

Variation of Levels of mRNA Coding for Antenna and Reaction Center Polypeptides in *Rhodospseudomonas capsulata* in Response to Changes in Oxygen Concentration

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The effect of oxygen tension on the transcription of genes coding for the photosynthetic apparatus of *Rhodospseudomonas capsulata* was determined by the Southern hybridization technique. Restriction endonuclease digests of the R-prime plasmid pRPS404 and a subcloned fragment thereof served as DNA probes for genetically defined regions. The results showed that transcripts corresponding to the genes for certain pigment-binding polypeptides increase in amount by about 40-fold after a drop in oxygen tension. Transcripts hybridizing to genes involved in bacteriochlorophyll biosynthesis increase to a much lesser extent, and several genes involved in carotenoid biosynthesis are not affected by pO₂.

Photosynthetic membrane synthesis is repressed by oxygen in many members of the family *Rhodospirillaceae*, but the molecular nature of this regulation is largely unknown (6). Unable to demonstrate changes in mRNA levels associated with changes in synthesis of the photosynthetic apparatus, some investigators have suggested that translational controls must be responsible (5, 13). It has recently become possible to probe mRNA populations with cloned fragments of *Rhodospseudomonas capsulata* DNA that bear the genes specifying various components of the photosynthetic apparatus (11). We report here that transcripts coding for the L and M reaction center polypeptides and the 12- and 8-kilodalton light-harvesting complex I (LHI) antenna polypeptides are greatly enhanced in cells grown at low oxygen tension, whereas transcripts from genes coding for bacteriochlorophyll biosynthetic enzymes and the H reaction center polypeptide respond to a lesser extent and transcripts encoding several carotenoid biosynthetic enzymes seem unresponsive to changes in oxygen concentration.

The R-prime plasmid pRPS404 carries all the genetic information known to be required for development of the photosynthetic apparatus (9), and the genetic contents of the *Bam*HI and *Eco*RI restriction fragments of pRPS404 have been established (Fig. 1; 11). We used the procedure developed by Southern (10) to examine the kinetics of transcription of this region of the *R. capsulata* chromosome during a transition from high to low oxygen tension, which stimulates synthesis of the photosynthetic apparatus. Figure 2 shows the pigment contents of *R. capsulata* cells grown at various oxygen concentrations. Synthesis of photosynthetic membranes is roughly proportional to the bacteriochlorophyll content in most *Rhodospseudomonas* species (8).

Cultures of *R. capsulata* SB1003 (11) were grown in malate minimal medium (12) for several generations with gassing sufficient to maintain the dissolved oxygen concen-

tration at 20%. Zero-time samples were harvested, the gassing mixture (oxygen, nitrogen, and 5% CO₂) was changed to 2% oxygen, and samples were taken at various times up to 1 h after the transition. RNA was extracted from each sample (2), 5'-³²P end-labeled (4), and hybridized to restriction fragments of pRPS404 that had been separated electrophoretically and blotted onto nitrocellulose paper (Fig. 3; 10). The identities of restriction fragments that bind RNA are indicated in Fig. 3. Although some fragments are not separated, most ambiguities can be resolved by comparing the *Eco*RI and *Bam*HI patterns. Densitometer tracings of the autoradiographs were used to quantify the data, and times of exposure of the autoradiographs were adjusted to remain within the linear response range of the X-ray film (Fig. 4). RNA transcripts binding to the *Bam*HI-C DNA fragment were present even during growth in 20% oxygen, but the concentration of these transcripts increased markedly in response to the drop in oxygen, reaching approximately 40 times the repressed concentration after about 40 min of incubation. These transcripts must hybridize to the portion of *Bam*HI-C that also occurs in *Eco*RI-B, since that band showed a similar enhancement in labeling, whereas *Eco*RI-H and -Q did not (see Fig. 3). This transcribed area corresponds to a region of the chromosome wherein map mutations that affect synthesis of reaction center and light-harvesting polypeptides, the *rxoA* locus (see Fig. 1). We cloned the fragment of DNA extending from the *Eco*RI site between *Eco*RI-Q and -B to the *Bam*HI site between *Bam*HI-C and -B (the EB2 fragment), and the resulting plasmid is pRPSEB2 (Fig. 5). We repeated the Southern hybridization experiment, using RNA from 20 min after induction and pRPSEB2 DNA digested with various combinations of restriction enzymes, and the three fragments indicated by stars in Fig. 5 bound the labeled RNA strongly and approximately equally. The *Sal*I-*Pst*I fragment that is only partially starred was only weakly labeled. DNA sequencing studies have shown that the region that binds the labeled RNA corresponds precisely to the genes for the two LHI polypeptides and the L and M reaction center polypeptides (D. C. Youvan, M. Alberti, H. Bergusch, E. J. Belina, and J. E. Hearst, Proc. Natl. Acad. Sci. U.S.A., in press). The sequencing data show that the genes for the LHI

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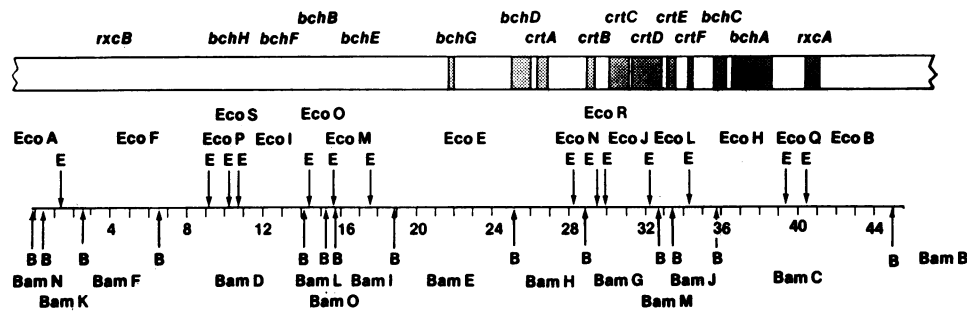


FIG. 1. Alignment of the genetic and restriction maps of the region of the *R. capsulata* chromosome coding for the photosynthetic apparatus. The *bch* genes affect bacteriochlorophyll synthesis; *crt* genes, carotenoid synthesis; and *rxc* genes, reaction center and antenna protein syntheses. The shaded areas indicate the genetically determined map position of groups of mutations conferring the same phenotype. The positions of genes for which no shaded area is indicated were determined by marker rescue, but they have not yet been mapped by genetic techniques capable of precise positioning. Genetic linkage between *bchB* and *bchE* has been established. The 46 kilobases of *R. capsulata* DNA carried in pRPS404 is indicated on the restriction map. Arrows labeled E and B indicate the sites of digestion by *EcoRI* and *BamHI*, respectively. The fragments produced by digestion with either enzyme are named alphabetically by size. The junction fragments, *EcoRI*-A, *EcoRI*-B, *BamHI*-A, and *BamHI*-B, carry regions of the R factor that are not indicated on this map. *EcoRI*-C, *EcoRI*-G, and *EcoRI*-K are composed entirely of DNA from the vector pBLM2 (1).

polypeptides begin what appears to be an operon and are contained on the *EcoRI*-*PstI* fragment, as is the 5' end of the L subunit gene. The rest of the L subunit is on the adjacent *PstI*-*SalI* fragment, and the adjacent *SalI*-*SalII* fragment carries most of the M subunit structural gene. Since we found approximately equal amounts of transcripts hybridizing to each of the first three subfragments indicated in Fig. 5, the 15:1 ratio of LHI to reaction center polypeptides (7) is not reflected in the amounts of transcripts available at 20 min postinduction. We do not know whether the RNA transcripts detected here are equally active in translation.

The *rxcB* locus is another site of mutations affecting the

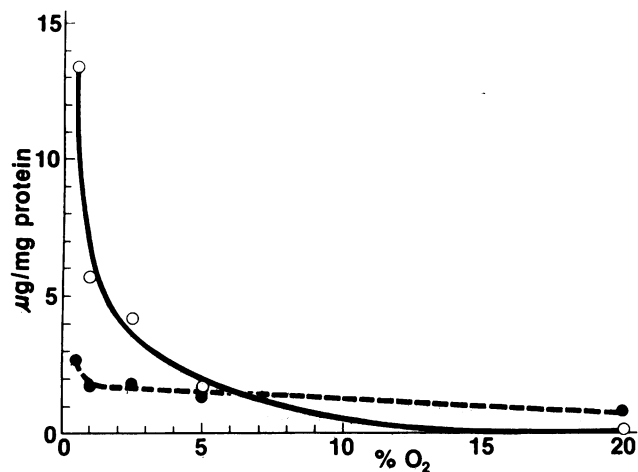


FIG. 2. The effect of growth at various oxygen tensions on pigment content of *R. capsulata*. Strain MR126 (D. Zannoni, S. E. Peterson, and B. L. Marrs, submitted for publication) was grown in malate minimal medium (11) for at least four generations in a VirTis model 43-100 fermenter with a dissolved-oxygen controller to maintain the indicated oxygen tension. Samples were removed at approximately 5×10^8 and 10×10^8 CFU/ml, and bacteriochlorophyll (○—○), carotenoid (●—●), and protein contents were determined (6). In each case, the specific pigment content was the same at the two cell densities, indicating that steady-state pigment synthesis had been achieved. Each point is the average of results from the 10^9 -CFU/ml sample from two fermenter runs.

synthesis of reaction centers and LHI complexes (11). The above-cited DNA sequence studies show that the H subunit of the reaction center is coded for by a part of the *BamHI*-F restriction fragment. The results of the Southern blotting analysis shown in Fig. 3 and 4 indicate that transcription of this region increased in response to a drop in pO_2 , but the

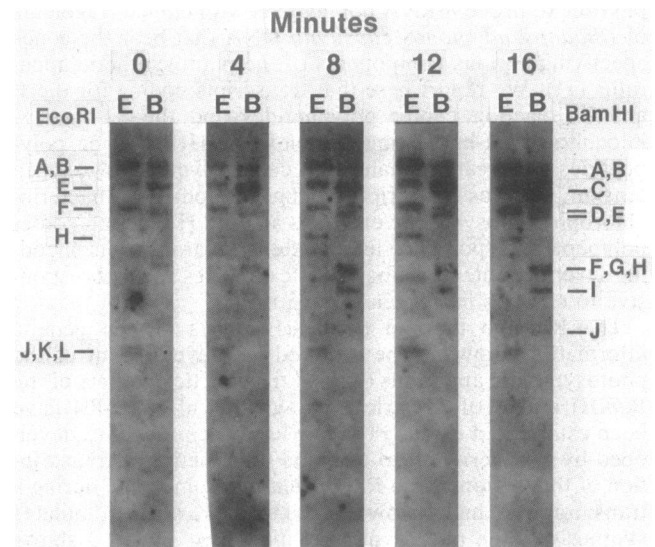


FIG. 3. Autoradiographs of Southern hybridizations of RNA, sampled at various times after a drop in pO_2 , to DNA fragments from the R-prime plasmid pRPS404. Ten identical samples of pRPS404 DNA were digested with either *EcoRI* or *BamHI* restriction endonucleases, electrophoresed through agarose gels, and blotted onto nitrocellulose filter paper (10). The filter was then cut into five replicate strips, each of which was incubated with an RNA sample that had been isolated from cells at the indicated times after a shift from 20 to 2% pO_2 . Each RNA sample had been 5' end labeled with ^{32}P in vitro after mild alkaline hydrolysis to decrease the RNA to an average chain length of 80 nucleotides. Hybridization was for 18 h at 42°C in 0.02% Ficoll-0.02% polyvinylpyrrolidone-0.02% bovine serum albumin-0.30 M NaCl-0.03 M sodium citrate (pH 7.0)-50% formamide, followed by three washes in 0.30 M NaCl-0.03 M sodium citrate.

transcripts did not accumulate to levels as high as those binding to the EB2 fragment. The ratio of transcripts binding to the EB2 fragment to those binding to *Bam*HI-F was approximately 8:1, which is consistent with the observed excess of LHI polypeptides over reaction center, but rigorous quantitative deductions about translation efficiencies may not be drawn from these data because of the semiquantitative nature of the Southern technique in this type of application.

Several of the DNA fragments that bind transcripts that respond to oxygen carry genes specifying bacteriochlorophyll biosynthetic enzymes, e.g., *Eco*RI-H, *Bam*HI-I, and *Bam*HI-D. The amounts of these transcripts did not increase dramatically after the drop in oxygen concentration. The lesser amount of these transcripts compared with that of the pigment-binding proteins can be rationalized in terms of the relative amounts of their respective protein products required for photosynthetic membrane synthesis. The biosynthetic enzymes catalytically produce bacteriochlorophyll, but the pigment-binding antenna and reaction center proteins bind bacteriochlorophyll stoichiometrically.

In a related set of experiments in which the Mu d1 phage was used (3), we found a three- to fourfold increase in β -galactosidase synthesis driven by promoters for the *bch* genes in cells grown in 1% O₂ compared with 20% O₂ (1). The modest increase in *bch* transcripts observed in the experiments reported here agrees with those findings.

The *Eco*RI-J and -L fragments contain only genes involved in carotenoid metabolism, and transcripts hybridizing to these fragments did not change during the course of the experiments. Since the carotenoid content varied by a factor of only about two when cells were grown in 2% as compared with 20% oxygen (Fig. 2), the lack of transcriptional response is not surprising.

These studies show that oxygen tension affects the amounts of transcripts from genes coding for bacteriochlorophyll biosynthetic enzymes and strongly affects those from genes coding for pigment-binding proteins. These studies do

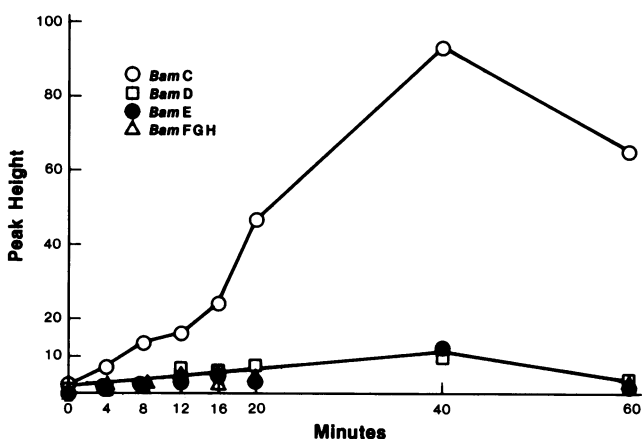


FIG. 4. Kinetics of the effect of a decrease in oxygen tension on the content of RNA transcripts hybridizing to restriction fragments bearing the genes encoding the photosynthetic apparatus. An experiment identical to that described in the legend to Fig. 3 was carried out, except additional time points were collected. The autoradiographs were subjected to densitometry, and the peak heights were measured. Time of exposure of the autoradiographs was adjusted to avoid saturation of the response of the X-ray film. Shown is hybridization to *Bam*HI-C (○), *Bam*HI-D (□), *Bam*HI-E (●), and *Bam*HI-F, -G, or -H (△).

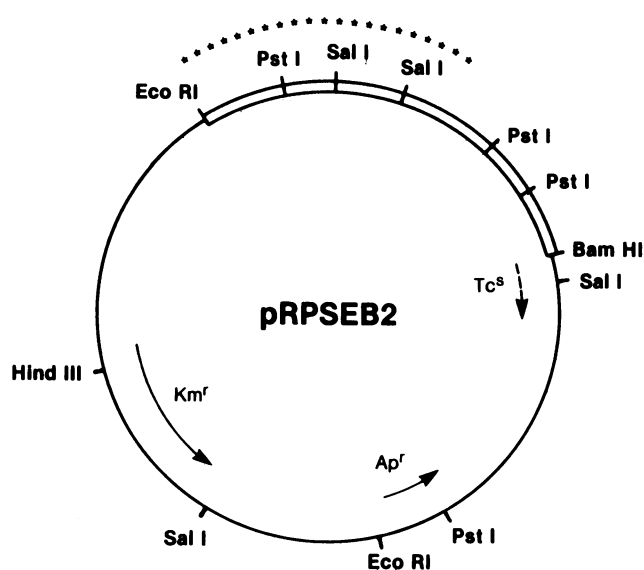


FIG. 5. Map of plasmid pRPSEB2. The plasmid was constructed as previously described (11). The 375-base-pair *Eco*RI-*Bam*HI fragment of pDPT42 (a derivative of pBR322 [11]) has been replaced by *R. capsulata* DNA, represented here by the open boxes. The size of pRPSEB2 is 15.4 kilobases. The stars indicate restriction fragments that bind RNA species found 20 min after a reduction in pO₂ in an *R. capsulata* culture. The partially starred *Sal*I-*Pst*I band is weakly labeled in this Southern hybridization experiment.

not differentiate between increased transcription and decreased degradation of these messages, and they do not rule out regulation at other points in the biosynthesis of the photosynthetic membranes. The present results, together with sequence analyses and complementation tests, strongly suggest that a regulatory element that is involved in the response to oxygen is located adjacent to the *rxcA* locus. Study of this control region is currently under way.

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