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Biological consequences of segmental alterations in mRNA stability: effects of deletion of the intercistronic hairpin loop region of the *Rhodobacter capsulatus puf* operon

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It has been proposed that intercistronic stem and loop structures located in the *puf* operon of the photosynthetic bacterium *Rhodobacter capsulatus* account for segmental differences in transcript stability and consequently, the differential expression of the B870 and reaction center (RC) proteins encoded by *puf*. We report here that deletion of these structures leads to a failure to detect as discrete fragments the B870-encoding 0.49 kb and 0.50 kb mRNA segments located upstream from the site of the hairpins. The absence of these stable transcript fragments is associated with altered stoichiometry of the B870 and RC pigment–protein complexes in the bacterial intracytoplasmic membrane and a decreased rate of cell growth under photosynthetic conditions. These results support the view that the hairpin loop structures of the *puf* intercistronic region function *in vivo* to impede exoribonucleolytic degradation of upstream mRNA and establish that segmental variations in mRNA stability have a biologically important role in regulating the expression of *puf* operon genes.

Key words: transcript stability/gene expression/photosynthesis/RNA secondary structure/RNA degradation

Introduction

The events leading to the absorption of light and the initiation of electron transport in the purple nonsulfur bacterium *Rhodobacter capsulatus* involve distinct and well-characterized pigment–protein complexes. Light harvesting complexes termed B870 (also known as LHI) and B800-850 (also known as LHII) serve as antennae to capture photons and funnel energy to the reaction center (RC) complexes, where electron transport begins (Drews and Oelze, 1981). Earlier investigations (Belasco *et al.*, 1985), have shown that the α and β peptide subunits of the B870 complex and the L and M subunits of the RC are derived from a single RNA transcript encoded by an operon previously termed *rxcA*, but recently renamed *puf*. The genes encoding the H subunit of the RC are located elsewhere on the chromosome (Youvan *et al.*, 1984). Belasco *et al.* (1985) have proposed that the molar excess of B870 complex versus RC complex in the intracytoplasmic membrane of *R. capsulatus* (Schumacher and Drews, 1978; Kaufmann *et al.*, 1982) results from differences in the rate of degradation of the *puf* transcript segments that specify the B870 and RC proteins. According to this view, highly stable stem and loop structures found at the boundary between the B870 and RC encoding segments of the *puf* transcript act as barriers to 3′ exoribonucleases, thereby impeding decay of the upstream mRNA segment and yielding an excess of B870 mRNA relative to RC mRNA.

To investigate directly the molecular effects and biological role of the region of secondary structure located at the junction of the B870 and RC-encoding components of *puf* mRNA, we deleted a 30 bp DNA segment that corresponds to the intercistronic stem and loop region of the transcript. We find that in the absence of this region, the stable mRNA that codes for the B870 peptides is not observed. Loss of protection of the B870-encoding mRNA segment of the *puf* transcript is associated with altered stoichiometry of the corresponding pigment–protein complexes in the intracytoplasmic membrane and consequent effects on cell growth under photosynthetic conditions.

Results

Effects of the intercistronic hairpin on stabilization of the B870-encoding mRNA segment

A 2.7 kb polycistronic mRNA species specifies both the B870 and RC peptides encoded by the *puf* operon of *R. capsulatus* (Belasco *et al.*, 1985). A region of intra-strand secondary structure located in the intercistronic region (Figure 1) and having two alternative stem and loop configurations, appears to act as a barrier to 3′ exoribonucleases, impeding degradation of the immediately 5′ transcript segment and yielding stable 0.49 kb and 0.50 kb *puf* mRNA fragments that encode only the B870 peptides (Belasco *et al.*, 1985). As seen in Figure 2, the control strain, RG304, shows the 2.7 and 0.49/50 kb RNA species reported previously for the wild-type operon (Belasco *et al.*, 1985). Deletion of the DNA corresponding to the hairpin loop region (strain RG607, Table I) resulted in disappearance of the B870-encoding 0.49/0.50 kb mRNA band, providing direct support for the notion that the intercistronic region of secondary structure is responsible for the stability of the short mRNA species and their consequent occurrence as discrete mRNA fragments. The relative amount of the 2.7 kb RNA species compared to total cellular RNA was not affected by deletion of the stem and loop structures, consistent with the view that these structures act primarily to protect the upstream RNA against degradation rather than to terminate transcription. The half-life of the 2.7 kb transcript in RG607 also was unaltered by removal of the stem and loop region (unpublished data and Chen *et al.*, in preparation). Results identical to those shown in Figure 2 were observed for strain UG607 versus UG304 (Table I), which fail to synthesize the B800-850 complex, and which were used for experiments involving spectral analysis of *puf* operon products (see below).

Several minor bands were also observed when *puf* mRNA was probed with a *Pst*I DNA fragment extending about 1.9 kb upstream from a site within the L gene of *puf* (cf. Belasco *et al.*, 1985). One of these, an RNA species about 2.0 kb in length, was present in the strain carrying the wild-type *puf* operon but was missing in the strain lacking the intercistronic hairpin loop region (Figure 2). This RNA species hybridized to a 0.25 kb *Sal*I–*Sph*I DNA fragment that carries the genes encoding the

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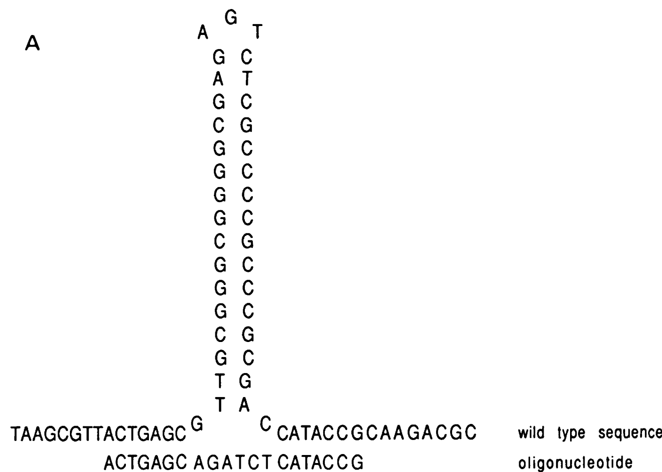

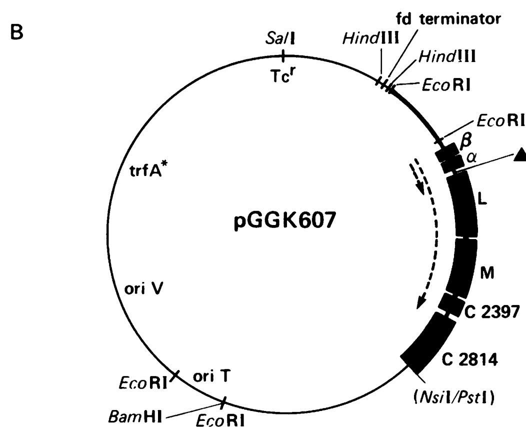

Fig. 1. A. Sequence of wild type and mutant DNA in the region corresponding to the intercistronic hairpin loop located between the B870 and RC segments of the *puf* transcript. **B.** Map of the pGGK607 plasmid. The dashed lines represent the 0.49/0.50 kb and 2.7 kb transcripts, and the filled triangle indicates the location of the stem and loop sequence that is present in pGGK304 but deleted in pGGK607.

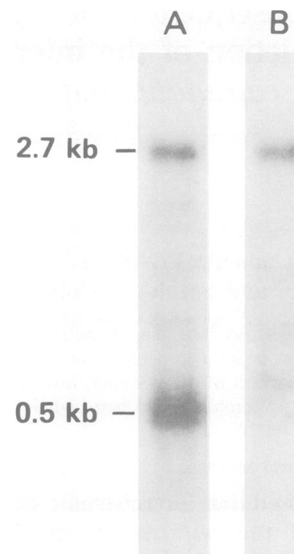

Fig. 2. Total RNA was isolated from *R. capsulatus* strains RG304 (**A**, wild type *puf* sequence) and RG607 (**B**, hairpin loop deleted from *puf* sequence), separated on a 1% agarose formaldehyde gel, transferred to nitrocellulose and hybridized against the 1.9 kb *Pst*I fragment of the *puf* operon.

amino terminal end of the B870 β peptide, plus part of the upstream DNA region – but not with a 2.5 kb, downstream *Pst*I DNA fragment that carries only the RC genes (data not shown). The molecular nature and source of the 2.0 kb transcript and of the other minor bands seen in the figure have not been determined.

Effect of hairpin-loop removal on the stoichiometry and photosynthetic capabilities of pigment–protein complexes

SDS–PAGE (Figure 3) indicated that the concentration of the B870 α (12k) and β (7K) peptides were reduced about 50% relative to other intracytoplasmic proteins in strain RG607, in which the region containing the intercistronic hairpin loop structure has been deleted from *puf* operon DNA. To determine the effects, in a mutant lacking the intercistronic hairpin loop region, of the observed alterations in the steady state levels of *puf*-encoded mRNA and protein, the stoichiometry of the B870 and the RC pigment–protein complexes in the intracytoplasmic membrane was investigated. Use of a mutant host strain lacking the B800-850 (LHII) complex, which overlaps spectrally with the B870 and RC complexes, facilitated our analysis.

In these experiments, the absorbance of the peak at 875 nm, which results from the combined absorbance of the complexes of the RC and B870 proteins with bacteriochlorophyll (Bchl) was compared to the 803 nm absorbance peak of RC–Bchl. In the presence of the hairpin loop region, the bulk of 875 nm absorbance results from the spectrum of B870-bound Bchl, which occurs in a 10–30-fold excess over the RC-bound Bchl (Schumacher and Drews, 1978; Kaufmann *et al.*, 1982). As seen in Figure 4, deletion of the hairpin loop region resulted in a decrease in 875 nm absorbance relative to 803 nm absorbance. Concomitant changes occurring in other absorbance peaks as a result of deletion of a 30 bp segment that includes the intercistronic hairpin loop region with the *puf* operon suggest that a complex balance exists between the steady state levels of B870 peptides and other components of the photosynthetic apparatus of *R. capsulatus*.

Table II presents quantitative data on the ratio of total Bchl to RC–Bchl isolated from *R. capsulatus* strains containing or lacking the intercistronic hairpin loop structures. Absorbance at 772 nm of acetone–methanol extracts of membranes or cells measured the total Bchl, which is the sum of Bchl bound to B800–850 proteins, B870 proteins, and RC proteins. In strains lacking the B800–850 complex, which constitutes the bulk of the protein-bound Bchl, the absorbance of acetone–methanol extracts at 772 nm measures the sum of the B870 and RC complexes. Photobleaching indicates the RC-bound Bchl, which is the only membrane component that undergoes a reversible change in absorbance at 870 nm when isolated membranes are illuminated (Clayton, 1978).

As seen in Table II, deletion of the intercistronic region containing the hairpin loop structures resulted in reduction of Bchl in the membrane, regardless of whether the strain contains or lacks the B800–850 proteins (cf. RG607 versus RG304, and also UG607 versus UG304). However, the nmol of RC per mg of membrane protein in the two strains deleted for the hairpin loop region (i.e. RG607 and UG607) was elevated over the corresponding strains containing the hairpin loop region (i.e. RG304 and UG304), presumably reflecting decrease in a non-RC component of the membrane protein (i.e. the B870 peptides). The result

Table I. Bacterial strains and plasmids

	Genotype/phenotype	Reference
Plasmids		
pTJS133	Tc^r	Schmidhauser and Helinski, 1985
pRK2013	Km^r	Figurski and Helinski, 1979
pRPS404	Km^r	Marrs, 1981
pGGK304	Tc^r	This work
pGGK607	Tc^r	This work
R. capsulatus strains		
B10	Wild type	Marrs, 1974; Weaver *et al.*, 1975
NK3	$Bchl^+,Crt^+,RC^+,B870^+,B800-850^-$	Kaufmann *et al.*, 1984
U43	Sp^r $Bchl^+,Crt^+,RC^-,B870^-,B880-850^-$	Youvan *et al.*, 1985
ΔRC6	$Bchl^-,Crt^+,RC^-,B870^-,B800-850^-$	Beatty *et al.*, unpublished See Materials and methods
RG304	Tc^r,pGGK304 in ΔRC6 $Bchl^+,Crt^+,RC^+,B870^+,B800-850^+$	This work
RG607	Tc^r,pGGK607 in ΔRC6 $Bchl^+,Crt^+,RC^+,B870^+,B800-850^+$	This work
UG304	Tc^r,pGGK304 in U43 $Bchl^+,Crt^+,RC^+,B870^+,B800-850^-$	This work
UG607	Tc^r,Sp^r,pGGK607 in U43 $Bchl^+,Crt^+,RC^+,B870^+,B800-850^-$	This work
E. coli strains		
HB101	$pro^-,leu^-,thr^-,lacY^-$,hsdM,HsdR,recA,Smr^r	Boyer and Roulland-Dussoix, 1969

R. capsulatus strain NK3 was derived from wild-type 37b4 (DSM938). All other mutant strains are derivatives of wild type B10.

is a decreased ratio of total Bchl versus RC in the strains lacking the hairpin loop region; because the Bchl complexed with the B800–850 proteins normally represents a major component of membrane-bound Bchl, the change in ratio was most evident in the strains that also contain a mutation that eliminates the B800–850 complex.

As also seen in Table II, a strain defective in the synthesis of B800–850 complex (NK3), but containing normal *puf* genes in the chromosome, showed a sharp reduction of nmol Bchl per mg of membrane protein in comparison with the wild-type strain, B10. An analogous reduction occurs in the $B800-850^-$ host, UG304, versus the wild type host, RG304, both of which carry wild-type *puf* genes on the pTJS133 plasmid; placement of the wild-type *puf* genes on a plasmid in a $B800-850^+$ strain (RG304) yields significantly higher Bchl per mg membrane protein in comparison with the Bchl measured for a $B800-850^+$ strain that carries the wild-type *puf* genes in the chromosome. While total Bchl per cell was not influenced by removal of the hairpin loop region and its consequent effects on the production of the B870 β and α peptides, total Bchl was reduced in cells unable to synthesize normal B800–850 proteins; in such cells deletion of the hairpin loop region yielded a further reduction in total Bchl. Thus, a decrease in B870 peptides apparently does not affect total cellular Bchl unless the B800–850 complex is also defective or absent.

The multi-faceted effects of removal of the intercistronic hairpin loop region were evident also from an analysis of ATP production. Earlier investigations with strain NK3, which lacks the B800–850 complex, have shown that this complex is not necessary to achieve the maximal photophosphorylation rate (measured in mol ATP/mol membrane/bound Bchl/min) when photophosphorylation by the intracytoplasmic membranes is measured under saturating light conditions (Reidl *et al.*, 1985). Thus, the photophosphorylation rate is increased under saturating

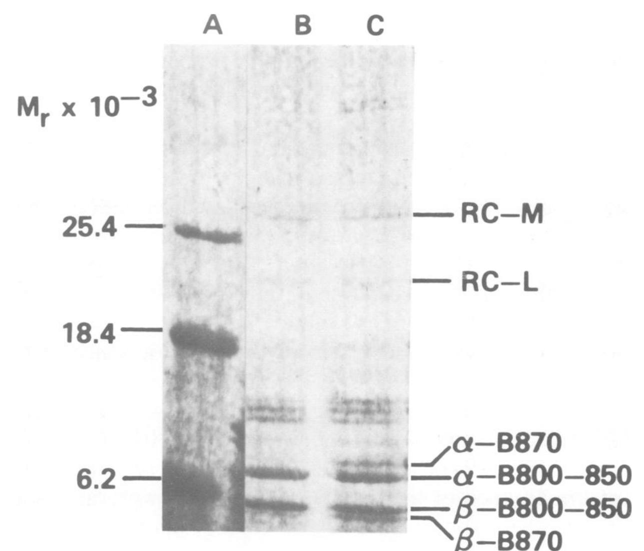

Fig. 3. Purified membranes of *R. capsulatus* strains RG304 (lane **C**, wild type *puf* genes) and RG607 (lane **B**, hairpin-loop deleted from *puf* sequence) were analyzed on 11.5–16.5% SDS polyacrylamide gels. Lane **A**: protein standards. 50 μg of total membrane protein isolated from phototrophic cultures was loaded onto lanes B and C. The pigment-binding proteins of the reaction center and of the B800–850 and B870 complexes are indicated.

light in a B800–850 mutant, which shows reduced membrane-bound Bchl. Data presented in Table II indicated that removal of the hairpin loop region results in an increased photophosphorylation rate in strains RG607 and UG607 versus the otherwise isogenic strains containing the cloned wild-type *puf* operon; since the B870 proteins are reduced in RG607 and UG607, not all of the B870 complex normally synthesized by the wild-type *puf* operon appears to be necessary for maximum photophos-

Table II. Amount of total Bchl and reaction center Bchl of cells and membranes of *R. capsulatus*, photophosphorylation rates of membranes, and doubling time of the cultures

	nMol Bchl per 10^8 cells	nMol Bchl per mg membrane protein	nMol RC per mg membrane protein	nMol Bchl per nMol RC in membrane	nMol ATP per nMol membrane bound Bchl per min	td chemotr. growth	td phototr. growth
B10	0.94 ± 0.014	41.8 ± 0.8	0.37 ± 0.02	113.0 ± 6.8	15.0 ± 2.2	2 h 25 min	2 h 30 min
NK3	0.37 ± 0.006	19.3 ± 0.4	0.70 ± 0.05	27.5 ± 1.9	77.0 ± 11.5	2 h 25 min	5 h 30 min
RG304	0.93 ± 0.014	54.8 ± 1.1	0.53 ± 0.02	103.4 ± 6.2	13.2 ± 2.0	2 h 25 min	4 h 20 min
RG607	0.95 ± 0.014	50.0 ± 1.0	0.60 ± 0.02	83.3 ± 5.0	36.4 ± 5.4	2 h 25 min	4 h 20 min
UG304	0.60 ± 0.009	34.6 ± 0.7	1.24 ± 0.05	28.9 ± 1.8	73.0 ± 11.0	2 h 25 min	5 h 15 min
UG607	0.23 ± 0.003	27.5 ± 0.6	1.80 ± 0.07	15.0 ± 1.2	93.0 ± 13.9	2 h 25 min	5 h 40 min

The values give the mean of four measurements carried out with identical membrane samples. The maximum deviation that was observed between two measurements using the same sample is indicated. RC: reaction center, td: doubling time. Time measures are indicated to the nearest 5 min point.

phorylation. The effects on photophosphorylation of removal of the hairpin loop region provide further evidence that significant effects on the stoichiometry of the pigment–protein complexes encoded by *puf* ensue when the barrier to rapid upstream mRNA degradation is deleted.

Effects of removal of the hairpin loop region on bacterial growth

While enhanced stability of an mRNA segment located upstream from a region of intra-strand secondary structure has been correlated with increased synthesis of protein encoded by the protected mRNA segment (Belasco *et al.*, 1985; Wong and Chang, 1986; Newbury *et al.*, 1987), segmental differences in mRNA stability previously have not been shown to have biologically important consequences. To determine whether impeding degradation of the *puf* mRNA segment 5′ to the hairpin loop barrier has detectable biological effects, the growth properties of *R. capsulatus* strains encoding wild-type or mutant *puf* transcripts were investigated.

During chemotrophic growth under high oxygen tension, the presence of the hairpin loop region made no difference in doubling time (Table II), consistent with the known repressed condition of the *puf* operon when *R. capsulatus* strains are cultured chemotrophically (Cohen-Bazire *et al.*, 1957). Under photosynthetic growth conditions, deletion of the hairpin loop region had no detectable effects in strains synthesizing the B800–850 complex (i.e. RG607 vs RG304). However, in the absence of the B800–850 complex, deletion of the intercistronic barrier was associated with a decrease in the bacterial growth rate (strain UG607 versus the otherwise isogenic strain UG304). Consistent with prior results (Reidl *et al.*, 1985), effects on bacterial growth rate of the absence of the B800–850 proteins were also seen in this series of experiments, as was a surprising inhibition of growth rate when either wild-type or mutated *puf* genes were carried by a plasmid (cf RG304 and B10); this latter effect was not additive to the prolongation of doubling time that resulted from absence of the B800–850 complex (cf. strains NK3 and UG304). The pTJS133 plasmid itself did not affect the growth of either B10 or NK3 (our unpublished data). Together, these observations suggest that the concentrations of *puf*-derived complexes relative to other membrane-bound pigment–protein complexes may require stringent regulation for optimal growth of *R. capsulatus* under photosynthetic growth conditions.

As seen in Figure 5A, deletion of the hairpin loop region prolonged the time required for cells precultivated at high oxygen tension to adapt from chemotrophic to phototrophic growth conditions; growth under high oxygen leads to a physiological reduction in the B800–850 complex (Schumacher and Drews, 1978). In a mutated host unable to make the B800–850 complex (Figure

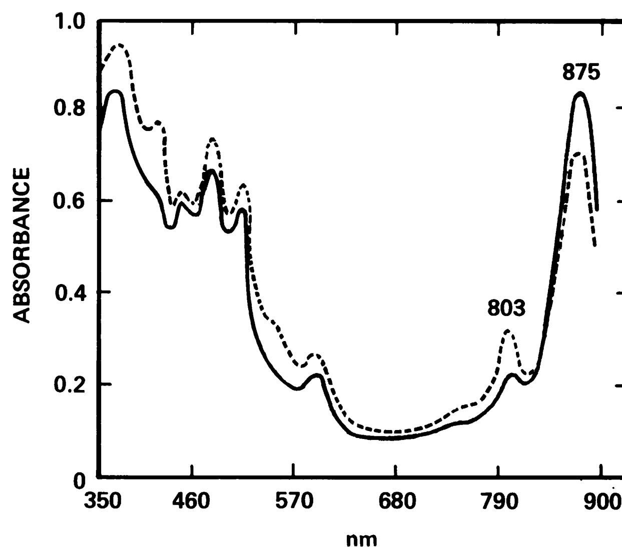

Fig. 4. *In vivo* absorption spectra of purified membranes from phototrophically grown *R. capsulatus* strains, UG304 (——, wild type *puf* genes) and UG607 (----, hairpin loop deleted from *puf* seuqence), both of which lack the B800–850 complex. The 875 nm absorbance results mainly from the B870 complex and to a lesser degree from the RC complex, while the 803 nm absorbance results from the RC complex. Carotinoid absorbance peaks at 444, 471 and 503 nm, and Bchl absorbance peaks at 370 and 591 nm are seen, as well as a peak at 413 nm, which reflects absorbance by cytochromes.

5B), the strain lacking the hairpin loop region showed a longer adaptation time than did an otherwise isogenic strain carrying the wild-type *puf* genes. While data shown in the figure also indicate that strain NK3, which is a B800–850$^-$ mutant carrying its wild-type *puf* genes on the chromosome, took longer to adapt to photosynthetic growth conditions than did a similarly B800–850$^-$ strain (UG304) that carries the wild-type *puf* operon on a plasmid, it should be noted that NK3 and UG304 are derived from different wild-type strains.

Discussion

Previous work has shown that 0.49 kb and 0.50 kb *puf* mRNA fragments that specify for the B870 α and β peptides are present in a ninefold molar excess over a 2.7 kb *puf* transcript segment that encodes both the B870 and the RC proteins in both *R. capsulatus* (Belasco *et al.*, 1985) and *R. spheroides* (Zhu *et al.*, 1986). Zhu *et al.* have proposed that 5′ processing is the initial step in the degradation of the 2.7 kb transcript in *R. spheroides*, that the 0.5 kb and 2.7 kb transcripts are initiated at different

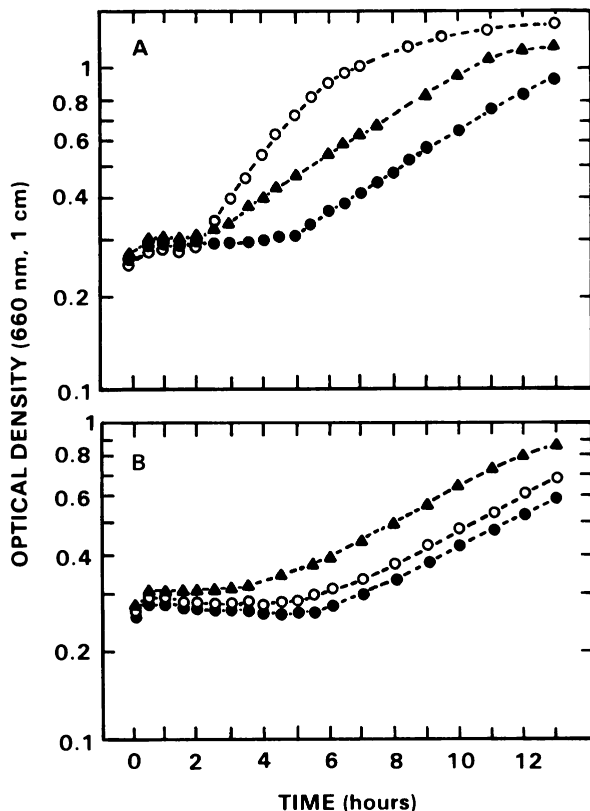

Fig. 5. *R. capsulatus* cultures were precultivated at high oxygen tension and shifted to phototrophic growth conditions at time 0. Panel **A** shows the growth curves for strains containing B800−850 complex: B10 (○, wild type *puf* genes in chromosome), RG304 (▲, wild type *puf* genes, on plasmid), RG607 (●, intercistronic hairpin loop removed from *puf* sequence, on plasmid). Panel **B** shows the growth curves of strains missing the B800−850 complex: NK3 (○, wild type *puf* genes in chromosome), UG304 (▲, *puf* wild type genes, on plasmid), UG607 (●), intercistronic hairpin loop removed from *puf* sequence, on plasmid).

sites, and that the molar excess of the short mRNA species in *R. spheroides* results from different rates of transcription for the short and long *puf* transcripts. However, pulse chase experiments have shown that in *R. capsulatus*, the 0.49 kb and 0.50 kb *puf* mRNA species are derived from the 2.7 kb transcript (Belasco *et al.*, 1985).

The experiments reported here provide strong evidence for the notion that segmental control of mRNA degradation is a mechanism by which the differential expression of the B870 and RC genes within the polycistronic *puf* operon normally is accomplished within *R. capsulatus*. They demonstrate directly that the intercistronic region containing the previously described stem and loop structures is responsible for the existence of the B879-encoding 0.49 kb and 0.50 kb *puf* mRNA fragments and support the view (Belasco *et al.*, 1985) that a biological function of these hairpin loops is to slow degradation of the upstream segment of the *puf* transcript by an exonuclease acting in a 3′ to 5′ direction.

Recently, analogous protection of 5′ mRNA segments by barrier hairpin loop structures has been shown for a transcriptional terminator located within a *B. thuringiensis* gene and for cloned REP sequences of *Escherichia coli*, leading in both cases to increased synthesis of the proteins encoded by the upstream mRNA segment (Wong and Chang, 1986; Newbury *et al.*, 1987). The intercistronic hairpin loop region of the *R. capsulatus puf* operon functions also in *E. coli* to accomplish differential stability of the upstream and downstream segments of *puf* mRNA (Belasco *et al.*, 1985). This hairpin region may also affect degradation, termination, or processing of a second *puf*-derived transcript (Figure 2).

Deletion of the region carrying the hairpin loop structures of the *puf* operon results in a change in the molar ratio of mRNA segments encoding the B870 and RC pigment-binding proteins and altered stoichiometry of the corresponding pigment−protein complexes in the membrane. However, the observed quantitative disparity between the changed stoichiometry of the *puf* transcript segments and the *puf*-derived membrane complexes suggests that factors in addition to the steady state levels of mRNA affect the synthesis of these proteins and/or their assembly into the membrane complexes. Jackson *et al.* (1986) recently have shown that reaction centers are not properly assembled into the photosynthetic membranes in the absence of the B870 gene products; potentially, the altered protein stoichiometry that results from removal of the intracistronic hairpin loop region might similarly affect RC assembly. Limited information is currently available about the stability of unbound B870 and RC proteins (Dierstein, 1983; Dierstein *et al.*, 1984).

The data presented here indicate that deletion of the intercistronic barrier to rapid mRNA degradation can affect the growth rate of *R. capsulatus* and its ability to adapt biologically to photosynthetic growth conditions. In strains able to synthesize the B800−850 complex, a decrease in the amount of B870 peptides, compared to the reaction center peptides, does not affect the bacterial growth rate even under photosynthetic conditions, indicating that the B800−850 complex is able to compensate for the effects of the loss of protection of the B870-encoding *puf* operon mRNA segment. However, a difference in growth rate secondary to removal of the hairpin loop structures was observed in a strain lacking the B800−850 complex. Additionally, when *R. capsulatus* strains that have the capacity for synthesizing B800−850 proteins were precultivated under high oxygen, which results in the repression of the genes synthesizing the B800−850 and B870/RC complexes — and then shifted to phototrophic growth conditions — the strain encoding wild-type *puf* transcripts showed a shorter adaptation time than an isogenic strain carrying a plasmid in which a segment containing the hairpin loops had been deleted from the *puf* operon. We conclude that the intercistronic hairpin loop structures in the *puf* operon are advantageous to the bacterial host during phototrophic growth and/or during environmental conditions that require a shift from a chemotrophic to phototrophic mode of ATP production. Presumably, the phenotypically important effects of the region of secondary structure have provided a continuing selective advantage for maintaining the hairpin loop in *R. capsulatus* transcripts.

Materials and methods

Bacterial strains, plasmids and growth conditions

The bacterial strains and plasmids used in this study are listed in Table I.

Plasmid constructions

The sequence encoding the stem and loop structure located between the B870 α gene and the L gene of the RC proteins was removed by oligonucleotide-directed *in vitro* mutagenesis essentially as described by Zoller and Smith (1983) (Figure 1). A synthetic oligonucleotide having the sequence shown in Figure 1A was annealed to single strand DNA containing the wild type *puf* sequence introduced as an *Eco*RI−*Pst*I fragment into bacteriophage M13mp9 (M13RC1, Belasco *et al.*, 1985). The desired clone, M13RC1ΔHP, contained a new *Bgl*II site. The mutated *Eco*RI−*Stu*I DNA fragment of M13RC1ΔHP was substituted for the *Eco*RI−*Stu*I fragment of pRC2 (Belasco *et al.*, 1985), a pBR322-derived construct that contains the structural genes of the *puf* operon. The normal sequence of genes was reconstituted by insertion of the upstream *Eco*RI fragment. In order

to allow its replication in *R. capsulatus*, the mutated operon was moved as an *NheI–NsiI* fragment into a derivative of pTJS133 (Schmidhauser and Helinski, 1985). As a portion of the *bla* promoter from pBR322 was transferred to pTJS133 during this construction, the promoter was removed by digestion with *HindIII* and religation. Finally, to preclude the effects of any transcription initiating from sequences within the vector continuing into the *puf* operon, the bacteriophage *fd* terminator (Beck *et al.*, 1978) was inserted into the *HindIII* site of the construct. The resultant clone, which is designated pGGK607, is diagrammed in Figure 1B. The plasmid pGGK304, containing the wild-type stem and loop structure, was constructed in a similar manner for use as a control.

E. coli strain HB101 was transformed with the pTJS derivatives pGGK304 and pGGK607, which were then conjugationally transferred to the *R. capsulatus* recipients using the helper plasmid pRK2013 (Figurski and Helinski, 1979) as previously described (Klug and Drews, 1984). Two different *R. capsulatus* strains were used, ΔRC6 (T.Beatty, unpublished) and U43 (Youvan *et al.*, 1985). Each of these strains, which are derivatives of the wild-type strain B10, contains a deletion of the chromosomal copy of the *puf* operon. In ΔRC6, the deletion extends from a *SalI* site 385 bp upstream from the translation start codon for the B870β protein to an *XhoII* site 682 bp downstream from the M subunit translation stop codon. In U43, the deletion comprises an *ApaI* fragment extending from 220 bp upstream from the B870β gene to 420 nucleotides downstream from the 3′ end of the coding sequence for the M subunit protein. In addition, U43 contains a point mutation which renders it unable to synthesize the B800–850 complex.

R. capsulatus strains were cultivated in RCVB minimal medium (Beatty and Gest, 1981), supplemented with potassium phosphate (10 mM, pH 6.8) and yeast extract (0.1%) at 35°C. Phototrophic cultures were grown in filled screw cap flasks in the light after 1 h of preincubation in the dark.

Isolation, blotting and hybridization of RNA

RNA isolation cultures of *R. capsulatus* were preincubated under high oxygen tension. At a cell density of 4×10^8/ml the bacteria were harvested, resuspended in fresh medium to give a density of 1.8×10^9/ml, and incubated under low oxygen tension (measured at 4.8% O_2 for UG304 and 5.2% O_2 for strain UG607). After 30 min of cultivation under low oxygen tension, samples were withdrawn for RNA isolations. Northern blot analysis was performed as described (von Gabain *et al.*, 1983).

Isolation and spectral and electrophoretic analysis of R. capsulatus membranes

Cells of *R. capsulatus* grown phototrophically under low oxygen were harvested, washed in P-buffer (25 mM Tris–HCl, pH 7.6, 5 mM EDTA, 15 mM NaN_3) and submitted to five periods of sonication on ice for 15 s with interruptions of 30 s. After centrifugation (20 min, 27 000 *g*, 4°C) the cell-free extracts were layered on a discontinuous sucrose gradient (5 ml each of 1.5, 1.2, 1.0, 0.6 M sucrose in P-buffer in 30 ml polycarbonate tubes). The colored bands were removed after 20 h of centrifugation (200 00 *g*), diluted with P-buffer and centrifuged for 4 h at 200 000 *g*. The sedimented membranes were resuspended in a small volume of P-buffer.

Spectra from diluted samples were recorded on a Beckman DU7 spectrophotometer. Membrane proteins were separated by SDS polyacrylamide electrophoresis on 1 mm slab gels (Laemmli, 1970) using a 11.5–16.5% continuous gradient of acrylamide, and were stained with Coomassie Brilliant Blue R250.

Quantitative determination of bacteriochlorophyll and proteins

The total amount of Bchl of *R. capsulatus* cells or membranes was calculated from the 770 nm absorbance of methanol/acetone (2:7/v:v) extracts using an extinction coefficient of 76 mM^{-1} cm^{-1} (Clayton, 1966). Bchl bound to the RC was determined by reversible reaction center bleaching at 870 nm and was carried out on a Perkin-Elmer spectrophotometer as described (Reidl *et al.*, 1983). An extinction coefficient of 113 mM^{-1} cm^{-1} (Strayley *et al.*, 1973) was used for calculation of the RC bound Bchl. Protein concentrations were assayed by the Lowry procedure (Lowry *et al.*, 1951).

Photophosphorylation

Cells of *R. capsulatus* cultures were sedimented, washed twice in S-buffer (100 mM glycylglycine, 2 mM $MgCl_2$, 0.5 mM succinate, 10% (w/v) sucrose, pH 7.5), and resuspended in S-buffer to give a density of 4×10^4 cells/ml. DNase I was added at 1 mg/g wet wt of cells and the cells were disrupted at 110 MPa using a French pressure cell. After centrifugation at 12 000 *g* for 20 min at 4°C the crude membranes were separated by centrifugation from the cell free extracts (200 000 *g*, 90 min). The sediment was resuspended in a small volume of 2 × S-buffer in a homogenizer. The samples were diluted with 2 × S-buffer to an $OD_{880\ nm}$ of 0.5–2.0. An enzyme test detecting the formation of NADPH independent of ATP production was used to determine the photophosphorylation activity as described (Cusanovich and Kamen, 1968). The reaction was carried out at 30°C in Thunberg cuvettes. Saturating excitation light of 80 W/m^2 was used, and the absorbance at 340 nm was determined using a Cary 14 spectrophotometer (Varian Instruments). An extinction coefficient of 6.22 mM^{-1} cm^{-1} was used for calculating the photophosphorylation rates.

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