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Cloning and expression of the *fbc* operon encoding the FeS protein, cytochrome b and cytochrome c_1 from the *Rhodopseudomonas sphaeroides* b/c_1 complex

N.Gabellini, U.Harnisch[1], J.E.G.McCarthy, G.Hauska[2] and W.Sebald

Department of Cytogenetics, GBF – Gesellschaft für Biotechnologische Forschung mbH., D-3300 Braunschweig, [1]Institute of Biochemistry, University of Düsseldorf, D-4000 Düsseldorf, and [2]Institute of Botany, University of Regensburg, D-8400 Regensburg, FRG

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The gene for the FeS protein of the *Rhodopseudomonas sphaeroides* b/c_1 complex was identified by means of cross-hybridization with a segment of the gene encoding the corresponding FeS protein of *Neurospora crassa*. Plasmids (pRSF1-14) containing the cross-hybridizing region, covering in total 13.5 kb of chromosomal DNA, were expressed *in vitro* in a homologous system. One RSF plasmid directed the synthesis of all three main polypeptides of the *R. sphaeroides* b/c_1 complex: the FeS protein, cytochrome b and cytochrome c_1. The FeS protein and cytochrome c_1 were apparently synthesized as precursor forms. None of the pRSF plasmids directed the synthesis of the 10-kd polypeptide found in b/c_1 complex preparations. Partial sequencing of the cloned region was performed. Several sites of strong homology between *R. sphaeroides* and eukaryotic polypeptides of the b/c_1 complex were identified. The genes encode the three b/c_1 polypeptides in the order: (5') FeS protein, cytochrome b, cytochrome c_1. The three genes are transcribed to give a polycistronic mRNA of 2.9 kb. This transcriptional unit has been designated the *fbc* operon; its coding capacity corresponds to the size of the polycistronic mRNA assuming that only the genes for the FeS protein (*fbc*F), cytochrome b (*fbc*B) and cytochrome c_1 (*fbc*C) are present. This could indicate that these three subunits constitute the minimal catalytic unit of the b/c_1 complex from photosynthetic membranes.

Key words: R. sphaeroides/b/c_1 complex/gene cloning/*in vitro* expression/polycistronic mRNA

Introduction

The electron transport chain of the phototrophic bacterium *Rhodopseudomonas sphaeroides* comprises a ubiquinol cytochrome c oxidoreductase or b/c_1 complex that catalyses cyclic photosynthetic electron transport together with the photochemical reaction center, ubiquinol and a soluble cytochrome c_2 (Dutton and Prince, 1978). In aerobic growth conditions the b/c_1 complex of *R. sphaeroides* functions in a respiratory chain of mitochondrial type (Baccarini Melandri and Zannoni, 1978). The b/c_1 complex isolated in its active form from photosynthetic membranes of *R. sphaeroides* GA (Gabellini *et al.*, 1982) and *R. sphaeroides* R26 (Yu and Yu, 1982), comprises four polypeptides: cytochrome b (40 kd), cytochrome c_1 (33 kd), FeS protein (24 kd) and a 10-kd polypeptide (Hauska *et al.*, 1983). Proton translocation activity and membrane potential generation are reconstituted when the enzyme is incorporated into liposomes (Hurt *et al.*, 1983). Although it has a simpler composition, this bacterial b/c_1 complex shows many functional similarities to the corresponding mitochondrial complex. The substrate specificity, sensitivity to inhibitors, spectral characteristics and redox properties are largely comparable (Gabellini *et al.*, 1982; Gabellini and Hauska, 1983a), suggesting that the essential features of the enzyme structure have been highly conserved. Whilst there have been recent advances in the biochemical characterization and kinetic analysis of the b/c_1 complex of *R. sphaeroides* (Crofts *et al.*, 1983), there has been no information up to now on the primary structure of this complex. Here we describe the initial steps towards a complete description of the sequence and mode of expression of the genes encoding the b/c_1 complex subunits of *R. sphaeroides*.

The gene encoding the Rieske FeS protein (Rieske, 1964) of *Neurospora crassa* has been cloned recently and sequenced (Harnisch *et al.*, in preparation). The observation that antibodies raised against the Rieske FeS protein of *N. crassa* cross-reacted with the FeS protein of *R. sphaeroides* b/c_1 complex (Gabellini and Hauska, unpublished) suggested the use of a segment of the gene encoding the *N. crassa* FeS protein as a probe for the corresponding gene of *R. sphaeroides*. This probe was successfully used to locate the FeS protein gene of *R. sphaeroides*, which was found to be included in an operon together with the genes for cytochrome b and cytochrome c_1. It is proposed to name this operon *fbc*, in accordance with the nature of the prosthetic groups FeS, heme b and heme c carried by the encoded subunit polypeptides and also with the order in which the genes occur in the operon. It is further proposed to name the gene for the FeS protein *fbc*F, for the cytochrome b *fbc*B and for cytochrome c_1 *fbc*C.

Results

Selection and analysis of RSF plasmids

The identification of the gene encoding the FeS protein of the b/c_1 complex of *R. sphaeroides* was achieved by means of cross-hybridization with part of the nuclear gene encoding the corresponding FeS protein of *N. crassa* (FeS protein probe, see Materials and methods).

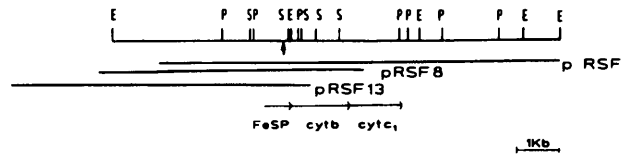

Fig. 1. Detailed restriction map of 10 kb of the cloned *R. sphaeroides* DNA including the *fbc* genes. The arrow indicates the position of the cross-hybridizing sequence. Restriction sites of the endonucleases *Eco*RI, *Pst*I and *Sal*I are indicated respectively as E, P, S. The regions cloned in three RSF plasmids analysed are indicated below the restriction map. pRSF1 and pRSF13 define the two ends of the 13.5-kb DNA region of the *R. sphaeroides* chromosome covered by the RSF plasmids. The locations of the genes for the FeS protein, cytochrome b and cytochrome c, included in the *fbc* operon are also indicated.

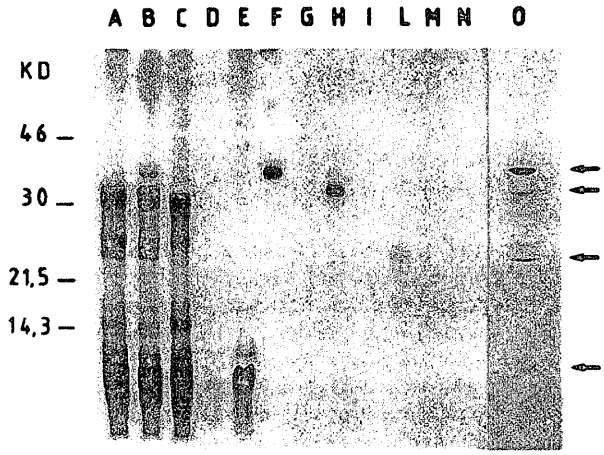

Fig. 2. Fluorogram of the [^{35}S]L-methionine-labelled polypeptides synthesized in the *R. sphaeroides in vitro* system and immunoadsorbed with specific antibodies to each subunit of the *R. sphaeroides* b/c_1 complex. **Lanes A** and **B** show the polypeptides synthesized under the direction of pRSF1. The samples loaded on **lane A** were incubated at 95°C for 3 min and those loaded on **lane B** were incubated at 20°C for 15 min in loading buffer. **Lane C** shows the polypeptides whose synthesis was directed by pBR322. The products loaded on **lane D** were obtained when 1% SDS was added simultaneously with pRSF1 at the beginning of the 75 min incubation at 37°C. **Lane E** shows the products obtained with the water blank. The labelled polypeptides of **lane B** were immunoadsorbed with antibodies specific to the cytochrome b (**lane F**) and respective control serum (**lane G**); with antibodies specific to the cytochrome c_1 (**lane H**) and respective control serum (**lane I**); with antibodies specific to the FeS protein (**lane L**) and respective control serum (**lane M**) and with antibodies specific to the 10-kd polypeptide (**lane N**). The distance of migration of the following marker proteins are indicated on the left hand side of the figure: lysozyme (mol. wt. 14 300), soybean trypsin inhibitor (mol. wt. 21 500), carbonic anhydrase (mol. wt. 30 000), ovalbumin (mol. wt. 46 000). The b/c_1 complex preparation from *R. sphaeroides* GA was run in parallel on the same gel. The polypeptide components indicated by the arrows are: cytochrome b (40 kd), cytochrome c_1 (33 kd), FeS protein (24 kd) and the 10-kd polypeptide. Reaction centre polypeptides are present in substoichiometric amount in the b/c_1 preparation (Gabellini and Hauska, 1983b).

Fe-S-protein

R. sphaeroides
EWLVILGVCSHLGCVPMGDKSGDFGGWFCPCHGSHYDSAGHIRKGPAPRNLDIP
EWLVMLGVCTHLGCVPIGE AGDYGGWFCPCHGSHYDISGRIRKGPAPLNLEIP
N. crassa (AA 134 - 183)

Cytochrome b

R. sphaeroides
PTPKNLNWWWLWGIVLAFTLVLQIVTGIVLAMHYTPHVACR
PQPSSINYWWNMGSLLGLCLVIQIVTGIFMAMHYSSNIELA
Yeast (AA 20 - 60)

Cytochrome c_1

R. sphaeroides
DQAQLRRGFQVYSEVCSTCHGMKFVPICTL
DHASIRRGYQVYREVCAACHSLDRVAWRTL
Yeast (AA 86 - 115)

Fig. 3. Some regions of homology between the *fbc* genes encoded polypeptides and corresponding eukaryotic polypeptides. The amino acid sequence of a region of the Rieske FeS protein of *R. sphaeroides* is compared with the corresponding sequence of the *N. crassa* FeS protein (Harnisch *et al.*, in preparation) encoded by the DNA fragment used as probe. The four cysteins included in the homologous sequence are indicated in bold type. The first region of homology from the N terminus between the cytochrome b of *R. sphaeroides* and yeast mitochondria (Nobrega and Tzagoloff, 1980) is presented. Finally the heme binding region of the cytochrome c_1 of *R. sphaeroides* and of yeast cytochrome c_1 (Sadler *et al.*, 1984) are compared, the two cysteins and the histidine (in bold type) participate in the covalent binding of the heme.

pBR322 derivatives, bearing partial *Sau*3A fragments (5–9 kb) of the *R. sphaeroides* genome, were used to transform *Escherichia coli*. Fourteen positive clones were selected from a total of 10 000 by means of colony filter hybridization. The plasmids (pRSF 1 – 14) isolated from the positive clones were shown, by restriction analysis, to contain a series of overlapping DNA fragments that covered a 13.5-kb region of the *R. sphaeroides* chromosome. The inserts of pRSF1 and pRSF13 defined the two ends of this region (Figure 1). A Southern blot of restricted *R. sphaeroides* genomic DNA, hybridized with all the pRSF plasmids, confirmed the identity and co-linearity of the cloned inserts with directly isolated genomic DNA. A detailed map of restriction sites for *Sal*I, *Pst*I and *Eco*RI endonucleases was constructed for 10 kb of *R. sphaeroides* DNA included in the RSF plasmids (Figure 1). The cross-hybridizing region was more precisely defined by further Southern blot analysis of restriction digests of pRSF8, hybridized with the *N. crassa* FeS protein probe. Cross-hybridization was obtained with *Eco*RI 4000-bp, *Pst*I 1100-bp, and *Sal*I 900-bp fragments. The determined DNA sequence showed (70%) homology with the corresponding region of the *N. crassa* gene chosen as probe. This region encoded an amino acid sequence that was 76% homologous with the related region of the *N. crassa* FeS protein (Figure 3).

In vitro expression of the genes of the RSF plasmids

RSF plasmids were used as templates for a cell free transcription-translation system prepared from *R. sphaeroides* L. (Chory and Kaplan, 1982) to determine whether the plasmids directed the synthesis of the whole FeS protein gene and also whether a single plasmid directed the synthesis of more than one b/c_1 complex subunit. A homologous *in vitro* system was chosen since it had already been reported that *R. sphaeroides* DNA is not expressed in *E. coli* systems (Kaplan *et al.*, 1982). It was confirmed that no expression of cloned *R. sphaeroides* genes can be obtained using an *E. coli in vitro* coupled transcription-translation system.

Plasmid RSF1 carrying 9 kb of *R. sphaeroides* DNA (Figure 1) was chosen for the experiment of *in vitro* protein synthesis in the presence of L-[^{35}S]methionine shown in Figure 2.

Plasmid RSF1 directed the synthesis of the three main subunits of the b/c_1 complex of *R. sphaeroides*: cytochrome b (40 kd), cytochrome c_1 (33 kd) and the FeS protein (24 kd). The three polypeptides can be seen amongst the total products of transcription-translation of pRSF1 (Figure 2, lanes A and B) together with other products whose synthesis was directed by pBR322 (lane C). When the samples were heated for 3 min at 95°C before loading on the gel, cytochrome b was no longer identifiable because of aggregation (see lane A). The identities of the b/c_1 complex polypeptides synthesized *in vitro*

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I  K  L  G  ▪                    M  S  G  I  P
ATCAAGCTGGGCTGAGGGGAAAGACACATGTCCGGAATTCCG
    fbc F                          fbc B

N  P  A  E  ▪                    M  K  N  L  L
AATCCGGCCGAGTGAGGAAAGGAACCGACATCATGAAAAACCTTCTG
    fbc B                          fbc C
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Fig. 4. The short intergenic distances between the *fbc* genes. Nucleotide sequences of the regions including respectively the TGA stop codon of the *fbc*F gene and the ATG start codon of the *fbc*B gene and the TGA stop codon of the *fbc*B gene and the ATG start codon of the *fbc*C gene are shown. Underlined are the Shine and Dalgarno-like sequences which are highly complementary to the 3′ end of the 16S rRNA of *R. sphaeroides*.

under the direction of pRSF1 (lane B) were confirmed by means of immunoadsorption using antibodies specific for each of the subunits of the *R. sphaeroides* b/c_1 complex (see lanes F, H, L). Antibodies raised against the 10-kd polypeptide of the b/c_1 complex of *R. sphaeroides* did not react with any polypeptide whose synthesis was directed *in vitro* by pRSF1 (lane N), suggesting either that the gene encoding the 10-kd polypeptide is not expressed in this system, or (in what seems to be the more likely explanation) that it is not located in the vicinity of the other genes.

The sizes of the cytochrome c_1 and of the FeS protein synthesized *in vitro* were slightly greater than those of the respective polypeptides of the isolated b/c_1 complex (Figure 2, lane O), indicating that these two subunits could be synthesized initially as precursors with mol. wts. 1 – 2 kd greater than those of the mature proteins. All RSF plasmids tested directed the synthesis of the FeS protein, while pRSF8 also directed the synthesis of incomplete cytochrome c_1 (data not shown). The fact that pRSF1, which bears more of the chromosomal DNA indicated on the right hand side of the restriction map (Figure 1) than the other plasmids, directed the full synthesis of all three b/c_1 complex polypeptides provided a first indication of the organization of the genes.

Mapping of the fbc genes on pRSF

Precise information about the organization and primary structure of the *fbc* genes was obtained by partial DNA sequencing of the RSF plasmids. Comparison of the encoded amino acid sequences with those of the homologous polypeptides from mitochondria and chloroplasts helped with the identification of the correct coding sequences. DNA sequencing of the 900-bp *Sal*I fragment identified by Southern blot analysis using the *N. crassa* FeS protein probe, revealed the existence of an open reading frame which was highly homologous to the *N. crassa* FeS protein probe sequence. The amino acid sequence encoded by this region of *R. sphaeroides* DNA which was homologous to the *N. crassa* probe showed 76% homology with the *N. crassa* FeS protein (Figure 3). This allowed initial identification and localization of the *R. sphaeroides* FeS protein gene (*fbc*F). Extending the partial DNA sequencing analysis further downstream from the 900-bp *Sal*I fragment led to the identification of the reading frames encoding cytochromes b and c_1. This relative location of these two genes had already been indicated by the *in vitro* expression data. A TGA stop codon in-frame with the FeS protein sequence was found in the adjacent 250-bp *Sal*I fragment (Figure 1). This stop codon was separated by 12 nucleotides from the initial ATG codon of a new reading frame (Figure 4), that showed high homology with the amino acid sequences of the b cytochromes of mitochondria and

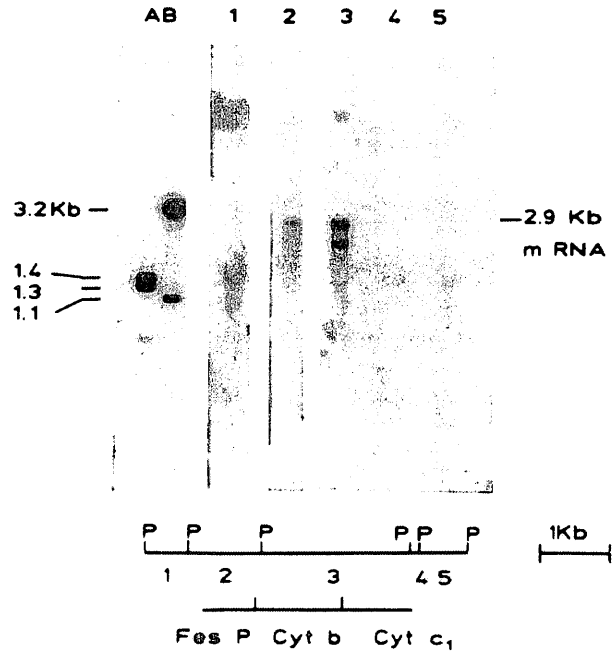

Fig. 5. Autoradiogram of a Northern blot derived from a 1.2% agarose gel indicating the polycistronic mRNA transcript of the *fbc* operon. In **lanes 1, 2, 3, 4** and **5** total *R. sphaeroides* RNA was separated by gel electrophoresis and used for hybridization respectively with pBR322 derived plasmids, containing *Pst*I fragments 1, 2, 3, 4, 5 numbered according to their order in the physical *Pst*I restriction map, also drawn in the figure. The positions of the *fbc* genes encoding the FeS protein, cytochrome b and cytochrome c_1 are indicated below the *Pst*I map. **Lanes A** and **B** were loaded respectively with *Taq* and *Hinc*II fragments of pBR322 which were used as size markers.

chloroplasts. The first stretch of homologous amino acid sequence of this reading frame, comprising the first hydrophobic α-helix of cytochrome b (Widger *et al.*, 1984; Saraste, 1984), is given in Figure 3, where it is compared with the yeast mitochondrial cytochrome b sequence (Nobrega and Tzagoloff, 1980). The proposed reading frame of the cytochrome b gene (*fbc*B) terminated with a TGA stop codon 359 bp downstream of the *Sal*I site in the *Sal*I-*Pst*I 1300-bp fragment (Figure 1). It was separated by 17 nucleotides from the initial ATG codon (Figure 4) of a third open reading frame which was shown to be that of the cytochrome c_1 gene (*fbc*C). Approximately 300 bp downstream from the start codon, the characteristic sequence of a covalent heme binding site of a c type cytochrome: *cys*-ser-thr-*cys*-*his* (Wakabayashi *et al.*, 1980) was found. There was also high amino acid sequence homology with the yeast cytochrome c_1 in the same heme binding region, as shown in Figure 3 (Sadler *et al.*, 1984).

The intergenic sequences between *fbc*F and *fbc*B, and between *fbc*B and *fbc*C (Figure 4), included Shine-Dalgarno like consensus sequences (Shine and Dalgarno, 1975) that were highly complementary to the sequence of the 3′ end of the 16S rRNA of *R. sphaeroides*: (5′)CUCCUUUCU(3′) (Gibson *et al.*, 1979). The sequence GGAAAGA lies three nucleotides upstream of the translational start codon of *fbc*B, and the sequence GGAAAG lies 11 nucleotides upstream of the *fbc*C start codon.

Identification of a 2.9 kb mRNA bearing all three fbc cistrons

The *in vitro* expression of RSF plasmids, taken together

with the DNA sequencing data, indicated that the genes *fbc*F, *fbc*B and *fbc*C, lie in a single operon. Confirmation of this was sought by performing Northern blot analysis with total *R. sphaeroides* RNA. The identification of a single mRNA species was achieved by hybridization with different segments of a 5-kb DNA region including the *fbc* genes (Figure 5). The 5-kb region was subcloned as five *Pst*I fragments in pBR322. The fragments were numbered 1 – 5 according to their order in the *Pst* restriction map as shown in the lower part of Figure 5. The nick-translated plasmids were used individually as hybridization probes. The evaluation of the size of the mRNA hybridizing with the *fbc* genes was estimated by comparing its migration rate against the rates of single-stranded *Taq*I and *Hinc*II DNA fragments derived from pBR322 (Figure 5, lanes A, B). Hybridization was clearly obtained between a 2.9-kb mRNA species and the *Pst*I fragment 2 of 1100 bp (Figures 5, lane 2). This fragment comprises all of *fbc*F (~600 bp), together with the first 170 bp of *fbc*B. Stronger hybridization was observed between the same mRNA species and the *Pst*I fragment 3 of 2300 bp (Figure 5, lane 3). Fragment 3 comprises the following 1100 bp of *fbc*B and approximately all of *fbc*C.

Only one mRNA species of 2.9 kb was also obtained by hybridization with the whole pRSF1 and pRSF8 (not shown). The smear in the 2.5-kb region, particularly evident in lane 3 of Figure 3, most likely reflects some partial degradation of mRNA during the isolation procedure.

The *Pst*I fragments 1, 4 and 5 flanking the *fbc* genes did not engage in detectable hybridization with any mRNA species. The shadow observed in the region of 1.3 kb, best visible in lane 1, represents some unspecific binding to the large rRNA subunit, the position of which was determined by ethidium bromide staining of the gel (not shown). The mRNA of 2.9 kb hybridizing with the *fbc* genes is concluded to be the transcriptional unit of the *fbc* operon. Its size coincides approximately with the total length of the three *fbc* genes.

Discussion

The identification of the *fbc* genes by heterologous hybridization was made possible by the existence of very strong homology between a region of the DNA sequences encoding the Rieske FeS proteins of *N. crassa* and *R. sphaeroides*. The hybridization of the two sequences was detected under low stringency conditions (see Materials and methods). A comparison of the two sequences revealed an overall homology of 70%; the longest perfect match occurs in one segment of 14 bp. Most notably, there is conservation of the segment containing a cluster of four cysteines that are presumably involved in the binding of the FeS cluster (Stout, 1982).

The deduced partial amino acid sequences of *R. sphaeroides* cytochrome b showed striking homology with the mitochondrial cytochrome b and chloroplast cytochrome b and 17-kd polypeptide (Heinemeyer *et al.*, 1984). The *R. sphaeroides* cytochrome b seems to be more of the mitochondrial type in the sense that it is a large mol. wt. polypeptide of 40 kd (Gabellini and Hauska, 1983b). The deduced sequence of *R. sphaeroides* cytochrome c_1 showed homology to the mitochondrial cytochrome c_1 (Figure 3) or chloroplast cytochrome f (Willey *et al.*, 1984; Alt and Herrmann, 1984) in the heme binding region.

A detailed comparison of the amino acid sequences must await the completion of the DNA sequence analysis of the operon. The lack of expression of *R. sphaeroides* genes in an *E. coli in vitro* system could reflect differences in promoter recognition and/or differential specificity of the translational machinery between the two species (cf. Chory and Kaplan, 1982). The two Shine-Dalgarno consensus sequences (Figure 4) identified upstream of the *fbc*B and *fbc*C genes respectively, showed high complementarity to the 3′ end of the 16S rRNA of *R. sphaeroides* (Gibson *et al.*, 1979), and also some similarity to the corresponding sequence of *E. coli*. This would suggest that the lack of expression of *R. sphaeroides* genes in *E. coli* systems is attributable to the absence of suitable promoter structures. It should be pointed out in this context that a search for any structures resembling a typical *E. coli* promoter in a 300-bp region upstream of the putative *fbc*F start codon was unsuccessful.

The apparent synthesis of precursor forms of the FeS protein and cytochrome c_1 *in vitro* could indicate that these two polypeptides may undergo processing before being integrated into the membrane. This might be expected from the location of the functional domains of the FeS protein and cytochrome c_1 on the P side (Hauska *et al.*, 1983) of the membrane, corresponding in *R. sphaeroides* to the outer surface of the cytoplasmic membrane. Thus the precursors may contain signal sequences responsible for directing transport across the cytoplasmic membrane.

The present paper shows that three subunits present in the b/c_1 complex preparation from *R. sphaeroides* are encoded by an operon. This observation leads to the question whether this operon alone encodes the functional ubiquinol-cytochrome c oxidoreductase and thus whether the 10-kd polypeptide present in the b/c_1 preparation plays, for example, only a regulatory role or is even a contaminant. In *E. coli* most of the defined multisubunit enzymes are fully encoded by operons such as the *atp* (Futai and Kanazawa, 1983; Walker *et al.*, 1984), *ace* (Guest and Stephens, 1980) and *sdh* (Wood *et al.*, 1984) operons. In photosynthetic bacteria, by contrast, the genes for the F_0 and F_1 portions of the ATP synthase are encoded in separate regions of the chromosome (Tybulewicz *et al.*, 1984). Moreover, the organization of the genes of the photosynthetic reaction center and light harvesting complex I of *R. capsulata* (Youvan *et al.*, 1984) and *R. sphaeroides* (Williams *et al.*, 1983), does not correspond to the polypeptide composition of the isolated enzyme complexes.

Thus the observation that the three *R. sphaeroides fbc* genes are organized in an operon does not constitute a sufficient basis to conclude that the functional unit of the b/c_1 complex comprises only three polypeptides. Rather, one can only draw analogy to the structure of the functional chloroplast b_6/f complex (Hurt and Hauska, 1982), which is effectively equivalent to a three subunit structure of the *R. sphaeroides* complex.

Materials and methods

Cloning of R. sphaeroides genes and selection of RSF plasmids

R. sphaeroides GA genomic DNA was isolated essentially as described by Barker (1982) and subjected to partial digestion by *Sau*3A. Restriction fragments of size 5 – 9 kb were isolated by 0.8% agarose gel electrophoresis and ligated into the (5′ dephosphorylated) *Bam*HI site of pBR322. The plasmids so obtained were used to transform *E. coli* 5K (Hanahan, 1983). The selection of RSF plasmids was performed by colony filter hybridization (Grunstein and Hogness, 1975). The DNA probe used for hybridization was isolated from a cDNA clone of *N. crassa* Rieske FeS protein mRNA. The probe (FeS protein probe) was a 149-bp *Bgl*I-*Xho*I, 5′ γ-^{32}P end-labelled cDNA fragment, encoding a cluster of four cysteines which are most probably involved in forming the FeS center (Figure 3; Harnisch *et al.*, in preparation). The probe was 5′ end-labelled with [γ-^{32}P] ATP (>5000 Ci/mmol, Amersham) and used at a concentration of ~3 μCi/20 ml

hybridization buffer. The hybridization conditions were kept at low stringency by incubation in 5 x NaCl-citrate (SSC), 0.1% SDS, 0.2% bovine serum albumin, Ficoll, polyvinylpyrrolidone, 50% formamide (reagent grade Merck) for 42 h at 20°C. After incubation the filters were washed several times in 5 x SSC at progressively increasing temperatures up to 45°C. Autoradiography was performed with Agfa-Gevaert X-ray film. Plasmid DNA was isolated (Maniatis *et al.*, 1982) from *E. coli* overnight cultures grown at 37°C in LB medium plus Ampicillin 50 μg/ml. Restriction enzymes were used according to manufacturers' specifications.

Southern blotting analysis was performed by standard techniques (Maniatis *et al.*, 1982) except that the condition of hybridization with the heterologous probe was kept at low stringency as described for colony filter hybridization.

In vitro transcription and translation system and immunoadsorption

The *in vitro* expression of *R. sphaeroides* cloned genes was tested in a S30 extract prepared from *R. sphaeroides* L, a strain that lacks the *Rsh*I restriction system (Chory and Kaplan, 1982; Zubay, 1973; Pratt, 1980). Cells from an exponentially growing photosynthetic culture were broken by passage through a Sorvall Ribi Cell Fractionator at 12 000 p.s.i. and 4°C. *In vitro* protein synthesis was carried out in the presence of 19 unlabelled amino acid (0.22 μM each) plus [^{35}S]L-methionine (Amersham); 50 μCi in a 50 μl reaction mixture. Aliquots of 5 μl of the assay mixture were loaded onto 12% SDS-polyacrylamide gels (Laemmli, 1970). Rabbit antisera were raised against each of the four SDS-denatured subunits of the *R. sphaeroides* b/c_1 complex. The subunits synthesized *in vitro* were immunoadsorbed from 40 μl of solubilized *R. sphaeroides in vitro* assay (Goldman and Blobel, 1978), using 5 mg of protein A-Sepharose CL-4B (Pharmacia) and 10 μl of antisera. Incubation of the samples at high temperature, causing irreversible aggregation of cytochrome b, was avoided. The ^{35}S-labelled polypeptides were visualized by fluorography (Bonner and Laskey, 1974) using Kodak X-Omat AR film.

DNA sequencing

DNA sequencing was performed as described by Maxam and Gilbert (1980) using 5' end-labelled fragments.

Isolation of RNA and Northern blot hybridization

R. sphaeroides GA total RNA was extracted from photosynthetic cultures in early exponential phase after lysozyme treatment by the guanidinium-isothiocyanate CsCl method (Maniatis *et al.*, 1982). Northern blots of glyoxylated RNA probes were performed as described by Thomas (1983) using nick-translated DNA probes. DNA fragments of pBR322 cleaved with *Taq*I and *Hinc*II used as size standards (Maniatis *et al.*, 1982) were denatured by incubation at 95°C for 3 min before incubation with glyoxal.

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