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Regulatory Factors Controlling Photosynthetic Reaction Center and Light-Harvesting Gene Expression in Rhodobacter capsulatus

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Summary

Most species of photosynthetic bacteria synthesize their photosynthetic apparatus only under conditions of reduced oxygen tension. To a large extent, this phenomenon is dependent upon anaerobic induction of photosynthesis gene expression. Here we report an example of a regulatory gene, *regA*, that is involved in transactivating anaerobic expression of the photosynthetic apparatus. We show that RegA is itself responsible for differential induction of light-harvesting and reaction center gene expression relative to operons for photopigment biosynthesis. Surprisingly, strains disrupted for *regA* were found to retain normal photosynthetic growth capabilities under high light intensities. We further show that photosynthetic growth in the absence of transactivating structural gene expression is a consequence of the superoperonal organization of the photosynthetic gene cluster.

Introduction

One of the first detailed analyses of environmental effects on bacterial photosynthesis was a classic study by Cohen-Bazire et al. (1957), which rather convincingly demonstrated that synthesis of the photosynthetic apparatus is very rapidly inhibited by the addition of molecular oxygen or by a sudden increase in light intensity. One conclusion drawn from their study is that the cell must possess a sensitive mechanism for detecting changes in the environment and have the ability of transducing this information to a system for regulating synthesis of various components of the photosystem. More recent studies have established that these stimuli repress synthesis of the photosynthetic apparatus by affecting expression of many of the genes involved in photosynthesis at both transcriptional (Biel and Marrs, 1983; Clark et al., 1984; Klug et al., 1985; Zhu and Hearst, 1986) and posttranscriptional levels (Belasco et al., 1985; Zhu et al., 1986; Klug et al., 1987; Chen et al., 1988; Zucconi and Beatty, 1988; Klug, 1991). The molecular mechanism of regulating photosynthesis gene expression has remained elusive, however, primarily due to a lack of regulatory mutants that exhibit altered expression of these genes.

The primary light-absorbing components of the Rhodobacter capsulatus photosynthetic apparatus are the membrane-bound light harvesting-I (LH-I) and light harvesting-II (LH-II) complexes, which are each composed of two small membrane-spanning structural polypeptides to which bacteriochlorophyll and carotenoids are noncovalently bound. The function of the LH complexes is to absorb visible and near-infrared radiation and to transfer this energy efficiently to the reaction center (RC) complex. The RC complex, for which the crystal structure has been solved (Deisenhofer et al., 1984, 1985; Michel et al., 1986; Allen et al., 1987), is composed of three membrane-spanning proteins to which pigment cofactors are also noncovalently bound. It is within the reaction center that light energy promotes a well-characterized charge separation of a specialized pair of bacteriochlorophyll molecules that ultimately leads to a cyclic flow of electrons through the electron transport chain, resulting in the production of a transmembrane potential that drives the synthesis of ATP.

The ability to utilize light effectively for energy requires that the cell coordinate the synthesis and assembly of the numerous components that compose the photosynthetic apparatus. For example, synthesis of the pigment and protein components is controlled in such a manner that there is rarely, if ever, any appreciable amount of bacteriochlorophyll synthesized in excess of the structural polypeptides of the LH and RC complexes (Beck and Drews, 1982; Klug et al., 1985). To a large extent, coordinate synthesis of these components is thought to be controlled at the level of expression of the *puf* operon. Besides encoding structural polypeptide components of the RC and LH-I complexes, this operon includes the *pufQ* gene, which controls the level of bacteriochlorophyll biosynthesis through an unknown mechanism (Bauer and Marrs, 1988). Under anaerobic conditions, expression of the *puf* operon is highly induced from a promoter that is embedded within an upstream gene (*bchA*) which encodes a bacteriochlorophyll biosynthetic enzyme (Bauer et al., 1988). More recently it has been shown that expression of the *puf* operon, as well as that of the *puh* operon, which encodes a third RC polypeptide, is also influenced by a complex set of overlapping transcripts, termed "superoperons" (Young et al., 1989; Bauer et al., 1991). As diagrammed in Figure 1, most of the structural and biosynthetic genes known to be necessary for development of the photosynthetic apparatus are tightly clustered into a region of the R. capsulatus genome termed the photosynthetic gene cluster (Marrs, 1981; Taylor et al., 1983; Zsebo and Hearst, 1984). This region is arranged such that centrally located operons containing carotenoid (*crt*) and bacteriochlorophyll (*bch*) biosynthetic genes are flanked by the *puf* and *puh* operons. (Unlinked to this region is the *puc* operon, which encodes structural polypeptides of the auxilliary LH-II complex.) One consequence of this organization is that there appears to be read-through coupling of the more weakly expressed upstream *crt* and *bch* transcripts with the strongly inducible downstream *puf* and *puh* operons (Young et al., 1989; Bauer et al., 1991; Wellington and Beatty, 1991; Wellington et al., 1991). Evidence from several laboratories suggests that the superoperonal organization is largely responsible for balancing low-level synthesis of pigments with that of the binding polypeptides during aerobic growth (Bauer et al., 1991; Wellington et al., 1991). How-

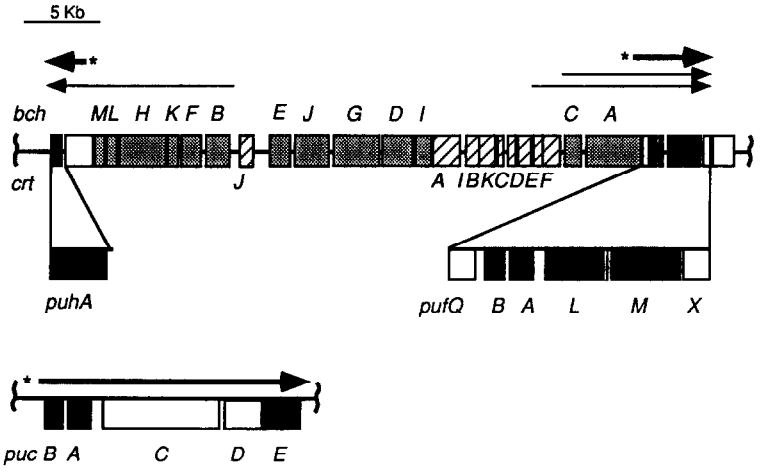

Figure 1. The Photosynthetic Gene Cluster

Structural genes encoded by the *puf*, *puc*, and *puh* operons are shown in the expanded regions as black boxes. Genes involved in bacteriochlorophyll and carotenoid biosynthesis are shaded and hatched, respectively, whereas genes containing regulatory or as yet unclear structural roles are unfilled. Arrows indicate transcripts that influence structural gene expression, with stars denoting the highly inducible transcripts influenced by the *regA* allele.

ever, the functional significance of this organization during photosynthetic growth is unclear.

In this study, we undertook a novel mutational approach to identify regulatory genes involved in controlling anaerobic induction of the LH and RC structural components of the R. capsulatus photosynthetic apparatus. The results of our analysis provide the first description of a regulatory factor, RegA, which is responsible for promoting high-level anaerobic expression of LH and RC structural genes. Interestingly, cells deficient in *regA* were found to retain their ability to synthesize significant amounts of the photosynthetic apparatus and, as a consequence, retain the ability to grow photosynthetically under conditions of moderate and high light intensities. We further show that retention of photosynthetic growth under these conditions is a consequence of the superoperonal organization of overlapping transcripts within the photosynthetic gene cluster, and that anaerobic induction of RC and LH gene expression is required for growth only in dim light.

Results

Isolation of Regulatory Mutants Deficient in LH and RC Gene Expression

We initially attempted to utilize penicillin selection for cells deficient in photosynthetic growth as an enrichment for mutants that fail to activate photosynthesis gene expression. However, these initial attempts failed to produce trans-acting mutants, presumably due to the ability of regulatory mutants to grow photosynthetically under standard laboratory growth conditions (see below). Subsequent enrichments, involving prolonged growth under dark anaerobic conditions, were more successful in obtaining spontaneous trans-acting regulatory mutants. Under these conditions, cells with reduced levels of photopigment biosynthesis outgrow wild-type cells that needlessly expend energy synthesizing photopigments (Madigan et al., 1982). Regulatory mutants were subsequently identified by visually screening colonies obtained from the enrichment cultures for two criteria indicative of trans-acting photosynthetic mutants. One criterion was a reduction in colony pigmentation caused by a decrease in expression from chromosomally encoded photosynthesis genes. These colonies were subsequently tested for a concomitant reduction in β-galactosidase activity expressed from the reporter plasmid pCB532Ω, which contains a fusion of *lacZ* to the *puf* operon (Bauer et al., 1988). Through the use of such a screen, over 50 independently isolated regulatory mutants were identified that fail to transactivate LH and RC gene expression. The results in Table 1 show the effect on expression of *puf* and *puh* operon reporter plasmids for 11 representative mutants. Since all of the mutants exhibited similar phenotypes with respect to expression of photosynthesis genes and mapped to the same locus (*regA*, see below), a detailed analysis of only one regulatory mutant, REG1, will be discussed herein.

Photosynthesis Genes Are Differentially Regulated

Plasmid-encoded translational fusions were used to assay REG1 for its ability to activate expression of different promoters involved in bacterial photosynthesis. As shown in Figure 2, the wild-type parent strain SB1003 exhibits a

Table 1. Reduced Anaerobic Expression of the *puf* and *puh* Operons by Trans-Acting Regulatory Mutants

Strain	pCB532Ω *puf::lacZ*	pCB701Ω *puh::lacZ*
SB1003 (wild-type)	1,694	848
REG1	49	32
REG2	36	68
REG3	33	47
REG4	62	31
REG5	46	23
REG10	29	65
REG11	4	68
REG12	38	143
REG16	26	157
REG25	30	124
REG29	48	98

Units of β-galactosidase activity are expressed as micromoles of O-nitrophenol-β-D-galactoside hydrolyzed per minute per milligram of protein in cell extracts prepared as described in Experimental Procedures. Translational fusions of *lacZ* to *pufQ* and *puhA* encoded by pCB532Ω and pCB701Ω have been described previously (Bauer et al., 1988, 1991).

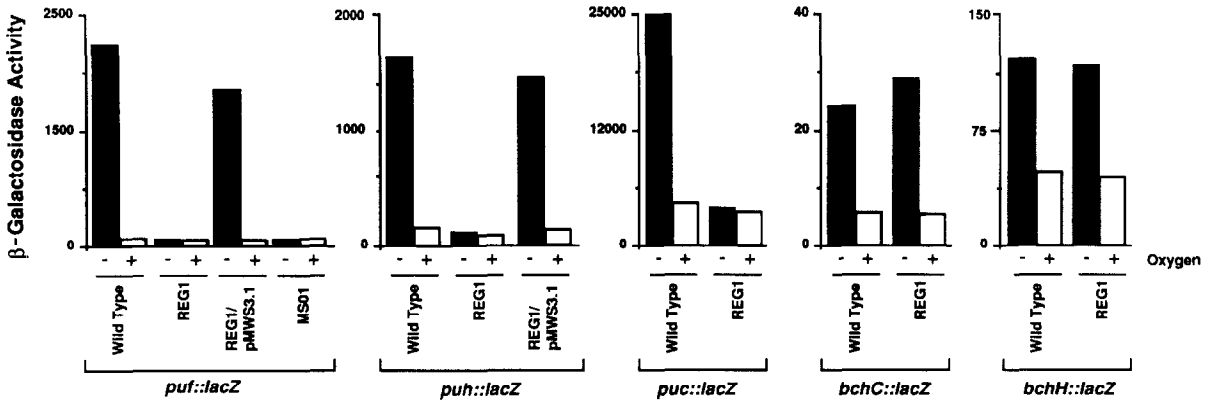

Figure 2. Specificity of the *regA* Allele for Photosynthetic Structural Gene Expression

Graphs represent expression of the *puf*, *puh*, *puc*, *bchCA*, and *bchB* operons as measured from transcriptional/translational fusions of these operons to *lacZ* encoded by pCB532Ω (Bauer et al., 1988), pCB701Ω (Bauer et al., 1991), pLHIIZ (Experimental Procedures), pDAY23 (Young et al., 1989), and pZY62Ω (Yang and Bauer, 1990), respectively. Both pCB532Ω and pCB701Ω lack the upstream *bch* operons, and thus the level of β-galactosidase expressed from these constructs represents transcription only from the previously characterized highly induced (*regA*-dependent; Bauer and Marrs, 1988) *puf* and *puh* operon promoters. The cells were grown either photosynthetically at 5380 lux light intensity or aerobically in the dark in PYS medium and assayed for β-galactosidase activity as described by Young et al. (1989). Units of β-galactosidase activity are as in Table 1.

30-fold induction of *puf*, *puh*, and *puc* expression and a 3-fold induction of *bchC* and *bchH* expression when shifted to anaerobiosis. In contrast, REG1 exhibits a virtual lack of anaerobic induction of the highly expressed *puf*, *puh*, and *puc* operon promoters, as demonstrated by the nearly identical values of β-galactosidase obtained from aerobic and anaerobic cultures. REG1 does exhibit normal induction of β-galactosidase activity expressed from *bchC*::*lacZ* and *bchH*::*lacZ* constructs, thereby demonstrating that these operons are regulated independently from those encoding structural polypeptides. We also note that oxygen-regulated operons that are unrelated to photosynthetic functions, such as *nifHDK*, appear to be unaffected by the mutation in REG1 (our unpublished data). From these results we conclude that the mutated allele in REG1 appears specifically to control the anaerobic induction of genes encoding structural components of the photosynthetic apparatus.

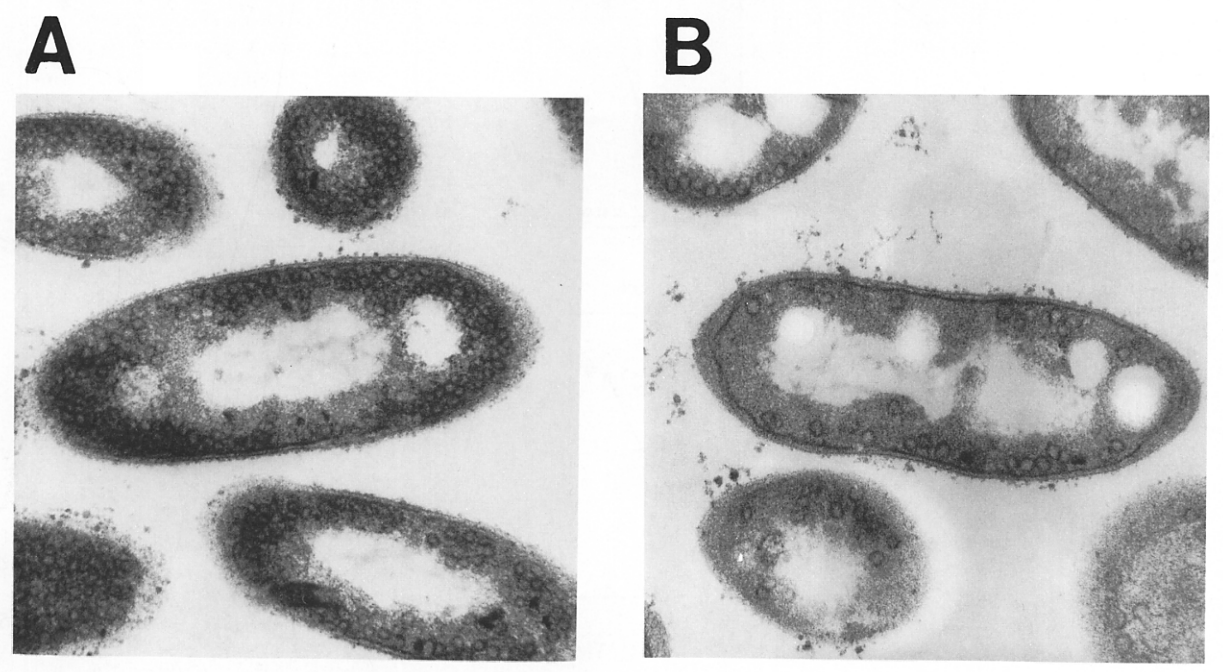

Figure 3. Ultrastructural Analysis of Regulatory Mutants

Transmission electron micrographs were taken of the wild-type cell line SB1003 (A) and the regulatory mutant REG1 (B). The cells were grown under anaerobic conditions to mid-log phase prior to being harvested and fixed as described previously (Gest and Favinger, 1983).

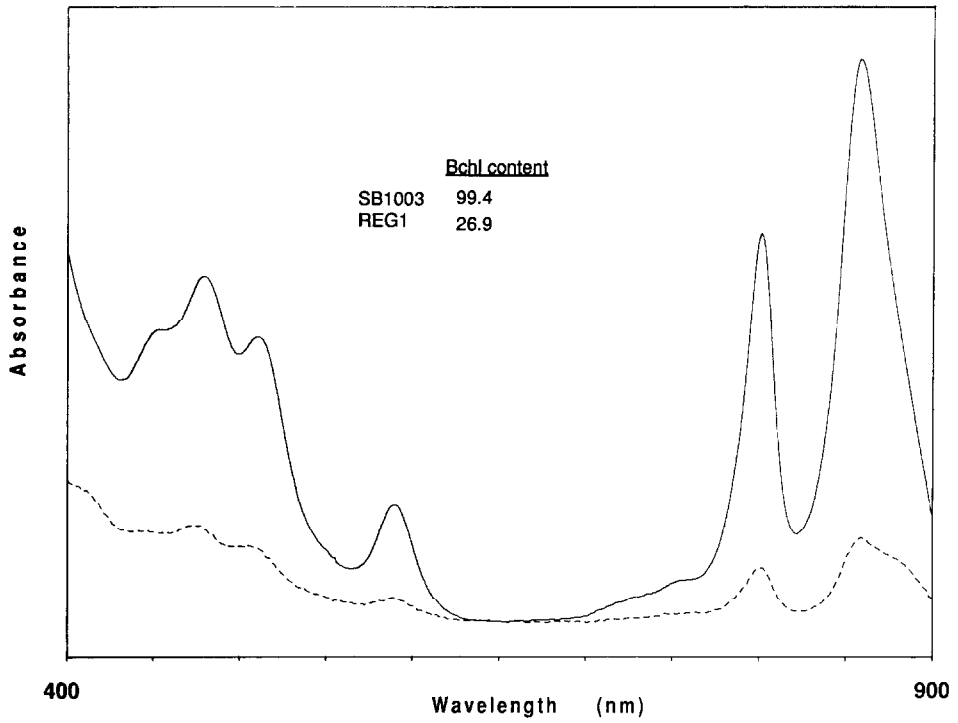

Figure 4. Spectral Analysis of Photopigment Production

Spectral analysis of the wild-type cell line SB1003 (solid line) and the regulatory mutant REG1 (dashed line). Both cultures were grown in the dark anaerobically to mid-log phase. Inset: Total bacteriochlorophyll content of these cells (μg of Bchl per mg of protein), determined as described in Experimental Procedures.

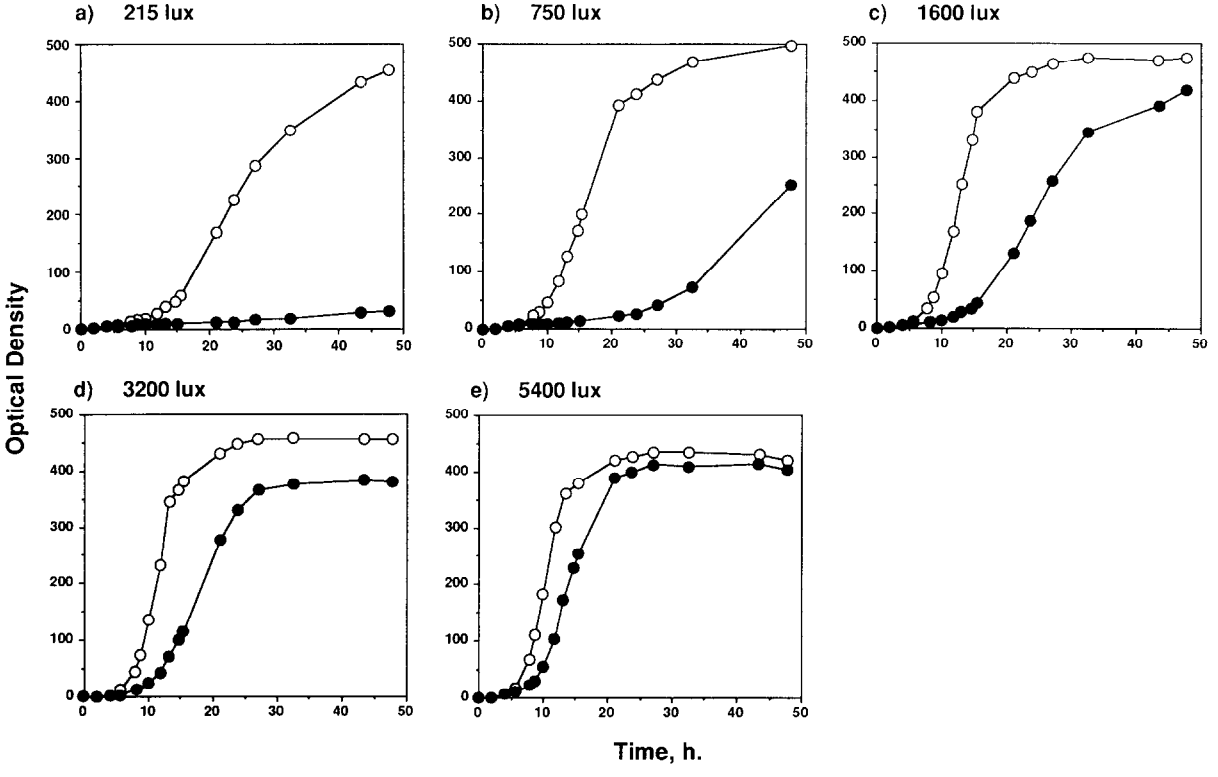

Figure 5. Photosynthetic Growth of Wild Type and REG1 at Different Light Intensities

Cultures were grown photosynthetically under the indicated light intensities in PYS medium and monitored by measuring turbidity with a Klett-Sumerson Spectrophotometer (red filter). Open circles denote the wild-type strain SB1003 and closed circles denote REG1.

Regulatory Mutants Retain Synthesis of a Functional Photosynthetic Apparatus

As shown in the transmission electron micrograph in Figure 3A, photosynthetically grown wild-type R. capsulatus produces a large number of intracytoplasmic membrane (ICM) vesicles within which the photosynthetic apparatus is housed. In contrast, aerobically grown wild-type R. capsulatus cells are essentially devoid of ICMs (Drews and Oelze, 1981). Analysis of anaerobically grown REG1 demonstrates that, even though the level of ICM production is quantitatively lower in this strain (Figure 3B), there is still a significant amount present. The ability of REG1 to synthesize a photochemical apparatus anaerobically is confirmed by spectral analyses of crude membrane fractions (Figure 4) that demonstrate that a significant amount of the RC, LH-I (875 nm), and LH-II (800–850 nm) photopigments are produced by REG1 even in the absence of transactivation of the *puf*, *puh*, and *puc* operons. Qualitatively, the *puc*-encoded LH-II apparatus appears to be affected to a greater extent, as indicated by a more severe reduction of the LH-II absorbance peaks relative to LH-I. A quantitative measurement of extracted bacteriochlorophyll shows that REG1 cells exhibit only a 5-fold reduction in total bacteriochlorophyll relative to wild-type cells under these conditions (Figure 4, inset).

In order to determine if the amount of photopigments produced by REG1 is physiologically significant, we analyzed the photosynthetic growth rates of wild-type and REG1 cells over a range of light intensities. The results shown in Figures 5 and 6B demonstrate that while growth of REG1 is severely impaired under dim light conditions (215–750 lux), the impairment is less severe under moderate light intensity (1,600–3,200 lux) and, except for shading effects in stationary phase, is insignificant under high light (5,400–21,500 lux). (For comparison, the solar constant, which is the maximum illumination reaching the earth, is approximately 90,000 lux.) We therefore conclude that high-level transactivation of LH and RC gene expression is required for growth only under low light conditions.

Superoperonal Clustering of Photosynthesis Genes Is Responsible for Promoting Photosynthetic Growth in the Absence of the LH and RC Transcriptional Activator, RegA

How does REG1 retain its ability to synthesize a photosynthetic apparatus without inducing anaerobic expression of *puf*, *puc*, and *puh* operons? Previous analyses have demonstrated that photosynthesis genes in R. capsulatus are tightly linked into "superoperons" with transcripts from upstream carotenoid and bacteriochlorophyll biosynthetic operons extending into and influencing expression of *puf* and *puh* (Figure 1; Young et al., 1989; Bauer et al., 1991; Wellington and Beatty, 1991; Wellington et al., 1991, 1992). Since expression of the upstream pigment biosynthetic operons is unaffected by the mutated allele in REG1, it is possible that the upstream *crt* and *bch* transcripts are providing sufficient *puf* and *puh* expression to promote photosynthetic growth under moderate and high light conditions. Indeed, the previous observation that there exists a linear relationship between the amount of bacteriochlorophyll biosynthesis and *pufQ* expression (Bauer and Marrs, 1988), coupled with our observation that a 30-fold reduction in the *puf* promoter activity (Figure 2) results in only a 6-fold reduction in bacteriochlorophyll levels (Figure 4), suggests that the upstream *crtEF/bchCA* promoters are providing a significant amount of *puf* operon (*pufQ*) expression in the absence of RegA. To test this possibility, we constructed strains that prevent *crtEF* and *bchCA* transcripts from influencing *puf* operon expression by placing a transcription terminator into the chromosomal copy of the *bchA* gene (Figure 6A). In confirmation of previ-

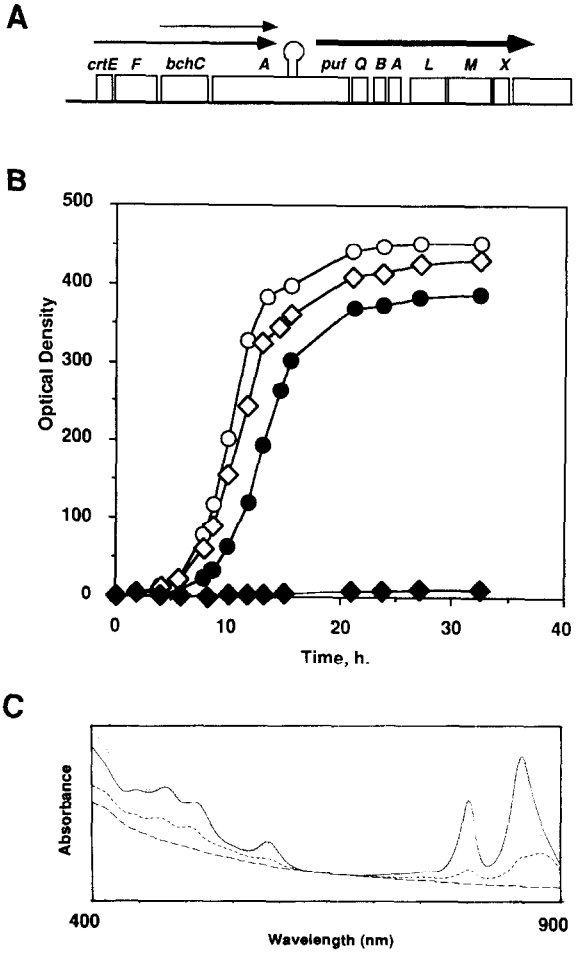

Figure 6. Physiological Consequences of Disrupting Read-Through Transcripts from Bacteriochlorophyll and Carotenoid Biosynthetic Operons

(A) Transcripts from the *crtEF* and *bchCA* operons were prevented from reading through the *puf* operon by the insertion of a transcription termination site (Ω) into the chromosomal copy of *bchA* in wild-type and REG1 derivatives of R. capsulatus. A wild-type copy of the *bchCA* operon was provided in trans on pDAY26 (Young et al., 1989).

(B) Photosynthetic growth under saturating light conditions of the wild-type strain SB1003/pDAY26 (open circles); DE333/pDAY26 (open diamonds), which is a derivative of SB1003 containing Ω in *bchA*; REG1/pDAY26 (closed circles); and REG1ΩA/pDAY26 (closed diamonds), which is a derivative of REG1 containing the *bchA* terminator.

(C) Absorption spectra of the above strains grown under dark anaerobic conditions. Solid line, SB1003/pDAY26; dotted line, DE333/pDAY26; small-dashed line, REG1/pDAY26; large-dashed line, REG1ΩA/pDAY26.

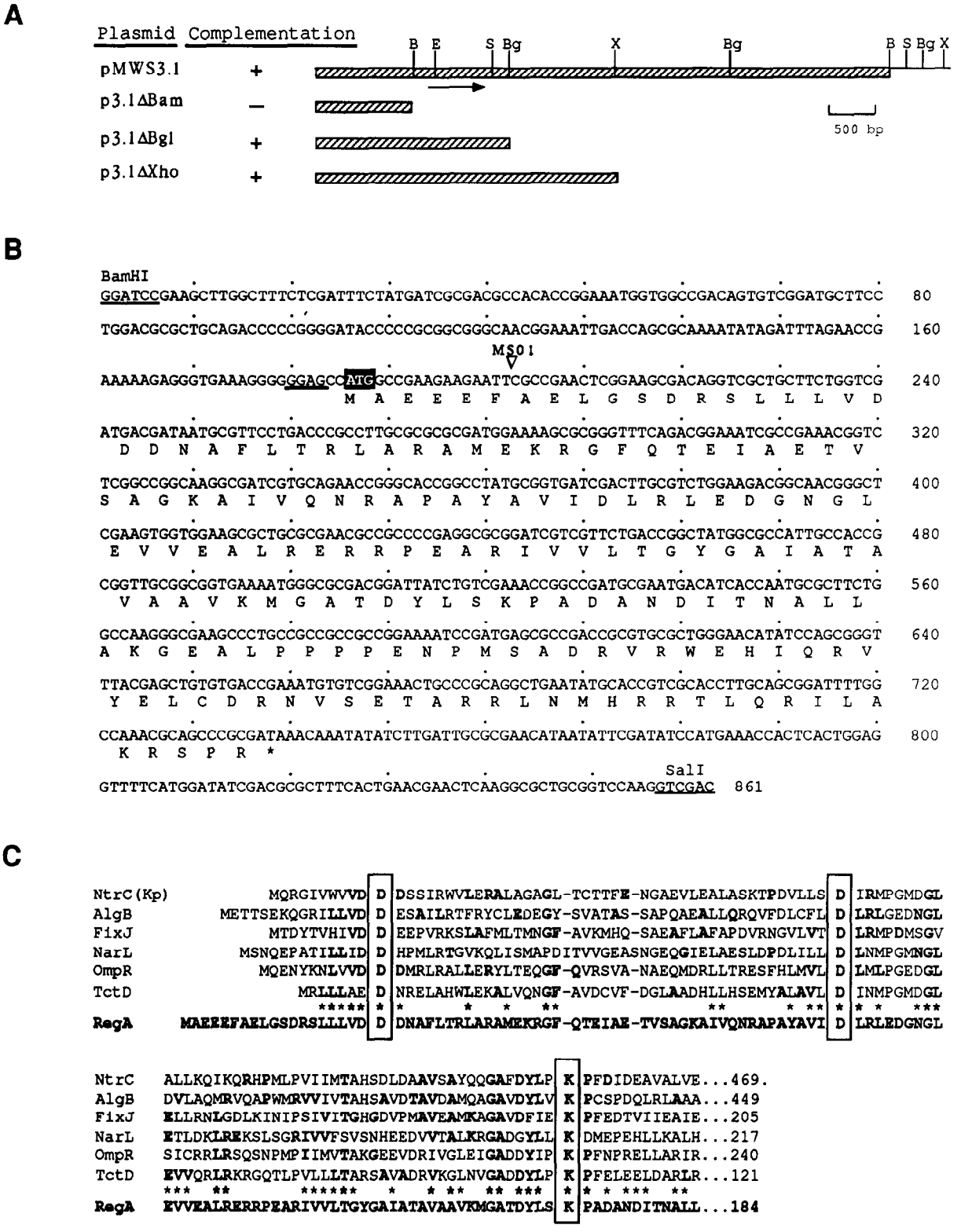

Figure 7. Physical Map and Sequence Analysis of *regA*

(A) Restriction maps of the insert in cosmid pMWS3.1 and deletion constructs used to localize *regA*. Constructs that restore pigmentation to REG mutants are noted. Restriction sites are abbreviated as B, BamHI; Bg, BglII; E, EcoRI; S, SalI; X, XhoI.

(B) Nucleotide and predicted amino acid sequences for the region between BamHI and SalI as denoted on the restriction map. The restriction sites used for subcloning and for constructing strain MS01 are noted, as are the Shine–Dalgarno sequence and the translation start codon of *regA*.

(C) Amino acid sequence homology between RegA and environmental response regulators. The sequences shown represent the most fully characterized and closest relatives within the three recognized subclasses of the response regulator family. Boxed residues are considered functional. Bold residues are amino acid residues identical to RegA, whereas starred residues indicate residues of similar function that are conserved between RegA and more than two-thirds of all of the response regulator sequences present in the GenBank–EMBL data base.

ous studies (Bauer et al., 1991; Wellington et al., 1991), we observed that prevention of the *crtEF/bchCA* transcripts from extending into the *puf* operon does not significantly affect photosynthetic growth in a strain containing a wild-type *regA* allele (Figure 6B). However, a similar construction in a REG1 derivative (REG1ΩA/pDAY26) results in a nearly complete inhibition of *puf* operon expression, as assayed by the virtual lack of *pufQ*-dependent bacteriochlorophyll biosynthesis (Figure 6C; Bauer and Marrs, 1988) and its concomitant inability to grow photosynthetically even under the highest light conditions tested (Figure 6B). This latter result suggests that read-through transcripts from upstream pigment biosynthesis operons in themselves afford sufficient expression of LH and RC structural genes (and *pufQ*) to support wild-type photosynthetic growth under higher light conditions.

Characterization of the *regA* Locus

The regulatory locus responsible for anaerobic induction of LH and RC genes was cloned by isolating a cosmid from a wild-type R. capsulatus genomic library that restores the ability of REG1 and the other REG mutants to grow photosynthetically under dim light conditions. One such cosmid, pMWS3.1, which complements REG1 with respect to expression of *puf* and *puh* operons (Figure 2), the level of photopigment biosynthesis, and induction of ICM formation (data not shown), was isolated. Localization of the regulatory gene within pMWS3.1 was undertaken by complementation analysis with subcloned deletion derivatives of pMWS3.1. As diagrammed in Figure 7A, an 1100 bp BamHI–BglII subclone of pMWS3.1 (p3.1ΔBgl) fully complemented all of the trans-acting regulatory mutants isolated during this study. Sequence analysis of this region revealed the existence of one contiguous open reading frame displaying codon usage indicative of a translated R. capsulatus gene (Figure 7B). To confirm that it is the locus responsible for transactivating the *puf*, *puh*, and *puc* operons, we constructed a strain of R. capsulatus, MS01, that contains a kanamycin resistance cassette inserted into the fifth codon of the same open reading frame. Strain MS01 displays an identical reduction in the level of *puf* expression (Figure 2) and absorption spectrum (data not shown) as that reported above for REG1. Inspection of the predicted amino acid sequence of the gene disrupted by MS01 (designated *regA*) shows that it is homologous to a well-characterized family of regulatory proteins that are known to control cellular functions in response to signals transduced from an environmental sensor (Figure 7C). Examination of the sequence does not reveal any obvious association of RegA with one of the three established subgroups of DNA-binding proteins into which about two-thirds of these response regulators have been classified (Stock et al., 1989, 1990). The significance of this will be discussed in more detail below.

Discussion

Numerous studies have demonstrated that synthesis of pigment, protein, and ICM components of the bacterial photosystem is coordinately regulated. For example,

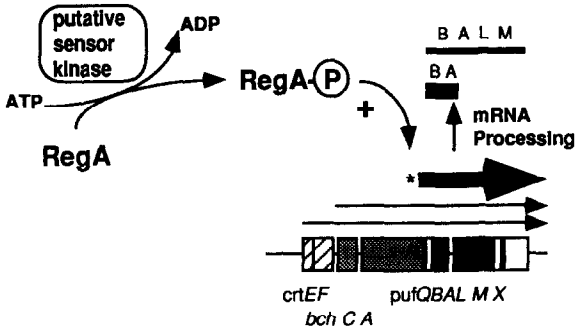

Figure 8. Regulatory Events Controlling *puf* Operon Expression

As discussed in the text, the *puf* operon is known to be expressed from low-level read-through transcripts from upstream *crtEF* and *bchCA* operons (thin arrows) as well as by a stronger transcript (bold arrow) under positive control by RegA. Analysis of *puf* transcripts has demonstrated that the full-length *puf* transcript is rapidly degraded at the 5′ and 3′ ends, giving rise to a predominant short *pufBA* transcript in addition to the longer *pufBALM* transcript. Sequence homology suggests the existence of an environmental sensor, which activates RegA via phosphoryl group transfer.

when cells are shifted from aerobic to anaerobic environments, synthesis of the LH and RC polypeptides is coordinated with pigment synthesis such that there is little if any detectable bacteriochlorophyll produced that is uncomplexed with structural polypeptides (Beck and Drews, 1982). To a large extent, this "coordinate regulation" is thought to involve control at the level of expression of various photosynthesis genes. This conclusion is based primarily on observations that both biosynthetic and structural genes display similar kinetics when they are induced by anaerobiosis or repressed by high light intensity (Biel and Marrs, 1983; Clark et al., 1984; Klug et al., 1985; Zhu and Hearst, 1986; Zhu et al., 1986; Bauer et al., 1988, 1991; Young et al., 1989) and has led to the suggestion that a "global regulator" might be responsible for controlling induction of all of the photosynthesis genes. On the other hand, the differing roles of their products (enzymatic vs. structural) suggest that the two classes of genes might be regulated differentially as well. Prior to this study, the implication of differential regulation was supported only by circumstantial evidence: the *puf*, *puc*, and *puh* operons are more highly inducible than are the bacteriochlorophyll biosynthetic operons (Biel and Marrs, 1983; Clark et al., 1984; Bauer et al., 1988, 1991; Young et al., 1989; Yang and Bauer, 1990), and limited information that exists on their promoter sequences suggests that bacteriochlorophyll and carotenoid operons have a canonical sigma-70 type promoter, whereas the *puf* and *puh* operons share a unique promoter sequence (Bauer et al., 1988, 1991; Armstrong et al., 1989; Wellington and Beatty, 1989; Young et al., 1989). Our study therefore provides direct evidence for a mechanism of differential regulation acting on photosynthesis genes.

How, then, does the cell coordinate induction of the various components of the photosystem? The answer to this question is likely to be very complicated, since the control of transcription initiation appears to be only one level of an

extensive set of regulatory events involved in controlling photosynthetic gene expression. For example, the level of bacteriochlorophyll biosynthesis appears to be controlled by the first gene product of the *puf* operon, PufQ (Bauer and Marrs, 1988). Thus, expression of the *puf* operon appears to have a critical posttranslational role in controlling the stoichiometry of bacteriochlorophyll to LH and RC structural polypeptide synthesis. Likewise, expression of individual genes within the *puf* operon is also known to be affected by the processing of *puf* transcripts into individual segments, which are thought to be responsible for providing differing levels of *pufQ*, *pufBA*, and *pufLM* expression (Figure 8; Belasco et al., 1985; Klug et al., 1987; Bauer et al., 1988; Chen et al., 1988; Klug, 1991). Posttranscriptional control has also been implicated in the formation of LH-I and LH-II complexes (Bauer et al., 1991; Tichy et al., 1991). The results of our study also demonstrate that an additional level of control, namely the superoperonal organization of overlapping transcripts within the photosynthetic gene cluster, ensures sufficient *puf* operon expression to promote growth under moderate and high light intensities. Collectively, these results suggest that controlling transcription initiation of the LH/RC structural genes is only one aspect of a highly complex system responsible for regulating synthesis of the photosynthetic apparatus.

Another conclusion from this study is that *regA* encodes a transcriptional regulator that is responsible for activating expression of the *puf*, *puh*, and *puc* operons in response to a decrease in oxygen tension. This conclusion is based not only on the phenotype of *regA* mutations but also on the amino acid sequence homology of RegA to a well-characterized group of proteins that function in two-component systems to regulate gene expression in response to environmental signals. In general, most of these proteins are thought to bind DNA and activate transcription when they become phosphorylated by the sensor kinase component of the system. The histidine protein kinases are typically membrane-bound proteins that detect an environmental signal, such as a change in oxygen tension, and transmit this information by phosphorylating a specific aspartate residue on a response regulator. As noted in the Results section, RegA has extensive sequence similarity in its amino-terminal region (amino acids 1–124) to sequences shared among all of these response regulators. However, the carboxy-terminal region does not display notable sequence similarity to any of the three subclasses (FixJ, OmpR, NtrC; Stock et al., 1989) that have been shown to bind DNA. Instead, RegA may be similar to the smaller response regulators, including CheY, SpoOF, and CheB, in that they do not function directly as transcriptional activators but act as intermediaries that interact with other proteins in more complex regulatory cascades (Stock et al., 1989; Burbulys et al., 1991; Bourret et al., 1991). It is intriguing therefore to consider that RegA may be only a single link in a more complex regulatory chain, responsible for controlling photosynthesis gene expression in response to many different environmental factors. Indeed, we have preliminary evidence from separate mutational screens that at least two additional loci linked to *regA* are involved in controlling *puf*, *puh*, and *puc* expression in response to oxygen and light intensity (data not shown). Further sequence and biochemical analyses of RegA and these additional regulatory proteins are under way and should provide some insight into whether or not RegA is directly involved in binding to the promoter regions in question, and to what specific environmental signal(s) it is responding.

Experimental Procedures

Bacterial Strains, Plasmids, and Culture Conditions

R. capsulatus strains were routinely grown at 34°C in the complex medium PYS, or in RCV 2/3 PY (Young et al., 1989). Spectinomycin and kanamycin were used at 10 μg/ml for maintenance of plasmids and construction of stable recombinants in R. capsulatus. Rifampicin was used as a counterselection for exconjugates at a concentration of 100 μg/ml. Highly oxygenated growth was achieved by shaking a 10 ml culture in a 250 ml flask at 250 rpm. Anaerobic conditions were achieved by filling 18 ml screw-capped tubes with medium. Illumination was provided by banks of incandescent Lumiline 60 W lamps, with intensity being controlled by shading cultures with neutral-density filters. Light intensity was measured using a Weston Instruments, Inc., Illumination Meter model 755. To avoid secondary effects, such as oxygen depletion in aerobically grown cultures or self-shading in photosynthetically grown cultures, all cells were harvested in early logarithmic phase, at a relatively low cell density of 1.5×10^8 cells per ml. Escherichia coli strains were routinely cultured at 37°C in Luria broth medium (Sambrook et al., 1989). Ampicillin and spectinomycin were each added to media at 100 μg/ml, while kanamycin and trimethoprim were each used at 50 μg/ml.

Genomic Library Construction and Selection of pMWS3.1

A library of wild-type R. capsulatus DNA was prepared by ligating Sau3A partially digested genomic DNA, obtained from wild-type R. capsulatus SB1003, into the cosmid vector pJRD215 (Davison et al., 1987), which had been digested to completion with BamHI and treated with bacterial alkaline phosphatase (BRL). After ligation, the DNA was packaged into λ phage particles (Stratagene), which were then used to infect the E. coli host VCS257. Approximately 3000 kanamycin-resistant (Km^R) colonies were pooled and amplified, then stored in a 16% glycerol solution at −80°C. Selection of the cosmid, pMWS3.1, that complements the REG mutations was accomplished by tripartite mating between the genomic bank, the plasmid mobilizing strain Tec5 (Taylor et al., 1983), and the R. capsulatus mutant strain REG3.1. Cosmid pMWS3.1 was selected by incubating the mating plate in an anaerobic Gas-PAK jar (BBL), which was illuminated at approximately 500 lux. Exconjugates were picked after a 4-day incubation period and purified by growth under selective conditions.

Plasmid Constructions

Deletion derivatives of pMWS3.1 (Figure 7A) were constructed by deleting, replacing, or subcloning restriction fragments as follows: p3.1ΔSal and p3.1ΔBam were constructed by deleting the insert 4447 bp SalI and 5300 bp BamHI restriction fragments of pMWS3.1, respectively; p3.1ΔBgl was constructed by replacing the two small (2138 and 2838 bp) BglII fragments of pMWS3.1 with the Km^RBamHI restriction fragment of pUC4-KIXX (Barany, 1985); p3.1ΔXho was made by replacing the 4584 bp internal XhoI fragment of pMWS3.1 with the Km^R XhoI fragment of pUC4-KIXX. A reporter plasmid for *puc* operon expression, pLHIIZ, was constructed by inserting the 2.0 kb EcoRI–ClaI restriction fragment of pRPSLH2 (Youvan and Ismail, 1985) into the EcoRI–AccI site of pNM482 (Minton, 1984). This construct contains the *puc* operon promoter region (Zucconi and Beatty, 1988) followed by a translational fusion of the 26th codon of *pucB* to the 12th codon of *lacZ*. To prevent vector-initiated mRNA from influencing expression of the *pucB*::*lacZ* fusion, an EcoRI fragment encoding a transcription and translation termination site (Ω) was subsequently subcloned from pBR322Ω (Prentki and Krisch, 1984) into the upstream EcoRI site of pLHIIZ.

Genetic Manipulations

Mobilization of plasmids containing a ColE1 origin of replication from E. coli into R. capsulatus was accomplished using the mobilizing strain Tec5 as described previously (Taylor et al., 1983; Young et al., 1989). Plasmid derivatives of pJRD215 were mobilized in a similar manner using the IncP mobilizing strain S17-1 (Simon et al., 1983). Reporter plasmids were cured from R. capsulatus by growing serial cultures in PYS media in the absence of selection. After five serial passages, the cells were plated onto PY agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), which allowed visualization of colonies that had lost the reporter plasmid. Plasmid curing was subsequently confirmed by assaying for loss of the plasmid-encoded drug resistance marker.

Interposon mutagenesis was used to disrupt the chromosomal copy of *regA* in R. capsulatus. For this disruption, an EcoRI fragment of pUC4-KIXX containing a Km^R gene was inserted into the EcoRI site present in the fifth codon of *regA*. A plasmid containing this disruption was subsequently mated into the gene transfer agent (GTA) overproducing strain CB1127 (Young et al., 1989) for use in GTA-mediated transduction as described previously (Scolnik and Haselkorn, 1984).

Sequence Analysis

A 1100 bp BamHI–BglII fragment of pMWS3.1 that complemented the REG mutants was subcloned into the BamHI site of both M13 mp18 and mp19 (Messing and Vieira, 1982). Nested deletions of each insert, which spanned an average distance of 200 bp, were constructed according to the procedure of Dale and Arrow (1987). Sequence information was obtained by the dideoxynucleotide chain termination method (Sanger et al., 1977) using both modified T7 DNA polymerase (Sequenase, U.S. Biochemical Corp.) and high-temperature Taq polymerase (TaqTrack, Promega) in combination with deazanucleotide analogs. DNA sequence data was analyzed on a μVax computer using programs from the Sequence Analysis Package (version 7.0) of the University of Wisconsin Genetics Computer Group, and submitted to GenBank under accession number M64976.

Spectral and Protein Analysis

In vivo absorption spectra of ICM were obtained by sonicating cells in RCV medium (Weaver et al., 1975), and scanning the soluble fractions from 400 to 900 nm using a Beckman DU-50 recording spectrophotometer. Bacteriochlorophyll content was measured by extracting pigments from cells with cold acetone–methanol (7:2) as described previously (Clayton, 1966). Protein concentrations were measured by the method of Bradford (1976) (Bio-Rad kit #500–001). β-galactosidase activity of R. capsulatus cells containing reporter plasmids for gene expression was determined as described by Young et al. (1989).

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