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Gene expression of pigment-binding proteins of the bacterial photosynthetic apparatus: Transcription and assembly in the membrane of *Rhodopseudomonas capsulata*

(antenna pigment complexes/reaction center/bacterial photosynthesis/membrane assembly/transcriptional control)

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ABSTRACT **Lowering of oxygen partial pressure in chemotrophic cultures or reduction of light intensity in phototrophic cultures of *Rhodopseudomonas capsulata* induced formation of the photosynthetic apparatus. A maximum of mRNA coding for the reaction center (RC) and the light-harvesting I B870 antenna complex polypeptides occurred 30 min after induction. Maximal expression of mRNA for B800–B850 antenna proteins appeared with a lag time of about 25 min after RC/B870 mRNA. Pigment-binding polypeptides were inserted into the membrane immediately after mRNA synthesis. It is concluded that the delayed formation of the B800–B850 complex compared to the RC and the B870 complex is caused by sequential expression of the corresponding genes. Biological activity of pigment–protein complexes increased after the incorporation of their polypeptides parallel to the maximum of bacteriochlorophyll synthesis. Studies on mutant strains defective in the formation of pigment–protein complexes suggested that pigment synthesis is of importance for assembly of stable complexes.**

Decreasing oxygen tension in chemotrophic cultures or decreasing light intensity in phototrophic cultures of facultative phototrophic bacteria induces formation of an intracytoplasmic membrane system and a preferential biosynthesis of the photochemical reaction center (RC) and light-harvesting complexes B870 and B800-B850 (reviewed in refs. 1–3). A stable assembly of these complexes takes place, provided that all polypeptides of the respective complexes and the pigments bacteriochlorophyll (BChl) and carotenoids are synthesized (4, 5).

Recently it has been shown that decreasing oxygen tension in cultures of *Rhodopseudomonas capsulata* induces an increase of mRNAs specific for the RC subunits and B870 antenna polypeptides (6) and for the B800–B850 antenna polypeptides (7). These observations support the idea that the synthesis of the complex-forming polypeptides is under transcriptional control. The level of RNA for pigment synthesis shows only a small increase when oxygen tension is reduced (6, 8).

The genes for the M and L subunits of the RC and for the polypeptides of B870 are cotranscribed from the *rxcA* locus of the *R. capsulata* genome (9). The differential expression of RC and B870 genes has been explained by segmental differences in stability within the polycistronic *rxcA* transcript (9). Genes for the B800–B850 polypeptides lie outside this operon (10, 11). The structural genes for the B800–B850 α and β polypeptides have been cloned, and the nucleotide sequences have been determined (12). Polypeptides of the B800–B850 light-harvesting complex appear with a lag time after those of the RC and B870 complex in the membrane of *R. capsulata* (13).

If the synthesis of complex-forming polypeptides is under transcriptional control, the genes for the B800–B850 polypeptides should be expressed sequentially after RC/B870 genes, and polypeptides should be detectable immediately after transcription of their genes. The results reported herein confirm these predictions. Experiments using wild-type and mutant strains underline an independent and sequential expression of RC/B870 and B800–B850 genes effected either by reduction of oxygen tension or illumination. Increase of BChl and biological activity of photosynthetic complexes are delayed compared to incorporation of pigment-binding polypeptides into the membrane, which immediately follows the transcription of the respective genes.

MATERIALS AND METHODS

Enzymes and Radiochemicals. Restriction endonucleases, DNA polymerase, calf intestine alkaline phosphatase, deoxyribonuclease I, and T4 DNA ligase were purchased from Boehringer Mannheim. Radiochemicals and polynucleotide kinase were from Amersham-Buchler (Frankfurt, FRG). All enzymes were used according to suppliers' instructions.

Bacterial Strains, Plasmids, and Growth Conditions. Bacterial strains and plasmids used in this study are listed in Table 1.

Cultivation of *R. capsulata* under low oxygen tension in Erlenmeyer flasks has been described (7). Alternatively, the bacteria were grown in a 12-liter New Brunswick fermenter Microferm under strong aeration and with stirring to a density of 6×10^8 cells per ml. The aerobically grown cells were harvested and resuspended in 3 liters of fresh medium in a Biostat (Braun, Melsungen, FRG) to a density of 2.4×10^9 cells per ml. Within the next 10 min, the oxygen partial pressure was reduced to 5% of saturation, and samples were taken at various times after the transition, mixed with chloramphenicol (100 μg/ml), and poured onto crushed, frozen 10 mM Tris·HCl/3 mM magnesium acetate/60 mM NH_4Cl/10 mM NaN_3, pH 7.6.

The experimental conditions for growth of bacteria anaerobically under different light intensities in a turbidostat have been described (21).

Cloning, Immobilization, and Hybridization of Nucleic Acids. The 9.2-kilobase *Bam*HI fragment C of pRPS404 was extracted from agarose gel (22) and ligated into the site for tetracycline resistance in pBR322, creating plasmid pBBC1. The vector had been previously treated with alkaline phosphatase. DNA fragments were transferred to nitrocellulose BA85 (Schleicher & Schuell) by the procedure of Southern

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Abbreviations: RC, reaction center; BChl, bacteriochlorophyll.
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Table 1. Bacterial strains and plasmids

Strain	Phenotype	Reference/source
R. capsulata		
37b4	Wild type	DSM938*
NK3	BChl$^+$, RC$^+$, B870$^+$, B800–B850$^-$, Crt$^+$	11
YS	BChl$^-$, RC$^-$, B870$^-$, B800–B850$^-$, Crt$^+$	14
Glpho$^+$	BChl$^+$, RC$^+$, B870$^+$, B800–B850$^-$, Crt$^-$	15
Ala$^+$	BChl$^+$, RC$^+$, B870$^+$, B800–B850$^-$, Crt$^-$	16
Y5	BChl$^+$, RC$^-$, B870$^-$, B800–B850$^+$, Crt$^+$	16
Plasmid		
pBR322	Tc^r, Ap^r	17
pRPS404	Km^r†	18
pBBC1	Ap^r‡	
pVK1	Tc^r§	19
pRC1	Ap^r¶	20

Tc, tetracycline; Ap, ampicillin; Km, kanamycin; r, resistant; Crt, carotenoid.
*German Collection of Microorganisms DSM938.
†Contains chromosomal DNA of *R. capsulata* St. Louis encoding synthesis of BChl, Crt, RC, and B870 polypeptides.
‡*Bam*HI fragment C of pRPS404 cloned into the Tc^r site of pBR322 encodes B870 polypeptides and RC subunits L and M.
§Contains chromosomal DNA of *R. capsulata* 37b4 encoding B800–B850 polypeptides.
¶Carries rRNA genes of *R. capsulata* 37b4.

(23). Isolation of polysomes from *R. capsulata* was as described by Dierstein (24). Total RNA (10–20 μg) was denatured for 5 min at 65°C in 50% formamide/5% formaldehyde in Mops buffer (20 mM Mops/5 mM Na acetate/1 mM EDTA, pH 7.0). Samples were made 12.2 M in NaI to reduce binding of ribosomal RNA (25) and were filtered onto the nitrocellulose by using a Schleicher & Schuell Minifold. Labeling and hybridization of nucleic acids and quantification of hybridization have been described (7). Blot-hybridization filters were prehybridized in the presence of cosmid pRC1 [DNA for ribosomal RNA (rDNA)] to avoid unspecific hybridization of bound rRNA to the labeled plasmid.

Isolation and Analysis of Intracytoplasmic Membranes. Cell-free extracts of *R. capsulata* were prepared as described (4). Intracytoplasmic membranes were purified from the extracts by a centrifugation through a 1.5–0.6 M sucrose density gradient (for 16 hr at 100,000 × *g*). Membrane proteins were separated by $NaDodSO_4$/polyacrylamide gel electrophoresis on 1-mm slab gels (26) with a 11.5–16.5% continuous gradient of acrylamide. Gels were stained with Coomassie brilliant blue and scanned in the Kontron Uvikon spectrophotometer, 810.

BChl content of whole cells and membranes was calculated from the 772-nm absorption of the methanol/acetone extracts using an extinction coefficient of 76 $mM^{-1}\cdot cm^{-1}$ (27). Protein concentration was measured as described (28). Concentration of the RC was assayed by reversible bleaching at 870 nm (21) using the extinction coefficient 113 $mM^{-1}\cdot cm^{-1}$ (29).

Quantitative Immunoassay of Proteins. Pigment-binding proteins were determined by a quantitative immunoassay (30) using a Schleicher & Schuell Minifold for blotting. Purified rabbit antibodies were used at a concentration of 50 μg/ml. Tritium-labeled protein A was diluted to 200,000 cpm/ml in blocking solution. The excised dots were submitted to liquid scintillation counting.

RESULTS

Differential Gene Expression. When *R. capsulata* wild-type cells grown under high-light conditions were shifted to low-light conditions, the growth rate was strongly reduced and membrane differentiation was initiated (21), visualized by the increase in the BChl content of the cells (Fig. 1).

A maximum of hybridization with pBBC1 encoding RC and B870 polypeptides was observed after a 30-min incubation at low-light intensity. The hybridization probe for B800–B850-specific RNA was plasmid pVK1 (19). Hybridization to oligonucleotides complementary to amino acid sequences of the B800–B850 α and β polypeptides showed that the structural genes for both pigment-binding polypeptides of this complex were localized on pVK1 (unpublished data). A maximum of hybridization of RNA to pVK1 was noticed at 50 min after transition to low-light conditions.

Lowering of oxygen partial pressure in the culture also caused growth retardation and induction of BChl synthesis (Fig. 1). The increase in mRNA for RC/B870 polypeptides

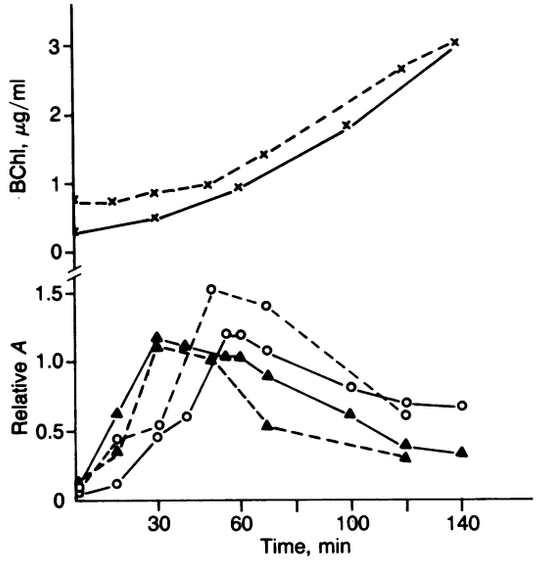

FIG. 1. Formation of the photosynthetic apparatus was induced in cultures of *R. capsulata* 37b4 by reduction of oxygen tension (——) or change of light intensity (– – – –) at time 0. Plasmids pBBC1 (genes for RC and B870) and pVK1 (genes specific for B800–B850) were hybridized against total RNA isolated at various times after transition. Equal amounts of plasmid DNA and total RNA were taken for blotting and labeling. Both Southern and RNA blot-hybridization techniques were used for identical probes. The plots represent an average of densitometrical analysis of autoradiograms of several single experiments. ▲, Relative absorbance of bands or dots hybridizing to *Bam*HI fragment C; ○, relative absorbance of bands or dots hybridizing to pVK1; ×, BChl content of cultures.

was similar to that caused by lowering the light intensity, and the maximal amount of B800–B850 mRNA appeared with a delay of about 30 min (Fig. 1).

Additional hybridization experiments, using identical RNA probes, were carried out with the *Bam*HI fragments G, J, and H of pRPS404 encoding genes for pigment synthesis. The maximal increase in hybridization to RNA isolated from induced *R. capsulata* cells was 2-fold (data not shown). We conclude that the increase of hybridization to *Bam*HI fragment C or pVK1 is not owing to the *BChla* and *BChlc* genes of pRPS404 or unknown genes on pVK1.

Escherichia coli lacZ structural genes were fused to the 2.7-kilobase *Eco*RI/*Apa* I fragment of pVK1 carrying the B800–B850 structural genes by using MudI (Ap^r *lac*) phage (unpublished data). When these modified plasmids were transferred to *R. capsulata*, clones were selected that showed an increase of β-galactosidase activity after reduction of oxygen tension. Kinetics of increase of β-galactosidase activity and of RNA hybridizing to pVK1 were nearly identical. These results show that no other transcripts from plasmid pVK1 were stimulated after reduction of oxygen tension with kinetic behavior different from that of the B800–B850 genes.

Kinetics of Incorporation of Proteins of Photosynthetic Apparatus in the Membrane Compared to mRNA Synthesis. If the synthesis of pigment-binding polypeptides were preferentially regulated on the transcriptional level, we should find a correlation between mRNA formation and synthesis of those polypeptides. A series of standard assays assured the applicability of the described immunoblotting procedure to our system (Fig. 2).

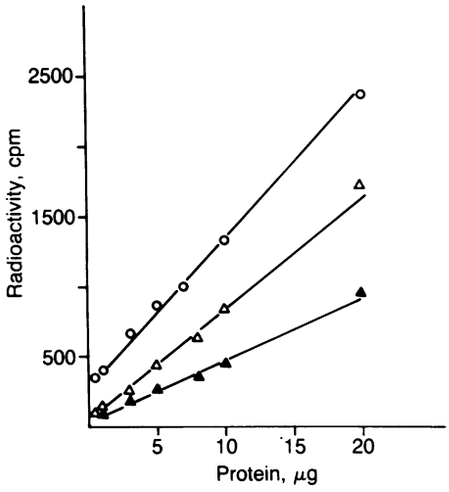

FIG. 2. One-half to 20 μg of intracytoplasmic membranes or isolated RC of *R. capsulata* was blotted to nitrocellulose. Immunobinding to specific antibodies was carried out as described, and radioactivity of antigen–antibody–protein A complexes was measured by scintillation counting. ○, Antibodies against B800–B850 reacting with intracytoplasmic membranes; ▲, antibodies against RC reacting with intracytoplasmic membrane; △, antibodies against RC reacting with isolated RC protein. Surprisingly, the lines for the quantitation of the RC proteins from either pure RCs or photosynthetic membranes were not parallel, and the line for reaction using the purified proteins was not higher, compared with the use of whole membranes, as expected from the relative amount of RC protein in membranes. However, the specificity of antibodies was proved by the immunoblotting technique, and dot blots against increasing amounts of membrane proteins from mutant YS resulted only in a negligible increase of radioactivity (data not shown). Possibly the reactivity of the RC proteins against the antibodies was reduced during the procedures of isolation and purification, whereas the membrane-bound RC proteins were less affected.

In parallel experiments all membrane fractions were also quantified by $NaDodSO_4$/polyacrylamide gel densitometry. Standard assays showed a linear correlation between the applied amount of total membrane protein between 10 and 100 μg and the peak height of single bands of photosynthetic

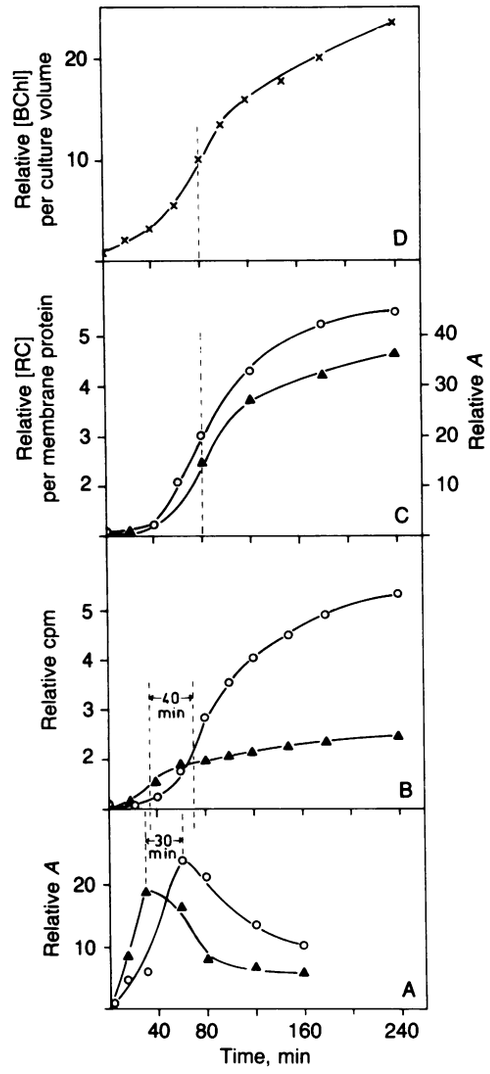

FIG. 3. Oxygen tension was reduced in cultures of *R. capsulata* at time 0, and total RNA and intracytoplasmic membranes were isolated and pigments were extracted at various times of induction. Determination of hybridizing RNA and BChl content are described in *Materials and Methods* and in the legend for Fig. 1. RC was determined in membrane suspensions containing about 5 μM BChl in 50 mM Tris·HCl/100 mM KCl, pH 7.5. Valinomycin and antimycin A were added to a final concentration of 5 μg/ml. The redox potential was adjusted to 400 mV by titration with potassium hexacyanoiron(III) and -(II) using a platinum calomel electrode. Photobleaching was measured at 870 nm; 64 pmol of RC per mg of membrane protein were observed in aerobically grown cells. The active B800–B850 pigment–protein complex was determined by measuring the increase of the absorption in membranes at 800 nm. The amounts of BChl, RNA, protein, and absorbance at time 0 of induction were assigned the numerical value 1. The relative increases of these values document the relative amount of hybridizing RNA as described in the legend for Fig. 1(*A*), relative increase of radioactivity of antigen–antibody–protein A complexes after immunobinding (*B*), relative increase of RC concentration ([RC]) per membrane protein and of absorbance at 800 nm (*C*), and relative concentration of BChl ([BChl]) in cells (*D*). ▲, Relative amount of RC/B870-specific mRNA, RC proteins, reaction center concentration; ○, relative amount of B800–B850-specific RNA, proteins and relative absorbance at 800 nm; ×, relative concentration of BChl in cells.

proteins (not shown). Immediately after mRNA formation induced by reduction of oxygen tension, pigment-binding polypeptides were incorporated into the membrane (Fig. 3 and Table 2). The maximal incorporation of B800–B850 polypeptides occurred about 40 min after insertion of RC polypeptides. This time sequence was nearly identical with sequential gene transcription of B800–B850 after RC/B870 genes.

For both pigment complexes, the maximal accumulation of polypeptides was 5 to 10 min after the maximal accumulation of the corresponding mRNAs, while the maximal rate of BChl synthesis and complex formation was found 80 min after transition to low oxygen tension (Fig. 3*D* and Table 2).

When the culture was switched from high-light to low-light conditions, the same kinetics in gene expression was obtained (not shown). The maximal rates of protein accumulation in the membrane were at 45 min for the RC and at 70 min for the B800–B850 complex, showing a retardation of 15 min compared to the maxima of the respective RNAs (Fig. 1). The maximal rate of BChl synthesis was found 90 min after transition of culture conditions. Compared to induction by reduction of oxygen tension, decreasing the light intensity resulted in a relatively lower increase of RC polypeptides.

Data from gel-scanning showed that the 14-kDa polypeptide of the B800–B850 complex (B800–B850) accumulated earlier than the 10-kDa (α) and 8-kDa (β) polypeptides, which arose together (not shown). The maximal incorporation of the 14-kDa polypeptide was observed 30 min after reduction of oxygen tension and at 70 min for both α and β pigment-binding polypeptides. The maxima of incorporation into the membrane were at 70 min for the B870 β polypeptide and at 40 min for the B870 α subunit, although the corresponding genes were clustered and cotranscribed. In contrast, the H and M subunits of RC appeared simultaneously with a maximal incorporation at about 35 min, although the respective genes were separated on the *R. capsulata* genome.

Biological Activity of Pigment–Protein Complexes. The *in vivo* absorption spectra (Fig. 4) and the reversible bleaching of RC indicated potentially active pigment complexes. The pigment complexes appeared in the membrane after the incorporation of pigment-binding polypeptides with a kinetics similar to that of BChl synthesis (Fig. 3). The quantitative determination of RC (Table 2 and Fig. 3) and the dominance of P870 during the initial phase of induction indicated that the assembly of pigment complexes in the membrane also was a sequential process (13, 32, 33).

Induction Experiments with Mutant Strains Defective in the Photosynthetic Apparatus. Cultures of mutant strains of *R. capsulata* (Table 1) were induced by decreasing oxygen tension as described for wild type. The results are summarized in Table 2.

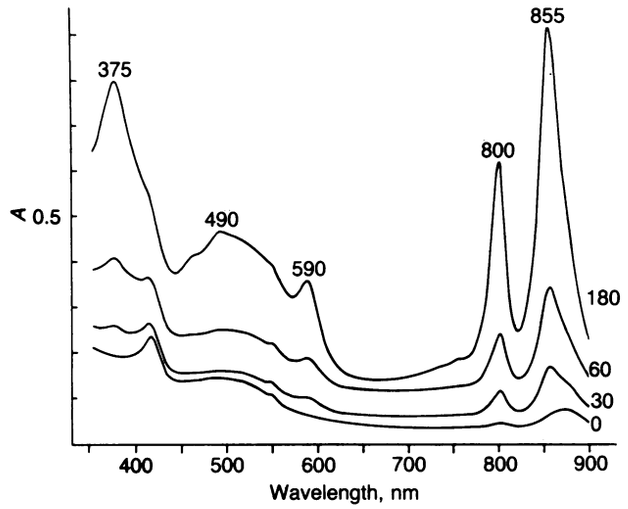

FIG. 4. Photosynthetic apparatus of wild-type *R. capsulata* was induced by reduction of oxygen pressure. At indicated times, growth was stopped and intracytoplasmic membranes were isolated. Equal aliquots of these membranes were diluted, and absorption spectra were recorded from 350 nm to 900 nm. The absorption of the bulk antenna complex at 800 and 850 nm in probes taken at later times cover the B870 absorption visible in aerobically grown cells (0 min). During induction, increase of absorbance of membrane-bound BChl (590 nm, 375 nm), carotenoids (490 nm), and pigment–protein complexes (800–850 nm, 870 nm) visualizes assembly of the photosynthetic apparatus.

In strains NK3 and Glpho$^+$, neither the B800–B850 complex nor its specific mRNA were formed after decreasing oxygen tension. In strain NK3, the kinetics and degree of increase of RC/B870-specific mRNA and incorporation of RC polypeptides were nearly identical as shown for wild type. No mRNA hybridizing to pVK1 accumulated. This observation confirmed that transcription of genes for the α and β polypeptides of B800–B850 was blocked by Tn*5* in NK3 and that no other operons were cloned on pVK1, whose transcription was stimulated by reduction of oxygen tension.

In strain Glpho$^+$, the maximal amount of RC/B870-specific RNA and maximal rates of incorporation of RC polypeptides and BChl synthesis appeared later than in wild-type strain 37b4. Although the BChl concentration increased only 3-fold after 120 min, the hybridization of RNA to *Bam*HI fragment C increased about 16-fold as in the wild-type strain.

In the mutant strains YS, Y5, and Ala$^+$, we observed stimulation of mRNA formation, which was not correlated with a concomitant assembly of the respective complexes. In YS none of the pigment–protein complexes were detectable

Table 2. Increase of photosynthesis-specific molecules in *R. capsulata* during induction

	Stimulation*				Time after induction for maximal effects, min				
								Increase of membrane-bound protein	
	RC/B870	RC-specific	B800–B850		Rate of BChl	Amount of specific RNA			
Strain	RNA	protein	RNA	Protein	synthesis	RC/B870	B800–B850	RC	B800–B850
37b4	+	+	+	+	90	30	55	35	65
NK3	+	+	−	−	90	30	—	40	—
Glpho$^+$	+	+	−	−	120	60	—	60	—
YS	+	−	−	−	—	120	—	—	—
Ala$^+$	+	+	+	−	90	ND	—	70	—
Y5	+	−	+	+	100	ND	ND	—	80

ND, not determined.

*+, Reduction of oxygen tension in cultures resulted in an increase of hybridizing RNA or protein, which was at least 1.5-fold; −, increase of RNA or protein was <1.5-fold.

in the membrane. Reduction of oxygen tension did not influence the amount of carotenoids in cells (not shown). The B800–B850 α polypeptide was synthesized but quickly degraded (4). RC/B870-specific RNA increased 5-fold in YS with a delay of 90 min compared to the wild-type strain, whereas transcription of B800–B850 genes was not stimulated.

Although the mutant Ala$^+$ showed spectra and protein patterns similar to those of phototrophically grown Glpho$^+$, gene expression was different in these two strains. Induction of RC/B870 genes was similar to that in Glpho$^+$. Additionally, a 4-fold increase of mRNA for B800–B850 was detected, although this complex was not synthesized in the mutant strain under semiaerobic conditions.

In strain Y5, mRNAs for RC/B870 and B800–B850 increased with similar rates as in the wild-type strain. However, accumulation of BChl and B800–B850 polypeptides was slightly delayed. We knew from immunoblotting experiments that the polypeptides of RC and B870 α and β were not (or only in traces) detectable in the membrane (ref. 34; unpublished results). Neither RC nor B870 complexes were assembled.

DISCUSSION

Earlier investigations reported a sequential accumulation of RC/870 and B800–B850 pigment–protein complexes in the membrane of *R. capsulata* after induction by decreasing the oxygen tension (13, 31) or light intensity (32). These results have been quantified in the present study and attributed to the sequential gene expression measured by RNA·DNA hybridization. Control experiments assured the practicability of the DNA probes used.

Maximal amounts of B800–B850-specific and RC/B870-specific RNAs appeared about 5 min before maximal rates of incorporation of the respective polypeptides into the membrane. Thus, the data support the idea that the synthesis of pigment-binding polypeptides is regulated at the level of transcription.

The genes for the B870 α and β polypeptides were cotranscribed (9). Probably posttranscriptional processes like sequential translation or incorporation cause a delayed appearance of B870 β protein in the membrane.

Induction experiments done by reduction of oxygen tension or illumination revealed similar results with respect to RNA as well as proteins. This supports the idea that at the transcriptional level, the same regulatory mechanism is active whether light intensity or oxygen tension is the trigger.

The rate of BChl synthesis was regulated transcriptionally to a small degree (6, 8). However, BChl influenced the stability of pigment-binding polypeptides in the membrane (4, 5). Biological activity of the complexes and BChl appeared simultaneously. Possibly BChl determined the rate of assembly process because the pool of free BChl and precursors was small in wild-type cells (35).

In mutant strains NK3 and Glpho$^+$, the transcription of the B800–B850 genes is affected by the mutation (Table 2; ref. 11). Strain Glpho$^+$ lacks carotenoid synthesis, which has pleiotropic effects on the synthesis of B800-B850 (11, 36). In both strains regulation of RC/B870 genes and assembly of respective complexes are unaffected. We conclude, that there is no influence of carotenoid synthesis on the regulation of the assembly of RC and B870 complexes.

In mutant strains YS, Y5, and Ala$^+$, the increase of photosynthetic mRNA species was not accompanied by incorporation of the respective polypeptides into the membrane or assembly of the complexes (Table 2). When single polypeptides of a pigment–protein complex in the presence or absence of BChl were incorporated into the membrane, no complex was formed and the polypeptides were quickly degraded (4, 5, 24). The assembly process and/or the stability of the complexes depend on all constituents. Although BChl synthesis influences the assembly and stability of the RC/B870 complexes, it has no influence on transcription of the genes of their pigment-binding polypeptides as shown by the formation of RC/B870 mRNAs in strain YS. In all mutant strains studied here, transcription of RC/B870 genes and B800–B850 genes are independently effected by mutations, suggesting an independent regulation of transcription in wild-type cultures.

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