

Effect of oxygen on levels of mRNA coding for reaction-centre and light-harvesting polypeptides of *Rhodobacter sphaeroides*

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The relative levels of mRNA for the reaction-centre L and M subunits, B875 (LH1) α and β polypeptides and B800–850 (LH2) α and β polypeptides, have been measured during pigment induction of *Rhodobacter sphaeroides*. Over the 6 h of the experiment, bacteriochlorophyll levels increased by at least 100-fold. No transcripts for photosynthetic components were detectable at the start of induction; after 2 h the levels of transcripts from the *puf* operon (encoding reaction-centre and B875 subunits) had reached the maximum; these transcripts were 2.7 and 0.5 kb respectively. The transcript for the *puc* operon (B800–850 complex) was estimated to be 0.55 kb and reached a maximum level after 6 h. These results are consistent with the proposal that, during the assembly of the photosynthetic apparatus, the synthesis of B875 reaction-centre aggregates precedes that of the major antenna, B800–850.

INTRODUCTION

The photosynthetic bacterium *Rhodobacter sphaeroides* provides an excellent model system for the study of photosynthetic membrane assembly, because the levels of photosynthetic components can be manipulated by light intensity or by oxygen tension. The photosynthetic apparatus is composed of three pigment proteins, the reaction centre, and light-harvesting antenna complexes B875 and B800–850. These pigment–protein complexes are absent in highly aerated cells, but, when transferred to low-oxygen growth conditions, the complexes appear and are found to be located in invaginations of the cytoplasmic membrane. The synthesis of reaction centres and B875 antenna predominates in the early stages of photosynthetic unit assembly, whereas later stages are characterized by the addition of the B800–850 complex [1].

The apoproteins for these complexes are encoded by the genes *puf* A, B, L and M (encoding B875 α and β polypeptides and reaction-centre L and M polypeptides respectively) and *puc* A and B (B800–850 α and β polypeptides respectively), with the *puf* genes organized into an operon [2].

Previous studies have reported that the ratio of B875 to reaction centres is relatively constant but that a variable amount of B800–850 antenna is present in the photosynthetic unit, depending on oxygen tension or light intensity [1,3,4]. The genetic basis for this differential response of the two antenna systems is unknown. The transcription of the *puf* operon has now been analysed in some detail [2,5], but there is no information on the relative levels of transcripts for *puf* and *puc* genes. Now that the *puc* genes have been cloned [6] it is possible to investigate the assembly of all of the pigment-binding components of the photosynthetic apparatus using specific gene probes to estimate the levels of transcripts for reaction-centre B875 and B800–850 apoproteins. The system we have chosen to examine is that used by Niederman *et al.* [1] in which a highly aerated non-

pigment suspension of *Rb. sphaeroides* is transferred to oxygen-limited growth conditions. This triggers the induction of photosynthetic membrane assembly over a period of up to 20 h. RNA has been prepared from cells at various times after transfer to low-aeration growth conditions and used in hybridization analyses with cloned gene probes. We find that mRNA levels for reaction-centre and B875 apoprotein genes reach a maximum early on in development, whereas mRNA levels for B800–850 apoprotein genes exhibit a more gradual increase.

MATERIALS AND METHODS

Introduction of pigment synthesis

This was performed as described by Niederman *et al.* [1]. Absorbance spectra of whole cells were recorded on a Perkin–Elmer 554 spectrophotometer. Bacteriochlorophyll levels were estimated using the absorption coefficient given by Clayton [7].

RNA preparation and analysis

Total RNA was prepared for inducing cells by centrifugation in CsCl following lysis in boiling SDS [8]. Northern blots were prepared as described in [8] and [9]. RNA sizes were estimated using the Bethesda Research Laboratories RNA ladder. Signals on the autoradiographs were quantified using densitometry with a Shimadzu CS-930 scanner.

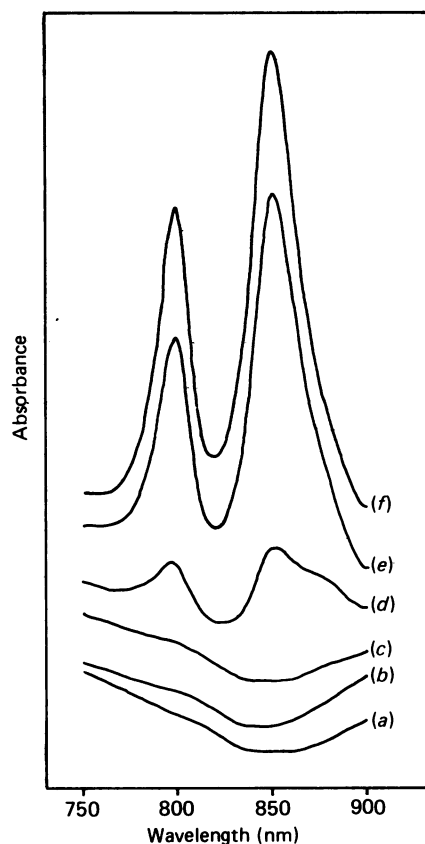
DNA probes

The following probes were used: (a) B800–850, 1.1 kb *Bam*H1 fragment of pMA81 [6]; (b) B875 and reaction-centre, 0.51 kb *Alu*I fragment of pJWI (Fig. 2a).

RESULTS AND DISCUSSION

Fig. 1 shows the induction of light-harvesting complexes in a low-aeration culture of *Rb. sphaeroides*; after

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2 h some pigmentation is apparent in the absorbance spectra of whole cells, with B875 present in nearly equal proportion to B800–850. Subsequently, the production of B800–850 outstrips that of B875; results similar to this have been analysed in more detail by Niederman *et al.* [1].

The structure of the gene probes is shown in Fig. 2. Fig. 2(a) shows pJW1, which contains the B875 and reaction-centre genes, with the 0.51 kb *AluI* fragment [2], which was used to assay mRNA encoding B875. pJW1 and a 1.2 kb *PvuII*–*NruI* fragment specific for reaction centres have also been used in a preliminary report of this work [10]. Fig. 2(b) shows pMA81, the 1.1 kb *BamHI* fragment and the location of the *puc A* and *B* genes. Their identity has been confirmed by DNA sequencing and comparison with the established amino acid sequence [6].

Northern blots of *Rb. sphaeroides* RNA were prepared and hybridized with the probes described above. The time points chosen were clustered at the beginning of pigment induction because preliminary studies had indicated that, in the case of the *puf* operon, there was a rapid early rise in the level of transcripts [10]. From Fig. 3(a) it is

Fig. 1. Absorbance spectra of whole cells showing induction of the photosynthetic apparatus in *Rb. sphaeroides*

(a)–(f), Samples were removed from the culture after 0, 0.5, 1, 2, 4 and 6 h of induction respectively. The same wet wt. of cells was used for each time point.

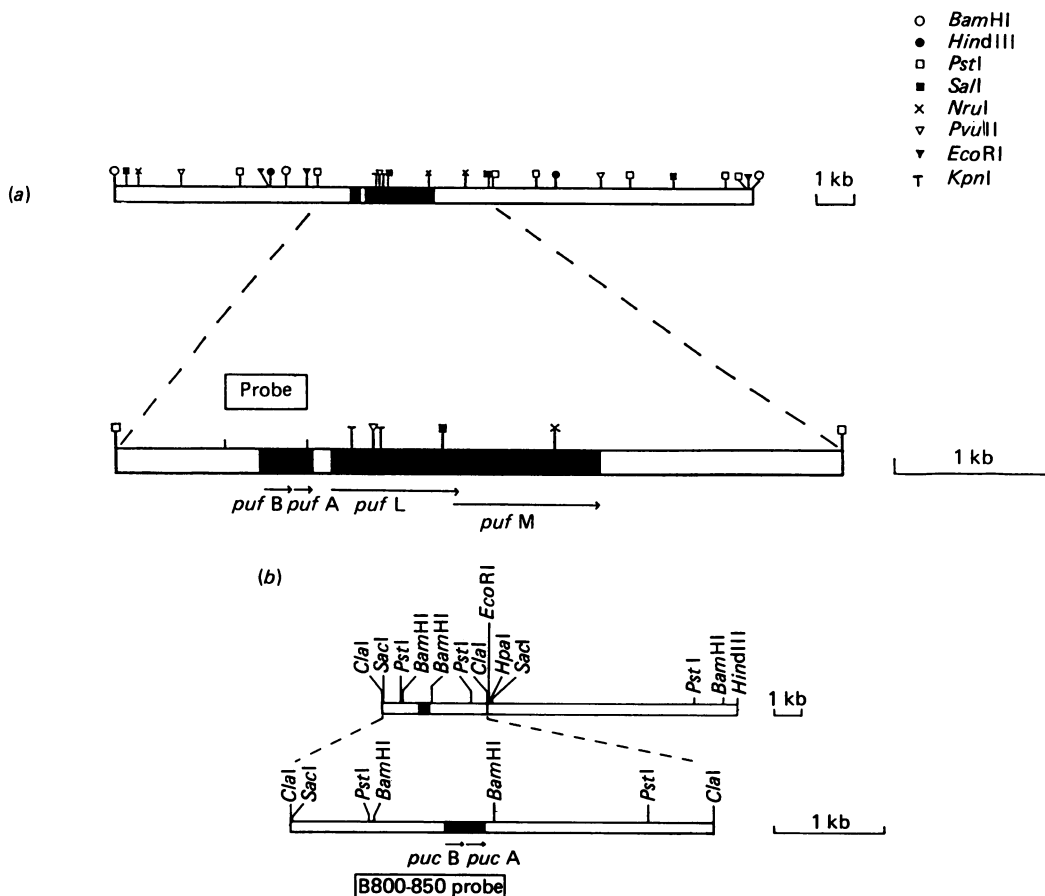


Fig. 2. Restriction maps of (a) pJW1 and (b) pMA81 showing probes used in subsequent hybridization studies and location of *puf* and *puc* genes

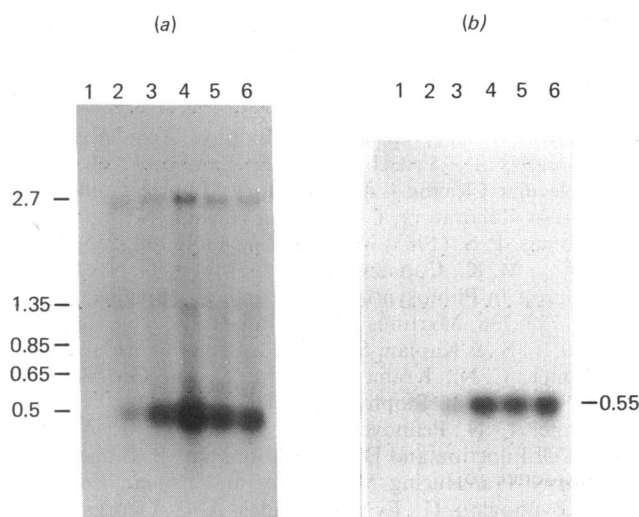


Fig. 3. Northern blots of *Rb. sphaeroides* RNA following pigment induction

Tracks 1 to 6, 0, 0.5, 1, 2, 4 and 6 h respectively. (a) *AluI puf* probe. (b) *BamHI puc* probe. Molecular masses are indicated alongside the bands in kbp.

apparent that there are two major transcripts of 2.7 and 0.5 kb plus three minor transcripts of 1.35, 0.85 and 0.65 kb. This is in close agreement with Zhu *et al.* [2,11], who reported two major transcripts (2.6 and 0.5 kb) plus a minor transcript of about 1.45 kb which is probably the 1.35 kb transcript reported here. The difference in the molecular mass of the two larger transcripts is not significant and may be due to the particular RNA markers used (with λ DNA markers we found the large transcript to be 2.6 kb [10]) or the different methods used for isolation and gel electrophoresis of the RNA. We have shown previously that the 2.7 kb transcript is the only mRNA species which encodes the reaction-centre L and M subunits [10] by probing Northern blots with a 0.7 kb *PvuII-NruI* fragment from pJW1 corresponding to the reaction-centre and M subunits only. The 0.51 kb *AluI* fragment from pJW1 which was used to probe this Northern blot contains the genes for the B875 α and β polypeptides plus about 200 base-pairs upstream [2]. This fragment hybridizes to give transcripts including the large 2.7 kb band. These results show that, for the two major transcripts at least, the 0.5 kb message encodes only the B875 polypeptides, whereas the 2.7 kb message encodes both the reaction centre and B875 polypeptides which is in agreement with the work of Zhu & Kaplan [11]. The Northern blot also indicates that the level of the 0.5 kb B875 message is 10–20-fold higher than that of the 2.7 kb B875 reaction-centre message, which would account for the ratio of B875 antenna to reaction-centre complexes found in the photosynthetic apparatus [1]. More detailed analysis of the transcripts [2] has shown that their 5' ends lie 29 base-pairs apart, and that the difference in the levels of the two major transcripts arises from differential transcript initiation.

In order to investigate the transcription of *puc* genes, a Northern blot was probed with the 1.1 kb *BamHI* fragment of pMA81 (Fig. 3b). It can be seen that B800–850 α and β polypeptides are encoded on a single transcript of approx. 0.55 kb.

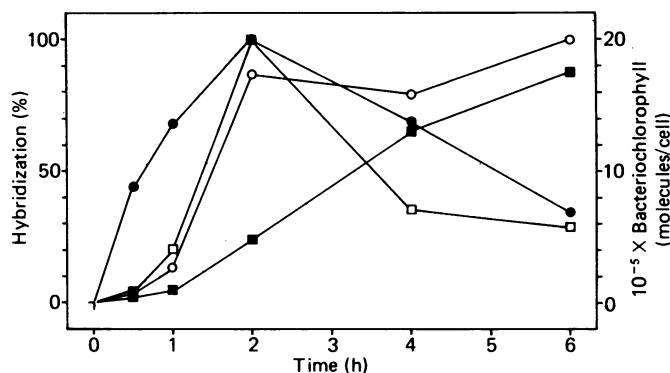


Fig. 4. Graph showing the change in levels of bacteriochlorophyll and mRNA for photosynthetic complexes

●, 2.7 kb transcript (reaction centre/B875); □, 0.5 kb transcript (B875); ○, 0.55 kb transcript (B800–850); ■, bacteriochlorophyll.

The levels of the transcripts encoding the subunits of the photosynthetic complexes and the number of bacteriochlorophyll molecules per cell during the induction of pigment synthesis are shown in Fig. 4: during the course of the experiment the amount of bacteriochlorophyll per cell increased at least 100-fold, broadly in line with the concentrations described previously [12]. The 2.7 kb transcript-encoding reaction centres and B875 [1,12,13]. Results from excitation–annihilation studies on this induction system show that, at the earliest appears more gradually, but still reaches a peak level after 2 h, and then falls away faster than the 2.7 kb transcript. The level of the B800–850 transcript reaches a maximum after 6 h, the level rising fairly rapidly between 1 and 2 h from 10% to 85% of maximum.

These observations are consistent with the proposal that the synthesis of the photosynthetic apparatus starts with the concomitant synthesis of reaction centres and B875 [1,12,13]. Results from excitation–annihilation studies on this induction system show that, at the earliest measurable stage in photosynthetic membrane assembly, energy-transfer domains are composed of 100–200 bacteriochlorophyll molecules (mainly B875 with 4–5 reaction centres [12]). More recently, it has been proposed that such domains form the building blocks for the mature photosynthetic apparatus. [14]. During the next stage of photosynthetic membrane assembly, B800–850 antenna is added to existing B875 reaction-centre ‘cores’, and probably forms aggregates containing a minimum of 40–50 bacteriochlorophyll molecules [14,15]. This sequential mechanism for the assembly of the photosynthetic unit has also been established for *Rb. capsulatus* [16]; hybridization analyses have demonstrated that a downward shift in oxygen tension in the growth medium results in increased levels of transcripts coding for reaction-centre and B875 complexes [17–19] and the B800–850 complex [18,19]. In agreement with the work of Klug *et al.* [18] we find a small lag in the rise of B800–850-specific mRNA in comparison with that for reaction centres and B875. This lag in mRNA levels occurs approx. 3 h into experiments where the induction of bacteriochlorophyll occurs over a period of 19 h [10]. Here, a similar eventual level of cellular bacteriochlorophyll was achieved in 6 h, which shortened the lag

considerably. However, these observations are consistent with respect to cellular bacteriochlorophyll, since in each case mRNA levels encoding B800–850 apoproteins rise rapidly as bacteriochlorophyll increases to $(6-8) \times 10^5$ molecules/cell. There is clearly a close regulatory relationship between the biosynthesis of pigments and proteins of the photosynthetic apparatus. In this regard, it has been shown that mRNA specific for genes controlling bacteriochlorophyll synthesis achieves a maximum level after 90 min of pigment induction [20], a rise which parallels that seen for reaction-centre- and B875-specific mRNA. Whether or not the lag in the rise of B800–850-specific mRNA with respect to reaction centres and B875 is indicative of different modes of control on *puc* and *puf* genes is not clear; hybridization studies such as these do not permit distinction between increased transcription of the genes or differential stability of mRNA species. Experiments to test these possibilities are currently in progress.

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