Regulation of the steady-state levels of B800-850 complexes in *Rhodobacter capsulatus* by Light and Oxygen

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ABSTRACT

Photosynthetic organisms exhibit a variety of responses to changes in light intensity, including differential biosynthesis of chlorophyll-protein complexes. Cultures of *Rhodobacter capsulatus* grown anaerobically with a low intensity of light (2 W/m²) contained about four times as much B800-850 light harvesting complex as cells grown under high light intensity (140 W/m²). The mRNA transcripts encoding B800-850 beta and alpha peptides were analyzed by Northern blot, S1 nuclease protection and capping with guanylyl transferase. It was found that the steady-state levels of B800-850 mRNAs in high light-grown cultures was about four times as great as in cells grown under low light intensity. Therefore the lesser amounts of mature B800-850 peptide gene products found in cells grown with high light intensity were the result of a posttranscriptional regulatory process. It was also found that there were two polycistronic messages encoding the B800-850 peptides. These messages shared a common 3' terminus but differed in their 5' end segments as a result of transcription initiation at two discrete sites. Moreover the half life of B800-850 mRNAs was about 10 minutes in cells grown with high light and approximately 19 minutes in low light-grown cultures. Transcriptional and translational fusions were constructed between the B800-850 transcription initiation region (from this point on referred to as the *puc* transcription initiation region; see Fig. 1) and the *Escherichia coli lacZ* gene. From these studies it was concluded that the rates of transcription initiation of the *puc* (B800-850) genes was higher in cells grown with high light illumination than in low light-grown cultures, and that the relative amount of B800-850
complexes under these conditions was controlled by a translational or a posttranslational mechanism. The translational and transcriptional fusions were also used for examination of oxygen regulated expression of the *puc* genes.
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Abreviations and Symbols

ATP  adenosine triphosphate
bch a  bacteriochlorophyll a
bp  base pair
Ci  Curie
CFU  colony forming units
dATP  deoxyadenosine triphosphate
dCTP  deoxycytosine triphosphate
dGTP  deoxyguanosine triphosphate
DNA  deoxyribonucleic acid
TTP  thymidine triphosphate
EDTA  ethylenediaminetetraacetic acid
kb  kilobase
kd  kilodalton
lacZ  E. coli beta-galactosidase gene
mA  milliampere
nt  nucleotide
pucA, pucB  structural genes of B800-850 peptides
pufA, pufB  structural genes of the B870 peptides
pufM, pufL, pufH  structural genes of the reaction centre peptides
CNPG  o-nitrophenyl-β-D-galactoside
RC  reaction centre
RC-M, L, H  peptide components of the reaction centre
RNA  ribonucleic acid
Tc  Tetracycline
TTP  thymidine triphosphate
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INTRODUCTION

*Rhodobacter capsulatus* (formerly known as *Rhodopseudomonas capsulata*; ref. 23) is a purple nonsulfur photosynthetic bacterium that is capable of growth by use of several different modes of energy generation, including aerobic respiration in darkness and anaerobic photosynthesis (20). During respiratory growth in the presence of high concentrations of oxygen, cells of *R. capsulatus* are in many respects indistinguishable from non-photosynthetic bacteria. When growing photosynthetically *R. capsulatus* produces metabolic energy by cyclic photosynthesis which results in protons being pumped across the cytoplasmic membrane, creating a proton gradient that drives ATP synthesis. Both aerobic and anaerobic forms of energy metabolism are coupled to distinct electron-transport pathways which are embedded in two ultrastructurally and biochemically distinct forms of the cytoplasmic membrane (20). Therefore this bacterium provides an attractive model to study procaryotic cell differentiation, since the type of energy generation mechanism (such as chemotrophy or phototrophy) and cellular composition are dependent on the culture growth conditions.

With a reduction in the amount of oxygen available to cultures, respiring cells develop an extensive intracytoplasmic membrane system (derived from the cytoplasmic membrane) into which is placed the photosynthetic apparatus. There are three pigment-protein complexes produced by *R. capsulatus* which make up the photosynthetic apparatus (Fig. 1): the reaction centre (RC), which is the site of charge separation, and two distinct light harvesting complexes (B870 and B800-850) which function to gather light energy and funnel it into
Fig. 1. Diagramatic representation of the reaction centre, B870 and B800-850 complexes in the membrane of *R. capsulatus*. The genes encoding the photosynthetic peptides are represented below as DNA fragments (the heavy segments represent the structural genes). Although the *putX* open reading frame is transcribed, it is not known if it encodes a peptide (Figure adapted from Drews *et al* ref. 18).
the reaction centre (19). The key pigment involved in photon capture is bacteriochlorophyll $a$ (bch $a$), which is noncovalently bound to the photosynthetic peptides and is held in an orientation such that light energy can be either trapped and transferred to another complex (as in the light harvesting complexes), or can be used to excite electrons to initiate cyclic photosynthesis (as in the reaction centre) (20).

The RC complex is made up of three subunits: designated M, L and H whereas the B870 complex is composed of two peptides called B870 alpha and beta (19). The genes encoding the B870 peptides and two of the three RC peptides have been cloned, sequenced and found to form an operon (6, 43). All genes in this operon are called put genes, therefore the RC structural genes are designated putM and putL whereas the B870 genes are referred to as putA and putB (Fig. 1). The RC-H peptide seems not to be involved in the process of photosynthesis (33, 43) and the gene encoding the RC-H subunit, designated puhH, is on a separate operon. The B800-850 light harvesting complex of R. capsulatus contains two peptides, referred to as the alpha and beta peptides, that bind bch $a$ (22). The genes encoding the pigment-binding peptides (designated pucA and pucB) have been cloned and their DNA sequence determined, but it is not known how far away the puc genes map from the put genes on the R. capsulatus chromosome (44). The two puc (B800-850) structural genes are located very close to each other and have been assumed to form an operon (47). A third peptide, the 14 kD peptide, has been found to co-purify with the B800-850 alpha and beta peptides, but it does not bind pigments and its function is obscure (20, 22).
When *R. capsulatus* cells are shifted from a highly aerated culture condition to an oxygen limited growth condition the synthesis of all of the components of the photosynthetic unit is initiated to facilitate the photosynthetic growth mode. The specific bch a content increases 80 fold and the number of reaction centres, B870 and B800-850 complexes is increased approximately 10 to 20 fold (36). Clark *et al* (14) and Belasco *et al* (6) determined that there was more mRNA encoding the RC and B870 peptides in oxygen limited cultures than in highly aerated cultures. As well Zhu and Hearst (47) found 40 fold more *puc* mRNA in low oxygen grown cells than cells grown with high levels of oxygen. In high to low oxygen shift experiments Klug *et al* (26) found a transient 10 to 20 fold increase in *puc* and *puf* mRNA which eventually dropped to a new higher steady state level. Bauer *et al* (4) constructed *pufl-lacZ* translational fusions on plasmids and conjugated them into *R. capsulatus* cells. When grown under high and low oxygen conditions the cells containing these plasmids were found to have 30 fold more beta-galactosidase activity in oxygen limited cultures. Since the amount of mRNA and the levels of *lacZ* expression both parallel the levels of photosynthetic peptides, oxygen is assumed to affect the transcription of the photosynthetic genes. Therefore oxygen regulated expression of the *puc* and *puf* genes appears to act at the level of transcription. Beatty *et al* (5) constructed transcriptional fusions between the *lac* operon and progressive *puf* promoter region deletions, and determined that the DNA sequence responsible for oxygen regulated transcription is several hundred nucleotides upstream from the first structural gene in the *pufl* operon.

Photosynthetically growing *R. capsulatus* cells have at least two mechanisms to adapt to variation in light intensity: cells can either change the number of photosynthetic units per cell, or alter the size of the photosynthetic
unit. Schumacher et al (37) found that in anaerobically growing cells shifted from high light to low light, the number of photosynthetic units doubled and their size had increased by 70%. As well bch a levels increased five fold and more intracytoplasmic membrane was produced. The relative amounts of RC and B870 complexes remain relatively constant with changes in illumination, whereas the amounts of B800-850, relative to RC and B870 complexes, increase in response to a reduction in light intensity during photosynthetic growth (21, 27, 37). Therefore the increased size of the photosynthetic unit in low light illumination is due to an increase in the number of B800-850 complexes. Because the light intensity has decreased the cell increases the efficiency of photon capture by producing relatively more B800-850 complexes, thus forming larger photosynthetic units.

As the photosynthetic peptides cannot function in the absence of bch a, the synthesis of this pigment provides a convenient site for regulation of photosynthetic gene expression. In cells grown in low light more bch a is present than in high light grown cells (37). Since the B800-850 apo-peptides must bind bch a to participate in photosynthesis, the increase in the number of B800-850 complexes may be related to the increase in bch a content.

The primary goal of my project was to determine how the anaerobically growing cell regulates the number of B800-850 complexes produced. In this investigation I used segments of the cloned puc (B800-850) genes as probes to determine the approximate transcriptional start and termination sites of puc mRNAs, and their relative steady state amounts, under photosynthetic conditions with either excess or growth rate-limiting levels of light. Also, puc-lac transcriptional and translational fusions were constructed on plasmids to
determine the relative rates of transcription and translation initiation of the *puc* genes in various growth conditions. The relative amounts of *puc* mRNAs were compared to the relative amounts of complexes, and a posttranscriptional regulatory mechanism was proposed to account for the relationship between mRNA levels and the amounts of mature B800-850 complexes.
1. Bacterial strains and growth conditions. The wild type strain
*Rhodobacter capsulatus* B10 (30) and the deletion mutant *Rhodobacter capsulatus* ΔRC6 (13)
were used throughout this study. The *Escherichia coli* strains JM108 (42),
JM83 (42), RB404 (10), and HB101(9) were used.

Cultures of *Rhodobacter capsulatus* B10 containing a plasmid were routinely
grown in RCV medium (40) supplemented with 0.5 µg tetracycline (Tc)/ml,
0.1% yeast extract and 10 mM phosphate buffer. For high and low oxygen
growth experiments *Rhodobacter capsulatus* cells were grown to stationary phase in 3 ml
of liquid medium at 30°C for 16 hours. The cells were then diluted to an O.D.650
of 0.003 and transferred to a 500 ml Erlenmeyer flask containing 40 ml of the
liquid medium. This culture was grown at 34°C at 300 rpm until it reached a
density of 150 to 200 Klett units. The sample was then diluted to 10 klett units
in two Erlenmeyer flasks. The highly aerated sample had 40 ml of medium in a
500 ml flask grown at 300 rpm, while the low oxygen culture had 40 ml of
medium in a 50 ml flask at 150 rpm. When both samples reached 80 Klett units
(about 3x10^8 colony forming units (CFU)/ml) the cells were harvested,
pelleted and frozen.

Inocula for both high and low light grown samples were grown to
stationary phase in 3 ml of the enriched RCV medium at 34°C under low aeration
to induce the synthesis of the photosynthetic apparatus. The cells were then
diluted to 10 Klett units and transferred to a completely filled 20 ml screw cap
tube and incubated at 34°C with either 140 W/m^2 (high light) or 2 W/m^2 (low
light) incandescent tungsten illumination. Cells were harvested at a density of $3 \times 10^8$ CFU/ml to minimize the effects of self-shading.

*R. capsulatus* B10 cultured with high and low light illumination for RNA isolation was grown as described in the previous section (except that no Tc was added to the culture medium). In experiments determining the half-life of the *puc* mRNA, 800 ml of cells were grown photosynthetically, with either high or low light illumination, in a ca. one litre Roux bottle at 34°C. The medium was bubbled continuously with filtered 95%N$_2$-5%CO$_2$ gas. When cells reached a density of $3 \times 10^8$ CFU/ml a 25 ml sample was removed, transcription initiation was stopped by addition of rifampicin (200 µg/ml) to the culture, and subsequent samples were then taken at 20, 40, 60 and 80 min. for RNA extraction.

*Escherichia coli* cultures were grown in LB medium as described (29).

2. **Determination of beta-galactosidase activities.** The *R. capsulatus* cell pellets were resuspended in 1 ml of Z-buffer (0.06 M Na$_2$HPO$_4$.7H$_2$O, 0.04 M NaH$_2$PO$_4$.H$_2$O, 0.01 M KCl, 0.001 M MgSO$_4$.7H$_2$O, and 0.05 M beta-mercaptoethanol, pH 7.0) (31) and sonicated to release the beta-galactosidase enzyme. The samples were centrifuged to pellet cellular debris. To measure the amount of beta-galactosidase enzyme in the supernatant fluid the substrate o-nitrophenyl-β-D-galactoside (ONPG) was used. When beta-galactosidase cleaves ONPG it releases o-nitrophenol which absorbs light strongly at 420 nm, therefore by following the increase in absorbancy at 420 nm one can quantitate the amount of enzyme produced. The assay mixture contained 500 mM ONPG, various amounts of the sonicated extract and Z-buffer to 1 ml.
3. Construction of the plasmid pJAJ103lac903. The broad
host range plasmid pJAJ103 (24) was cut with BamHI and ligated to the 7.6 kb
BamHI to BglI fragment from pMC931 (12) to give pJAJ103lac903 (Fig. 2).
This 7.6 kb segment has part of the trp operon fused upstream of the lacZYA
structural genes. The 7.6 kb fragment contains the last 24 codons of the trpB
gene, starting from a HpaI site which has been converted to a BamHI site (11),
and downstream of the trpB gene is the entire 0.9 kb trpA gene plus 47 nt
beyond the trpA stop codon (J. T. Beatty personal communication). These trp
sequences had been fused in vivo (12) to the lac operator region 23 nt from the
start codon of the lacZ structural gene, such that the lac promoter had been
deleted. Upstream from the trp genes on pJAJ103lac903 there are unique
PstI and BamHI sites which were used to insert promoter fragments from the
puc operon. Transcription initiated from the cloned promoter would then
proceed through the trp genes and into the lac operon (see Fig. 2).

4. Construction of the transcriptional fusion between the
puc promoter and the lacZ operon. The R. capsulatus pucA and pucB
structural genes were previously isolated on a 5.75 kb EcoRI fragment cloned
into pBR322 to give pRPSLHII (44). The putative puc promoter was isolated
from pRPSLHII on a 2.9 kb PstI-ClaI fragment which contained the first 73 nt
of the pucB structural gene and approximately 2.0 kb of upstream sequence as
outlined in Figure 2. This 2.9 kb PstI-ClaI fragment from pRPSLHII was
inserted into AccI - PstI cut pUC13 to give pAZII. The 2.9 kb piece was cut out
of pAZII with PstI and BamHI and ligated with pJAJ103lac903 (see above)
that had also been cut with PstI and BamHI. The resultant plasmid, pAZIII, now
had the puc promoter transcriptionally fused to the lac operon. pAZIV was
Fig. 2. Construction of the *puc-lacZ* transcriptional fusions pAZIII and pAZIV. The open boxes represent the *lacZ* gene, the hatched boxes are the *trp* genes (carried by pJAJ103) and the closed boxes are the *puc* genes. Abbreviations: A-Acc 1; B-Bam H1; C-Cla 1; E-Eco R1; P-Pst 1.
constructed by deleting the 1.5 kb \textit{PstI} segment from pAZIII.

5. Construction of a translational fusion between the \textit{lacZ} and the \textit{pucB} structural genes. A plasmid containing a translational \textit{lacZ} gene fusion to the \textit{pucB} structural gene was constructed by first isolating a 6.8 kb \textit{BamHI} fragment from pMC931 (12). This fragment contained the \textit{lac} operon with a truncated form of the \textit{lacZ} gene, which has the first eight codons of the amino-terminal end removed and a unique \textit{BamHI} site inserted adjacent to the eighth codon. This segment was cloned into the \textit{BamHI} site on pAZII, 3' of the \textit{pucB} stuctural gene, to give pAZII(lacOF) (see Fig. 3). pAZII(lacOF) contains the \textit{puc} promoter and the first 73 nt of the \textit{pucB} structural gene fused to \textit{lacZ} such that the \textit{pucB} gene was in a different translational reading frame from \textit{lacZ}. An in-frame (translational) fusion was created between the \textit{pucB} and the \textit{lacZ} genes by subjecting pAZII(lacOF) to a \textit{BamHI} partial digest and isolating plasmid molecules that had been cut only once. The linear fragments were digested with mung bean nuclease, which degraded the 5' protruding \textit{BamHI} ends, and then religated to form the translational fusion, pAZII(lacIF). This in-frame fusion was isolated from pAZII(lacIF) on an 8 kb \textit{PstI} - \textit{BamHI} fragment and inserted into pJAJ103 (see above) to give pAZV, which was conjugated from \textit{E.coli} JM108 into \textit{R.capsulatus} B10 (see below). The final construct contains the \textit{puc} transcription initiation region, \textit{pucB} ribosome binding site and its first 25 codons fused inframe to the \textit{lacZ} gene.

6. Conjugation of recombinant plasmids into \textit{R. capsulatus} B10. To transfer the plasmid constructs into \textit{R. capsulatus} B10 a triparental mating was done (17). \textit{E. coli} containing the recombinant plasmid was mixed with \textit{R. capsulatus} B10 and \textit{E. coli} HB101(pRK2013). The plasmid pRK2013
Fig. 3. Construction of the *puc-lacZ* translational fusion pAZV. The open box represents the *lacZ* gene whereas the closed box represents the *puc* genes. Abbreviations: B-*Bam* H1; E-*Eco* R1; P-*Pst* 1.
encodes the mobilizing factors which allow the transfer of the recombinant plasmid from *E. coli* to *R. capsulatus* B10. The three strains were mixed together, spotted onto an RCV plate and incubated overnight at 30°C. The cells in the spot were then streaked on another RCV plate containing 0.5 μg/ml Tc. The *E. coli* strains used were multiply auxotrophic and therefore could not grow on the RCV minimal medium. In addition, the plate contained Tc which allowed only *R. capsulatus* cells containing the plasmid to grow. To check for *E. coli* contamination an isolated *R. capsulatus* colony was restreaked onto an RCV medium which in addition to Tc contained 0.1% yeast extract. The pure *R. capsulatus* colony was picked, grown up and frozen as a stock culture.

7. **Probe constructions.** The 5' probe used in S1 protection experiments was an anti-sense RNA molecule, which was transcribed by T7 RNA polymerase. The 270 nucleotide (nt) Smal-Cla I fragment of pRPSLHII (44) (labelled 5' in Fig. 5) was sub-cloned into the pT7-2 vector (Pharmacia), which contains the T7 promoter. The 3' probe was obtained as the 800 nt ApaLI-Smal segment (indicated as 3' in Fig. 5) from pRPSLHII. The ApaLI site was filled in (29) by incubation with the Klenow fragment of DNA polymerase I, and with dATP, dCTP, dGTP and 50 μCi of alpha-32P-TTP (600 Ci/mmole; New England Nuclear).

8. **In vitro transcription of RNA probes.** To produce homogeneously labelled 5' RNA probes, 1-2 μg of linearized DNA template were used with T7-RNA Polymerase as recommended by the commercial supplier (Pharmacia), and incubated at 37°C for one hour. Samples were then extracted with phenol:chloroform (1:1), and the nucleic acids were precipitated twice from ethanol. The pellet was washed with 70% ethanol, dried, dissolved in 100
μl of DNase I buffer (20 mM sodium acetate, 10 mM MgCl₂ and 10 mM sodium chloride, pH 4.5) with 23 units of DNase I (Boehringer Mannheim) and incubated at 22°C for 30 min. The sample was then phenol extracted and the RNA was precipitated with ethanol. Usually 10-20 μg of RNA were obtained from 1-2 μg of linearized DNA template, with a specific activity of 0.5-1.0 x 10⁶ dpm/μg RNA.

9. RNA isolation, blotting and hybridization. Isolation of RNA from *R. capsulatus* was done as described previously (39). The blotting procedure was done as follows: 5 μg of *R. capsulatus* RNA were dissolved in running buffer (20mM MOPS, 1 mM EDTA, 5 mM sodium acetate, pH 7.0) supplemented with 50% formamide and 2.2 M formaldehyde, heated to 70°C for 3 min., cooled, and loaded onto a 1% agarose-6.6% formaldehyde gel and run at 20-40 mA. The gel was then blotted for 16 hours onto a sheet of nitrocellulose paper which had been soaked in 20xSSC (29). The filter was baked for 2 hours at 80°C. Prehybridizations were done in 200 μg/ml denatured salmon sperm DNA, 5xSSPE (29), 0.3% SDS and 50% formamide at 42°C for 1-2 hours. This mixture was supplemented with 1x10⁶ dpm of radioactive probe for hybridizations of 12-16 hours at 42°C. The filters were washed three times, for 7 min each, at 70°C in 0.1xSSPE, 0.1% SDS.

10. Capping of RNA with guanylyl transferase. Unfractionated RNA (10 μg) from *R. capsulatus* cells grown with high light was incubated in guanylyl transferase buffer (25mM Tris-HCl, 2 mM MgCl₂, 1 mM DTT, pH 7.5) with 150 μCi alpha-³²P-GTP (600 Ci/mmmole) and 10 units of guanylyl transferase (BRL) in a total volume of 100 μl at 37°C for 30 min (32). The RNA was extracted twice with phenol:chloroform, ethanol precipitated four times, washed and dried. The pellet was dissolved in 100 μl
diethylpyrocarbonate-treated (29) sterile distilled water.

11. **S1 nuclease protection experiments.** A 5 µg sample of *R. capsulatus* RNA was ethanol precipitated with 0.2-0.4 µg of the 5' probe or 5 ng of the 3' probe and 5 µg of yeast tRNA. The pellet was washed in 70% ethanol and dried. The sample was dissolved in 30 µl of hybridization buffer (0.4 M sodium chloride, 0.04 M sodium phosphate, 0.4 mM EDTA, pH 6.5), heated to 95°C for 5 min., quickly transferred to 70°C and incubated for 16 hours under parafin oil. S1 nuclease (1500 units) in 300 µl of S1 buffer (0.28 M sodium chloride, 0.03 M sodium acetate, 4.5 mM zinc sulphate, pH 4.5) was added and the sample was incubated at 37°C for one hour. Then 50 µl of a solution of 4 M ammonium acetate, 0.1 M EDTA, with 20 µg carrier tRNA were added, followed by extraction with 350 µl of buffered phenol:chloroform (1:1). After the phenol extraction, an ether extraction was performed to remove the parafin oil. 400 µl of isopropanol were then added to precipitate the nucleic acids. The RNA pellet was washed, dried and dissolved in sample buffer (80% formamide, 0.5xTBE (25), 0.02% bromphenol blue and 0.02% xylene cyanol), heated to 95°C for 5 min., cooled and loaded onto a 5% polyacrylamide-7M urea gel in 0.5xTBE. After electrophoresis the gel was dried and exposed to X-ray film.

When capped RNA and unlabelled probe were used in S1 protection experiments the procedure was modified slightly. 10 µg of *R. capsulatus* RNA and 0.2 µg of the 5' probe were ethanol precipitated and then dissolved in hybridization buffer, heated to 95°C, cooled to 70°C and incubated for 12-16 hours under parafin oil. The sample was then digested with 1500 units of S1 nuclease for 1 hour, followed by incubation with 25 ng of RNase A for 15 min. at 20°C. The RNA was phenol extracted, ether extracted, ethanol precipitated and
subsequently dissolved in sample buffer. It was then heated to 95°C for 3 min, cooled to 0°C and loaded onto a 5% polyacrylamide-7M urea gel.

12. Analytical Methods. The light intensity was measured with a Li-Cor Quantum Sensor (Model Li-185B). To measure total cell protein a version of the Lowry (28) protein assay was performed in which cells were boiled in 0.05 M NaOH for 1 min. and then cooled before adding the other reagents. The protein standard used was crystalline bovine serum albumin. Spectral scans were done on 5 ml. of cells (1.5 x10⁹ CFU) that had been resuspended in 1 ml of 22.5% BSA (SIGMA). Autoradiograms were scanned with a Quick Scan Jr. (Helena Laboratories Corp.) densitometer, and the area under peaks was either measured by an integrator or the appropriate peaks were cut out and weighed.
RESULTS

1. Use of puc-lac fusions to estimate the relative levels of puc transcription and translation under different growth conditions. To measure the relative amounts of puc transcription the puc promoter and the first 73 nt of the pucB structural gene were fused to the lac operon on the plasmids, pAZIII and pAZIV (Fig. 2), and conjugated into R. capsulatus (see Materials and Methods). To evaluate the role of translation of the puc mRNA an in-frame fusion was made between the pucB structural gene and the lacZ gene on the plasmid pAZV (Fig. 3). The cells harbouring these plasmids were grown under four separate conditions: high oxygen, low oxygen, anaerobic high light and anaerobic low light. Once the cultures reached a predetermined cell density the cells were harvested, lysed and assayed for beta-galactosidase.

As the pJAJ103 plasmid is a slightly unstable vector that is lost from a R. capsulatus culture at a rate of about four percent per generation (24), a correction factor was obtained which compensated for plasmid loss. To determine the degree of plasmid loss for each growth experiment cell samples were plated onto RCV (40) medium with and without Tc. Since only those cells with the pJAJ103 derivative would grow in the presence of Tc, it was possible to calculate the ratio of the total number of cells plated to the number of cells that had retained the plasmid. The enzyme activities were multiplied by this ratio to obtain a value corrected for plasmid loss. Tables 2 and 4 have the raw data whereas Tables 1 and 3 give the values corrected for plasmid loss.
A. Oxygen effects

i. Expression of the pucB-lacZ transcriptional fusions in R. capsulatus cells grown with either high or low oxygen tension.

The specific activities of beta-galactosidase in cultures of R. capsulatus B10(pAZIII) were 86 under low oxygen tension and 54 under high oxygen tension (Table 1, line 1). The specific activities of beta-galactosidase in extracts of R. capsulatus B10(pAZIV) were also higher in low oxygen growth conditions (Table 1, line 2). The uncorrected values also show more enzyme activity in low oxygen grown cells (Table 2, lines 1 and 2). Zhu and Hearst (47) have shown that there is more puc mRNA in cells grown in oxygen limited conditions than in highly aerated cultures, so these lacZ transcriptional studies support a theory that the increased amount of puc mRNA in oxygen limited cultures is partially due to increased transcription initiation.

pAZIII was also conjugated into R. capsulatus ΔRC6 (13), which has the puf structural genes replaced by the neomycin phosphotransferase structural gene (knT). R. capsulatus ΔRC6 is therefore unable to grow photosynthetically since the reaction centre structural genes are missing. As well, this strain produces extremely low amounts of B800-850 complexes because one of the puf genes (pufQ) is necessary for synthesis of B800-850 light harvesting complexes (unpublished results). The pufQ gene is encoded on the puf operon and is upstream of the pufB gene. It is not known what role pufQ has in the regulation of photosynthetic gene expression however when it was deleted from R. capsulatus, the cell could no longer produce B800-850 complexes, therefore pufQ may effect the transcription of the puc genes. To estimate the rates of transcription the puc-lacZ transcriptional fusion was conjugated into
TABLE 1. Specific activities of beta-galactosidase in cultures grown in either highly aerated or oxygen limited growth conditions, (corrected for plasmid loss)\(^1\).

<table>
<thead>
<tr>
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<th>HIGH OXYGEN</th>
<th>LOW OXYGEN</th>
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<tbody>
<tr>
<td>B10(pAZIII)(^2)</td>
<td>54 (^4) (14)</td>
<td>86 (8.4)</td>
</tr>
<tr>
<td>B10(pAZIV)(^2)</td>
<td>45 (3.6)</td>
<td>75 (11)</td>
</tr>
<tr>
<td>B10(pAZV)(^3)</td>
<td>7.4x10(^3) (3.0x10(^3))</td>
<td>2.2x10(^4) (9.0x10(^3))</td>
</tr>
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</table>

\(^1\) The values given are the mean of several experiments, and the numbers in parentheses represent the standard deviation of the enzyme activity values.

\(^2\) pAZIII and pAZIV are the puc-lac transcriptional fusions (Fig. 2)

\(^3\) pAZV is the puc-lac translational fusion (Fig. 3)

\(^4\) Specific activities are expressed as nm ONPG cleaved per min. per mg protein.
TABLE 2. Specific activities of \textit{beta}-galactosidase in cultures grown in either highly aerated or oxygen limited growth conditions (uncorrected for plasmid loss)\textsuperscript{1}.

<table>
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<th>HIGH OXYGEN</th>
<th>LOW OXYGEN</th>
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<tbody>
<tr>
<td>B10(pAZIII)</td>
<td>20 (4)</td>
<td>44 (7)</td>
</tr>
<tr>
<td>B10(pAZIV)</td>
<td>39 (5)</td>
<td>68 (4)</td>
</tr>
<tr>
<td>B10(pAZV)</td>
<td>$3.6 \times 10^3$ ($3.0 \times 10^2$)</td>
<td>$9.6 \times 10^3$ ($5.0 \times 10^2$)</td>
</tr>
<tr>
<td>ΔRC6(pAZIII)</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>ΔRC6(pAZV)</td>
<td>$4.3 \times 10^3$</td>
<td>$1.0 \times 10^4$</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data expressed as in Table 1.
R. capsulatus ΔRC6. The specific activities of beta-galactosidase of
R. capsulatus ΔRC6(pAZIII) were 15 in a high oxygen growth condition and 40
in low oxygen tension (Table 2, line 4). Therefore transcription of puc genes in
the absence of the pufQ structural gene appeared to be the same as when this
gene was present in the wild type R. capsulatus B10 strain. This indicated a
posttranscriptional mechanism for regulation of puc expression by pufQ.

ii. Expression of the pucB-lacZ translational fusions in R. capsulatus grown in either high or low oxygen conditions. As translation may play a key role in regulating pucA and pucB gene expression, a translational fusion was constructed between the pucB and lacZ structural
genes (Fig. 3). Extracts of R. capsulatus B10(pAZV) grown in a high oxygen
environment yielded a beta-galactosidase specific activity of 7.4x10^3 whereas
low oxygen grown cells gave 2.2x10^4 (Table 1, line 3). The uncorrected values
showed the same trend (Table 2, line 3). The most striking difference between
the transcriptional and translational fusions was that the levels of beta-
galactosidase activity were about one hundred fold higher for the translational
fusions.

A possible reason for the 100 fold difference in beta-galactosidase
expression between the transcriptional and translational fusions was that the R.
capsulatus ribosomes may initiate more efficiently from the pucB ribosome
binding site on the translational fusion than the lacZ ribosome binding site on
the transcriptional fusions. A second possibility could be that the trp genes
which are between the lac operon and the puc promoter on the transcriptional
fusions might interfere with lacZ gene expression. Within the trp sequence is
an E. coli transcriptional terminator, which may be recognized by the
R. capsulatus RNA polymerase and therefore prematurely terminate transcription. However in spite of the vast differences in absolute amounts of beta-galactosidase produced, the translational fusion showed about the same degree of oxygen regulated production of beta-galactosidase as did the transcriptional fusions.

As the pufQ gene appears not to affect the transcription of the puc genes, (see transcriptional fusion studies) it was of interest to determine if translation was regulated by pufQ. Therefore the translational fusion pAZV was conjugated into R. capsulatus ΔRC6 and cultured in high and low oxygen conditions. In oxygen limited cultures the specific activity of beta-galactosidase was 4.0x10^3 whereas highly aerated cultures gave 1.0x10^4 (Table 2, line 5). Both the transcriptional and the translational fusions gave the same relative amounts of lacZ expression in ΔRC6 as in the wild type strain. Therefore translation does not seem to be involved in regulation of puc expression by oxygen, or by pufQ.

B. LIGHT EFFECTS:

i. Expression of the pucB-lacZ transcriptional fusions in R. capsulatus grown anaerobically with high or low light intensity. The specific activities of beta-galactosidase in anaerobically grown cultures of R. capsulatus B10(pAZIII) were 152 in high light illumination and 110 in low light illumination (Table 3, line 1). As well B10(pAZIV), grown photosynthetically, yielded more beta-galactosidase enzyme activity when grown with saturating light intensity (Table 3, line 2). The uncorrected values also show more enzyme activity in high light grown cells. (Table 4, lines 1 and 2). These results showed that there may be an increased
TABLE 3. Specific activities of beta-galactosidase in cultures grown with either high or low light illumination (corrected for plasmid loss)\(^1\).

<table>
<thead>
<tr>
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<th>HIGH LIGHT</th>
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<tbody>
<tr>
<td>B10(pAZIII)</td>
<td>152 (38)</td>
<td>110 (21)</td>
</tr>
<tr>
<td>B10(pAZIV)</td>
<td>212 (17)</td>
<td>110</td>
</tr>
<tr>
<td>B10(pAZV)</td>
<td>9.7x10(^3)</td>
<td>2.8x10(^4) (6.0x10(^3))</td>
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</table>

\(^1\) Data expressed as in Table 1.
TABLE 4. Specific activities of beta-galactosidase in cells grown with either high or low light illumination (uncorrected for plasmid loss)\(^1\).

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<thead>
<tr>
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<th>HIGH LIGHT</th>
<th>LOW LIGHT</th>
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</thead>
<tbody>
<tr>
<td>B10(pAZIII)</td>
<td>56 (1)</td>
<td>28 (6)</td>
</tr>
<tr>
<td>B10(pAZIV)</td>
<td>88 (6)</td>
<td>38 (11)</td>
</tr>
<tr>
<td>B10(pAZV)</td>
<td>(5.1 \times 10^3) (1.2( \times 10^3))</td>
<td>(7.5 \times 10^3) (6.0( \times 10^2))</td>
</tr>
</tbody>
</table>

\(^1\)Data expressed as in Table 1.
rate of transcription initiation of \textit{puc} genes in cells grown with high light illumination.

\textbf{ii. Expression of the \textit{pucB-lacZ} translational fusion in cells grown with either high or low light illumination.} The levels of \textit{lacZ} expression from the translational fusion in \textit{R. capsulatus} B10 grown photosynthetically with saturating or growth rate limiting light intensity were 9.7x10^3 in high light illumination and 2.8x10^4 in low light illumination (Table 3, line 3). The specific activities of beta-galactosidase are higher in cells grown with growth rate limiting light intensity which is in contrast to the results obtained with the transcriptional fusions, in which the levels of beta-galactosidase activities were slightly higher in cells grown with high light illumination. Therefore it appeared that the rates of translation initiation might have been four fold greater in cells grown with low light illumination. These results taken as a whole suggest a different mechanism for oxygen and light regulation of expression of the \textit{puc} genes.

\textbf{2. Studies on the relative levels of \textit{puc} mRNAs and B800-850 complexes in \textit{R. capsulatus}.}

\textbf{A. Effects of different levels of illumination on photosynthetic growth of \textit{R.capsulatus} cells.} As can be seen in the spectral scans of intact cells shown in Fig. 4, substantially greater amounts of pigments were found in cells grown in low light than with high light illumination. The absorption at about 860 nm is due to a combination of B800-850, B870 and reaction centre complexes, whereas nearly all the 800 nm peak
Fig. 4. Spectral scans of high (a) and low (b) light grown *R. capsulatus* cells.
is due to B800-850 light absorption (45). Therefore the area under the 800 nm band is proportional to the number of B800-850 pigment-protein complexes in the *R. capsulatus* membrane. A comparison of the 800 nm peaks from the spectral scans shown in Fig.4 revealed that there were four fold more B800-850 complexes in cells grown with low light than with high light.

The culture turbidity and protein content of equal numbers of colony forming units in cultures grown under both levels of illumination were within 10% of each other, indicating that there were no major differences in cell size. However the growth rate, expressed as the time required for a doubling in cell number, was twelve hours for cultures grown with low light and two hours for cultures grown with high light.

It has been observed that the RNA content of *R. capsulatus* cells is proportional to growth rate (2), and it was found that cells grown with high light contained about three times as much total RNA as cells grown with low levels of illumination (that is, 7.5 µg total RNA/3x10^8 CFU in high light and 2.5 µg total RNA/3x10^8 CFU for low light grown cells).

**B. Sizing and end-mapping of puc transcripts.** The approximate length of *puc* transcripts was estimated by Northern blot analysis of RNA from *R. capsulatus* cultures grown with either high or low light intensities. A radioactive anti-sense RNA probe was obtained by *in vitro* transcription of the 270 bp DNA fragment designated 5' in Fig. 5, which extends from a *Clal* site in the *pucB* gene to a *SmaI* site 190 nt before the start codon of this gene. The results of a Northern blot experiment are shown in Fig. 6, and indicate that transcripts of about 530 nt in length were present under both growth conditions tested. Although this transcript was long enough to encode both
Fig. 5. Representation of the 3' and 5' probes used in S1 protection experiments. At the top of the figure is a representation of a 1.25 kb Sma I fragment from pRPSLHII(44). The B800-850 *pucA* and *pucB* genes, which are transcribed from left to right, are shown as heavy regions. The endpoints of the 5' probe (270 nt) and the 3' probe (800 nt) are shown below. S-Sma I, A-Apa L1, C-Cla I.
Fig. 6. Northern blot of RNA isolated from *R. capsulatus* grown with either high or low light illumination. The blot was hybridized with the *in vitro* transcribed 5' RNA probe. Radioactively labelled *Hae* III cut M13mp11 single stranded DNA fragments were used as molecular weight markers. H-high light, L-low light
the alpha and beta B800-850 peptides, it was possible that a second transcript was derived from the \textit{pucA} gene, for the probe was complementary only to the \textit{pucB} gene. To distinguish between these possibilities and learn more about the transcription of these genes, 5' and 3' end-mapping experiments were done.

The 5' probe (Fig. 5) was protected from S1 nuclease digestion by hybridization with \textit{R. capsulatus} RNA, and the protected fragments were visualized by gel electrophoresis and autoradiography. The results from a typical experiment are shown in Fig. 7A: two distinct RNA molecules hybridized with the 5' probe, suggesting that both of the \textit{puc} genes might be encoded by polycistronic transcripts with different 5' ends. The two 5' ends would be separated by approximately 15 nt, with the end of the longer segment being about 125 nt upstream of the start codon of the \textit{pucB} structural gene.

The probe used to map the 3' end of the \textit{puc} mRNA in S1 protection experiments was an end-labelled DNA molecule, which extends from an \textit{ApaI} site within the \textit{pucA} structural gene to a \textit{SmaI} site 700 bp downstream of the \textit{pucA} stop codon (see Fig. 5). This 800 bp fragment was labelled at the \textit{ApaI} site. As shown in Fig.7B, one DNA segment, of about 120 nt, was protected from nuclease digestion by the RNA in both high and low light samples.

There seemed to be slightly more RNA from low light grown cells that was homologous to the 5' probe in the Northern blot experiment shown in Fig. 6, but in the end-mapping experiments (Fig. 7) the amount of protected probe in the two growth conditions was about equal. To quantify the relative amounts of \textit{puc} mRNA in high and low light grown cells saturation hybridization experiments were performed as described in the following section.
Fig. 7. End-mapping analysis of B800-850 mRNA isolated from high and low light grown *R. capsulatus* cells. The results from the 5' end-mapping are given in panel A, which showed two protected fragments of 200 and 185 nt. The undigested probe (seen in all three lanes) ran as a 280 nt fragment. The 3' end-mapping, in panel B, showed a single 120 nt protected segment and the 800 nt end-labelled probe. Molecular weight markers were *Hae* III cut M13mp11 radioactively labelled single stranded DNA molecules. HL-high light, LL-low light, C-control(probe incubated with Yeast tRNA prior to S1 nuclease treatment).
C. Saturation hybridization and half-life determination of *puc* mRNAs. Although the end-mapping experiments implied that cells cultured in both growth conditions contained equal amounts of *puc* mRNA, it was possible that the concentration of the probe was too low to drive the homologous hybridizations to completion. In Fig. 8 are shown the results of an experiment in which either 0.2 μg or 0.4 μg of $^{32}$P-labelled 5' probe were used in an S1 protection experiment with 5 μg of high and low light RNA samples. Because there was no increase in the amount of RNA hybridized to the probe when the amount of probe was increased, it was concluded that in this experiment the amounts of *puc* mRNAs detected were accurate measurements of the relative steady state levels of these transcripts in cells grown with the two conditions of illumination. Densitometric scans showed that there was approximately 1.4 fold more *puc* mRNA (per μg total RNA) in cells grown with high light as compared with low light grown cells. Because the faster-growing high light cells contained about three times as much total RNA as low light grown cells, the steady-state levels of *puc* mRNA in cells grown with high illumination would then seem to be four times as great as cells grown under low levels of light.

Since the steady state levels of RNAs are in part a function of their decay rates it was of interest to determine the half-lives of the *puc* mRNAs. The decay rates of these transcripts were derived from densitometric scanning of autoradiograms such as the one shown in Fig. 8, in which the 5' probe was used. The half-lives were found to be 10 min. (±1.0 min.) for RNAs from high light cells and 19 min. (±2.5 min.) for RNAs from cells grown with low levels of light. The rate of decay of both of the transcripts detected with this probe were the same. Thus, although the steady-state levels of *puc* mRNAs are
Fig. 8. Determination of the relative steady-state levels and half-lives of B800-850 mRNAs. S1 protection experiments were performed on 5 μg of *R. capsulatus* RNA from each time point, using 0.2 μg of the 5' RNA probe (0.4 μg on lanes labelled 0x2). Although only one representative autoradiogram is shown here, the half-lives of the B800-850 mRNA were determined by averaging the measurements from three separate experiments. *Hae* III cut M13mp11 radioactively labelled single stranded DNA fragments were used as molecular weight markers. HL-high light, LL-low light, C-control (probe incubated with Yeast tRNA prior to S1 nuclease digestion).
four fold greater in cells grown with high light, the rates of decay are also greater, so there must be more frequent initiations of transcription than in cells grown with low levels of illumination.

D. Localization of puc transcription initiation sites. The RNA end segments detected with the 5' probe could be from either a pair of overlapping 5' transcripts or they could derive from 3' and 5' RNA ends. To specifically identify the 5' ends of molecules that resulted from transcription initiations, total RNA was incubated with \( \alpha^{32P} \)-GTP and guanylyl transferase, in order to transfer \( 32P \)-GMP to di- and triphosphates on 5' mRNA ends (32). The resultant labelled RNA was then hybridized to the unlabelled 5' probe and digested with S1 nuclease. Only those transcripts that hybridized to the probe and had a labelled 5' end (that is, a transcription initiation site) would be detected by gel electrophoresis and autoradiography after S1 digestion. It was found that the 5' probe protected two capped RNA molecules that must correspond to the two 5' ends detected in the previous S1 experiment, as shown in Fig. 9. There was greater variation between independent experiments in the relative intensities of the two capped transcripts (perhaps due to variation in the efficiency of capping) than there was in the standard S1 experiments. Therefore, it was concluded that the standard S1 protection experiments provided a more accurate estimate of the relative amounts of the two transcripts. The difference in sequence composition and the presence of the additional 5'-5' linked (32) nucleotide probably account for the differences in electrophoretic mobility of these RNA segments, relative to the segments of the S1 protected RNA probe run in the adjacent lane. These results show that there are two sites, separated by 10 to 15 nt, at which transcription of the puc genes initiates.
Fig. 9. Autoradiogram of the gel used for separation of capped mRNA segments protected from S1 digestion by hybridization with the 5' probe. Lane 1 contains the radioactive probe and the two segments protected by hybridization with the puc mRNAs (compare with Fig. 7A). In lane 2 are the two radioactively capped mRNAs (arrows) protected from S1 digestion by hybridization with non-radioactive 5' probe.
DISCUSSION

1. Analysis of *puc-lac* plasmid fusions.

A. Regulation of expression of the *puc* genes by oxygen. The transcriptional and translational fusions of the *puc* promoter to the *lacZ* gene have provided data on oxygen regulated transcription of the *pucB* and *A* genes. It has been estimated that there are at least 40 fold more *puc* transcripts in oxygen limited cultures than in highly aerated cells (47). Zhu and Hearst (46) proposed that the decreased amount of *puc* mRNA in highly aerated samples is due to a decrease in the rate of transcription initiation of the *puc* genes as well as an increase in the rate of decay of the message. In the transcriptional and translational fusions described in this thesis (Tables 1 and 2) there is less beta-galactosidase activity in cells grown in highly oxygenated samples than oxygen limited cultures. My results support the idea that the rate of transcription initiation of the *puc* genes in cells grown with low levels of oxygen is greater than in highly aerated cells. The half-life of the transcript also appears to play a role in regulating the levels of B800-850 complexes, for I have found approximately a two fold difference in the rate of transcription initiation of the *puc* genes in high and low oxygen grown cells but there is 40 fold more *puc* mRNA in low oxygen grown cells. Therefore exposure of the cell to oxygen appears to decrease the rate of transcription initiation as well as increase the rate of decay of the *puc* message.
B. Regulation of expression of the *puc* genes by incident light intensity. It was also shown that when *R. capsulatus* cells were grown anaerobically, incident light intensity affected the expression of the *puc* genes. Although the transcriptional fusion gave more beta-galactosidase activity in cells grown with high light illumination than low light illumination, the translational fusion produced more enzyme activity in cells grown with low light intensity (Tables 3 and 4). This implied that initiation of translation of the *puc* mRNA was more efficient in cells grown with low light illumination. As there was four fold more *puc* mRNA in high light grown cells (Fig. 8) but four fold more B800-850 complexes in low light grown cells (Fig. 4), one might expect there to be an elevated level of translation of the *puc* genes in cells grown with low light intensity.

C. Problems associated with plasmid borne gene fusions. A potential source of error with plasmid borne transcriptional and translational *lacZ* fusions is that a change in copy number can affect the amount of beta-galactosidase enzyme produced. For example, the copy number of pBR322 in *E. coli* HB101 decreases as growth rate increases (25). Since the growth rate of *R. capsulatus* is substantially different in the four growth conditions tested it is possible that the plasmid copy number could vary significantly. In addition to growth rate, promoter strength can also affect plasmid copy number. If transcription from a promoter proceeds through the origin of replication of a plasmid it can interfere with plasmid replication. Wong *et al* (41) observed that when a strong promoter was placed on pBR322 such that transcription ran through the origin of replication the plasmid copy number dropped three to five fold. If the promoter was placed in the opposite orientation then no decrease in
plasmid copy number was seen. The theory is that RNA polymerase while transcribing the DNA template hinders RNA primer production (in the case of pBR322 derivatives). Since the *puc* promoter is more active transcriptionally in oxygen limited cultures than highly aerated growth conditions it is possible that the higher rate of transcription initiation might interfere with plasmid replication thus lowering copy number. This would result in an underestimate of the degree of oxygen or light regulated transcription. To date no experiments have been done to determine the copy number of pJAJ103 derivatives in *R. capsulatus* grown under these conditions.

Another problem with plasmid borne gene fusions is that transcription of the *puc* genes could be affected by DNA topology. It has been shown previously that transcription of some genes (for example *lac* in *E. coli*) is partially controlled by the degree of supercoiling of the DNA template (8). To test the *lac* promoter's response to DNA supercoiling *in vitro* transcriptions were performed on templates that were either relaxed or partially negatively supercoiled. As the negative superhelicity increased the rate of transcription initiation also increased, until it reached a maximum above which introduction of new supercoils had the effect of lowering transcription initiation rates. In this system, the more negatively supercoiled the template the more efficiently RNA polymerase can melt apart the complementary strands until the template becomes so distorted with additional supercoils that RNA polymerase can no longer recognize the promoter thus accounting for the decrease in transcription rates at very high superhelicities.

It has been observed by Balke and Gralla (3) that the linking number of the plasmid pBR322 changes when *E. coli* shifts from growth on glucose to
acetate. Balke and Gralla state that "When the external environment changes, global changes in metabolism are accompanied by changes in the topological state of the DNA...". In the growth studies of my project the same medium was used, but the cells were grown under different environmental conditions. The cellular metabolism of *R. capsulatus* cells grown with high light, low light, high oxygen and low oxygen are very different. For example, cells growing in oxygen limited cultures produce a vast intracytoplasmic membrane apparatus, large amounts of bch a, reaction centre and light harvesting peptides and grow extremely slowly. Highly aerated cells have no internal membrane apparatus, grow rapidly, and do not produce any of the components of the photosynthetic unit. The cellular environment within these two cells is very different and therefore the linking number of any plasmid could be different in cells grown under these conditions. If transcription of the *puc* genes is sensitive to supercoiling, there is no guarantee that the plasmid copy of the *puc* genes has the same superhelical density as the *puc* genes in the chromosome of *R. capsulatus*.

The use of multicopy gene and operon fusions may give artefactual values when there is a trans-acting factor involved in regulation of gene expression. If in the *puc* system there exists a trans-acting regulatory factor its function might be obscured by the high number of binding sites available on plasmid copies.

One way to avoid the problems of plasmid instability, variations in copy number and topology differences, would be to insert the transcriptional and translational *lacZ* fusions into the *R. capsulatus* chromosome at or near the *puc* locus. Once inserted into the genome the fusion will be maintained as a single stable copy. If DNA topology effects transcription of the *puc* genes, the fusion should have the same conformation as the wild type genes.
Another way to measure the levels of transcription of genes is to measure mRNA encoding the genes of interest. Due to the inherent problems with plasmid borne gene and operon fusions it was decided for the remainder of my thesis project to investigate the regulation of the levels of \textit{puc} mRNA in cells grown with high and low light intensity.

2. Analysis of \textit{puc} mRNA in cells grown with high and low light illumination. The mapped 3’ and 5’ ends of the two \textit{puc} mRNAs gave overall transcript lengths of about 505 and 491 nt, which corresponds well to the size of the mRNA species found in the Northern blot (Fig. 6). Since the resolution of nucleic acids of this size in agarose gels is poor compared to polyacrylamide gels, the Northern blot could not distinguish between the two RNA transcripts, which may be why previous reports have only mentioned a single transcript of the \textit{puc} genes (46, 47).

Two transcription initiation sites of the \textit{puc} genes have been identified. Both transcripts had the same decay rate and their relative amounts, although slightly variable between different experiments, were usually about equal. There could either be two promoters (one for each transcript) or a single promoter that directs transcription initiation at two sites. Most bacterial dual promoters examined, such as \textit{E. coli} rRNA promoters, have two transcription initiation sites separated by a large distance, each mRNA being transcribed from separate -10 and -35 regions (35). The \textit{puc} genes however have two transcription initiation sites separated by only 10 to 15 nucleotides. As an \textit{R. capsulatus} promoter consensus sequence is not available and the resolution of the endmapping experiments was +/- 5 nt, it was not possible to positively identify -10 and -35 regions.
In other tandem promoter systems the individual promoters are usually regulated differently from each other. For example, the \textit{rrnA} gene in \textit{E. coli} (which encodes one of the rRNAs) is transcribed from two promoters (35). Transcription initiation from the most upstream promoter, P1, is strongly growth rate dependent whereas transcription initiation from the downstream promoter, P2, is only weakly dependent on growth rate. Therefore in rapidly growing cells the majority of the transcripts originate from P1, but in slow growing cells the rate of transcription initiation from both promoters is approximately equal (34). The two \textit{puc} transcripts however behave identically in the two growth conditions tested (see Figs. 7 and 8). In both high and low light growth conditions the ratio of the longer transcript to the shorter was approximately 1.0. Therefore the reason for having two transcription initiation sites for the \textit{puc} genes was not immediately apparent.

Upon examination of the nucleotide sequence upstream of the start sites no \textit{E. coli} like -10 or -35 regions could be found. This was expected, as to date all \textit{R. capsulatus} promoters tested have failed to function in \textit{E. coli} (24). However, there exists a direct repeat of "ACACTTG" in the DNA sequence upstream of where the 5' end of the longer \textit{puc} mRNA transcript maps (Fig 10). The centres of these repeated sequences are separated by 14 nt, which was the approximate distance between the 5' ends of the two \textit{puc} mRNA's. Moreover, the centre of the most upstream repeated sequence is approximately 25 nt from the start site of the longer transcript while the centre of the downstream sequence is about 26 nt from where the initiation site of where the shorter transcript maps. The spacing of the repeated sequences in relation to each other and to the \textit{puc} 5' mRNA ends suggested that they may be involved in transcription initiation.
Fig. 10. Sequence of the 5' and 3' regions flanking the B800-850 *pucA* and *pucB* genes (44). The approximate positions of the 5' and 3' ends of the B800-850 transcript are shown by the lines above the sequence. Since there was some uncertainty in the interpretation of the sizes of the S1-protected segments of the probes the dashed lines represent a possible error of +/− 5 nt. The repeated sequence of "ACACTTG", which may be involved in transcription initiation, is designated by two heavy arrows pointing in the same direction. The 3' end of the B800-850 mRNA maps near a palindromic sequence that is indicated by two arrows pointing towards each other. The last four codons of the *pucA* gene are underlined.
CCGACTTTACTGACCTGACGCTCTCGGGCTCCCATAGTGCGTCTCACGAGGTCGGATCACAGACGGCCCGCTCAGTAATCTGCTGACCTTTGGGCCCACCGGCACCCGTCGGTCGGCATTCCCTCCGG

5' ........................................ 3'

CCGACTTTACTGACCTGACGCTCTCGGGCTCCCATAGTGCGTCTCACGAGGTCGGATCACAGACGGCCCGCTCAGTAATCTGCTGACCTTTGGGCCCACCGGCACCCGTCGGTCGGCATTCCCTCCGG

→ → → ← ←
The 3' terminus of the *puc* transcript is in the region of a potential stem-loop structure (Fig. 10) which has been shown to serve as a transcriptional terminator (13).

When *R. capsulatus* cultures were grown photosynthetically, four times as much *puc* mRNA was found in cells grown with high light as in cells grown with low light illumination. The steady state levels of mRNAs depend on both the rates of transcription and mRNA decay. In this study the *puc* mRNAs isolated from cells grown with high light illumination had a half-life of 10 min. whereas the same transcripts found in low light grown *R. capsulatus* cells had a half-life of 19 min. (Fig. 8). As the *puc* mRNAs isolated from high light grown *R. capsulatus* were degraded more rapidly yet were present at greater steady state amounts than in low light grown cells, the frequencies of transcription initiation must be greater in cells exposed to saturating light intensity. The *puc-lac* transcriptional fusions also showed that there was more transcription initiation in cells grown in high light intensity. In spite of this difference in the amount of *puc* mRNAs per cell, low light grown cells were found to contain four times as many B800-850 complexes.

In some systems regulation of expression of cotranscribed genes is a result of segmental differences in stability of the transcript. For example, the ten to one stoichiometry of the B870 to reaction centre complexes in *R. capsulatus* is determined primarily by the different stabilities of segments of the polycistronic mRNA encoding the genes (6). The 2.7 kb *puf* primary transcript contains the *pufA* and *pufB* genes (B870 peptides) as well as the *pufL* and *pufM* structural genes (reaction centre peptides). The segment of the transcript which encodes the *pufAB* structural genes is approximately ten times
more stable than the \textit{pufML} segment. As a result of this difference in half-life the fragment of mRNA encoding the \textit{pufA} and \textit{pufB} genes is ten times more abundant than the \textit{pufML} fragment. The steady state levels of the \textit{puc} mRNA were initially measured using a probe specific for the \textit{pucB} gene (Fig. 5). Therefore it could only be said that the segment of mRNA encoding the \textit{pucB} structural gene was more abundant in cells grown in saturating light intensity than in cells cultured with growth rate limiting light intensity. Subsequently the relative amount of the 3' end of the \textit{puc} messages was measured by densitometric scans of the autoradiogram of the 3' end mapping experiments (Fig. 7B), in which the probe used was specific for the \textit{pucA} structural gene. The scans of the 3' end mapping experiments showed that the high light : low light ratio of \textit{puc} mRNA in 5 \( \mu \)g of RNA is approximately 1.1 : 1.0 which is close to the ratio of 1.4 : 1.0 found with the 5' probe. Therefore this demonstrates that the entire \textit{puc} transcript is more abundant in cells grown with high light intensity than low light intensity, and that there are no significant segmental differences in the half-life of the transcript.

If it is assumed that the amounts of B800-850 peptides parallel the amounts of the mRNAs, then in high light grown cells there would have to exist a pool of these light harvesting peptides that had not yet bound bch a. It has been shown by Dierstein that the turnover rate of the B800-850 \textit{alpha} peptide in a bch-less mutant strain of \textit{R. capsulatus}, and in wild type cells exposed to inhibitors of bch a synthesis, is much greater than in untreated wild type cells (15, 16). Therefore in the presence of bch a the B800-850 peptides may be protected from degradation, perhaps because the apopeptides exist in a protease-sensitive conformation that changes to a protease-resistant conformation after binding bch a(15, 16). I have found that cells grown with saturating light
intensity have the capacity to produce four fold more B800-850 peptides than cells grown with low light illumination (due to the higher levels of puc mRNA). However, since less bch a is produced in high light grown *R. capsulatus*, more of the newly translated peptides would be turned over as they would be unable to bind bch a molecules. No free bch a is detected in normal cells of photosynthetic bacteria, so the ratio of pigment-binding peptides to bch a must be equal to or greater than 1. It has been well established that the total bch a content of anaerobically grown *R. capsulatus* increases as the light intensity decreases (2, 7, 20, 27, 37); therefore when cells are shifted from a high to a low light environment more bch a is produced, which could bind to pre-existent components of a B800-850 apopeptide pool. These protein-pigment complexes would be more resistant to proteolytic degradation and could then function in light absorption.

However, it is also possible that there was control of translation of *puc* mRNAs, so that translation would be less efficient in cells grown with high light intensity. The *lacZ* translational fusions gave some evidence that translation was more efficient in cells grown with low light illumination. However since there are four fold more B800-850 complexes in cells grown with low light illumination but only one-quarter the amount of *puc* mRNA as cells grown with high light intensity one would expect translation to be sixteen times more efficient in cells grown with low light intensity. But only a three to four fold increase in translation efficiency was detected by the *lacZ* translational fusion which implies that expression of the *puc* genes may also be regulated posttranslationally. Regulation may occur at the level of bch a synthesis, bch a binding or insertion of the mature B800-850 complexes into the *R. capsulatus*
membrane. Additional experiments would be necessary to better distinguish between translational and posttranslational regulatory mechanisms. It should be noted that with either model the amount of B800-850 complexes in the membranes of anaerobically grown *R. capsulatus* is limited by the availability of bch a. The regulation of bch a biosynthesis by light also seems to be largely posttranscriptional (7).

It has been found that cells grown in darkness with high aeration contained significant levels of B870 and reaction centre peptide mRNAs although the cells did not absorb in the 800-900 nm range (6; and unpublished observations), since Dierstein has shown that the reaction centre L peptide, although more rapidly degraded in the absence than in the presence of bch a, can be immunologically detected in cells in the absence of bch a (15). Therefore it seems that a translational or posttranslational mechanism may be significant for both oxygen and light regulation of biogenesis of other photosynthetic complexes in *R. capsulatus*, as a supplement to the previously described transcriptional and posttranscriptional control processes (6, 14, 26, 46, 47). An advantage of this sort of posttranscriptional regulation might be that it would allow a very rapid increase in light harvesting capability, because the rate of that increase would be limited by the catalytic activity of the enzymes in the bch a biosynthetic pathway, and would not require *de novo* initiation of transcription of genes encoding pigment-binding peptides.
REFERENCES


