AerR, a Second Aerobic Repressor of Photosynthesis Gene Expression in *Rhodobacter capsulatus*

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Open reading frame *orf192*, which is located immediately upstream of the aerobic repressor gene *crtJ*, was genetically and biochemically demonstrated to code for a second aerobic repressor (AerR) of photosynthesis gene expression in *Rhodobacter capsulatus*. Promoter-mapping studies indicate that *crtJ* has its own promoter but that a significant proportion of *crtJ* expression is promoted by read-through transcription of *orf192* (AerR) transcripts through *crtJ*. Disruption of *aerR* resulted in increased photopigment biosynthesis during aerobic growth to a level similar to that of disruption of *crtJ*. Like that reported for *CrtJ*, β-galactosidase assays of reporter gene expression indicated that disruption of *aerR* resulted in a two- to threefold increase in aerobic expression of the *puc* and *puf* operons. However, unlike *CrtJ*, AerR aerobically represses *puf* operon expression and does not aerobically repress *bchC* expression. Gel mobility shift analysis with purified AerR indicates that AerR does not bind to a *bchC* promoter probe but does bind to the *crtJ*, *puc*, and *puf* promoter probes. These results indicate that AerR is a DNA-binding protein that targets genes partially overlapping a subset of genes that are also controlled by *CrtJ*. We also provide evidence for cooperative binding of AerR and *CrtJ* to the *puc* promoter region.

Oxygen tension is an important factor regulating synthesis and assembly of the photosynthetic apparatus in photosynthetic bacteria (7). Oxygen suppression of photosystem synthesis is mainly attributed to the activation or inactivation of many transcription factors that regulate photosynthesis gene expression. One well-characterized, oxygen-regulated transcription factor is the aerobic repressor *CrtJ*, which in the presence of oxygen represses bacteriochlorophyll (*bch*), carotenoid (*crt*), and respiratory (29) and light harvesting-II (*puc*) (22) gene expression. It has recently been demonstrated that exposure of *CrtJ* to oxygen results in the formation of an intramolecular disulfide bond and that formation of this bond is needed for binding of *CrtJ* to its target promoters (S. Masuda, C. Dong, D. Swem, and C. E. Bauer, unpublished data). Another well-characterized redox regulator is the sensor kinase RegB, which, together with its cognate response regulator RegA, is responsible for the global control of many aerobically and anaerobically regulated cellular processes (3). This includes photosynthesis (17, 26), nitrogen regulation (8), hydrogen utilization (8), carbon fixation (32), respiration (29, 30), and cytochrome biosynthesis (29, 30).

In this study, we have identified a new transcription factor coded by an open reading frame *(orf192)* located just upstream of *crtJ* in the previously sequenced *Rhodobacter capsulatus* photosynthesis gene cluster (1). Disruption of *orf192* indicates that it codes for an aerobic repressor (AerR) of carotenoids and bacteriochlorophyll and for the reaction center and light-harvesting apoproteins. The results of gel mobility shift assays indicate that AerR binds to the *pufQ-crtA-crtJ*, and *pucB* promoters. Additionally, we show that AerR and *CrtJ* cooperatively interact with a subset of promoters that are aerobically repressed by both of these repressors.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The wild-type *R. capsulatus* strain SB1003 (33), the regA-disrupted strain MS01 (26), and the *crtJ*-disrupted strain CD-2 (Dong et al., unpublished) have been described previously (33). *E. coli* strain DH5α (Novagen) was used for routine DNA manipulations, and BL21(DE3)/pLysE was used for protein overproduction. *E. coli* strains S17-1 λ pir (27) and HB101/pDPT51 (31) were used to mobilize plasmids into *R. capsulatus*. *R. capsulatus* strains were cultured photosynthetically under anaerobic conditions or aerobically at 34°C in PYS medium (34).

Construction of aerR and *crtJ* reporter plasmids. Several lacZ-based reporter plasmids were constructed to assay the transcription of aerR and *crtJ*. The initial construction involved PCR amplification of a 3.2-kb aerR-crtJ DNA segment using primers Ose5 (5′-AGTGTCCATCTGCGTCCCGGT-3′) and Ose6 (5′-GTTGAAACGGTCCTGGAGCA-3′) which amplified a 2.2-kb DNA segment that was subsequently inserted into *E. coli* strains (C. Bauer, unpublished data). The amplified product was cloned into pUC119 at the EcoRI and XbaI sites of pPHU234 (11). Reporter plasmids were constructed to assay the transcription of aerR and *crtJ* by inserting the 0.74-kb EcoRI fragment from pES12 into the *E. coli* strains, and the 1.9-kb EcoRI fragment from pES13 into the *R. capsulatus* strains. Reporter plasmids were constructed to assay the transcription of aerR and *crtJ* by inserting the 0.64-kb XbaI fragment from pES14 into the *R. capsulatus* strains, and the 0.4-kb XbaI fragment from pES15 into the *R. capsulatus* strains.

Construction of aerR and *crtJ* reporter plasmids. Several lacZ-based reporter plasmids were constructed to assay the transcription of aerR and *crtJ*. The initial construction involved PCR amplification of a 3.2-kb aerR-crtJ DNA segment using primers Ose5 (5′-AGTGTCCATCTGCGTCCCGGT-3′) and Ose6 (5′-GTTGAAACGGTCCTGGAGCA-3′) which amplified a 2.2-kb DNA segment that was subsequently inserted into *E. coli* strains (C. Bauer, unpublished data). The amplified product was cloned into pUC119 at the EcoRI and XbaI sites of pPHU234 (11). Reporter plasmids were constructed to assay the transcription of aerR and *crtJ* by inserting the 0.74-kb EcoRI fragment from pES12 into the *R. capsulatus* strains, and the 1.9-kb EcoRI fragment from pES13 into the *R. capsulatus* strains. Reporter plasmids were constructed to assay the transcription of aerR and *crtJ* by inserting the 0.64-kb XbaI fragment from pES14 into the *R. capsulatus* strains, and the 0.4-kb XbaI fragment from pES15 into the *R. capsulatus* strains.
Gene disruptions. A deletion of aerR, as well as a deletion of both aerR and ctfJ, was made in the homologous region of one of the target loci (Kan'). The resulting construct had an in-frame FLAG epitope to the carboxyl terminus of CrtJ (Scientific Immunizing Systems; Eastman Kodak Co.). For epipode plasmid construction, the CrtJ coding sequence was PCR amplified using primers 5'-CCGAGTCTCGGCTCAGCCGAGTCTGGTCACT GC and 5'-CCCTCTGATCATGATCGACGGCTGTAGATC GA-3'. With this primer set in the presence of the ctfJ coding region and an XbaI site added to the primer furthest from ctfJ (underlined). The two DNA fragments were subcloned into pBluescript II SK(+) separately to generate the pBluescript:uporf192 between XbaI and BglII sites that was isolated from pBS115 were then subcloned into pBluescript:uporf192 between BglII and XbaI sites to generate the plasmid pCD3, which has the Kan' cassette in the same orientation as aerR-ctfJ. This construct has the Kan' DNA fragment in the same orientation as aerR-ctfJ.

For disruption of the chromosomal copy of aerR, plasmids pES7 and pES8 were introduced into HB101/pJPT51 and then transferred into SB1003 by bi-parental conjugation as described earlier by Young et al. (34). Allelic replacement of the aerR-Kan' DNA segments from plasmids pES7 and pES8 into the genome of SB1003 generated strains ES7 and ES8, respectively. The aerR-ctfJ deletion strain was constructed by homologous recombination using gene transfer agent-mediated allelic exchange (25) of the aerR:Kan' aerR DNA segment from plasmid pCD3 into the chromosome of SB1003, resulting in strain CD3. Each of the gene disruptions was confirmed by PCR amplification and DNA sequence analysis.

Immunodetection analysis. In vivo levels of CtfJ in the wild-type strain SB1003 and in the aerR-deleted strains ES7 and ES8 were measured by the addition of an in-frame FLAG epitope to the carbonyl terminus of CtfJ (Scientific Imaging Systems; Eastman Kodak Co.). For epipode plasmid construction, the CtfJ coding sequence was PCR amplified using primers 5'-CAGAAGTCTCGGCTCAGCCGAGTCTGGTCACT GC and 5'-CCGAGTCTCGGCTCAGCCGAGTCTGGTCACT GC, which contain HindIII and BglII sites, respectively (underlined). The amplified segment was cloned into pBluescript II SK(+), from which a HindIII-BglII fragment was then subcloned into HindIII and BamHI sites in pMP23 (12). The resulting construct had ctfJ fused in frame with a 7-amino-acid-long FLAG epitope followed by a stop codon. A CtfJ-FLAG DNA fragment was then ligated into the chromosome of strains SB1003, ES7, and ES8 by homologous recombination by selecting for gentamicin resistance. Proper integration was confirmed by DNA sequence analysis and DNA fragment analysis of each of the plasmid DNA fragments of each of the aerR-ctfJ deletion strains with each other and with aerR-ctfJ (28), and a His6-Trx-AerR fusion protein was overexpressed in 4 liters of Terrific broth by induction with 0.5 mM isopropyl-

Galactosidase assay and spectral analysis. Cells were grown photosynthetically or aerobically in PYS medium to a cell density of 65 Klett units. For analysis of ctfJ, ctfJ, ctfJ, and ctfJ expression, cell extracts were prepared and assayed for β-galactosidase activity as described by Young et al. (34). For measuring ctfJ and aerR expression, β-galactosidase activity was measured as described by Miller (16) using modifications described by Eelsen et al. (10). Protein concentration was determined by the method of Bradford (6).

For spectral analysis, cells were grown to a density of approximately 50 Klett units, chilled to 0°C, harvested by centrifugation at 8,000 × g for 10 min, and resuspended in 1/10 volume of 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA. The cells were then disrupted by sonication and centrifuged at 8,000 × g for 10 min, the supernatant was removed, and the absorbance (280 nm) was measured. AerR and CtfJ purification. Forward and reverse primers 5'-CCATGGACC TGTGGTTCGACG-3' and 5'-GAACGTCATGACCGAGAAGACG-3' were used to amplify the aerR coding sequence that contains NcoI and BamHI sites (underlined), respectively. The amplified fragment was cloned into pBluescript II SK(+) and was subsequently introduced into pET32(a) (+) (Novagen Corp.) between NcoI and BamHI to generate the overexpression plasmid pET32(a) (+):aerR. The plasmid was transformed into E. coli strain BL21(DE3) pLysE (28), and a His6-Trx-AerR fusion protein was overexpressed in 4 liters of Terrific broth with induction by 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 25°C for 4 h. Cells were harvested and resuspended in 50 ml of ice cold 1× loading buffer (5 mM imidazole, 0.5 mM NaCl, and 20 mM Tris-HCl, pH 7.9) and were lysed by three passages through a chilled French pressure cell at 18,000 lbm(2). The lysate was centrifuged by clarification at 26,000 × g for 30 min, after which the supernatant was filtered through a 0.45-μm-pore-size Acrodisc (Hercules filter membrane; Gelman Sciences). The clarified supernatant was loaded on a Ni2+ column, washed with at least 40 volumes of 1× loading buffer, and eluted with 1× washing buffer (20 mM imidazole, 0.5 mM NaCl, and 20 mM Tris-HCl, pH 7.9). Elution fractions were pooled and dialyzed overnight at 4°C against TPAE buffer containing 50 mM Tris-HCl (pH 8.0), 200 mM potassium acetate, and 1 mM EDTA. AerR protein was further purified by incubation with S-proteinagarose beads at 4°C overnight and was eluted with 3 M MCl(2). The purified protein was dialyzed against TPAE buffer containing 20% glycerol overnight at 4°C and was stored at −80°C. A His6 derivative of CtfJ that was in used for gel mobility shift assays in this study was isolated from E. coli as described by Ponnampalam and Bauer (21). The percent active protein fraction was assayed as described by Ponnampalam and Bauer (21).

Mobility shift assay. [32P]-Labeled DNA probes containing either the behC, pucB, pufQ, or ctfJ promoter region were prepared by PCR amplification as previously described (21). Primers 5'-CAATTCGAGCTAAAATCTTGACGAC and 5'-AAGCTTCTCTGGTAAACTTGTC-3' were used to amplify a 640-bp pucB promoter region containing two TGT-N12-ACA CrtJ-binding palindromes. Primers 5'-GCGACATTATGAGCAGCTGGG-3' and 5'-TTGCCAAAGGGTGGACCGACC-3' were used to amplify a 262-bp ctfJ promoter region, and primers 5'-GGCCGGTTGATCCTCGGACCA-3' and 5'-GAGACACGGTCATCCCTTACG-3' were used to amplify a 263-bp pufQ promoter region. A 300-bp ctfJ-ctfJ promoter region containing two CrtJ palindromic sequences was amplified by using primers 5'-CGCGGGCGACGATCGATCTGG-3' and 5'-CCGCAAGGGCCGACCGAT-3', which contain HindIII and BglII sites, respectively (underlined). The amplified segment was cloned into pBluescript II SK(+), from which a HindIII-BglII fragment was then subcloned into HindIII and BamHI sites in pMP23 (12). The resulting construct had ctfJ fused in frame with a 7-amino-acid-long FLAG epitope followed by a stop codon. A CtfJ-FLAG DNA fragment was then ligated into the chromosome of strains SB1003, ES7, and ES8 by homologous recombination by selecting for gentamicin resistance. Proper integration was confirmed by PCR amplification and DNA sequence analysis.

The expression levels of FLAG-tagged CtfJ in the recombinant strains SB1003-FLAG, ES7-FLAG, and ES8-FLAG were examined by Western blot analysis by using the FLAG epitope-specific monoclonal antibody M2 as a primary antibody as described by the supplier (Sigma) and a chemiluminescence-based horseradish peroxidase-linked secondary antibody (ECL Western blotting detection system; Amersham Pharmacia Biotech). Cells were harvested at a culture density of 100 Klett units by centrifugation at 7,649 × g for 5 min. The cell pellets were resuspended in a buffer containing 50 mM Tris-HCl (pH 8.0), with equal volumes of cell suspensions subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analysis.

β-Galactosidase assay and spectral analysis. Cells were grown photosynthetically or aerobically in PYS medium to a cell density of approximately 65 Klett units. For analysis of aerR, aerR, and aerR expression, cell extracts were prepared and assayed for β-galactosidase activity as described by Young et al. (34). For measuring aerR and aerR expression, β-galactosidase activity was measured as described by Miller (16) using modifications described by Eelsen et al. (10). Protein concentration was determined by the method of Bradford (6).
pattern as well as to address the issue of promoter location (Fig. 1). The location of the aerR promoter was determined by constructing three plasmids, pESM16, pES15, and pES16. Plasmid pESM16 contains 162 bp of DNA upstream of the aerR coding region. SB1003 cells harboring this plasmid do not exhibit β-galactosidase activity above that which was observed with the vector alone, indicating that this segment does not contain the aerR promoter region. In contrast, SB1003 cells containing plasmids pES15 and pES16, which contain 590 and 1,080 bp of DNA upstream of aerR, respectively, exhibit significant amounts of β-galactosidase activity. The expression pattern of both strains is the same, with 2.5-fold-higher levels of aerR::lacZ expression in photosynthetically (anaerobically) grown cells over that observed with aerobically grown cells (Fig. 1A). We also addressed whether AerR, CrtJ, or RegA may be responsible for oxygen-regulated expression of aerR by assaying expression of the aerR::lacZ reporter plasmid pES15 in various regulatory mutants. As seen in the bar graph in Fig. 1B, oxygen-regulated expression of aerR is retained in strains that are disrupted for AerR, CrtJ, and RegA. This indicates that there is an additional unidentified oxygen-responding regulator that controls expression of aerR.

Plasmids pES17, pESM18, and pES18 were constructed to measure the expression pattern of crtJ. Plasmid pES17, which contains a crtJ::lacZ fusion preceded by the aerR-crtJ intergenic region as well as a large portion of the aerR coding region,
exhibits significant crtJ expression. This indicates that crtJ has its own promoter, which is located either in the intergenic region between aerR and crtJ or within the aerR coding sequence (Fig. 1A). Unlike the aerR promoter, the presence or absence of oxygen (Fig. 1A) does not affect the crtJ promoter. aerR transcripts clearly affect crtJ expression, since the β-galactosidase activity of the crtJ::lacZ fusion plasmid pES18, which contains both the aerR and crtJ promoter regions, is reproducibly higher than the level of expression observed with plasmids pES17 and pESM18, which contain only the crtJ promoter region. This suggest that read-through of the aerR transcript may impart an important contribution to crtJ expression.

Phenotypic effects of aerR disruption. The phenotypes of two aerR-disrupted strains were characterized. Strain ES7 was constructed; it replaced codons 59 to 175 of aerR with a DNA fragment coding for a KanR gene that is in the same orientation as aerR and crtJ. Strain ES8 has the same aerR DNA segment (codons 59 to 175) replaced with the same KanR gene, which is in the orientation opposite to that of aerR and crtJ (Fig. 2A). Phenotypic differences between aerobically grown colonies

FIG. 2. Assays for polarity of the aerR::KanR insertion mutations. (A) Strains ES7 and ES8 contain the same KanR gene insertion in two different orientations. (B) Spectral analysis of membrane fractions from the wild-type strain SB1003, the two aerR-disrupted mutants ES7 and ES8, and the crtJ-disrupted strain CD2-4, grown under anaerobic conditions. (C) Immunoblot analysis of the amounts of FLAG CrtJ synthesized in strains SB1003-FLAG, ES8-FLAG, and ES7-FLAG.
of strains ES7 and ES8 are striking. Strain ES7 exhibits pale, poorly pigmented colonies, whereas strain ES8 produces colonies that are slightly more pigmented in the central region of the colony than are observed with wild-type cells (data not shown). Spectral analysis of photopigments present in membrane fractions of dark anaerobically grown cells indicated that strain ES7 synthesized significantly lower amounts of bacteriochlorophyll and carotenoid photopigments than did wild-type SB1003 cells (Fig. 2B). Indeed, pigment production in ES7 is reduced to the extent that this strain is incapable of photosynthetic growth under low-illumination conditions of 6.8 μM/m². Strain ES7 also accumulates significant amounts of an uncharacterized pigment at 666 nm. In contrast to the spectral alterations observed with strain ES7, anaerobically grown ES8 cells exhibited wild-type levels of photopigment production (Fig. 2B) and normal photosynthetic growth capabilities under anaerobic conditions.

To address the different phenotypes exhibited by the aerR-disrupted strains, we explored the possibility of polar effects on expression of crtJ. The in vivo expression levels of CrtJ were directly assayed by constructing epitope-tagged chromosomal versions of crtJ in the wild-type strain SB1003 as well as in the aerR-disrupted strains ES7 and ES8, leading to strains SB1003-FLAG, ES7-FLAG, and ES8-FLAG, respectively. Western blot analysis was then performed on whole-cell extracts to measure CrtJ-FLAG levels using a monoclonal antibody to the FLAG epitope tag. Strain ES7-FLAG, which has the Kan cassette in the orientation opposite to that of crtJ, had a level of CrtJ-FLAG that was indistinguishable from that of SB1003-FLAG, which had no disruption of aerR (Fig. 2C). This indicates that elevated aerobic pigment biosynthesis observed in strain ES8 is not a consequence of polarity on crtJ expression. In contrast, the amount of CrtJ in strain ES7-FLAG, which has the Kan' gene inserted in aerR in the same orientation as is crtJ, is approximately 20-fold higher, as measured by densitometric scanning of the autoradiograph, than the level observed in strain SB1003-FLAG (Fig. 2C). Presumably the Kmr' gene promoter inserted into aerR in strain ES7 drives increased transcription of crtJ, which leads to its overexpression and subsequent reduction of pigment levels.

As discussed below, genetic evidence indicates that bchC expression is regulated by CrtJ but not by AerR. Thus, the effect of increasing CrtJ concentration on bch gene expression can readily be assayed by measuring β-galactosidase activity in SB1003 and ES7 cells that harbor the bchC::lacZ expression plasmid pDAY23Ω (34). The β-galactosidase levels in the bar graph in Fig. 3A show that overexpression of CrtJ in strain ES7 resulted in a 50-fold reduction in bchC::lacZ expression. This also indicates that reduced pigment biosynthesis observed in ES7 is likely caused by increased repression of bch gene expression by overexpressed CrtJ.

To definitively test whether overexpression of CrtJ superrepresses bch gene expression, we selected for photosynthetically competent (PS+) suppressors of ES7 and then sequenced the crtJ coding sequence from two randomly selected PS+ suppressors. As shown in Fig. 3B, both PS+ suppressors of ES7 contained point mutations in the helix-turn-helix DNA-binding motif of CrtJ at bases that are conserved with the Rhodobacter sphaeroides CrtJ homolog PspR (20). One suppressor (ES7B) converted the Arg codon (cGg) at position 451 to a Gln codon (cAg). When grown aerobically, both point mutation strains exhibited a dark red colony phenotype indistinguishable from that of the crtJ deletion strain CD2-4 (5, 22).

We also assayed bchC::lacZ expression in the two suppressor strains ES7B and ES7C, which harbored the bchC expression plasmid pDAY23Ω. The bar graph in Fig. 3A shows that bchC::lacZ expression was significantly elevated in the two suppressor strains relative to that observed in ES7. Indeed, the level of bchC::lacZ expression observed in the suppressor strains is comparable with that observed for strain CD2-4, which contains a deletion of crtJ (Fig. 3A). We can conclude from these suppressor studies that reduced pigment production in strain ES7 is indeed a consequence of constitutive suppression of bch and crt gene expression, which is caused by overproduction of CrtJ.

**Disruption of aerR leads to elevated photosynthesis gene expression.** β-Galactosidase activity was analyzed from aerobically grown derivatives of strain ES8, which contained plasmids with lacZ translational fusions to different photosynthesis genes. For comparison, we assayed in parallel the gene expression patterns of both wild-type cells, cells of the crtJ-disrupted strain CD2-4 (5), and cells of strain CD3, which contains a deletion of both aerR and crtJ. As shown in Fig. 4, disruption of aerR in strain ES8 resulted in elevated expression of the puc::lacZ and crtI::lacZ reporter genes to a level that is slightly above that of...
by disrupting deletion strains, we can conclude that the phenotype exhibited used 2.0 shift assays using af expression.

AerR with the bchC promoter region. These in vitro DNA-binding results are consistent with the in vivo ß-galactosidase activities as described for Fig. 3. The upstream CrtJ DNA-binding site is located at −279 to −296 bp, and the downstream one is located at −39 to −56 bp relative to the start site of puc transcription, as described by Olsen et al. (9). For the cooperative studies, we PCR amplified one probe containing both CrtJ palindromes that extends from −392 to +72 bp relative to the puc transcription initiation site. A second probe encompassing only the upstream palindrome was PCR amplified; it extends from −332 to −112 bp. The third probe extends from −149 to +29 bp and contains only the downstream CrtJ DNA-binding site.

Figure 6A shows the results of a typical gel mobility shift cooperativity experiment for the puc promoter probe that contains only the downstream CrtJ DNA-binding site. Lanes 2 through 4 demonstrate that CrtJ does not bind to this probe at relatively low concentrations of 141 and 281 nM and binds only a slight percentage of shifted probe at a CrtJ concentration of 656 nM. Lanes 5 to 7 demonstrate that there is also no shift of the probe in the presence of AerR at a concentration of 24, 48, or 72 nM. However, lanes 8 to 10 show that incubation of 141 nM CrtJ, with as little as 24 nM AerR, results in a noticeable gel shift. There is nearly a complete shift of the DNA probe when 141 nM CrtJ is incubated with 72 nM AerR (lane 10). In the presence of 281 and 656 nM CrtJ (lanes 11 to 16), even less AerR is required to observe a shift of the probes. Therefore, the presence of both proteins clearly enhanced their individual DNA-binding affinities.

To quantitate cooperation between AerR and CrtJ, different amounts of CrtJ were incubated with the three pucB promoter probes in the presence or absence of AerR. For each of the probes, the percentage of the probe that was shifted was calculated using a PhosphorImager with the results plotted with a

![Fig. 5. Gel mobility shift assays with purified AerR.](http://jb.asm.org)
SigmaPlot graph. The DNA-binding curves obtained with the probe containing just the upstream CrtJ DNA-binding site (probe, −332 to −112 bp) (Fig. 6B, upstream) and with the probe containing just the downstream CrtJ DNA-binding site (probe, −149 to +29 bp) (Fig. 6B, downstream) exhibited very similar EC_{50}s for CrtJ binding to these probes in the presence or absence of AerR (EC_{50} is defined as the effective concentration of CrtJ needed to obtain a shift of 50% of the probe).

When no AerR was added (graphs of filled circles), the upstream probe had an EC_{50} of 710 nM and the downstream probe had an EC_{50} of 875 nM CrtJ. With the addition of 24 nM AerR (graphs of open circles), the EC_{50}s decreased to 580 and 530 nM for the upstream and downstream probes, respectively. With the addition of 48 nM AerR (graphs of inverted filled triangles), there was a further decrease in the EC_{50}s to 185 and 155 nM for the upstream and downstream probes, respectively.
The enhancing effect of AerR on CrtJ binding was increased to 15-fold for the pucB probe containing both palindromes (Fig. 6B, both). For this probe, the EC50 was reduced from 355 nM with no AerR (filled circles) to 85 and 31 nM in the presence of 24 and 48 nM AerR (empty circles and solid inverted triangles, respectively). Consistent with the β-galactosidase assay, which showed that AerR did not regulate expression from the bchC promoter (Fig. 4), the presence of AerR did not enhance CrtJ binding to the bchC probe (data not shown). This implies that AerR may cooperate with CrtJ for binding to a specific subclass of CrtJ-controlled promoters.

**DISCUSSION**

This study demonstrates that the open reading frame that is located immediately upstream of the aerobic repressor CrtJ (orf192) also codes for a repressor of photosynthesis gene expression. Expression studies indicate that some of the genes repressed by AerR are also aerobically repressed by CrtJ. For example, as shown in Fig 7, the puc and crtI promoters are both corepressed by AerR and CrtJ. In contrast, expression of the puf operon is repressed only by AerR, while the bchC operon is repressed only by CrtJ. Indeed, the different photosynthesis gene expression patterns exhibited by the aerR-disrupted strain ES8 (Fig. 4), relative to those of the crtJ-disrupted strain CD2-4, indicate that the aerR::Kan' disruption in ES8 does not exert a polar effect on crtJ expression. This conclusion is also supported by our immunoblot data (Fig. 2C), which indicate that ES8 has normal amounts of CrtJ expression.

In contrast to the lack of polarity observed in strain ES8, strain ES7 (which contains the Kan' gene in the same orientation as crtJ) does have a significant polar effect on crtJ expression. In this strain, the amount of CrtJ is increased approximately 20-fold, which results in a significant reduction in photosystem biosynthesis. Since the difference in DNA-binding affinities between oxidized and reduced CrtJ to its target promoters is approximately fivefold (21), it seems likely that the 20-fold increase of CrtJ observed in strain ES7 is causing constitutive repression of photosynthesis gene expression. This conclusion is supported by the observation that photosynthetically competent suppressors of ES7 map to the helix-turn-helix DNA-binding motif of CrtJ, which presumably abolishes or significantly reduces the DNA-binding activity of CrtJ.

Previous studies have indicated that CrtJ-repressed promoters fall into two classes. One class, represented by the bchC promoter, has two closely spaced CrtJ recognition palindromes located just 8 bp apart, with one overlapping the −35 promoter region and the other the −10 promoter region (23). Mutational studies have demonstrated that binding of CrtJ to the bchC promoter involves cooperative interactions between CrtJ bound at the −35 palindrome and CrtJ bound at the −10 palindrome (23). Mutations that increase or decrease the spacing between these palindromes disrupt cooperative interactions between CrtJ repressors that are bound to these two sites. AerR does not appear to affect CrtJ-mediated repression of this class of promoters. The second class of CrtJ-repressed promoters also has two CrtJ recognition palindromes, with the difference being that these palindromes are distantly removed (9). In the puc operon, one CrtJ palindrome is located at the −35 promoter region and the second palindrome is located 240 bp upstream (9). In the crtA-crtI promoter region, the palindromes are 76 bp apart, with one palindrome overlapping the −35 region of the crtI promoter and the other overlapping the −10 region of the crtI promoter (9). One model is that AerR may have a role in stabilizing the binding of CrtJ, which is known to exist in solution as a tetramer, to one of the distant binding sites. Previous mutational and in vitro DNA-binding

![Fig. 7](http://jb.asm.org/) A diagram depicting CrtJ and AerR circuits that repress individual transcripts. Both proteins affect transcription initiation (lines with a single arrowhead) at promoters that contain distantly removed CrtJ recognition palindromes (lines with double arrows). Only CrtJ represses the bchC promoter that contains two closely spaced palindromes (8 bp apart), and only AerR represses the puf promoter, which does not contain CrtJ recognition palindromes.

![Fig. 8](http://jb.asm.org/) A model depicting possible interactions between CrtJ and AerR at promoters that contain distantly spaced palindromes. The number of AerR symbols drawn relative to that of CrtJ symbols is arbitrary.
studies have indicated that CrtJ cooperatively binds to the distantly removed palindromes in the puc and crtA-crtF promoters (9). This suggests that the DNA must loop around, allowing coordinate interaction of CrtJ to these distant sites. Figure 8 depicts a model that we favor, in which AerR may have a role in bending the DNA that would facilitate the interaction of CrtJ tetramers to distantly removed recognition sites. The DNA-binding curves for CrtJ binding to the puc probe in the presence and absence of AerR initially indicate that AerR is capable of stimulating CrtJ binding to the DNA probes at substoichiometric levels. However, studies have indicated that E. coli-overexpressed, purified CrtJ contains a significant fraction that is inactive for DNA binding. When correcting for the percent active fraction of CrtJ that is capable of DNA binding (Table 1 [21]), we find that an excess of AerR is actually needed to promote maximal binding of CrtJ to the full-length puc probe that contains both palindromes. Thus, it is possible that AerR may be binding to the intervening sequence between the CrtJ recognition palindromes to facilitate DNA bending. Additional DNA-binding and DNase I footprint studies are ongoing with AerR and AerR plus CrtJ to specifically address aspects of this model.

In addition to functioning as a corepressor of puc expression with CrtJ, AerR also functions on its own to repress light harvesting-I and reaction center (puf) gene expression. Mutational analysis of the puf promoter region has indicated that this promoter may be under control of a repressor, as evidenced by increased aerobic expression of some promoter point mutations (18). There has also been a report of an uncharacterized protein that binds to the puf operon promoter in an oxygen-dependent manner (13, 14, 15). The identification of AerR as an aerobic repressor of the puf operon confirms that puf expression undergoes complex regulation involving aerobic repression by AerR as well as anaerobic activation by RegA. Indeed, the puf and puc operons are both aerobically repressed and anaerobically activated by unique sets of regulatory proteins. Presumably this allows for differential expression of light harvesting-I and puc under various oxygen and light intensities. Forthcoming details of how AerR interacts with its target promoters should provide an understanding of the mechanism of repression that is affected by AerR.

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<th>Probe</th>
<th>Corrected CrtJ EC50 (nM) with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 nM AerR</td>
</tr>
<tr>
<td>Upstream palindrome</td>
<td>14.8</td>
</tr>
<tr>
<td>Downstream palindrome</td>
<td>12.4</td>
</tr>
<tr>
<td>Both palindromes</td>
<td>2.48</td>
</tr>
</tbody>
</table>

Values are corrected for percent active fraction of CrtJ as described by Ponnampalam and Bauer (21).

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REFERENCES


