The expression of genes encoding proteins of B800-850 antenna pigment complex and ribosomal RNA of *Rhodopseudomonas capsulata*

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The synthesis of the photosynthetic apparatus was induced in chemotrophically grown cultures of the wild type strain 37b4 of *Rhodopseudomonas capsulata* by lowering of oxygen partial pressure. In these induced but growth-limited cultures the amount of total RNA per cell increased. Using specific DNA-probes for genes of the light-harvesting complex B800-850 and for ribosomal RNA it was shown that the levels of mRNA for the proteins of the light-harvesting complex B800-850 and for ribosomal RNA increased. In the mutant strain G1pho$^+$, missing the B800-850 complex, but synthesizing small amounts of the M_r 10000 polypeptide of the complex, the B800-850-specific mRNA was not increased during induction. It is concluded that the synthesis of the B800-850 complex is under transcriptional control and that, under these conditions, the level of rRNA also increased.

Rhodopseudomonas capsulata *Oxygen partial pressure* *Ribosomal RNA* *B800-850 Antenna pigment complex* *Transcriptional control* *Gene expression*

1. INTRODUCTION

Early investigations on the physiology of phototrophic bacteria revealed that light intensity and oxygen partial pressure influence the synthesis of the photosynthetic apparatus, in that a lowering of oxygen partial pressure in dark grown chemotrophic cultures caused an immediate hyperexponential increase of the cellular bacteriochlorophyll (Bchl) content and a coordinated synthesis of pigment-binding polypeptides, which are assembled in the intracytoplasmic membrane in order to form the pigment–protein complexes of the photochemical reaction center (RC) and the light-harvesting (LH) complexes B870 and B800–850 [1–7]. This process is accompanied by an enlargement of the intracytoplasmic membrane system [8–11]. After lowering of oxygen tension, RC and B870 LH complex are the first detectable Bchl species which are synthesized [6,12,13]. The B800-850 LH complex appeared after a lag phase of 90 min and dominated after 130 min of incubation [12,13]. The B800-850 LH complex contains two pigment binding polypeptides of apparent M_r 10000 and 8000 and one polypeptide (M_r 14000) which is not Bchl bound.

The signal chain between variation of oxygen partial pressure in the medium and regulation of gene expression is unknown. In prokaryotes control mechanisms very often act on the level of transcription. A recent report showed that the pigment binding polypeptides of the light-harvesting complex B800-850 of *Rhodopseudomonas capsulata* are encoded by long-living mRNA [14].

Here, we describe the formation of mRNA of genes responsible for formation of the B800-850 complex and of the ribosomal RNA of *R. capsulata* induced by lowering the oxygen tension. For the detection of mRNA specific for the B800-850 LH complex the plasmid pVKl was used as hybridization probe. This plasmid was constructed by cloning chromosomal DNA of *R. capsulata* into the wide host range vector pRK290 [15].

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and biosynthetic processes in *R. capsulata* revealed a correlation between variation of external factors and total RNA content of cells [16]. We studied the variation of ribosomal RNA during induction by hybridization of total RNA to pRCl [17], a cosmid carrying rRNA genes of *R. capsulata*.

2. MATERIALS AND METHODS

2.1. *Enzymes and radionucleotides*

T4 DNA ligase, calf intestine alkaline phosphatase and restriction endonuclease were purchased from Boehringer, Mannheim or Bethesda Research Laboratory, Neu-Isenburg, FRG. Radionucleotides and polynucleotide kinase were from Amersham-Buchler, Frankfurt. All enzymes were used according to suppliers instructions.

2.2. *Bacterial strains, plasmids and growth conditions*

R. capsulata strain 37b4 (wild type, German collection of microorganisms, Göttingen, DSM 938) and the photosynthetic mutant strain Glpho$^+$ [18] were cultivated in a malate salt medium supplemented with 0.05% yeast extract (Difco) [19]. The induction of the photosynthetic apparatus was performed semiaerobically in the dark according to [6] in 100-ml Erlenmeyer flasks filled with 80 ml of culture. The cell concentration was about 1 mg cell protein per ml.

Plasmid pVK1 is a pRk290 [15] derivative containing an insert of chromosomal DNA of *R. capsulata* encoding proteins responsible for formation of the B800-850 LH complex. Cosmid pRCl has been constructed by inserting chromosomal DNA of *R. capsulata* encoding rRNA into the vector pHC79 [17].

2.3. *Isolation of nucleic acids*

Plasmid DNA was isolated from *Escherichia coli* using a modified method of [20]. Isolation of total RNA from *R. capsulata* has been described previously [14]. Concentrations of the isolated nucleic acids were calculated from their absorption at 260 nm.

2.4. *Immobilization and hybridization of nucleic acids*

Restriction fragments of plasmids were separated on 0.8% agarose gels and transferred to nitrocellulose BA 85 (Schleicher and Schüll, Dassel) by the Southern technique [21]. Total RNA was separated on denaturing formaldehyde agarose (1.2%) gels [22] and transferred to nitrocellulose as in [23]. Plasmid DNA was labeled with [α-^{32}P]-ATP by nick translation [24]. Total RNA was treated with alkaline phosphatase for 1 h at 35°C to eliminate 5'-phosphate, afterwards incubated for 5 min at 95°C and labeled with [γ-^{32}P]ATP using polynucleotide kinase at 37°C for 1 h. Hybridization was carried out in 50% formamide at 42°C, following [23]. For evaluation filters were dried and exposed on Kodak X-Omat films. For quantification the developed films were measured densitometrically using a Kontron Uvikon spectrophotometer, type 810. Alternatively, hybridizing bands were excised from the filters and subjected to liquid scintillation counting.

3. RESULTS AND DISCUSSION

3.1. *Expression of genes encoding proteins of B800-850 in wild type and mutant strain Glpho$^+$* of R. capsulata

Wild type strain *R. capsulata* 37b4 was induced to synthesize the photosynthetic apparatus by lowering the oxygen partial pressure in dark grown cultures. Although the oxygen partial pressure could not be measured, we know from studies under controlled p_{O_2} that at 70 Pa growth is inhibited but membrane differentiation is fully induced [5]. Increase of Bchl content of cells in the Erlenmeyer flasks indicated the induction of the photosynthetic apparatus (fig.1a). Growth of the cultures was negligible under the experimental conditions (fig.1a). At different times during induction samples were taken and total RNA was isolated as described. Equal amounts of the isolated RNA were labeled to a specific activity of about 2×10^6 cpm/μg and hybridized against plasmid pVKl digested with *Eco*RI and *Xho*I. Autoradiogram (fig.2) and densitometric analysis of the blackened areas (fig.1b) showed a strong increase of mRNA complementary to the 6 kb *Eco*RI/*Xho*I fragment of pVKl within the first 60 min of incubation, indicating a stimulation of transcription of B800-850 genes. The amount of hybridizing RNA decreased during the following 70 min.

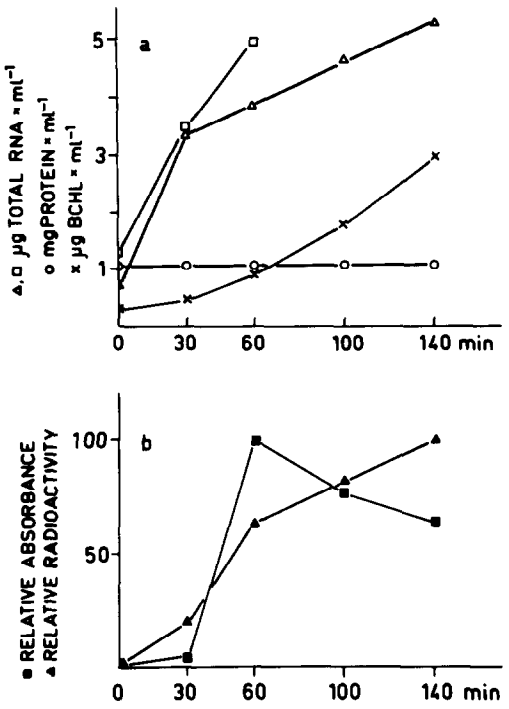


Fig.1. Formation of the photosynthetic apparatus, induced in cultures of *R. capsulata* by reduction of oxygen partial pressure (time 0), as described in [6]. At the times indicated cultures were cooled on ice and the biosynthetic processes stopped [14]. (a) ×—×, Bchl content; ○—○, protein content; △—△, $\mu g \cdot ml^{-1}$ total RNA isolated from *R. capsulata* strain 37b4; □—□, $\mu g \cdot ml^{-1}$ of total RNA isolated from *R. capsulata* strain Glpho$^+$. (b) Strength of hybridization from autoradiographs in figs 2,3 were quantified. ■—■, Relative absorbance of hybridizing bands of fig.2; ▲—▲, relative radioactivity of hybridizing bands of fig.3.

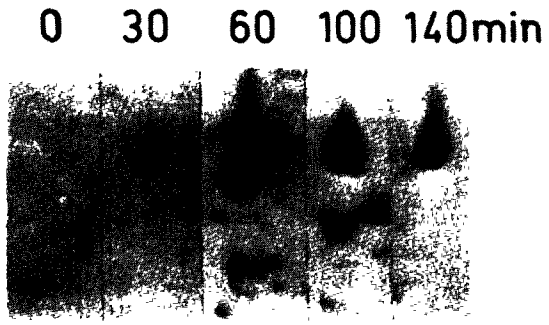


Fig.2. Fragments of plasmid pVK1 (genes for B800-850 LH complex), digested with *Eco*RI and *Xho*I, were separated on agarose gel and transferred to nitrocellulose as in section 2. Equal amounts of total RNA isolated at 0, 30, 60, 100 and 140 min after lowering of oxygen tension were end-labeled and hybridized against the immobilized plasmid. The 6 kb *Eco*RI/*Xho*I fragment of pVK1 showed a maximum of hybridization after 60 min of induction. There is a strong increase of hybridizing RNA within the first 60 min of induction and a slow decrease within the following 70 min. Relative absorbances of the bands are indicated in fig.1b. The film was exposed for 20 h.

It has been shown recently that mRNA for Bchl-binding polypeptides of RC and antenna complex B870 of *R. capsulata* increased with similar kinetics after induction [26]. However, the induction of the transcription of B800-850-specific genes seems to be delayed compared to genes for RC and B870, as has been observed in earlier experiments for the incorporation of complex specific proteins into the membrane [6,13].

The experiment was repeated using the mutant strain Glpho$^+$. This strain, a reconstituent of the mutant strain Ala$^+$pho$^-$, is unable to form the B800-850 LH complex, although the 10-kDa polypeptide of the complex is present in the membrane [18]. Total RNA isolated from aerobically grown cells was compared to RNA isolated at 30 and 60 min after induction. Equal quantities of the isolated RNA were labeled to 3×10^6 cpm/μg and hybridized against the *Eco*RI/*Xho*I digest of plasmid pVK1. Although the Bchl content of cells was doubled within the 60 min of incubation, no clear differences in the strength of hybridization were found at the 3 time points (not shown). These data suggest that transcription of B800-850 specific genes in the strain Glpho$^+$ is not regulated as in the wild type.

3.2. *Expression of genes encoding rRNA of* R. capsulata

After lowering of oxygen tension the amount of total RNA isolated from equal culture volumes increased up to 7-fold compared to aerobically grown cells of wild type (fig.1a). In the mutant Glpho$^+$ the amount of total RNA increased 4-fold within the first 60 min of induction. Since the main part of bacterial RNA species consists of ribosomal RNA, it was expected that the synthesis of ribosomes was stimulated during induction to photo-

trophic growth. To test this hypothesis equal aliquots of total RNA isolated from equal volumes of cultures at various times of induction were separated on a formaldehyde agarose gel. Cosmid pRCl carrying ribosomal genes of *R. capsulata* was labeled by nick translation and hybridized against the immobilized RNA probes. The autoradiographs revealed that rRNA increased during induction to phototrophic growth (fig.3). Surprisingly, the 14 S rDNA of *R. capsulata* occurred later than the 16 S rRNA fraction. *E. coli* rRNA, taken as marker on the formaldehyde agarose gel, did not hybridize to pRCl. The relative radioactivity of the hybridizing bands increased (fig.1b). From these results we conclude that induction of the photosynthetic apparatus is correlated with an increase of ribosomes under the chosen experimental conditions. It is supposed that the differentiation process in cells of *R. capsulata*, induced by switching from chemotrophic to phototrophic energy metabolism, includes stimulation of the synthesis of ribosomes. Increase of ribosomes has been observed in shift-up experiments increasing the growth rate [27]. In the experiments described here the growth rate was strongly reduced. Thus, it is not self evident, that the amount of ribosomes is increased.

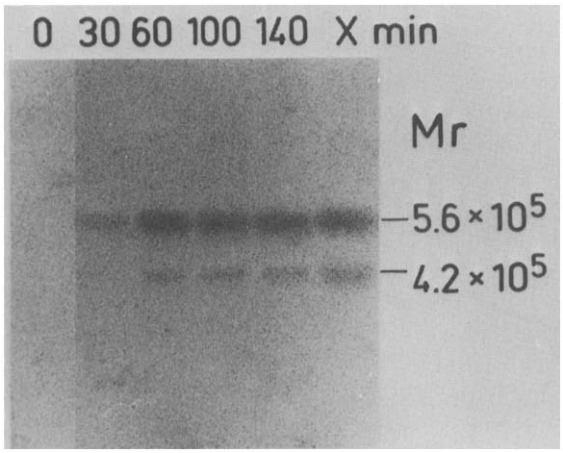


Fig.3. Equal aliquots of total RNA isolated at different phases of induction were separated on formaldehyde agarose gel, transferred to nitrocellulose and hybridized against labeled pRCl (genes for rRNA). The autoradiogram was exposed for 12 days. A continuous increase of hybridizing RNA is visible. The rRNA pattern of *R. capsulata* is different from that found in other bacteria [27]. The 23 S rRNA is processed and the rRNA species visible on the autoradiograph show 16 S and 14 S fractions. On lane X the total RNA, isolated from a culture grown anaerobically in the light, was blotted. The amount was equal to that of the 140 min induction.

4. CONCLUSIONS

We observed that in chemotrophically grown cells of *R. capsulata* the steady-state concentrations of mRNA specific for the B800-850 light-harvesting complex and the level of rRNA increased after lowering of oxygen partial pressure. This indicates a transcriptional control. The observed increase of discrete RNA species can be caused by an increase of transcription rates or by a decrease of turnover rates. Earlier experiments in this laboratory have shown that the mRNAs for polypeptides of the B800-850 complex of *R. capsulata* have half-lives between 16 and 22 min [14]. This low turnover time, which has been determined under similar culture conditions, lets us suggest that the increase in hybridizing specific mRNA during the first 60 min of induction (fig.2) is due to an increase of the rate of transcription. The increase of rRNA shows that besides the biosynthesis of the photosynthetic apparatus other biosynthetic processes are induced. The mechanism of oxygen control, which has also been observed in *E. coli* and other facultative anaerobic bacteria, is unknown at present but is under study.

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