of the various compounds per ml, and harvested at early log phase.

<sup>b</sup> Expressed as micromoles of ONPG hydrolyzed per minute per milligram of protein.

when the cells are grown under high-light conditions (6,000 lx) compared with when the cells are grown under low-light conditions (100 lx). Thus, light-mediated *ahcY* regulation does correlate well with the known two- to threefold light regulation of bacteriochlorophyll synthesis which is observed when cells are grown under similar lighting conditions.

We next assayed gene expression from *orf5*, which is transcribed divergently from *ahcY* in strains grown under both high- and low-light conditions. We therefore constructed reporter plasmid pCB800 $\Omega$ , in which the sequence encoding the first 142 amino acids of the *orf5* gene product is fused to *lacZ*. Measurement of  $\beta$ -galactosidase expression from pCB800 $\Omega$  in wild-type strain St. Louis demonstrated that *orf5::lacZ* expression is indeed regulated fivefold by light intensity (Fig. 4). However, as also indicated in Fig. 4, this regulation is opposite to that observed for *ahcY* expression, in that *orf5* is maximally

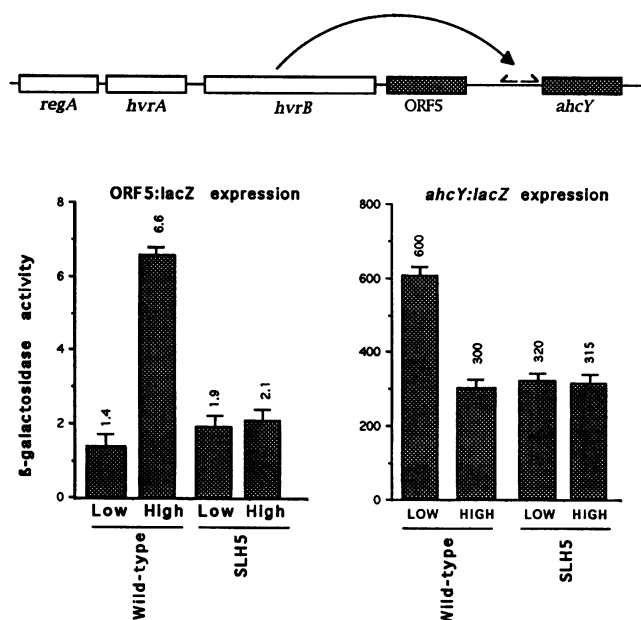


FIG. 4. Regulation of the *orf5* and *ahcY* genes. Expression was measured by assaying  $\beta$ -galactosidase activity from plasmid pCB800 $\Omega$ , harboring a fusion of *orf5* to the *lacZ* gene, and plasmid pCB801 $\Omega$ , which harbors an *ahcY::lacZ* fusion. In strain SLH5 (*hvrB*), light-specific induction of *ahcY* and *orf5* is abolished. Values represent averages of three independent measurements. Shown above the bar graphs is a model in which HvrB binds to and regulates expression from the *orf5* and *ahcY* light-regulated promoters.

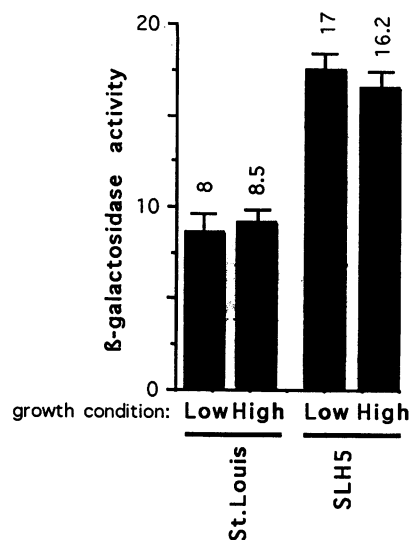


FIG. 5. Autoregulation of *hvrB*. The bar graphs show the relative levels of  $\beta$ -galactosidase activity from plasmid pJB802, which harbors a fusion of the *hvrB* structural gene to *lacZ*. Assays were performed with cells grown anaerobically under either high (8,000 lx)- or low (250 lx)-light conditions. Values represent averages of three independent measurements.

induced under high-light conditions, whereas *ahcY* is maximally induced under low-light conditions.

***hvrB* affects light-mediated regulation of *ahcY* and *orf5*.** We next constructed a derivative of strain St. Louis (SLH5) which contains a Km<sup>r</sup> gene inserted into the 157th codon of *hvrB* (see Materials and Methods). This strain was then used to address whether *hvrB* is involved in the observed light-mediated regulation of the *ahcY* and *orf5* genes. For this analysis, *ahcY* and *orf5* expression was measured by assaying  $\beta$ -galactosidase activity from derivatives of SLH5 that harbored pCB801 $\Omega$  and pCB800 $\Omega$ . The results of this analysis (Fig. 4) demonstrated that the observed light-mediated regulation of *ahcY* and *orf5* is abolished in SLH5. Both of these genes are expressed at a constitutively decreased level, indicating that *hvrB* functions both as a low-light activator of *ahcY* expression and as a high-light activator of *orf5* expression.

**The *hvrB* gene is itself negatively autoregulated.** A further aspect of LysR family members is that they typically autoregulate their expression as a means of maintaining appropriate intracellular levels (17). To determine whether *hvrB* expression is similarly autoregulated, we constructed plasmid pJB802, which contains an in-frame fusion of *hvrB* to *lacZ*.  $\beta$ -Galactosidase expression from pJB802 was assayed in strain St. Louis and in *hvrB*-disrupted strain SLH5. As diagrammed in Fig. 5, *hvrB* expression does indeed appear to regulate itself, as evidenced by the twofold-higher level of  $\beta$ -galactosidase expression observed in SLH5 compared with the parent strain, St. Louis. Furthermore, *hvrB* expression does not appear to be regulated by light intensity, as is the case for *ahcY* and *orf5* expression (Fig. 4).

**Primer extension analysis.** To better understand the characteristics of a light-regulated promoter, the 5' end of the *ahcY* transcript was mapped by primer extension (Fig. 6). The 5' end of the *ahcY* message was mapped to a G residue 23 nucleotides upstream of the AUG initiation codon of *ahcY* by use of a 24-base synthetic oligonucleotide primer complementary to the *ahcY* message. It could also be argued, however, that the location of the primer extension product corresponds to the

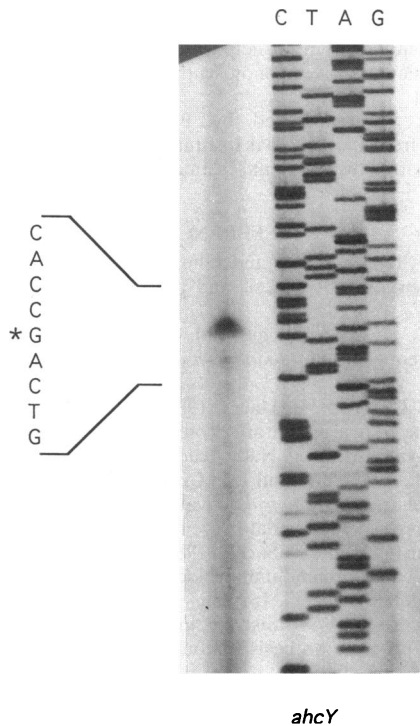


FIG. 6. Mapping of the 5' end of *ahcY* mRNA. A 5'-<sup>32</sup>P-labeled primer complementary to *ahcY* was hybridized to total cellular RNA from wild-type *R. capsulatus*. A strand complementary to the specific transcript was then synthesized by use of avian myeloblastosis virus reverse transcriptase and separated by electrophoresis on a 6% sequencing gel. A DNA sequencing ladder was generated from the same primer in a recombinant M13 template. The position of the resulting band, indicating the start site of transcription, is indicated in the sequence with an asterisk.

adjacent A residue located 3' to the aforementioned G residue. While this ambiguity does not hinder the overall conclusions of our analysis, we note it here nonetheless. *ahcY* promoter elements similar to *E. coli*  $\sigma^{70}$  consensus -10 and

-35 regions, as well as to other *R. capsulatus* promoter elements (20), are indicated in Fig. 7. Also indicated in Fig. 7 is a 26-bp region of dyad symmetry that overlaps the *ahcY* -35 promoter region and that could possibly serve as a binding site for the *hvrB* gene product.

DISCUSSION

In this study, we demonstrate that the expression of two adjacent and divergently transcribed genes, *ahcY*, encoding the enzyme AdoHcyase, and *orf5*, a gene of unknown function, is differentially regulated in response to environmental light intensity. Evidence is also presented for the existence of a regulatory gene, *hvrB*, the product of which is required for the light-dependent activation of both *ahcY* and *orf5*.

The predicted amino acid sequence of HvrB exhibits a high degree of sequence similarity to the family of bacterial transcriptional regulatory proteins known as the LysR family (17). This family is characterized by several criteria (10, 17, 32). First, these proteins typically function as transcriptional regulators of nearby, usually divergently transcribed promoters. Second, members of this family of regulatory proteins tend to have a similar mass (30 to 40 kDa). Third, they exhibit sequence similarity within the amino terminus, which is also the region that contains a helix-turn-helix DNA binding motif. Finally, they negatively autoregulate their own expression. As demonstrated by our analysis, HvrB meets all of these criteria for a transcriptional activator that belongs to the LysR group.

Despite the similarities of HvrB to LysR members, HvrB is somewhat atypical, in that most LysR-type regulators utilize compounds which are somewhat directly involved with the activities of the target operons as coregulators. For example, homocysteine is a coactivator for MetR (26), flavonoids are coactivators for NodD (10), and indoleglycerol phosphate is a coactivator for TrpI (4), etc. HvrB-regulated expression of *ahcY*, however, does not appear to be affected by the intracellular ratio of AdoMet to AdoHcy, the presence of a variety of metabolites associated with the activated methyl cycle, or the metabolite analogs that we have tested (Table 2). The only reproducible effect that we found for *ahcY* and *orf5* expression is light intensity, which is abolished in an *hvrB*-disrupted

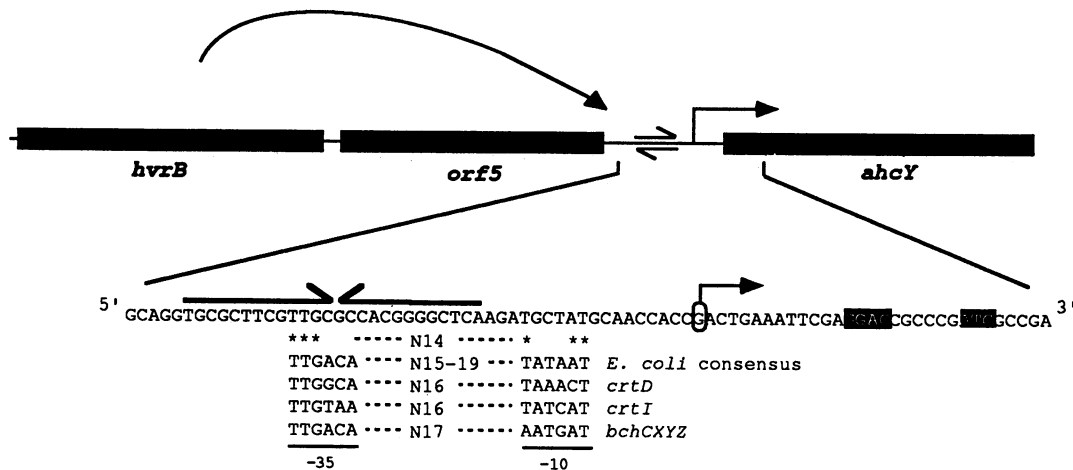


FIG. 7. Arrangement of the *ahcY* promoter region. The start site of transcription, as determined by primer extension analysis, is indicated by an arrow within the sequence. Asterisks indicate sequences identical to *E. coli*  $\sigma^{70}$  consensus promoter elements. Convergent arrows indicate a 26-bp region of dyad symmetry. The ATG initiation codon and consensus GGAG ribosome binding site are highlighted. Sequences of *R. capsulatus* promoter elements have been described elsewhere (21).

background. There still remains, however, a formal possibility that HvrB regulation of *ahcY* expression responds to some metabolite in the activated methyl pathway and that we have not yet determined the correct conditions under which this regulation can be observed.

If we assume that light intensity is indeed being sensed by HvrB, then we need to address why *R. capsulatus* cells have developed a mechanism of regulating *ahcY* expression in response to light intensity. Numerous studies have indicated that photosynthetic bacteria have a complex set of overlapping regulatory factors that tightly control the synthesis of bacteriochlorophyll in response to such factors as oxygen tension, light intensity, and protein synthesis (2, 3, 34, 35). Surprisingly, several early studies also indicated that alterations in the ability of the cells to synthesize AdoMet have profound effects on the capability of the cells to synthesize bacteriochlorophyll (13, 14, 20). For example, early inhibitor studies demonstrated that the inhibition of methionine biosynthesis, either by feedback inhibition by threonine or by the addition of the methionine analog ethionine, severely reduced bacteriochlorophyll biosynthesis, even at concentrations that did not impair growth (13, 14). Similarly, methionine auxotrophs were also shown to lack the capability to synthesize bacteriochlorophyll, even when supplemented with high levels of methionine (20). A conclusion derived from these early studies was that these treatments interfered with the intracellular level of AdoMet, which consequently resulted in the inhibition of bacteriochlorophyll synthesis. The mechanism of this inhibition is thought to occur at the methylation of Mg-protoporphyrin IX, which is the first committed step of the Mg-tetrapyrrole branch of the bacteriochlorophyll pathway. The enzyme responsible for this reaction, MPMT, is known to be noncompetitively inhibited by AdoHcy at a  $K_i$  that is similar to the  $K_m$  for AdoMet (18, 29). Thus, the ability of the cell to alter the AdoHcy/AdoMet ratio could be a controlling factor in the synthesis of bacteriochlorophyll. On the surface, it would appear that high-light repression of *ahcY* expression could be an important mechanism for controlling bacteriochlorophyll biosynthesis in response to light intensity. However, it should be noted that strains which are disrupted for *hvrB* appear to exhibit normal, 1.5- to 2-fold in vivo light regulation of bacteriochlorophyll synthesis over the range of intensities that we have tested (6,000 to 120 lx; unpublished data). Thus, although we believe that light regulation of *ahcY* expression may be a mitigating factor in regulating the synthesis of bacteriochlorophyll, it most likely is only a minor component in a more complex multifaceted process that is responsible for the control of photopigment biosynthesis.

To further our understanding of light-regulated *R. capsulatus* promoters, of which little is known, we mapped the 5' end of the *ahcY*-specific mRNA by primer extension. The results (Fig. 6 and 7) indicate that the start site of *ahcY* transcription lies in a region with a good match to previously characterized promoter elements in *R. capsulatus*. Interestingly, at a position 18 to 44 bases upstream of the 5' end of the *ahcY* mRNA lies a 26-bp region of dyad symmetry which overlaps the *ahcY* -35 promoter region. Since many DNA binding proteins interact with regions of dyad symmetry, it is possible that this region serves as a binding site for HvrB and subsequently affects the transcription of *ahcY* and *orf5* under different light conditions. Further biochemical studies are needed to determine whether there is a chromophore associated with purified HvrB and if, in fact, HvrB binds to DNA in a light-dependent manner.

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