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Effects of Light, Oxygen, and Substrates on Steady-State Levels of mRNA Coding for Ribulose-1,5-Bisphosphate Carboxylase and Light-Harvesting and Reaction Center Polypeptides in *Rhodopseudomonas sphaeroides*

YU SHENG ZHU AND SAMUEL KAPLAN*

Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

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The mRNA levels specific for ribulose-1,5-bisphosphate carboxylase, light-harvesting I polypeptides α and β, and reaction center polypeptides L and M were assayed by use of a series of DNA probes specific for each cognate mRNA. Both the steady-state amounts and sizes of the specific mRNAs were measured as a function of the light intensity incident to the culture, the presence or absence of oxygen, and the type of substrate present in the growth medium. Northern hybridization revealed at least two and possibly three transcripts for ribulose-1,5-bisphosphate carboxylase. The cellular level of mRNA specific for ribulose-1,5-bisphosphate carboxylase increased in consort with enzyme activity as a function of both light intensity and reducing state of the substrate. Neither mRNA nor enzyme activity was detectable in aerobically grown cells. For the light-harvesting I and reaction center polypeptides there exist two transcripts, the larger of which appears to be a polycistronic mRNA possessing information for all four polypeptides and a smaller transcript specific for only the α and β polypeptides of the light-harvesting I complex. The regulation of each of these mRNAs was affected by light and oxygen, but was not significantly affected by the oxidation-reduction state of the substrate.

The process of photosynthesis in photosynthetic organisms, whether plants, algae, or procaryotes, is strongly affected by environmental factors. Among these fators, light is certainly one of the most important. The regulation of photosynthetic genes by light has been well established in green plants, algae, blue-green bacteria, and photosynthetic bacteria (1, 7, 25), but the molecular basis for this regulation is largely unknown.

The purple nonsulfur photosynthetic bacterium *Rhodopseudomonas sphaeroides* provides an attractive system for studying the regulation of the activity of photosynthetic genes by light, O_2, and other environmental parameters. Upon the removal of oxygen, the cell develops an extensive intracellular membrane that comprises the photosynthetic apparatus (5, 6, 11, 12, 16, 17). Further, the amount of intracellular membrane per cell and the composition of the intracellular membrane are functions of the light intensity (11, 16, 17). Therefore, the synthesis and composition of the intracellular membrane are under at least two different levels of control, the pO_2 and the light intensity.

In addition to the photosynthetic membrane components, ribulose-1,5-bisphosphate (RuBP) carboxylase (RuBPCase) in *R. sphaeroides*, a key enzyme of the Calvin cycle and a major soluble protein in the cytoplasm (21), represents another model system for studying the regulation of photosynthetic gene expression. Two structurally and immunologically distinct forms of RuBPCase, forms I and II, exist in *R. sphaeroides* (27). Form I is composed of eight large and eight small subunits and resembles the eucaryotic enzyme in its kinetic parameters (27). Form II, which lacks small subunits, is reported to be a hexamer of large subunits and resembles the procaryotic dimer enzyme as typified by *Rhodospirillum rubrum* (13, 14, 21). The cellular levels of both forms of the enzyme are dependent on light, oxygen, and type of carbon substrate (29). The light- and oxygen-dependent regulation of the synthesis of soluble and photosynthetic membrane proteins of *R. sphaeroides* is also clearly demonstrated by recent experiments involving shifts in these environmental parameters (5, 7).

With the advent of recombinant DNA techniques, DNA segments defining many of these important membrane and soluble proteins have become available. The authors of a recent paper have begun to examine specific mRNA levels in a related organism, *Rhodopseudomonas capsulata* (8). The availability of specific DNA probes defining the genetic regions for the RuBPCase form II (22) and for both the light-harvesting polypeptides (LH-1) (P. Kiley and S. Kaplan, unpublished data), and the reaction center polypeptides (RC-L and RC-M) (30, 31) of *R. sphaeroides* has made it possible to qualitatively and quantitatively probe specific mRNAs by hybridization with these unique cloned fragments. The study presented here demonstrates the identification and regulation in the levels of mRNA coding for RuBPCase, LH-1, RC-L, and RC-M polypeptides in *R. sphaeroides* grown under defined steady-state conditions of light, oxygen, and carbon source.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. *R. sphaeroides* wild-type strain 2.4.1 was used throughout. The cells were grown on modified Sistrom medium A (26) supplemented with 0.4% butyrate and 0.1% $NaHCO_3$ as the carbon source unless otherwise noted. The cells were fully adapted to the butyrate medium before inoculation. Those cells undergoing photoheterotrophic growth were grown at 30°C on the butyrate medium in completely filled flat screw-cap bottles (100 ml) normally at near-saturating light intensity (10 W/m^2), provided by a bank of lumiline lamps (General Electric Co.). When light intensity was to be a variable, the cells were also grown under conditions designated low

* Corresponding author.

light intensity at 3 W/m^2 in comparison with 10 W/m^2, designated as the high-intensity light. The light energy was measured with a Yellow Springs-Kettering model 6.5A radiometer through a Corning colored-glass filter (CS no. 7-69; 620 to 1,100 nm). Dark-shift experiments were performed by wrapping the growth vessels from high light-grown cells with aluminum foil for 1 h at 30°C. Partial aerobic growth was maintained by chemoheterotrophic culturing of the cells (100 ml in a 500-ml flask) on a gyratory shaker at 30°C. In some cases the culture (100 ml) was sparged with a gas mixture of O_2-N_2-CO_2 (25:74:1) to obtain high O_2 partial pressure; such conditions are designated full aerobic growth (5). In addition to butyrate, malate and succinate (0.4%) were also used as carbon sources in certain experiments. Cell growth was routinely monitored turbidimetrically with a Klett-Summerson colorimeter (Klett Manufacturing Co., Inc.) equipped with a no. 66 filter. One Klett unit is equivalent to a cell density of approximately 10^7 cells per ml. Chemoheterotrophically grown cells and photoheterotrophically grown cells were harvested at the cell densities indicated below.

RuBPCase, protein, and bacteriochlorophyll assay. RuBPCase activity was assayed with toluene-treated cells based on the method of Tabita et al. (28), with some minor modifications. Samples (20 ml) of cells were harvested, washed twice in 10 ml of cold Tris (50 mM)–EDTA (10 mM) buffer (pH 8.0), and then suspended in 2 ml of the same buffer. A 1-ml amount of cold toluene was added and mixed, and the cells were kept on ice for 10 min. After centrifugation at 5,000 rpm for 10 min, the cells were washed with Tris-EDTA buffer and suspended in 1 ml of the same buffer. Reactions were performed in scintillation minivials with rubber stoppers and contained the following: 0.05 ml of 50 mM $MgCl_2$, 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.2), 0.05 ml of 100 mM $NaH^{14}CO_3$ (specific activity, 300 to 1,000 cpm/nmol), 0.05 ml of toluene-treated cell suspension, 0.025 ml of 10 mM RuBP, and 0.075 ml of water. All components minus the RuBP were mixed and preincubated for 10 min at 33°C to activate the enzyme. Prewarmed RuBP was added to initiate the reaction. The assay was performed at 33°C for 3 to 5 min and stopped with the injection of 0.025 ml of 6 N HCl. The reaction mixture was dried down at 80°C under N_2 in a fume hood, allowed to cool, and suspended in 0.1 ml of water. The samples were then counted in Tritosol (22). One unit of RuBPCase is defined as the amount of enzyme needed to carboxylate 1 μmol of RuBP in 1 min at 23°C.

Protein concentrations were determined by a modified Lowry procedure (19). The total bacteriochlorophyll (Bchl) content of the cells was estimated by absorbancy measurements at 775 nm of acetone-methanol (7:2, vol/vol) extracts with an extinction value of 75 mM (9).

Extraction of RNA. A 100-ml sample of fresh cell culture was rapidly poured onto 100 ml of crushed, frozen extraction buffer containing 80 mM Tris-chloride (pH 7.5), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol (freshly added), 25 mM NaN_3, and chloramphenicol (200 μg/ml), rapidly mixed, and centrifuged at 7,000 rpm for 3 min. The cells were lysed by adding 5 ml of lysis buffer containing 0.5% sodium dodecyl sulfate, 10 mM EDTA, 2 mM *O*-phenanthroline, 0.2 mg of heparin per ml, and 0.25 mg of proteinase K per ml and then frozen in a dry ice-ethanol bath and thawed at 37°C for three successive cycles. The cell lysates were incubated at 37°C for 20 min and then extracted with hot (65°C) phenol (redistilled)-chloroform (1:1, vol/vol, three or four times until a clear aqueous phase was obtained. DNA and RNA were precipitated by adding 2 volumes of ethanol to the aqueous phase and dissolved in 5 ml of 50 mM Tris-chloride–5 mM $MgCl_2$ (pH 7.8). DNA was digested by incubation with RNase-free DNase I (50 μg/ml) at 37°C for 20 min. DNase I was removed by phenol extraction, and the RNA was finally collected by ethanol precipitation. About 1 mg of RNA was obtained from 100 ml of cells. The ratio of absorbancy at 260 nm to that at 280 nm was 1.8 to 2.0. RNA was used either for dot blots (5 μg per dot) after denaturing with formaldehyde or for Northern blot (15 μg per lane) hybridization after glyoxylation and agarose gel electrophoresis.

Isolation of chromosomal DNA. The bulk DNA of *R. sphaeroides* was isolated basically by the procedure of Saito and Miura (23). The total bulk DNA was digested with *Bam*HI and separated on an agarose gel.

Radioactive labeling of RNA and DNA. Total *R. sphaeroides* RNA was radiolabeled in vitro by phosphorylating the 5'-OH groups produced by partial alkaline hydrolysis of RNA with ^{32}P from [γ-^{32}P]ATP with T4 polynucleotide kinase by the method of Bogorad et al. (4). RNA samples for Southern hybridization were adjusted to equal specific radioactivity (10^6 cpm/μg). The plasmid DNA or DNA fragment used for dot blot and Northern hybridizations were labeled with [α-^{32}P]dCTP by nick translation by the protocol of Bethesda Research Laboratories, Gaithersburg, Md. The labeled DNA and [α-^{32}P]dCTP were separated with a spun column of Sephadex G-50 by the method of Maniatis et al. (18).

Dot blots and Northern and Southern hybridizations. Dot blots and Northern and Southern hybridizations were performed as previously described (4). The plasmids used as probes for hybridization are listed in Table 1. To quantitatively assess mRNA in either the dot blots or Northern hybridizations, the films were scanned with a GS300 transmittance-reflectance scanning densitometer (Hoefer Scientific Instruments) linked to a recording integrator (Waters M 730 data module). For quantitative comparisons, the dots on the filters were sometimes cut out and counted in 2,5-diphenyloxazole–1,4-bis-(5-phenyloxazolyl)benzene cocktail with a Beckman liquid scintillation counter.

Chemicals. The DNA from plasmids pLI10, C-2-C, pUI135, and pUI128 were generous gifts of P. Hallenbeck and S. Kuhl, respectively. The DNA from plasmid pJW1 and cloned *KpnI-PstI*, *AluI*, *PvuII-SalI*, and *XhoI-NruI* fragments derived from pJW1 were kindly supplied by P. Kiley. [γ-^{32}P]ATP was made by T. J. Donohue by the Johnson and Walseth technique (15). RuBP was obtained from Sigma Chemical Co., St. Louis, Mo. $NaH^{14}Co_3$ (53 mCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass. Agarose, restriction enzymes, and a nick translation reagent kit were purchased from Bethesda Research Laboratories. Gene Screen (New England Nuclear) was used for all hybridizations. Proteinase K was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. RNase-free DNase I was obtained from Worthington Diagnostics, Freehold, N.J. Phenol was redistilled, and all other chemicals were reagent grade.

RESULTS

Effects of light, oxygen, and substrates on RuBPCase and Bchl. RuBPCase is a major soluble cytoplasmic enzyme characteristic of photosynthetic growth and was chosen for study. Further, Bchl levels were used as a measure of photosynthetic development, since the level of Bchl is a measure of intracellular membrane content and composition (16). Four different growth conditions were used to study the

TABLE 1. Plasmids used for probing photosynthetic genes of *R. sphaeroides*

Plasmid	Vector	Insert	Size (kb)	Gene encoded	Reference
pLI10	pAS621	*Bam*HI fragment of *R. sphaeroides* DNA	3.0	RuBPCase II	(22)
C-2-C	M13mp10WRF	*Sal*I-*Bam*HI fragment	1.2	RuBPCase II	Unpublished[a]
pJW1	pBR322	*Bam*HI fragment of *R. sphaeroides* DNA	12.5	LH-1, RC-L, RC-M	(30)
		*Kpn*I-*Pst*I fragment	1.2	LH-1, portion of RC-L	Unpublished[b]
		*Alu*I-*Alu*I fragment	0.51	LH-1	Unpublished[b]
		*Pvu*II-*Sal*I fragment	0.46	RC-L	Unpublished[b]
		*Xho*I-*Nru*I fragment	0.37	RC-M	Unpublished[b]
pUI135	pBR322	*Sal*I fragment of pJW1	7.3	LH-1, RC-L, portion of pJW1 vector	Unpublished[c]
pUI128	pBR322	*Sal*I fragment of pJW1	2.35	RC-M, portion of RC-L	Unpublished[c]

[a] P. Hallenbeck and S. Kaplan.
[b] P. Kiley and S. Kaplan.
[c] S. Kuhl and S. Kaplan.

effects of light and O_2 on RuBPCase and Bchl. (i) In the high-light conditions, *R. sphaeroides* cells were photosynthetically grown under near-saturating high intensity light (10 W/m^2). (ii) In the dark conditions, the cells were grown under high light intensity and were shifted to the dark for 1 h. (iii) In the low-light conditions, cells were photosynthetically grown under low-intensity light (3 W/m^2). (iv) In partial O_2, the cells were aerobically cultured on a shaker. Because cells growing under these different growth conditions had different growth rates, an effort was made to harvest the cells at comparable cell densities (from 110 to 125 Klett units) unless otherwise noted. After the cells were harvested, the RuBPCase activity and Bchl content were determined. Both RuBPCase and Bchl were affected by light and O_2 (Table 2). The shift of cells from high-intensity light to the dark resulted in a 25% reduction in RuBPCase activity, but only a 10% reduction in the level of Bchl, after 1 h. Under steady-state low-intensity light, the RuBPCase activity was approximately 50% of its high-light level, whereas the level of Bchl was approximately 80% greater than its high-light level, as expected. The partially aerobically grown cells had negligible RuBPCase activity, whereas the Bchl content, although low, was detectable. At 110 to 125 Klett units on a rotary shaker the cells were beginning to go through a low-O_2 phase followed by the induction of the photosynthetic apparatus (see below).

The cellular levels of RuBPCase and Bchl were also compared among the cells growing on the different substrates, namely, butyrate, malate, and succinate (Table 3). The cells were grown at high light and harvested during the late log phase (between 200 and 220 Klett units). Under these conditions the cells are undergoing severe shading; however, such conditions are useful in seeing maximal RuBPCase levels. The levels of Bchl showed only slight, but reproducible, differences on the different substrates, whereas the RuBPCase activity showed a marked decrease when the cells were grown on malate (20-fold) and succinate (8-fold) relative to butyrate-grown cells. The relative order of RuBPCase levels (Table 3) is the reverse of the relative growth rates.

Light-, O_2-, and substrate-regulated levels of mRNA coding for RuBPCase, LH-1, RC-L, and RC-M. Since RuBPCase levels and Bchl content are regulated by light, O_2, and substrates, these parameters were varied to learn more about the nature of gene regulation in *R. sphaeroides*. The RNA isolated from cells grown under the different conditions was hybridized with ^{32}P-labeled DNA probes for genes specifying RuBPCase, LH-1, RC-L, and RC-M, and semiquantitative measurements were made by dot hybridization. The Northern hybridization procedure affords additional information about the numbers and sizes as well as the amounts of the transcripts.

Dot hybridization. Figure 1 presents the results obtained from dot hybridizations of DNA probes against bulk RNA isolated from cells grown under the conditions designated. The quantitative measurements obtained from scintillation counting of the filters are summarized in Tables 4 and 5. The quantitative results were similar when obtained by densitometric scans of the dot blots. The C-2-C clone contains a 1.0-kilobase (kb) *Sal*I-*Bam*HI fragment of pLI10 cloned into M13 phage (P. Hallenback, personal communication). This 1.0-kb fragment contains most of the gene for RuBPCase. The *Kpn*I-*Pst*I fragment of pJW1 contains the LH-1 gene as well as a portion of RC-L, and the subclones pUI135 and pUI128 are the probes for LH-1 plus RC-L and for RC-M

TABLE 2. Effects of light and O_2 on the Bchl content and RuBPCase activity of *R. sphaeroides*[a]

Growth condition[b]	Bchl		RuBPCase	
	μg/mg of protein	%	U/mg of protein	%
HL	3.87	100	0.127	100
D	3.52	90	0.095	75
LL	6.90	178	0.071	75
O_2	0.48	12	0.001	0.7

[a] Cells were grown on Sistrom medium containing 0.4% butyrate and 0.1% sodium bicarbonate.
[b] HL, high-intensity light (10 W/m^2); D, shift from high-intensity light to dark for 1 h; LL, low-intensity light (3 W/m^2); O_2, partially aerobic growth.

TABLE 3. Effects of carbon substrates on Bchl content and RuBPCase activity of *R. sphaeroides*

Substrate	Bchl		RuBPCase	
	μg/mg of protein	%	U/mg of protein	%
Butyrate	8.4	100	0.141	100
Malate	7.2	85	0.007	5
Succinate	7.9	94	0.019	13

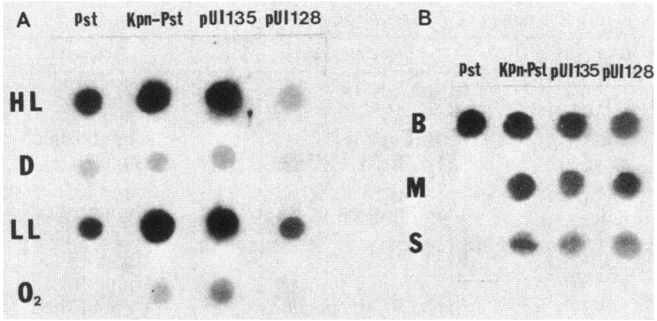

FIG. 1. Dot blot hybridization of *R. sphaeroides* RNA with cloned probes for RuBPCase, LH-1, RC-L, and RC-M. Cell cultures were grown and RNA was isolated as described in the text. Equal amounts of RNA (5μg) after denaturation were spotted onto Gene Screen, hybridized with ^{32}P-labeled probes, and exposed to X-ray film. Each probe employed is listed above the figures and described in Table 1, and the culture conditions are listed to the left of the figures. Abbreviations: HL, high-intensity light; D, shift from high-intensity light to dark for 1 h; LL, low-intensity light; O_2, partially aerobic growth; B, butyrate; M, malate; S, succinate.

genes, respectively. It can be determined from the data in Fig. 1A and Table 4 that the shift of cells from high light to dark for 1 h resulted in the degradation of greater than 70% of the mRNA for RuBPCase. Similarly, cells grown under steady-state low light intensity (3 W/m^2) had a 20% reduction in the level of mRNA specifying RuBPCase when compared with cells growing at high light. Under aerobic growth conditions, no mRNA specifying RuBPCase was detectable. Although mRNA specifying LH-1, RC-L, and RC-M responded to light and O_2, several major differences were observed when we compared these results with those observed for RuBPCase. First, the decreased light intensity used for growth from 10 to 3 W/m^2 resulted in an increased level of mRNA specifying these membrane proteins; second, cells grown under partial aerobiosis still had a measurable level of mRNA (30 to 40%). This is not unusual, since detectable levels of Bchl were present. However, under high pO_2, LH-1 mRNAs were still present despite the absence of Bchl. On the other hand, a very different pattern of responses was observed when the same mRNA levels were measured in cells grown on different substrates (Fig. 1B, Table 5). The cells grown on butyrate synthesized substantially higher levels of mRNA for RuBPCase as compared with that synthesized when cells were grown on either malate (16-fold) or succinate (7-fold). However, relatively small differences were observed in the levels of mRNA for the specific membrane proteins when cells were grown on the different carbon substrates.

TABLE 4. Effects of light and O_2 on levels of mRNA coding for RuBPCase, LH-1, RC-L, and RC-M of *R. sphaeroides*[a]

Growth condition[b]	Relative amt (%)			
	RuBPCase	LH-1	LH-1 plus RC-L	RC-M
HL	100	100	100	100
D	34	30	28	25
LL	80	183	152	147
O_2	<1	25	28	25

[a] The relative amounts of mRNA were measured by dot hydridization, quantified by scintillation counting of the filters, and expressed as the percentages of mRNA level under high-intensity light (set as 100). Only the results within a column can be compared, not those among columns.
[b] See footnote *b* of Table 2.

Northern hybridization. Figure 2 shows a Northern hybridization of isolated RNA with DNA probes for the RuBPCase, LH-1, RC-L, and RC-M. The RNA species were extracted from *R. sphaeroides* cells grown on butyrate under different conditions of light and O_2 as described above. After glyoxylation, the RNA was separated by agarose gel electrophoresis. Two strong bands of rRNA, 16S and 14S, can be seen on the agarose gel (Fig. 2A and B, right lanes). The ratio of 16S rRNA to 14S rRNA is roughly 2.1:1, indicating that there has been little or no degradation of rRNA during isolation (20). The RNA was transferred to Gene Screen and hybridized with ^{32}P-labeled nick translated C-2-C DNA. Three transcripts (2.3, 2.0, and 1.4 kb) were found when fragment C-2-C was used as the probe (Fig. 2A). Each of these hybridization complexes was very stable, resisting melting out up to a wash temperature of slightly over 90°C. The ratio of 2.3-, 2.0-, and 1.4-kb transcripts is 1:4:2.5. Each of the three transcripts is coordinately light and oxygen regulated. Further, the amount of transcript apparent in low light-grown cells relative to high light-grown cells is precisely what one would expect from the dot blot results (Fig. 1). When a 0.91-kb internal *Pst*I fragment of the RuBPCase gene was used as a specific probe, the same three transcripts were obtained (data not shown). No transcripts for RuBPCase were detected in aerobically grown cells. Even more interesting are the transcripts for the LH-1 polypeptides. The probe for LH-1 is the 1.2-kb *Kpn*I-*Pst*I fragment of pJW1 containing the LH-1 genes as well as a portion of the RC-L gene. Two major transcripts (2.6 and 0.8 kb) and two minor transcripts (2.0 and 1.45 kb) are seen in Fig. 2B, panel 1. Under high-intensity light with the *Kpn*I-*Pst*I probe, the ratios of the major transcripts (2.6 and 0.8 kb) to one another were approximately equal. However, after 1 h in the dark or in the presence of O_2 the amount of larger transcript (2.6 kb) decreased, but the concentration of the small transcript (0.8 kb) changed only slightly; therefore, the ratio of the 0.8-kb transcript to the 2.6-kb transcript reached a value of 12, suggesting a greater rate of turnover of the 2.6-kb transcript relative to the 0.8-kb transcript. Under low-intensity light the ratio of the small transcript to the large transcript increased to a value of 1.9.

Northern hybridizations were performed with probes specific for RC-L (Fig. 2B, panel 2) and RC-M (Fig. 2B, panel 3), which indicated that these genes were expressed on the large transcript (2.6 kb), but were absent from the small transcript (0.8 kb). Since these four structural genes coding for LH-1, RC-L, and RC-M are closely linked on the chromosome (31; P. Kiley and S. Kaplan, unpublished data), it seems reasonable to assume that the larger transcript represents a single polycistronic transcript specifying the four polypeptides: LH-1 α and β, RC-L, and RC-M. The LH-1 polypeptides appear to be specified by the small transcript. Unlike the response observed when the C-2-C probe for RuBPCase was used, no significant changes were

TABLE 5. Effects of carbon substrates on levels of mRNA coding for RuBPCase, LH-1, RC-L, and RC-M of *R. sphaeroides*[a]

Substrate	Relative amt (%)			
	RuBPCase	LH-1	LH-1 plus RC-L	RC-M
Butyrate	100	100	100	100
Malate	6	78	64	86
Succinate	14	58	63	79

[a] See footnote *a* of Table 4.

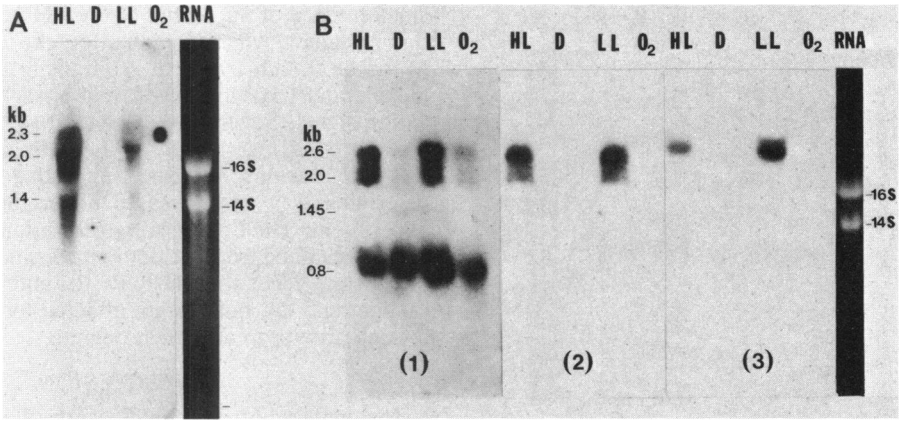

FIG. 2. Northern hybridization of *R. sphaeroides* with cloned probes for RuBPCase, LH-1, RC-M, and RC-L. Samples of glyoxylated RNA from *R. sphaeroides* cells, grown photosynthetically on the buryrate medium under the conditions described in the legend to Fig. 1, were fractionated on 1.0% agarose gel (rightmost lanes of A and B), transferred to Gene Screen, and hybridized with ^{32}P-labeled probes for RuBPCase (A) and LH-1 (B, panel 1), RC-L (B, panel 2), and RC-M (B, panel 3). The probes for RuBPCase and LH-1 are listed in Table 1. The cloned fragment of *Pvu*II-*Sal*I (0.46 kb) and *Xho*I-*Nru*I (0.37 kb) were used as the probes specific for RC-L and RC-M, respectively. Both are the internal portions of their respective genes.

found in the relative abundance of the two major transcripts for LH-1 when the cells were grown on the different substrates (data not shown).

Because the *Kpn*I-*Pst*I probe contains, in addition to the LH-I genes, a portion of the distal RC-L gene, the observed ratio of the small transcript (0.8 kb) to the large transcript (2.6 kb) (1:1 to 2:1) is not an accurate measure of the true steady-state ratio of each of these transcripts, since the RC-L and RC-M coding regions reside only on the large transcript. Therefore, an *Alu*I DNA fragment containing only the information for the LH-1 polypeptides as well as a small amount of proximal DNA was used for Northern hybridization analysis, and the relative levels of the small and large transcripts were determined (Fig. 3). From the densitometer tracings of Fig. 3, the steady-state ratio of the small transcript to the large transcript was 10:1. From five independent determinations of this kind, the average ratio for the small to the large transcript was 11.2:1, ranging from 7:1 to 18:1.

Southern hybridization. The results from the dot blots and Northern hybridization experiments were further confirmed by Southern hybridization. Figure 4 shows the results of Southern hybridization of a *Bam*HI digest of plasmid pLI10 DNA with ^{32}P-labeled bulk RNA, as described above.

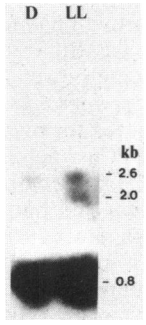

FIG. 3. Northern hybridization of *R. sphaeroides* with a specific probe for LH-1. The hybridization conditions were the same as in Fig. 1, except a 0.51-kb *Alu*I-*Alu*I fragment of pJW1 was used as a specific probe for LH-1.

Plasmid pLI10 is a pBR322 derivative containing a *trp* promoter and having an insert of a 3.0-kb fragment of *R. sphaeroides* DNA containing the gene coding for RuBPCase II (Table 1, Fig. 4C). The results, employing different conditions of light and O_2 are shown in Fig. 4A; the right lane is an agarose gel on which the 4.9-kb vector and the 3.0-kb

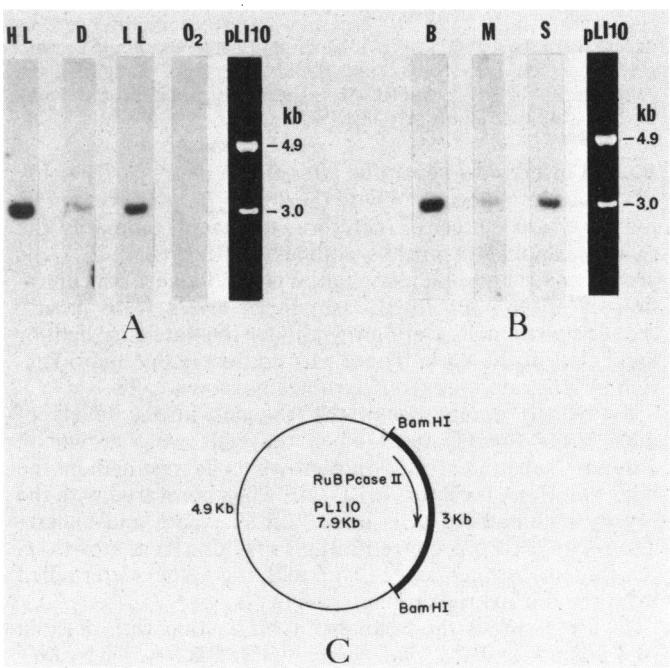

FIG. 4. Southern hydridization of pLI10 *Bam*HI digest with ^{32}P-labeled RNA. The pLI10 DNA was disgested with *Bam*HI, separated on a 1.0% agarose gel, transferred to Gene Screen, and hybridized with ^{32}P-labeled RNA from *R. sphaeroides* cells. The cells were grown on butyrate medium under different lighting conditions (A) or on different substrates (B) as described in the legend to Fig. 1. The rightmost lanes of A and B are agarose gels, and the remaining lanes are autoradiograms of these gels hybridized with the indicated probes, where the 4.9-kb vector and 3.0-kb *Bam*HI insert are indicated. Panel C shows the restriction map of pLI10 indicating the location of the gene coding for form II RuBPCase.

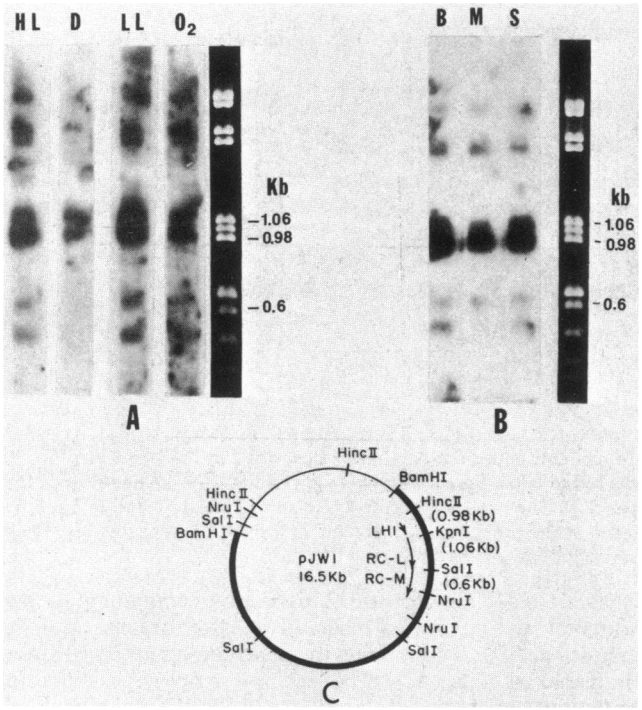

FIG. 5. Southern hybridization of pJW1 with ^{32}P-labeled RNA. The pJW1 DNA was digested with *Hinc*II, *Kpn*I, *Sal*I, and *Nru*I and blotted onto Gene Screen after fractionation on an agarose gel. The hybridization results employing different light and O_2 treatments (A) and different substrates (B) are shown. The conditions were as described in the legend to Fig. 1. The rightmost lanes are the agarose gels on which 1.06-, 0.98-, and 0.6-kb fragments containing the genes for RC-L, LH-1, and RC-M, respectively, are indicated. Panel C shows the restriction map of pJW1.

*Bam*HI insert were separated after electrophoresis. The bulk RNA was homologous only to the 3.0-kb *Bam*HI insert. The hybridization intensity, therefore, represents primarily the relative amount of mRNA coding for RuBPCase II. Cells grown under high-intensity light had the highest concentration of mRNA for RuBPCase; these levels were greatly reduced when cells were grown under low-intensity light or incubated in the dark. There was no observable hybridization of RNA derived from aerobically grown cells.

Figure 4B demonstrates the changes in the levels of mRNA for RuBPCase II when the cells were grown on different substrates. Butyrate-grown cells resulted in the highest mRNA level for RuBPCase when compared with the results obtained for cells grown on succinate and malate. The relative mRNA concentrations resulting from growth on the three substrates agreed well with the values determined from the dot hybridization experiments.

Figure 5 shows the Southern hybridization with plasmid pJW1 DNA and *R. sphaeroides* derived RNA. The pJW1 DNA was digested with *Sal*I, *Kpn*I, *Nru*I, and *Hinc*II. Approximately 13 electrophoretic bands were obtained, as shown on the agarose gel on the right lanes of Fig. 5A and B. A partial map of the restriction sites located on pJW1 is presented in Fig. 5C. The 0.98-kb *Hinc*II-*Kpn*I, 1.06-kb *Kpn*I-*Sal*I, and 0.6-kb *Sal*I-*Nru*I fragments contain genes coding for LH-1, RC-L, and RC-M, respectively (P. Kiley and S. Kaplan, unpublished data). The autoradiographs demonstrated that the LH-1 genes (0.98 kb) had the strongest hybridization signal. The 1.06-kb fragment had a rather strong hybridization signal, probably due to the fact that a small portion of the 3′ end of the LH-1 genes extended into this fragment, which contains the entire RC-L gene. However, the 0.6-kb fragment, containing the RC-M gene and lacking any LH-1 fragment, had a relatively weak hybridization signal. Several other bands that were located on the 12.0-kb DNA insert of *R. sphaeroides* also hybridized with RNA to various degrees, suggesting that other genetic determinants were expressed under the conditions of this experiment. The Southern hybridization results verified the results obtained from the dot hybridization experiments, that is, that the genes for LH-1, RC-L, and RC-M are regulated by light and O_2, but are not affected by the substrates used for cell growth to any great degree.

DISCUSSION

The metabolic, morphological, and biophysical changes induced by the imposition of different environmental regimens on *R. sphaeroides* have been well studied (11, 16, 17); nevertheless, information specifying the molecular events governing such changes with respect to gene regulation is limited. The present investigation with carefully defined probes and methods provides substantial, detailed information about the regulation of mRNA coding for several photosynthetic membrane proteins as well as RuBPCase in cells subjected to a variety of cultural conditions.

The hybridization experiments reported here demonstrate the importance of light as a trigger in the regulation of photosynthetic gene expression. Cells grown under low-light conditions revealed significantly reduced levels of mRNA for RuBPCase. However, under similar conditions the level of LH-1-specific mRNA was shown to be very high, as might be anticipated because more photosynthetic membrane is produced to compensate for the decreased light intensity and because there is a greater density of photosynthetic units per unit of membrane (32). Based on the total radioactivity added for hybridization, the specific radioactivity of the RNA (3.6×10^7 cpm/μg of RNA), and the amount of radioactivity shown to hybridize, we calculated that the mRNA specific for LH-1 was about 0.006% of total RNA, but 0.2% of the total mRNA.

Growth in the presence of O_2 completely abolished the expression of the gene for RuBPCase, whereas a considerable level of mRNA specifying LH-1 was still synthesized in the presence of O_2, although no Bchl was detected under high O_2 partial pressure. On the other hand, the amounts of mRNA fro RC-L and RC-M significantly decreased (Fig. 2B), indicating that the mRNAs for RC-L and RC-M are very unstable.

As might have been anticipated, the substrates used to support photosynthetic growth had a major influence on the mRNA levels for RuBPCase. Butyrate has been shown to be the best source of reducing power and shows a very high level of RuBPCase in *R. sphaeroides* (27). The high level of RuBPCase can be accounted for by the increased transcriptional activity of the RuBPCase gene, which shows a 17-fold increase in cognate mRNA accompanied by a 20-fold increase in RuBPCase activity on butyrate as compared with that on malate. Although the data presented here cannot exclude the possibility of translational regulation of RuBPCase expression, the data are consistent with a transcriptional control mechanism. On the other hand, the substrates had relatively small effect on the expression of the genes for LH-1, RC-L, and RC-M.

The Northern hybridization experiments revealed that multiple transcripts may exist for both RuBPCase and LH-1. A single gene for the large subunit of RuBPCase from maize

yields two differentially regulated mRNAs with sizes of 1.6 and 1.8 kb (10). More complex patterns of RNA are seen for other photoregulated genes (3). In this study, three *R. sphaeroides* transcripts for RuBPCase of 2.3, 2.0, and 1.4 kb were found, with either C-2-C or an internal *Pst* fragment of the RuBPCase gene (data not shown) as a probe. The 1.4-kb transcript was the minimal size for the structural gene of RuBPCase. However, the 2.0-kb transcript was the major transcript observed. We also found that the levels of these three transcripts were regulated coordinately when the light and O_2 pressure were changed.

Northern hybridization of the genes for LH-1, RC-L, and RC-M revealed that two major transcripts of 2.6 and 0.8 kb and two minor transcripts of 2.0 and 1.45 kb existed. The genes coding for RC-L (30) and RC-M (31) of *R. sphaeroides* have been sequenced. The LH-1 genes in *R. capsulata* have been sequenced and code for two polypeptides, B870α and B870β. In *R. sphaeroides*, the LH-1 genes have approximately 350 base pairs, with a spacer of about 150 base pairs, and are linked to the RC-L and RC-M genes (P. Kiley and S. Kaplan, unpublished data). Although these four genes may have separate start codons, they probably constitute the same operon (33). Since the 2.6-kb transcript is long enough to code for LH-1, RC-L, and RC-M, it probably gives rise to a polycistronic mRNA expressing at least these four genes. The 0.8-kb transcript is long enough to code for LH-1 and may be a single transcript of only the LH-1 genes. This assumption is confirmed by the results of the Northern hybridization of RNA with probes specific for RC-L and RC-M (Fig. 2B) (2, 3), because only the 2.6-kb transcript, but not the 0.8-kb transcript, hybridized under these conditions. If we assume that each of these transcripts is initiated from the same site and ignore the mechanism by which the smaller as opposed to the larger is generated, then this may be the basis for the existence of a relatively fixed, 15:1 stoichiometry between LH-1 and the RC polypeptides (7). The average ratio of the small transcript (0.8 kb) to the large transcript (2.6 kb) (11.2:1) is within reasonable error identical to the fixed ratio observed for the gene products of the LH-1, RC-L, and RC-M genes. Therefore, the relative steady-state abundance of these two transcripts may be the sole basis for the stoichiometry of the gene products. The exact mechanism, initiation, termination, or selective degradation leading to the steady-state abundance of each of these transcripts in *R. sphaeroides* remains to be determined. In a paper that appeared recently, Belasco et al. (2) made essentially identical observations for the presence and relative abundance of two mRNA species for the LH-1, RC-L, and RC-M genes in *R. capsulata*. These authors concluded that selective degradation from the 3′ end of the large transcript is responsible for the steady-state level of the small and large transcripts. The 2.6-kb transcript greatly diminished when the cells were shifted from light to dark or when cells were grown aerobically, whereas the concentration of the 0.8-kb transcript was maintained at almost the same levels under identical conditions (Fig. 2). The different stability of two transcripts may be involved in the regulation of the LH-1, RC-L and RC-M genes in *R. sphaeroides* and, as suggested by Belasco et al. (2), for *R. capsulata*. This result will be described in more detail in a separate paper.

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