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Transcription of oxygen-regulated photosynthetic genes requires DNA gyrase in \textit{Rhodobacter capsulatus}

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\textit{Communicated by Martin Gellert, February 22, 1988 (received for review November 9, 1987)}

\textbf{Abstract}

The regulation of the photosynthetic genes by DNA supercoiling in \textit{Rhodobacter capsulatus} has been studied by using gyrase inhibitors \textit{in vivo} and by measurement of mRNA levels of more than a dozen genes. The results demonstrate that the levels of mRNA for light-harvesting (I, II) and reaction center (L, M, H) proteins, bacteriochlorophyll biosynthetic enzymes, ribulose-bisphosphate carboxylase (EC 4.1.1.39), and the mRNAs from the open reading frames Q and R decreased immediately and dramatically upon addition of novobiocin and coumermycin. In contrast, the mRNAs for carotenoid biosynthetic enzymes, the cytochrome \textit{bc}$_3$ complex, and constitutively expressed mRNA under aerobic conditions for light-harvesting I and for reaction center (L, M) proteins are less sensitive to the inhibitors. In accordance with these results, the biosynthesis of bacteriochlorophyll is markedly repressed by gyrase inhibitors novobiocin, coumermycin, nalidixic acid, and oxolinic acid either under anaerobic conditions or during a shift from aerobic to anaerobic conditions. The synthesis of light-harvesting (I, II) bacteriochlorophyll complexes is also inhibited by novobiocin and coumermycin. The kinetics of specific mRNA changes and the differential sensitivity of anaerobic and aerobic genes to the gyrase inhibitors strongly suggest that DNA supercoiling is involved in the differential expression of photosynthetic genes in response to the level of oxygen in \textit{R. capsulatus}.

\textit{Rhodobacter capsulatus} is a facultative purple nonsulfur Gram-negative bacterium capable of growing either aerobically in the dark (chemotrophic) or anaerobically in the light (phototrophic), primarily depending on the oxygen tension in the environment (1). In the presence of oxygen, cells grow aerobically with an active respiratory electron transport chain as an energy source. Lowering the oxygen tension induces anaerobic growth with a photosynthetic electron transport chain producing energy. In the latter case the photosynthetic intracytoplasmic membrane composed of pigment–protein complexes including two light-harvesting (LH) antennae (I, II) and three reaction center (RC) polypeptides (L, M, H) develops. The pigments bacteriochlorophyll (BChl) and carotenoid (Crt) bound to the LH (I, II) polypeptides capture photons and funnel this energy into the RC. In the special membrane protein environment of the RC containing BChl, Crt, bacterioopheophytin, quinone, and iron, the charge separation occurs and the electron transport begins. The energetic photoelectron drives a cyclic series of redox complexes consisting of quinones and cytochromes (\textit{bc}$_3$, \textit{c}$_2$) in the membrane. The resultant proton gradient across the membrane drives ATP synthesis by way of an ATPase. The switch of the growth modes by oxygen is the result of activation and inactivation of a large number of genes responsible for different metabolic pathways (2).

Earlier studies have indicated that the biosynthesis of BChl in purple nonsulfur bacteria is inhibited by oxygen (3). Several enzymes in the BChl biosynthetic pathway, such as \textit{\alpha}-aminolevulinate synthase, magnesium protoporphyrin methyltransferase (4), and magnesium protoporphyrin chelatase (5), appear to be repressed by oxygen. The transcription of a few genes in the BChl biosynthetic pathway has been demonstrated to be regulated by oxygen using \textit{lac} fusion (6). The synthesis of pigment binding proteins involved in LH and the photochemical reaction in the photosynthetic membranes is also repressed by oxygen (7). Recently, a number of studies (8–15) have shown that the levels of mRNA for LH (I, II) and RC (L, M, H) complexes in \textit{R. capsulatus} or \textit{Rhodobacter sphaeroides} are reduced in response to oxygen. Although most photosynthetic genes are repressed by oxygen, the Crt genes, particularly the \textit{crtA} gene, are activated by oxygen, perhaps for the protection of the cells from photooxidation (9). It has been reported that an open reading frame (ORF) designated as Q upstream from the \textit{puf} operon may function as a regulatory gene to control the \textit{puf} operon and BChl biosynthesis in response to oxygen (16). Recently, the same authors have modified their hypothesis suggesting that the Q gene may code for a BChl binding protein.\textsuperscript{1} We have found that two ORFs exist in the \textit{EcoQ} fragment of \textit{R. capsulatus}. The additional ORF [\textit{I743} base pairs (bp)] designated as R is located upstream from the Q gene (225 bp) with a space of 1 bp (M. Alberti and J.E.H., unpublished data). The R gene in \textit{R. sphaeroides} seems to regulate the insertion of LH-I in the membrane.\textsuperscript{1} Furthermore, we have found that the expression of both putative genes is also regulated by oxygen and light (see below). This raises the further question: What is the fundamental mechanism for oxygen regulation? Since most of the photosynthetic genes are coordinately expressed in response to the depletion of oxygen, there might be a common mechanism for their coordinate expression. Here we present \textit{in vivo} evidence using the gyrase inhibitors novobiocin and coumermycin that the expression of the photosynthetic genes in \textit{R. capsulatus} in response to oxygen is mediated by the supercoiling of DNA.

Materials and Methods

Bacteria and Anaerobic and Aerobic Growth Conditions. \textit{Rhodobacter capsulatus} SB1003 was grown either photoheterotrophically or chemoheterotrophically as described (9, 10). The photoheterotrophically grown cells were cultured at 30°C and at a light intensity of 30 W/m² in 15-ml screw-cap tubes. The

Abbreviations: LH, light harvesting; RC, reaction center; BChl, bacteriochlorophyll; Crt, carotenoid; ORF, open reading frame; PuP, case, ribulose-bisphosphate carboxylase.

\textsuperscript{1}Bauer, C. E. & Marrs, B. L., Symposium on Molecular Biology of Photosynthetic Procytoles, June 8–10, 1987, University of Wisconsin–Madison, p. 2 (abstr.).

\textsuperscript{2}Kaplan, S., Symposium on Molecular Biology of Photosynthetic Procytoles, June 8–10, 1987, University of Wisconsin–Madison, p. 1 (abstr.).
tubes were filled completely to the top with malate-minimal RCV medium (17). The doubling time of cells under these conditions is about 3 hr. The gyrase inhibitors novobiocin, coumermycin, nalidixic acid, and oxolinic acid were added when the cells were grown to midlogarithmic phase (5-7 x 10⁶ cells per ml). The cells were harvested at various time points and used for pigment, chromatophore, and RNA preparation (9, 10).

For the experiments involving a shift from aerobic to photosynthetic growth, the aerobic growth was maintained by chemoheterotrophic culture (10 ml of medium in a 120 ml Erlenmeyer flask) on a Gyraatory shaker (200 rpm). Induction of the photosynthetic genes was performed by pouring the midlogarithmic-phase aerobic cells into 15-ml screw-cap tubes filled to the top and by culturing them under the anaerobic photosynthetic conditions. Novobiocin or coumermycin was immediately added to cell cultures after the shift from the aerobic to the anaerobic conditions. The cells were harvested after 3 hr for pigment and RNA extraction.

**Assay of Pigments and Chromatophores.** Phototrophic pigments were extracted with acetone/methanol (7:2). The total BCHl content of the cells was estimated by absorbancy measurements at 775 nm (10). Chromatophores were prepared from the cell lysate (10). The absorption spectra of the pigments and the chromatophores were measured as described (9,10).

**RNA Isolation and Blot Hybridization.** Total RNA was extracted as described (8). After purification, the RNA was fractionated by electrophoresis and transferred to the membranes. Procedures for dot blots and RNA transfer hybridizations and the preparation of hybridization probes (plasmid DNA, M13 phage DNA, and synthetic oligonucleotides) for various photosynthetic genes have been described (9,10). To quantify hybridization, either the autoradiograms were scanned with a GTS 300 transmittance-reflectance scanning densitometer (Hoefer, San Francisco) or the filters were assayed for radioactivity in 2,5-diphenyloxazole-1,4-bis(5-phenyloxazolyl)benzene cocktail with a Packard model 3385 liquid scintillation counter.

**RESULTS**

**Differential Inhibition by Novobiocin and Coumermycin of the mRNAs for LH and RC Subunits, BCHl and Cytobiosynthetic Enzymes, Ribulose-Bisphosphate Carboxylase (RuP₂Case), and Cytocobyrinic Acid.** The superhelicity of DNA in bacteria is determined by a dynamic balance between gyrase, which supercoils the DNA, and topoisomerase I, which relaxes the DNA. Thus, inhibition of gyrase leads to a decrease in negative superhelicity (18). If the expression of the photosynthetic genes in *R. capsulatus* is dependent on the supercoiling of the DNA, inhibitors of gyrase would repress transcription and reduce the levels of these gene products. We have measured the mRNA levels in *vivo* of various genes in *R. capsulatus* cells with or without novobiocin and coumermycin by dot or RNA transfer hybridization using probes for specific genes. To determine whether or not the inhibition is specific to anaerobic genes for photosynthesis, we have also examined the effect of gyrase inhibitors on the levels of mRNA for the cytochrome *bc₁* complex. The cytochrome *bc₁* consists of an FeS protein, a cytochrome *b*, and a cytochrome *c₁*; the genes coding for these three subunits are on the *fbc* operon (19). Fig. 1c shows that the *fbc* operon is expressed in anaerobic and aerobic conditions.

Dot hybridization (Fig. 1) shows that the levels of the various mRNAs were constant in the absence of the drugs over the course of the 3-hr incubation. The levels of various mRNAs, however, changed in different patterns after drug treatment. The response of the various RNA species to gyrase inhibition roughly falls into one of two broad classes: gyrase-dependent, oxygen-regulated mRNAs; and gyrase-less dependent, constitutive mRNAs. The first group includes the genes for the structural components of the photosynthetic apparatus (LH-1, LH-11, RC), BCHl biosynthesis, and CO₂ fixation (RuP₂Case). These mRNAs are immediately and dramatically reduced after the addition of gyrase inhibitors as shown in Fig. 1a. The patterns of mRNA for LH-11, RC-M, and RC-H (data not shown) were similar to LH-1 and RC-L, respectively. The half-lives of mRNAs for LH proteins following addition of the gyrase inhibitor were ~20 min, and those for RC proteins were about 10 min, a result similar to the data observed following addition of proflavin as an inhibitor of transcription (9). (See last column, Table 1.) The levels of mRNA from *bc₁*, *fbc*, and *RuP₂* in *BamHI-E* fragment (Fig. 1a) and mRNA from *rbcl* and ORF Q, R (not shown) were also significantly reduced by the inhibitors within 30 min.

The second class of genes that are less sensitive to gyrase inhibitors includes genes for cytochromes and genes for Cytobiosynthetic enzymes typified by *crta*, *I* in *BamHI-H* fragment (Fig. 1a) and *crn* in *BamHI-M* fragment (not shown). The levels of mRNAs from these genes and from the *fbc* genes decreased at a slower rate and to smaller extents, reaching half of their initial values after ~3 hr (Fig. 1a).

The reduction of the levels of mRNA from the genes coding for LH-11, RC-L, and RC-H and from the genes *rbcl*, Q, R, and *bc₁* in *BamHI-E* under photosynthetic conditions can be detected by dot hybridization in as short a time as 15 min following the inhibitor treatment, as shown in another set of experiments (see top three rows of Fig. 1b, marked as PS). The sensitivity of different classes of genes to the inhibitors has also been tested in the shift from aerobic to photosynthetic conditions. The cells were grown aerobically and then shifted to anaerobic photosynthetic conditions in the presence or the absence of novobiocin and coumermycin. RNA was extracted 3 hr after the shift. The formation of the photosynthetic membrane is induced in the cells during the transition period characterized by no growth at the early stage. The measurement of mRNA has also revealed the differential sensitivity of different classes of genes to the gyrase inhibitors as shown in the bottom three rows of Fig. 1b, marked as (O₂ → PS). The effects of oxygen and light intensity on the mRNA levels from the Q and R genes are shown in Fig. 1c.

The decrease in levels of mRNA for LH-11 and RC (L, M) has been observed by using RNA transfer (Fig. 2a) and dot (Fig. 2b) hybridization even when the novobiocin concentration is reduced to 5 and 10 µg/ml. However, the basal mRNA levels of the same genes from aerobic cells, which is much lower than that in photosynthetic cells, are not affected by novobiocin (Fig. 2b).

**Effects of Novobiocin and Coumermycin on the Biosynthesis of BCHl and LH-I and LH-II Complexes.** Since the transcription of various photosynthetic genes including the genes involved in BCHl biosynthesis and the genes for LH proteins is inhibited by novobiocin and coumermycin, the biosynthesis of BCHl and LH complexes must also be inhibited by the drugs, but over a longer time scale. We have examined BCHl content in the presence of the gyrase inhibitors and have shown that the accumulation of BCHl in photoheterotrophically grown cells is strongly inhibited by novobiocin and coumermycin (data not shown). The BCHl accumulation is inhibited by novobiocin and coumermycin at concentrations as low as 5 µg/ml. Photosynthetic growth was gradually inhibited as the concentration of the inhibitors was increased from 5 µg/ml to 100 µg/ml, although viability of the cells was not affected up to 150 µg/ml (see also ref. 21). The time course of the inhibition of BCHl accumulation indicates that a significant inhibition is observable 30 min after addition of the drugs.
Fig. 1. Dot blot hybridization of *R. capsulatus* RNA with gene probes for LH and RC subunits, BChl and Crt biosynthetic enzymes, RuP₃Case, the Q and the R products, and cytochrome bc₁ complex. (a) Time course of RNA levels in the presence of inhibitors. Novobiocin (50 μg/ml) (marked as C) and coumeyracin (50 μg/ml) (marked as B) were added to photosynthetically growing cells. A represents RNA from the photosynthetic cells without the inhibitor treatment. The total RNA was extracted from an equal number of cells at various times (0, 30, 60, 90, 120, 180 min) as indicated on the left. The RNA was denatured in formaldehyde, blotted onto a GeneScreen membrane, hybridized with 32P-labeled DNA probes, and exposed to X-ray film as described (8). The probes are marked on the top of each panel. (b) Dot hybridization. The top three rows represent the RNA from photosynthetically growing cells (marked as PS). The RNA was isolated from the cells 15 min after addition of coumeyracin and novobiocin. The bottom three rows represent the RNA isolated from the cells 3 hr after a shift from aerobic to anaerobic conditions (marked as O₂₋→ PS). A: control; B: coumeyracin (50 μg/ml); C: novobiocin (50 μg/ml). Most probes have been described (9, 10). The probe for Q is an M13 clone, ESL1, containing an EcoRI–Sal I fragment in the ORF Q from 142 to 342 bp 5′ to the pufB gene. The probe for R is an M13 clone, S357, containing an internal Sau3A insert within the ORF R from 339 to 740 bp 5′ to the pufB gene; it may partially overlap with the Q mRNA, based on S1 nuclease analysis. * ESL1 and S357 are within the EcoRI-Q fragment of pRPS404. The probe for the rbcl gene is a plasmid, pLJ10, carrying a 0.3-kilobase (kb) heterologous DNA insert coding for form II RuP₃Case of *R. sphaeroides* (20). The same size of major transcript of rbcl and its same response to oxygen and light were observed in *R. capsulatus* (unpublished data) as in *R. sphaeroides* (8). The probe for fbc genes is a plasmid, pRSF1, containing an insert coding for the entire fbc operon of *R. capsulatus* only one 2.9-kb transcript from this operon was found (19). (c) Dot hybridization of mRNAs from fbc and the Q and R genes. The mRNA was isolated from cells grown under different oxygen and light conditions as described (10). The anaerobic photosynthetic conditions are designated as HL (high light), D (dark), and LL (low light). The aerobic condition is designated as O₂.

The inhibition of BChl biosynthesis by the drugs has also been observed in the cells shifted from aerobic to anaerobic photosynthetic conditions. Such a shift is described in the former section. We could detect no growth in our cultures based on optical density measurements at 680 nm during the first 3 hr of the induction period with or without the drugs. The BChl is absent under aerobic conditions. However, the synthesis of BChl is initiated by the shift to anaerobic conditions in the absence of the drugs and fails to initiate when novobiocin or coumeyracin is present. The BChl content in the control cells was estimated at 1.0 μg/mg of protein after a 3-hr induction, whereas BChl in either novobiocin- or coumeyracin-treated cells was not detectable.

The spectra of pigment extracts and chromatophores (data not shown) have also demonstrated that the accumulation of LH-I, II complexes as well as BChl is markedly inhibited by novobiocin and coumeyracin.

To further verify the involvement of DNA supercoiling in BChl accumulation, we have tested two additional inhibitors of gyrase: nalidixic acid (50 μg/ml) and oxolinic acid (50 μg/ml). A similar inhibition of BChl accumulation by these drugs was observed (not shown).

**Discussion**

*R. capsulatus* carries genes for aerobic and anaerobic metabolic modes, whose expression and regulation are determined by environmental oxygen tension. One of the features of this regulation is the coordinated expression of many groups of genes essential for LH and photochemical reactions (8–12, 14, 15) upon removal of oxygen. It is reasonable to suggest that a common mechanism for oxygen-specific regulation exists that is superimposed on the individual regulation of some of these genes. Although several hypotheses, such as redox potential control (22) or aporepressor-corepressor interactions (23), have been proposed as mediators of the environmental signal, the exact mechanism of oxygen regulation has not been clarified. Recent results in Gram-negative bacteria, however, have shed some light on this question (21, 24). Yamamoto and Droffner (24) have hypothesized that the expression of genes required for anaerobic growth in *Salmonella typhimurium* depends on the supercoiling of chromosomal DNA, whereas the expression of genes required for aerobic growth depends on the relaxation of DNA. Kranz and Haselkorn (21) have shown by lac fusion
that the anaerobic expression of the nif operon in R. capsulatus is controlled by the supercoiling of DNA. They also noticed a decrease in cellular pigmentation after gyrase inhibitor treatment. The effect of DNA topology on the transcription of rbcL and atpB in maize (25) and pea (26) and on hydrogenase synthesis in Bradyrhizobium japonica (27) has been recently reported using gyrase inhibitors. The results presented in this report, using the gyrase inhibitors novobiocin, coumermycin, nalidixic acid, and oxolinic acid in vivo, indicate that the regulation by oxygen of the mRNA levels of genes for the LH peptides, RC proteins, and RuPCase in R. capsulatus is mediated by the supercoiling of DNA. Superhelical tension may be involved in the regulation of expression of numerous prokaryotic and eukaryotic genes (24–28). The topology of DNA is mainly determined by two enzymes, topoisomerase I and gyrase, in bacteria. DNA gyrase catalyzes the conversion of relaxed DNA to a superhelical form at the expense of ATP; topoisomerase I catalyzes the reverse reaction, converting supercoiled DNA to a more relaxed form (18, 28, 29). There has been speculation that topoisomerase and gyrase might act at specific sites on the chromosome to effect the supercoiling of some genes more than for others (30). DNA gyrase has been extensively studied in bacteria. It is made up of two subunits, A and B. Novobiocin and coumermycin are specific inhibitors of the bacterial gyrase B subunit, whereas nalidixic acid and oxolinic acid are inhibitors for the A subunit (18, 28, 29). We have shown that the ATP/ADP ratio of a cell extract from R. capsulatus is strongly inhibited by these drugs. The measurement of a decrease in psoralen photo-reactivity with the DNA in BamHI-C,F and K fragments upon treatment of cells with novobiocin has provided direct evidence that novobiocin is affecting the superhelicity of genomic DNA in vivo (D. Cook, G. Armstrong, and J.E.H., to be published elsewhere). It is therefore our position that novobiocin and other gyrase inhibitors can be used as useful tools in the study of the superhelicity of the chromosome. In this study we have shown that novobiocin and coumermycin have strong inhibitory effects on the levels of mRNA for BChl biosynthesis, LH, and RC complexes and on their respective mRNAs. The levels of mRNA for LH and RC, however, were not reduced in two novobiocin-resistant mutants, RC232 and RC233, following novobiocin treatment (data not shown). We have also demonstrated a significant inhibition of mRNA from rbcL and the ORFs Q and R. This inhibition occurs on a time scale of minutes and is observed only for oxygen-sensitive genes. The concentrations of the inhibitors used (50 μg/ml) in this study do not irreversibly damage R. capsulatus cells since cell growth is recovered after washing away the novobiocin. The reduction of mRNA for LH-I and RC (L, M) has been observed after treatment with novobiocin even at concentrations as low as 5 or 10 μg/ml when the cell growth undergoes only a slight inhibition. We, therefore, believe that the inhibition of transcription upon addition of the antibiotics is a specific consequence of the selective repression of transcription of a large number of photosynthetic genes. As we reported earlier (9), the half-lives of mRNAs for LH-I, LH-II, and RC (L, M, H) in R. capsulatus are 19, 24, and 10 min, respectively, when proflavin is used as a specific transcription inhibitor. The half-lives of respective mRNAs in the presence of novobiocin or coumermycin measured in this study agree with these values. The similarity of the kinetics of in vivo mRNA levels of this class of genes in response to the gyrase inhibitors and to proflavin provides

<table>
<thead>
<tr>
<th>Probe</th>
<th>Positions*</th>
<th>Size, bp</th>
<th>Gene or gene product</th>
<th>Ref.</th>
<th>Half-life of corresponding mRNA, min</th>
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<tbody>
<tr>
<td>M13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T319</td>
<td>BamHI-C</td>
<td>422 to 342</td>
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<tr>
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<tr>
<td>S331</td>
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<td>1704 to 1607</td>
<td>97</td>
<td>RC-M</td>
<td>10</td>
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<tr>
<td>T210</td>
<td>BamHI-F</td>
<td>3647 to 3413</td>
<td>234</td>
<td>RC-H</td>
<td>10</td>
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<td>ESL1</td>
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<tr>
<td></td>
<td>BamHI-C</td>
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<td>201</td>
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<td>pFL205</td>
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<td>crtA, -I, -bchI</td>
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<td>LH-II</td>
<td>10</td>
<td>24</td>
</tr>
</tbody>
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*The sequence addresses of these probes refer to positions on sequences first published by Youvan et al. (34).

1Specific for crtA.

![RNA transfer](image-url)
strong evidence that the primary effect of the gyrase inhibitors is on the transcription. A second class of genes, whose mRNAs have more or less similar half-lives as the first class does upon addition of proflavin, displays vastly different kinetic response to gyrase inhibitors in comparison to proflavin (Table 1). This differential sensitivity of the anaerobic genes tested and constitutive genes to the gyrase inhibitors supports the hypothesis that the gyrase inhibitors selectively alter the transcription of specific genes, presumably by changing the DNA conformation at or near the respective gene. Two classes of genes, therefore, can be distinguished based on their response to gyrase inhibitors: strongly gyrase-dependent anaerobic genes, including those for LH and RC complexes, BChl biosynthetic enzymes, RuP₃Case, and the Q and R genes; and genes less dependent on gyrase, such as genes for Ctr biosynthesis, cytochromes, and the constitutive genes for LH-I and RC complexes that are expressed under aerobic conditions. Thus, we propose that the change in oxygen levels induces changes in DNA topology, possibly locally, which, in turn, lead to selective changes in transcription of genes.

It is interesting to note that the oxygen-regulated and constitutive mRNAs for LH-I and RC (L, M) have different sensitivities to novobiocin and coumermycin. The genes for LH-I (β and α subunits) and RC (L and M subunits) plus an ORF, C2397, comprise a single operon designated as pufo operon (10, 11). Two transcripts (0.5 and 2.6 kb) originate from the pufo operon (10, 11, 13). The 0.5-kb transcript codes for LH-I (β and α) polypeptides, whereas the 2.6-kb transcript codes for LH-I (β and α) as well as RC (L, M) and C2397. Two pufo promoters, oxygen-regulated and constitutive, have been postulated recently in R. capsulatus on the basis of lac fusions. A simple model would be that the different promoters have different sensitivities to DNA topology. The alternative possibility would be a more complex interaction between the promoter, some upstream DNA regulatory sequence (31), or regulatory protein and gyrase. The different sensitivities of promoters to DNA topology has been demonstrated in other organisms (28, 31, 32). The *Escherichia coli* promoters for DNA gyrase subunits (32) and the *Chlamydomonas* chloroplast promoter for a dark-induced transcript A (31) are even stimulated by novobiocin. Recently, Menzel and Gellert have found that a DNA sequence 20 bp long is responsible for DNA relaxation-stimulated transcription of the *E. coli* gyrase gene (33). The mechanism by which supercoiling of DNA may lead to enhanced transcription during photosynthetic growth in *R. capsulatus* is unknown.

We thank J. Willson for kindly providing the mutants RC233 and RC233, S. Kaplan for providing his clone pLI10 as probe for *rbcL*, and N. Gabellini for providing us with her clone pRSF1 for probing *fbc*. We thank M. Gellert, N. Cozzarelli, and J. Wang for their valuable comments and D. Cook, G. Armstrong, M. Alberti, F. Leach, and D. Burke-Aguero for their help during writing. Most of this work was supported by the Office of Basic Energy Sciences, Biological Energy Research Division of the Department of Energy under Contract DE-AC03-76SF00098. The probes for the Crt genes and BChl genes were obtained from a project supported by National Institutes of Health Grant GM30786. The Biosearch Sam One was leased with Program Development Funds from the Director of Lawrence Berkeley Laboratory.