MINI-REVIEW

Organization and Structure of the Genes for the Cytochrome b/c_1 Complex in Purple Photosynthetic Bacteria. A Phylogenetic Study Describing the Homology of the b/c_1 Subunits Between Prokaryotes, Mitochondria, and Chloroplasts[1]

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Abstract

The cytochrome b/c_1 complex is an ubiquitous energy transducing enzyme, part of the electron transport chain of prokaryotes, mitochondria, and chloroplasts (b_6/f). In the ancient purple photosynthetic bacteria, the b/c_1 complex occupies a central metabolic role, being part of their photosynthetic and respiratory electron transport chain. In *Rhodobacter* the three subunits of the b/c_1 complex are FeS protein, cytochrome b, and cytochrome c_1, and they are encoded by a constitutively expressed operon named *fbc*. The organization of the genes for the cytochrome b/c_1 complex, the modality of transcription, and the biogenesis of the encoded polypeptides will be described. The *Rhodobacter* species used to isolate the *fbc* genes, previously reported as *R. sphaeroides* was identified as *R. capsulatus*. Further biochemical characterization of the prokaryotic b/c_1 complex indicated that the three polypeptides encoded by the *fbc* operon comprise the entire catalytic structure: ubiquinol–cytochrome-c reductase.

The amino acid sequences of the three b/c_1 subunits from the photosynthetic bacterium *Rhodobacter capsulatus* were compared with the corresponding sequences from yeast mitochondria and spinach chloroplasts. The high homology found between the sequences of all three redox polypeptides from *R. capsulatus* and yeast mitochondria (cytochrome b 41%, FeS protein 46%, cytochrome c_1 31%) provided further evidence that mitochondria arose from the phylogenetic line of purple bacteria. The structure of cytochrome b also exhibited considerable homology to chloroplast cytochrome b_6 plus subunit IV (26%). The amino acid sequence of the Rieske FeS protein from *R. capsulatus*

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[1] Abbreviations: kb, kilobase; kDa, kilodalton; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.
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and chloroplasts were found to be conserved only in the C-terminal part (14% total identity), whereas the homology between cytochrome c_1 and cytochrome f is very weak (12%), despite similar topology of the two polypeptides.

Analysis of the homology suggested that the catalytic sites quinol oxidase (Q_o) and quinone reductase (Q_i) arose monophonetically, whereas cytochrome c and plastocyanin reductase sites are not homologous and could derive from diverse ancestral genes by convergent evolution.

Key Words: Purple bacteria; *Rhodobacter*; *fbc* operon; promoter; cytochrome b; cytochrome c_1; Rieske FeS protein; phylogenesis.

Introduction

The photosynthetic bacteria from which most is known about the molecular genetics of electron transport chains are the purple bacteria of the genera *Rhodobacter* and *Rhodopseudomonas*. These are anoxygnetic photohetero-trophs able to synthesize ATP photosynthetically and to fix N_2 and CO_2 in the light. In the dark most of them are aerobic heterotrophs that may use O_2, or several other substrates, as terminal electron acceptor (Pfennig, 1978).

The application of molecular genetic techniques, such as transposon mutagenesis, to photosynthetic bacteria allowed the physical mapping of oxygen-regulated genes like the photosynthetic gene cluster (Zsebo and Hearst, 1984) and the nitrogenase genes (Avtges *et al.*, 1983). Constitutively expressed genes, coding for multisubunit enzymes, involved in photo-synthesis and respiration, have been highly conserved during evolution. The strong conserved sequences of the α and β subunits of the F_1-ATPase from *Escherichia coli* were used to identify the corresponding genes of *Rhodo-pseudomonas blastica* and *Rhodospirillum rubrum* (Tybulewicz *et al.*, 1984; Falk *et al.*, 1985). In these *Rhodospirillaceae*, however, only the genes for the F_1-ATPase form an operon, named *atp*, and the genes for the F_0 are not part of this transcriptional unit as in the case of *E. coli unc* operon (Gibson, 1982).

The high structural homology between the polypeptides carrying redox centers of the b/c_1 complex of *Rhodobacter capsulatus* and mitochondria allowed the identification of the genes for the prokaryotic b/c_1 complex by heterologous hybridization with a highly conserved sequence part of the nuclear gene for the Rieske FeS protein from *N. crassa*. All three subunits of the b/c_1 complex or ubiquinol–cytochrome-c reductase are encoded by an operon named *fbc* (Gabellini *et al.*, 1985).

The b/c_1 complex is an ubiquitous energy-transducing enzyme, and its structure and distribution have been most remarkably conserved. Besides the purple bacteria, including nonphotosynthetic phenotypes as *Paracoccus*, this enzyme occurs in cyanobacteria, green sulfur bacteria, in chloroplasts (b_6/f), and in mitochondria (for a review see Hauska *et al.*, 1983).

The elucidation of the primary structure of the cytochrome b, cytochrome c_1, and Rieske FeS protein (Rieske *et al.*, 1964) by determination of the DNA sequence of the *fbc* operon identified regions of functional significance in relation to the catalytic structures, chromophore ligands, topology, and biogenesis of these polypeptides (Gabellini and Sebald, 1986).

After a short overview of the taxonomy of the organisms, and of the photosynthetic electron transport, this review will examine the comparative molecular genetic data available for the membrane-bound electron and proton carrier cytochrome b/c_1 complex. The discussion of the structural relationships of the b/c_1 polypeptides within the genera *Rhodobacter* and among members of *Rhodospirillaceae* will be based on the sequence homology and immunological and biochemical evidence. The degree of homology between the species investigated was found to be in agreement with their taxonomic position. Furthermore, the results indicated that the *fbc* sequence reported (Gabellini and Sebald, 1986) is from a green derivative of *R. capsulatus* (Davidson and Daldal, 1987b).

The high sequence homology found between the b/c_1 polypeptides of *Rhodobacter* and mitochondria suggested that the b/c_1 complex of the ancient purple photosynthetic bacteria is the ancestor of mitochondrial complex III. The conservation of the structure of the cytochrome b polypeptide among purple photosynthetic bacteria, cyanobacteria, mitochondria, and chloroplasts further supported the hypothesis that the prokaryotic lines arose from a common organism.

The double metabolic role occupied by the b/c_1 complex in photosynthesis and respiration suggests that this enzyme is one of the most ancient components of respiratory chains (Baccarini-Melandri and Zannoni, 1978).

Taxonomic Background

The photosynthetic bacteria are mostly represented in the eubacterial kingdom and comprise purple bacteria, green sulfur bacteria, and cyanobacteria (Fox *et al.*, 1980). The cyanobacteria carry out oxygenic photosynthesis using two photosystems with water as electron donor and oxygen as ultimate oxidation product. The purple and green sulfur bacteria, in contrast, carry out anoxygenic photosynthesis with only one photosystem. They require electron donors of lower redox potential than water, such as reduced sulfur compounds, molecular H_2, or simple organic compounds. Based on the subcellular organization of the photosynthetic apparatus, green bacteria and purple bacteria are remarkably different (Pfennig, 1978). Purple bacteria have a greater metabolic versatility, and in addition to bacteriochlorophyll a, or b, they have a large number of carotenoid pigments. The

purple photosynthetic bacteria are differentiated in *Chromatiaceae* and *Rhodospirillaceae*. The major difference between the two families is the ability of *Chromatiaceae* to use the oxidation of sulfur to sulfate as a source for the phototrophic assimilation of CO_2 (Pfennig, 1978).

Some members of archebacteria, such as the genera *Halobacterium*, are also capable of photosynthesis but use a quite different mechanism that involves the photoactivation of the proton pump bacteriorhodopsin (Oesterhelt and Stoeckenius, 1973).

The purple photosynthetic bacteria comprise three major subdivisions called alpha, beta, and gamma. The alpha subdivision contains the most thoroughly studied species of *Rhodospirillaceae* and also includes nonphoto-synthetic phenotypes, e.g., *Paracoccus* (Woese *et al.*, 1984). This subdivision, made on the basis of ribosomal RNA sequences by the oligonucleotides cataloging method, is also in agreement with the metabolic phenotype of its members. The genera *Rhodopseudomonas*, which is included in the alpha subdivision, has recently been rearranged on the basis of morphological and physiological properties (Imhoff *et al.*, 1984), so that some members have been put in the new genera *Rhodobacter*, including *R. capsulatus* and *R. sphaeroides*. These species share many similarities in their metabolism and in the components of their photosynthetic apparatus, and in anaerobic con-ditions both of them develop vesicular cytoplasmic invaginations. The remaining *Rhodopseudomonas*, like *R. viridis*, have more specialized mem-brane systems of thylakoid type. From calculaton of the s_{AB} value, or associ-ation coefficient in Eubacteria, which permits the estimation of the temporary scale of evolution, it has been deduced that the most ancient phenotypes are related to anaerobic organisms like purple bacteria, cyanobacteria, and Clostridia (Fox *et al.*, 1980). A phylogenetic consideration emerging from these studies is that the chloroplasts originated from prokaryotic entities like cyanobacteria, while mitochondria arose from purple photosynthetic bacteria, in accordance with the endosymbiotic theory.

Photosynthetic Electron Transport

The mechanism of electron transport has been extensively studied in purple photosynthetic bacteria. The photosynthetic vesicles of *Rhodobacter*, termed chromatophores, comprise a complete system for the conversion of light into chemical energy (Niederman and Gibson, 1978). The radiant energy collected by the light-harvesting complexes is transferred to a special dimer of bacteriochlorophyll *a* (Bchl)$_2$ bound to the reaction center that becomes photooxidized. This primary reaction is followed by a series of electron-transfer reactions to the primary electron acceptor quinone

molecule $(Q)_A$ and to the secondary electron acceptor $(Q)_B$. This process induces transmembrane charge separation and membrane potential generation. The $(Bchl)_2$ left oxidized is reduced in turn by a water-soluble cytochrome c_2 while the reducing potential is accumulated in the freely diffusible pool of quinone. The mechanism of photosynthesis is best understood in photosynthetic bacteria, as a result of the extensive biochemical and biophysical characterization of the photochemical reaction center of *Rhodospirillaceae* (see Okamura *et al.*, 1982). This membrane protein complex has also been obtained in the crystalline form from *R. viridis* and *R. sphaeroides*, and the three-dimensional structure successfully resolved (Deisenhofer *et al.*, 1985; Chang *et al.*, 1986). At the same time, the DNA sequence analysis of the photosynthetic gene cluster from *R. capsulatus*, *R. sphaeroides*, and *R. viridis* has provided the amino acid sequences of the polypeptides binding the photopigments (Youvan *et al.*, 1984; see also Williams *et al.*, 1984; Weyer *et al.*, 1987).

During photosynthesis the reaction center is functionally coupled with the ubiquinol cytochrome c reductase in a cyclic electron transport system. Under aerobic conditions, when the synthesis of the photosynthetic apparatus is repressed, the b/c_1 complex is part of a respiratory chain of mitochondrial type and functions between dehydrogenase and terminal oxidase complexes (Baccarini-Melandri and Zannoni, 1978). The electron transport through the b/c_1 complex results in the vectorial translocation of protons across the membrane and generates electrochemical potential coupled to ATP synthesis.

There are a number of common features between bacterial and mitochondrial b/c_1, e.g., electron donor and acceptor specificity, sensitivity to inhibitors like antimycin A and myxothiazole, and midpoint potentials of the redox centers (see Hauska *et al.*, 1983). These observations supported the general conclusion that although the b/c_1 complex of bacteria has a simpler composition, it is operationally identical to that of mitochondria.

The electron transport scheme proposed for the b/c_1 complex of *R. sphaeroides* (Crofts *et al.*, 1983) is in agreement with the Q cycle mechanism (Mitchell, 1976). The ubiquinol is oxidized on the outer positive side of the membrane (Q_z or Q_o) through a concerted reaction that involves the 2Fe–2S cluster (Em_7 $+285$ mV) and the low-potential cytochrome b (b_L, Em_7 -90 mV). The oxidation of one ubiquinol molecule results in the release of two protons and delivery of two electrons that enter a branched pathway. One electron is transferred to cytochrome c_1 (Em_7 290 mV) that finally reduces cytochrome c_2. This is the high-potential arm of the electron transport chain that forms the cytochrome c reductase site of the complex. The second electron from cytochrome b_L is transferred to the heme b_H (Em_7 $+$ 50 mV) located near the opposite (cytoplasmic) side of the membrane. This
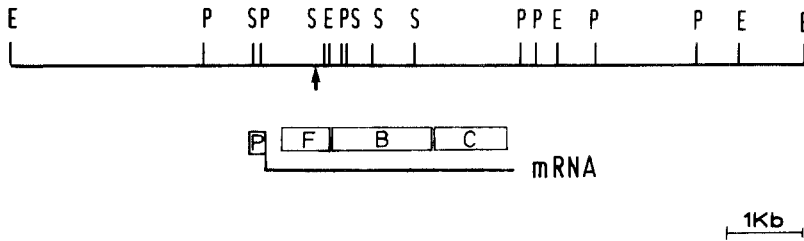
Fig. 1. Organization of the genes for the cytochrome b/c_1 complex in *Rhodobacter capsulatus*. Detailed restriction map of cloned genomic DNA from *R. capsulatus* including the *fbc* genes. Restriction sites of the endonuclease EcoRI, PstI, and SalI are indicated as E, P, and S, respectively. The arrow indicates the position of the sequence cross-hybridizing with the probe from *Neurospora crassa*. The position of the genes F for the FeS protein, B for cytochrome *b*, and C for cytochrome c_1 are indicated, as is the region of the promoter P and the length of the mRNA.

site catalyzes the reduction of ubiquinone to ubiquinol (Q_c or Q_i site) via a semiquinone intermediate and the concomitant uptake of two protons.

Molecular Genetics of the Cytochrome b/c_1 Complex from Purple Bacteria

Identification of the fbc Genes

The high structural homology existing between the prokaryotic and mitochondrial b/c_1 complex had enabled the identification of the *fbc* genes by means of heterologous cross hybridization (Gabellini *et al.*, 1985). A short DNA probe, part of the nuclear gene for the Rieske FeS protein of *Neurospora crassa* (Harnisch *et al.*, 1985) encoding the highly conserved region that binds the 2Fe–2S cluster, was used to locate the corresponding sequence of cloned *Rhodobacter capsulatus* DNA. The selected plasmids (pBR322 derivatives bearing 5–9 kb inserts) covered a large region of *Rhodobacter* chromosomal DNA including the cross hybridizing sequence (Fig. 1). To determine whether the plasmids included the whole FeS protein gene and also the genes for the other b/c_1 subunits, they were expressed in a cell-free transcription and translation system derived from *R. sphaeroides* (Gabellini *et al.*, 1985). By means of immunoadsorption with specific antibodies against each subunit of the b/c_1 complex, it was found that the template DNA encoded all three subunits FeS protein, cytochrome *b*, and cytochrome c_1. These findings provided the first indications that the genes were clustered in the prokaryotic genome. The DNA sequence analysis starting from the cross-hybridizing region immediately revealed the sequence of the FeS protein of *Rhodobacter* to be 70% homologous with that of the *Neurospora* probe.

Organization of the Genes for the b/c_1 Complex

The short intergenic distance strongly indicated that the genes lie within a single operon. The confirmation of this was achieved by the identification of a single polycistronic mRNA species covering the total length of the *fbc* genes (Gabellini *et al.*, 1985).

This operon was named *fbc* in accordance with the nature of the prosthetic groups FeS, heme *b*, and heme *c* carried by the encoded polypeptides and also with the order in which the genes occur in the operon (Fig. 1): *fbc*F for the FeS protein, *fbc*B for the cyrocrome *b*, and *fbc*C for the cytochrome c_1.

The elucidation of the complete DNA sequence of 3874 bp, comprised in three PstI restriction fragments (Fig. 1) of *R. capsulatus* genomic DNA including the *fbc* genes, provided more precise information on the organization and structure of the operon (Gabellini and Sebald, 1986).

The first gene in the order of transcription is *fbc*F which includes 576 nucleotides and encodes 191 amino acid residues of the FeS protein. The deduced relative molecular mass of 21 kDa is slightly smaller than that estimated from its migration in SDS-PAGE (Hauska *et al.*, 1983).

The following long reading frame of gene *fbc*B encodes 437 amino acid residues of the cytochrome *b* corresponding to a relative molecular mass of 48.1 kDa, larger than that predicted from its electrophoretic mobility (Gabellini and Hauska, 1983). The *fbc*C gene is the last gene in order of transcription. It includes 843 nucleotides, encoding 280 amino acid residues of the pre-apo-cytochrome c_1 with a relative molecular mass of 30.1 kDa. When determined by SDS-PAGE, the mature cytochrome c_1 exhibited an M_r of 34 kDa, whereas the size of the primary translation product was found to be 1–2 kDa larger (Gabellini *et al.*, 1985).

The availability of clones bearing the *fbc* genes helped the identification of the corresponding genes in other prokaryotes. The genes for the b/c_1 complex of *R. viridis* were identified by means of heterologous hybridization with the *fbc* genes from *R. capsulatus*. Also in the genera *Rhodopseudomonas* the three genes are clustered and probably form one transcriptional unit (Lang, unpublished).

The selection of clones bearing the cytochrome c_1 of *Paracoccus* was achieved by means of immunological screening (Paetow and Ludwig, 1986). The genes for all three subunits of *Paracoccus* b/c_1 complex were isolated via hybridization with the homologous DNA fragments from *Rhodobacter*. It was found that in *Paracoccus* the genes for the b/c_1 complex form an operon similar to that of *Rhodobacter* (Paetow *et al.*, 1986). As expected from their taxonomical position, the primary structure of the b/c_1 polypeptides has been found to be highly conserved between these two species of purple bacteria (Kurowski and Ludwig, 1987).

The *fbc*F gene was also used to locate the gene for the FeS protein of the cytochrome b_6/f complex of cyanobacteria, while the genes for the cytochrome *b* and *f* were identified by means of hybridization with the corresponding genes of chloroplasts (Kallas *et al.*, 1986). In *Nostoc*, however, the genes encoding the four subunits of the complex are arranged in three unlinked groups. Only the genes for the cytochrome *b* and subunit IV lie close to each other. In the chloroplast genome the genes for these two subunits are also clustered (Heinemeyer *et al.*, 1984). The amino acid sequence of the cytochrome *b* and subunit IV of chloroplasts is homologous with the sequence of the larger cytochrome *b* polypeptide of mitochondria (Widger *et al.*, 1984) and *Rhodobacter*. It is possible that a unique ancestral gene for the cytochrome *b* was split in the phylogenetic line of cyanobacteria.

Among the polypeptides of the b_6/f complex of chloroplasts only the FeS protein is encoded by the nuclear DNA (Alt *et al.*, 1983). While the cytochrome *b* is encoded in the mitochondrial genome, the numerous other subunits of the mitochondrial oxidoreductase are encoded by the nuclear DNA and imported post-translationally (Von Jagow and Sebald, 1980).

In spite of the high sequence conservation of the b/c_1 polypeptides, the organization of the genes differs markedly also among prokaryotes; while in purple bacteria all three subunits of the b/c_1 complex are encoded by an operon, in cyanobacteria only the genes for cytochrome *b* and subunit IV are clustered.

Transcription and Translation of the fbc Operon

The *fbc* genes are transcribed in a polycistronic mRNA of 3.1 kb from a promoter located upstream of the *fbc*F gene (Gabellini *et al.*, 1985). Since the b/c_1 complex takes part in both photosynthetic and respiratory metabolism in purple photosynthetic bacteria of the genera *Rhodobacter*, it can be assumed that the *fbc* genes are constitutively expressed in these organisms. This was confirmed by Northern blot hybridization of the total RNA with the *fbc* genes. The same RNA species (3.1 kb) was detected from aerobically and photosynthetically grown cells (Gabellini, unpublished).

The site of initiation of transcription of the operon, investigated by S_1 protection analysis, was located about 240 nucleotides upstream from the start codon of the *fbc*F gene (Gabellini and Sebald, 1986). The DNA sequence upstream from this point was compared with the sequence of the promoter region of other constitutively expressed genes, and indeed a strong homology was found with the DNA sequence of the *ATP* operon of *R. blastica* (Tybulewicz *et al.*, 1984). In Fig. 2 is shown the alignment of the DNA sequence upstream of the main transcription start of the *ATP* operon of *R. blastica* with the sequence upstream of the start of transcription of the

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a: GGACCCGT TGCCGCCAGCGCCG  AGGATGCCCGG GCGA  CCGCGCCGTCGACGT
b: TCACCCGTCTGC GCCGCAAGATCGAGCCCGACCCGCGCGA GCCGCGCTACCTGCAG
c: CAACCCGT TGC GGCGCTGCAA  AGCCTCTGCTAAG GACGCCGCAAAATCGG
                       |                              |
                     -35                            -10
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Fig. 2. Alignment of the nucleotide sequence in the promoter regions of *R. capsulatus fbc* operon and *R. blastica atp* operon. The nucleotide sequences in the region of the *fbc* promoter upstream of (a) T-549 located in the vicinity of the start of transcription and (b) G-610 beginning the repeated sequence are aligned with the nucleotide sequence (c) of *R. blastica atp* operon upstream of the main start of transcription. Positions − 10 and − 35 refer to the *atp* operon. Identical nucleotides are underlined.

fbc operon of *R. capsulatus*. The two sequences showed 60% homology from position − 1 to − 50, postulating only minor insertions or deletions. Remarkably the sequence around position − 10 GCCGC is conserved as well as the sequence ACCCGT-TGCG-CGC occurring after position − 35. This indicated that in *Rhodospirillaceae*, as in *E. coli*, the nucleotide sequences at positions − 10 and − 35 from the mRNA start are consensus sequences for the initiation of transcription, although they have a different composition (see McClure, 1985). Differences in promoter sequences between these two organisms are consistent with the general observation that *Rhodospirillaceae* genes are not expressed in cell-free transcription and translation systems from *E. coli*, and vice versa (Chory and Kaplan, 1982).

Interestingly the putative *fbc* promoter includes tandemly repeated consensus sequences. The second homologous sequence is found 60 bases upstream the putative start of transcription (Fig. 2). Such geometry has already been described in some *E. coli* promoters, where two or more repeats oriented in the same direction transcribe the same operon. The significance of this type of organization, which could play a regulatory role in the initiation of transcription, still remains to be elucidated (McClure, 1985). It is possible that the two series of consensus sequences carry different affinity for the RNA polymerase.

Another DNA sequence was pointed out as a possible promoter of the genes for the b/c_1 complex of *R. capsulatus* (Davidson and Daldal, 1987a). This sequence, GCGAAATATCT, which shows some homology with the region around the starts of transcription of *R. blastica atp* operon, is located about 220 bases upstream from the start of transcription of the *fbc* operon. It is therefore unlikely to be a consensus sequence for the initiation of transcription. Only a functional test on the promoter activity of these sequences, however, would provide the unambiguous identification of the nucelotide sequences of the promoter.

The 3′ end of *fbc* mRNA was determined by S_1 nuclease mapping (Gabellini and Sebald, 1986). The transcription of the *fbc* genes terminates

170 bases downstream of the TGA stop codon of gene *fbc*C. Two possible secondary structures are found in this region. One stable hairpin resembling the *Rho*-independent *E. coli* terminators (Platt, 1981) can be formed in the sequence located 118 bases upstream of the actual 3' end of the transcript. This secondary structure does not seem to act as terminator in *R. capsulatus*. A more complex secondary structure, composed of two loops connected by seven G.C pairs, includes the 3' end of the *fbc* transcript and most probably function as terminator.

Consensus sequences for the initiation of translation, which include a ribosomal binding site (Shine and Dalgarno, 1975), have been identified in the DNA sequence upstream of the start codons of the *fbc* genes (Gabellini and Sebald, 1986). These sequences are highly complementary to the sequence of the 3' end of the 16S rRNA of *R. sphaeroides* (Gibson *et al.*, 1979) and are homologous with the Shine and Dalgarno consensus sequences of *E. coli* (see Fig. 3).

Post-Translational Processing

Of the three redox polypeptides of the b/c_1 complex, the cytochrome c_1 and the FeS protein are functionally located on the periplasmic side of the membrane (see Hauska *et al.*, 1983); during their biogenesis, therefore, they are secreted through the cytoplasmatic membrane.

A precursor form of *R. capsulatus* cytochrome c_1 was demonstrated by the determination of the N-terminal sequence of the mature subunit (Gabellini and Sebald, 1986). The sequence started from the Asn 22 of the DNA deduced sequence. The pre-apo-cytochrome c_1 has a leader sequence of 21 amino acids including two positive charges (Lys 2–Lys 3) that address the polypeptide across the cytoplasmic membrane (see Fig. 7). The signal sequence Ala-Leu-Ala for the proteolytic cleavage is homologous to that of other prokaryotic secreted proteins (Rapoport and Wiedmann, 1985).

The existence of a precursor form of about 1 kDa greater than the mature FeS protein of *R. capsulatus* has been suggested by cell-free trans-lation of the *fbc* genes (Gabellini *et al.*, 1985). Indeed, the composition of the N-terminus of the FeS protein, as deduced from DNA sequence, resembles the leader sequence of cytochrome c_1 and that of other prokaryotic secreted proteins (Michaelis and Beckwith, 1982). It includes six charged residues, with a net charge of $+2$, followed by a hydrophobic sequence (see Fig. 6). Although the N-terminus of the mature FeS protein could not be detected, it is supposed that only the first hydrophilic segment is cleaved off during secretion, whereas the following hydrophobic part is retained in the mature polypeptide as hydrophobic anchor. Recent studies on the transport pathway of the Rieske FeS protein of *Neurospora* have suggested that the biogenesis

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                                                 Met Ser His Ala Glu Asp Asn Ala Gly Thr Arg Arg Asp Phe Leu
R.c.  AAT TTC AAG TTC GGG GAA GGG AGA AGA CAC GTG TCC CAC GCA GAA GAC AAC GCA GGC ACT CGG AGG GAT TTC CTC

                                                 Met Ser Asn Ala Asp Glu Ala His Gly Thr Arg Arg Asp Phe Leu
R.s.  CTG CAG CGG CCC GAG GAG GGG AGA AGT TCTCGTG TCC AAC GCA GAT GAA GCA CAC GGC ACT CGC AGG GAT TTC CTG
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Fig. 3. Alignment of the nucleotide and amino acid sequences from *Rhodobacter capsulatus* (GA), R.c. and *Rhodobacter sphaeroides* Ga, R.s. (Davidson and Daldal, 1987b), in the DNA region encoding the N-terminal sequence of the Rieske FeS protein. The Shine–Dalgarno-like consensus sequences and the nonconserved amino acids are underlined.

of this polypeptide has been conserved, as well as its function and structure (Hartl *et al.*, 1986). In eukaryotes this protein is encoded in the nucleus, synthesized in the cytoplasm as a precursor, and transported to the outer surface of the inner mitochondrial membrane. The leader sequence of *Neurospora* contains two different messages; the first addresses the transport to the matrix through a translocation contact site, which is then cleaved off. From this stage the biogenesis of the polypeptide follows the ancestral route. The second message is homologous to the prokaryotic leader sequence and promotes the secretion of the polypeptide through the inner mitochondrial membrane where, by a second cleavage, it is assembled in the mature form.

A Reevaluation of the Rhodobacter Species under Investigation

The *fbc* genes have been isolated from a green mutant thought to be *R. sphaeroides* Ga. This strain is a green derivative of *R. sphaeroides* 2.4.1, able to synthesize only two types of carotenoids, neurosporene and chloroxanthine (see Sistrom, 1978). It has been extensively characterized by spectroscopic investigations on the redox kinetics of the b/c_1 by several groups (e.g., see Baccarini-Melandri *et al.*, 1982; Crofts *et al.*, 1983), and was chosen for the isolation and biochemical characterization of the b/c_1 complex (Gabellini *et al.*, 1982) as well as for the isolation of the *fbc* genes. The original strain Ga, which was a gift of A. R. Crofts, was probably lost already in 1980. In that time the culture had a very long growth lag and was subsequently repurified starting from single green colonies (strain GA, Gabellini).

The DNA sequence of the operon encoding the b/c_1 complex from *R. capsulatus* SB1003 (Davidson and Daldal, 1987a) was found to be very similar to that reported for *R. sphaeroides* GA (Gabellini and Sebald, 1986). Over 98% of the FeS protein, 99% of the cytochrome *b*, and 90% of the cytochrome c_1 amino acid sequences were found identical. This result confirmed the determined sequence of the operon (Gabellini and Sebald, 1986) and suggested that the two sequences could be derived from the same species.

Strain GA was compared with other strains of *R. capsulatus* such as SB1003, St. Louis and with a genuine *R. sphaeroides* Ga by Southern blot hybridization with the *fbc* genes (Davidson and Daldal, 1987b). These authors concluded that strain GA was more related to *R. capsulatus* and cloned the *fbc* genes from a genuine *R. sphaeroides* Ga. The partial DNA sequencing data available for the FeS protein of *R. Sphaeroides* (Davidson and Daldal, 1987b) showed that the two sequences from *Rhodobacter* are very similar, and the determination of the DNA sequence coding for the N-terminal region of the FeS protein provided a conformation of the putative GTG start codon of the *fbc*F gene (Gabellini and Sebald, 1986). As shown in Fig. 3, although

the ribosome binding site (Shine and Dalgarno, 1975) is conserved in the two species, the nucleotide sequence in the noncoding region is less homologous. The Southern blot analysis of genomic DNA from other wild type strains of *R. sphaeroides* and *R. capsulatus* indicated that the restriction pattern of the DNA fragments including the *fbc* genes of strain GA is identical with that of *R. capsulatus* Kbl (Gabellini, unpublished).

Other diagnostic features, such as the vitamin requirement (Weaver *et al.*, 1975) and the behavior of cytochrome c_2 and c' on ion exchange chromatography (Bartsch, 1971), indicated that the strain GA investigated is a typical *R. capsulatus*. In agreement with the results of Davidson and Daldal (1987b), it is concluded that the *fbc* operon described (Gabellini *et al.*, 1985; Gabellini and Sebald, 1986) is from a green derivative of *R. capsulatus* most probably derived from strain Kbl. Therefore, from now on, strain GA will be referred to as *Rhodobacter capsulatus* (GA).

The proposal to rename the *fbc* operon as *pet* operon (Davidson and Daldal, 1987a), to recall the photosynthetic electron transport genes of chloroplasts (Hallick and Bottomley, 1983), is not appropriate because the b/c_1 complex encoded by the operon is not only involved in photosynthesis but also in the respiratory electron transport. Furthermore the b/c_1 of *Rhodobacter* is more similar to the mitochondrial b/c_1 complex than to the b_6/f complex of chloroplasts (see sequence homology).

Composition of the b/c_1 Complex from *Rhodobacter* and *Rhodopseudomonas*. Comparison with the R126 Mutant

In order to investigate the structural relationships of the b/c_1 polypeptides among the members of *Rhodospirillaceae,* both biochemical and immunological approaches were used. The enzyme was isolated from *Rhodobacter capsulatus* (GA), *Rhodobacter sphaeroides* 2.4.1, and from *Rhodopseudomonas viridis*, using octyl glucoside solubilization essentially as previously reported (Gabellini *et al.*, 1982). The b/c_1 complex from *R. capsulatus* R126, a nonphotosynthetic mutant of strain Y11 defective in the electron transport between cytochrome b and c_1 (Zannoni and Marrs, 1981), and from the revertant MR126 (constructed using a gene transfer agent), were a gift from B. A. Melandri (Fernandez Velasco and Melandri, unpublished data).

The composition of the b/c_1 was compared after SDS-PAGE by Coomassie staining (not shown) and by Western blot (Fig. 4) using rabbit antibodies directed against each subunit of the b/c_1 complex from *R. capsulatus* (GA). These experiments show that the compositions of all preparations from *R. capsulatus* are largely comparable. The mutant R126 has
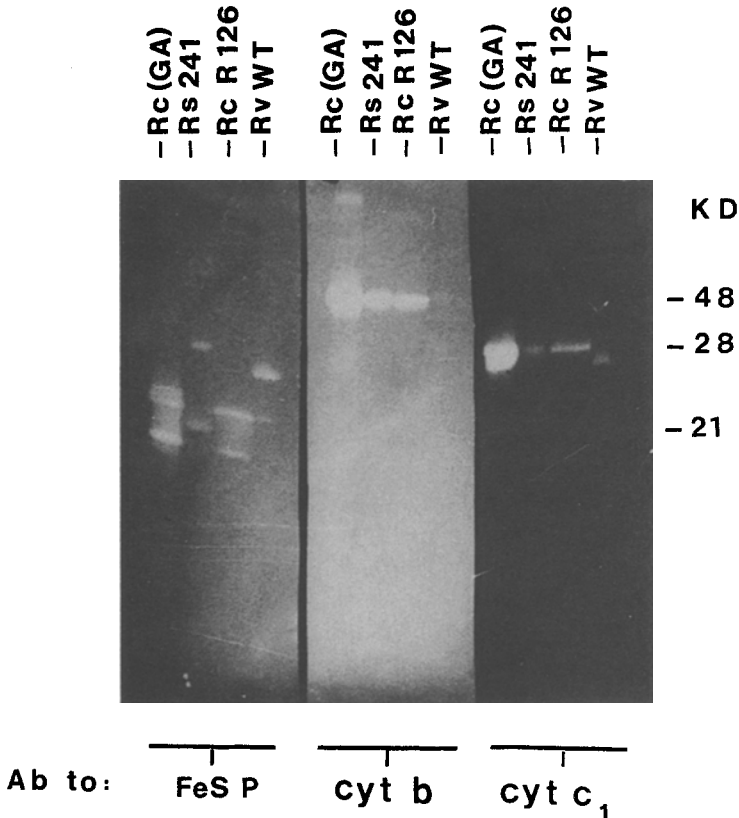
Fig. 4. Immunoblot from a 15% polyacrylamide SDS-gel. The b/c_1 preparations from *Rhodobacter capsulatus* (GA), Rc (GA) *Rhodobacter sphaeroides* 2.4.1, Rs 2.4.1, *Rhodobacter capsulatus* R126, Rc R126; and *Rhodopseudomonas viridis* wild type, Rv WT were loaded in triplicate. Rabbit antibodies directed against each of the three subunits of *R. capsulatus* (GA) b/c_1 complex were used separately. The left part of the blot was incubated with antibodies (Ab) directed to the FeS protein, (FeSP); the central part with Ab to the cytochrome *b* (Cyt *b*); the right part with Ab to the cytochrome c_1. The immunoreaction was visualized by using anti-rabbit antibodies coupled with a fluorescent marker. The position and the size of the b/c_1 subunits of *Rhodobacter* are indicated.

been demonstrated by means of spectroscopic kinetic characterization to be impaired in the cytochrome $c_1 + c_2$ reduction, and the lesion has been located in the Q_Z site of the oxidoreductase (Robertson *et al.*, 1986). The polypeptides involved in the formation of this catalytic site, FeS protein and cytochrome *b* (see also electron transport section), showed an identical electrophoretic migration with strain GA, and only a small difference in the electrophoretic mobility of cytochrome c_1 can be observed in the mutant (Fig. 5) and in the revertant (not shown). The significance of this variability

Cytochrome b / b₆

```
                    10                  20                  30
R.c.    M S G I P H D H Y E P K T G I E K W L H D R L P I V G L V Y D T I M - I P T P K
S.c.    . . . . . . . . . . . . . . . . M A F R K S N V Y L S L V N S Y I I D S P Q P S
S.o.    . . . . . . . . . M S - K V Y D W F E E R L E I Q A I A D D I T S K Y V P P H

        40                  50                  60                  70
R.c.    N L N W W W I W G I V L A F T L V L Q I V T G I V L A I D Y T P H V D L A F A S
S.c.    S I N Y W W N M G S L L G L C L V I Q I V T G I F M A M H Y S S N I E L A F S S
S.o.    V N I F Y C L G G I T L T C F L V - Q V A T G F A M T F Y Y R P T V T D A F A S

        80                  90                  100                 110
R.c.    V G H I M R D V N G G W A M R Y I H A N G A S L F F L A V Y I H I F R G L Y Y G
S.c.    V E H I I R D V H N G Y I L R Y L H A N G A S F F F M V M F M H M A K G L Y Y G
S.o.    V Q Y I M T E V N F G W L I R S V H R W S A S M M V L M M I L H V F R - V - Y L

        120                 130        ▲         140            ▲  150
R.c.    S - - Y K A P R E I T W I V G M V I Y L L M M G T A F M G Y V L P W G Q M S F W
S.c.    S - - Y R S P R V T L W N V G V I I F I L T I A T A F L G Y C C V Y G Q M S H W
S.o.    T G G F K K P R E L T W V T G V V L G V L T A S F G V T G Y S L P W D Q I G Y W

        160                 170                 180                 190
R.c.    G A T V I T G L F G A I P G L G P S I Q A W L L G G P A V D N A T L N R F F S L
S.c.    G A T V I T N L F S A I P F V G N D I V S W L W G G F S V S N P T I Q R F F A L
S.o.    A V K I V T G V P D A I P V I G S P L V E L L R G S A S V G Q S T L T R F Y S L

        200                 210                 220                 230
R.c.    H - Y L L P F V I A A L V A I H I W A F H T T G N N N P T - - G V E V R R T S K
S.c.    H - Y L V P F I I A A M V I M H L M A L H I H G S S N P L - - G I T G N L - - -
S.o.    H T F V L P L L T A V F M L M H F L M I R K Q G I S G P L / M G V T - K K P D L

        ▲          240                 ▲
R.c.    A D A E - K D T L P - - - F W P Y F - - - - V I K D L F A L A L V L L G F F A V
S.c.    - - - - - - D K I P - - - M H S Y F - - - - I F K D L V T V F L F M L I L A L F
S.o.    N D P V L R A K L A K G M G H N Y Y G E P A W P N D L L Y I F P V V I L G T I A

        270                 280                 290                 300
R.c.    V A Y M P N Y L G H P D N Y I Q A N P L S T P A H I V P E W Y F L P F Y A I L R
S.c.    V F Y S P N T L G H P D N Y I P G N P L V T P A S I D P E W Y L L P F Y A I L R
S.o.    C N V G L A V L E P S M I G E P A D P F A T P L E I L P E W Y F F P V F Q I L R

        310                 320                 330                 340
R.c.    A F A A D V W V V I L V D G L T F G I V D A K F F G V I A M F G A I A V M A L A
S.c.    - - - - - - - - - - - - - - - - - S I P D K L L G V I T M F A A I L V L L V L
S.o.    - - - - - - - - - - - - - - - - - T V P N K L L G V L L M A S V P A G L L T V

        350                 360                 370                 380
R.c.    P W L D T - S K V R S G A Y R P K F R M W F W F L V L D F V V L T W V G A M P T
S.c.    P F T D R - S V V R G N T F K V L S K F F F I F V F N F V L L G Q I G A C H V
S.o.    P F L E N V N K F Q N P F R R P V A T T V F L V G T V V A L - W L G I G A T L P

        390                 400                 410                 420
R.c    E Y P Y D W I S L I A S T Y W F A Y F L V I L P L L G A T E K P E P I P A S I E
S.c.    E V P Y V L M G Q I A T F I Y F A Y F L I I V P V I S T I E N V L F Y I G R V N
S.o.    I D K S L T L G L F

        430
R.c.    E D F N S H Y G N P A G
S.c.    K
```

Fig. 5. Alignment for maximal homology fo the amino acid sequence of cytochrome *b* from *Rhodobacter capsulatus* (R.c.), *Saccharomyces cerevisiae* (S.c.), and *Spinacea oleracea* (S.o.). Amino acid residues are given in single letter code, and identical residues are placed in boxes. Hydrophobic sequences predicted as trans-membrane spans are underlined, and the fourth hydrophobic segment that probably does not span the membrane is underlined by broken lines. The position of the four histidines ligands of the two hemes *b* is marked by triangles. The number refers to R.c. sequence.

is at present uncertain, and molecular characterization of the genes can provide more precise information about the physiological block of strain R126.

The two species *Rhodobacter sphaeroides* and *capsulatus*, in accordance with their taxonomical position, show strong immunological cross-reactivity for all three b/c_1 polypeptides. The major difference is observed in the electrophoretic mobility of the FeS protein, which is slightly retarded in *R. sphaeroides* (Fig. 5). The Rieske FeS protein from *Rhodobacter* runs as a double band if the enzyme is not denatured at high temperature before electrophoresis. Between the b/c_1 complexes of the two genera *Rhodobacter* and *Rhodopseudomonas* the conservation of the antigenic determinants seems to be lower, and the cytochrome b of *R. viridis* is not recognized by antibodies directed against the polypeptide of *R. capsulatus* (Fig. 5), while the cytochrome c_1 reacts only weakly. Stronger cross-reactivity is detected with the FeS protein antibodies, indicating that the structure of this polypeptide is most conserved, in agreement with sequence homology data (see below).

A b/c_1 preparation from *R. viridis*, obtained by dodecyl maltoside solubilization, which was depleted of the Rieske polypeptide and had lost the reductase activity, has previously been reported (Wynn *et al.*, 1986). The present preparation contains the full complement of b/c_1 polypeptides and retains the antimycin-sensitive oxidoreductase activity (Cully *et al.*, in preparation).

During the biochemical characterization of the b/c_1 complex from *Rhodobacter*, it was found that a small polypeptide of about 10 kDa copurified with the main redox polypeptides cytochrome b, cytochrome c_1, and FeS protein (Gabellini *et al.*, 1982; Yu and Yu, 1982). The gene for this protein was not found in the vicinity of the other *fbc* genes. The observation that this protein was not present in stoichiometric amounts with the other subunits and was in variable amounts in different b/c_1 preparations suggested that it could have been a contaminating protein. This 10-kDa protein was isolated from the b/c_1 complex of *R. capsulatus* (GA) by preparative SDS-PAGE and submitted to Edman degradation (Gabellini and Hoppe, unpublished). The detected sequence of 18 amino acid residues from the N-terminus was Met-X-X-Phe-Asp-X-Ile-Trp-His-Val-Phe-Asp-Glu-X-Pro-Val-Phe-Val. This sequence was found highly homologous with the sequence of the light-harvesting complex I, B870 α-polypeptide from another strain of *R. capsulatus* (Youvan *et al.*, 1984). This results clearly indicated that the polypeptide associated with the b/c_1 was a contaminating light-harvesting protein.

Moreover, purer b/c_1 preparations, composed of the three redox polypeptides only, exhibited the same oxidoreductase activity as the four-subunit preparation previously obtained (see Hauska *et al.*, 1983). It is concluded that the three subunits encoded by the *fbc* operon alone compose the

ubiquinol-cytochrome c reductase of *Rhodobacter*. This implies that the postulation of extra Q binding proteins (QP-c) (Yu and Yu, 1981), by analogy with the mitochondrial reductase, is not necessary in prokaryotes. These conclusions are not in agreement with the evidence of an antimycin binding site located in a 11-kDa polypeptide of *R. sphaeroides* (Wilson *et al.*, 1985). The three-subunit composition is also suggested for the b/c_1 complex of *R. viridis*, while a similar composition, but with a larger cytochrome c_1, was reported for the isolated b/c_1 of *Paracoccus* (Yang and Trumpower, 1986). Similarly the four subunits of the cytochrome b_6/f complex (Hurt and Hauska, 1981) are functionally equivalent to the three subunits of *Rhodobacter* b/c_1 complex.

Sequence Homology and Topology of the Three Redox Carriers

The following discussion on the primary structure of the b/c_1 subunits, derived from the DNA sequence analysis of the *fbc* operon, is based on the hydropathy profiles of the deduced amino acid sequences (Gabellini and Sebald, 1986) and on the homology with the corresponding polypeptides of mitochondria, chloroplasts, and other prokaryotes. Recently the comparison of the b/c_1 and b_6/f sequences available was extended to 22 cytochrome b, 12 cytochrome c_1 (or f) and 8 Rieske FeS protein (Hauska *et al.*, 1987). This study was particularly useful to identify essential residues and to evaluate evolutionary distances.

Cytochrome b

The structure of the cytochrome b has been most remarkably conserved among bacteria, mitochondria, and chloroplasts, such that the cytochrome b of *Rhodobacter* is similar to that of mitochondria, being a large polypeptide of 48 kDa, and is homologous with two subunits of chloroplasts cytochrome b_6/f complex, cytochrome b + 17-kDa polypeptides (Heinemayer *et al.*, 1984; Widger *et al.*, 1984). In Fig. 5 is shown an alignment for the maximal homology of the amino acid sequence of cytochrome b from *R. capsulatus* (Gabellini and Sebald, 1986) with the sequence of cytochrome b from yeast mitochondria (Nobrega and Tzagoloff, 1980) and with the sequence of cytochrome b_6 plus subunit IV of spinach chloroplasts (Heinemayer *et al.*, 1984). The N-terminal sequences of spinach b_6 and subunit IV have been corrected (Westhoff and Herrmann, unpublished).

There is 41% homology between the cytochrome b sequence from *R. capsulatus* with that from yeast mitchondria. The identity between the cytochrome b_6 and subunit IV from spinach chloroplasts is only 26%. This

result indicated that the cytochrome *b* of purple bacteria is more related to that of mitochondria than to the chloroplast one. The comparison of the cytochrome *b* sequence from two taxonomically related species of purple bacteria, *R. capsulatus* and *P. denitrificans* (Woese *et al.*, 1984), revealed 84% identity (Kurowski and Ludwig, 1987; not shown).

The polarity profile of the cytochrome *b* indicated nine possible membrane-spanning regions, which are marked in Fig. 5. The peaks of hydrophobicity of the prokaryotic sequence correspond to the position of homologous hydrophobic sequences from mitochondria and chloroplast cytochrome *b* and subunit IV, predicted to be transmembrane segments in α-helical conformation (Widger *et al.*, 1984; Saraste, 1984). In the chloroplast cytochrome b_6, the IX hydrophobic segment is missing. The fourth predicted membrane-spanning segment, corresponding to Pro-161 to Val-184 of *R. capsulatus* sequence, is rather polar and might not span the membrane. This is also suggested from the mapping of antibiotic resistant in the yeast cytochrome *b* gene (Colson and Slonimski, 1979). In the model including nine membrane-spanning segments, the resistance to diurone maps on regions located on both sides of the membrane. Supposing that the IV hydrophobic segment is not a trans-membrane span, all amino acids involved in diurone resistance would then become grouped together on one side of the membrane, in agreement with the Q-cycle mechanism were all the diurone resistance should be close to the Q_z binding pocket (see Crofts, 1987).

The comparison of the cytochrome *b* sequences from mitochondria and chloroplasts allowed the identification of the four conserved histidines postulated to be the ligands for the two hemes (Widger *et al.*, 1984; Saraste, 1984). These histidines are also conserved in the prokaryotic sequences (see Fig. 5). The histidines are located in two hydrophobic domains corresponding to membrane-spanning segments II and V, and are separated in both segments by 13 amino acid residues, with the exception of cytochrome b_6 in which there is an additional Thr in helix V. This variability could be the cause of the lower redox potential of cytochrome b_6 (Hauska *et al.*, 1987). The structural relationships of the four histidine ligands suggested that the two hemes are coordinately bound between hydrophobic spans II and V, and are located near opposite sides of the membrane, with the heme planes perpendicular, rather than parallel, to the plane of the membrane (Widger *et al.*, 1984; Saraste, 1984). An invariant proline residue might induce a twist between the heme ligand histidines of helix V, so that the helix II and V are arranged to an angle of approximately 30° to each other (Link *et al.*, 1986). This type of organization provides an explanation for the redox heterogeneity of the two hemes *b*.

It has been suggested that the highly conserved sequences Pro-Glu-Trp-Tyr 297 and Ile-Leu-Arg 306 (Fig. 5), occurring between the hydrophobic
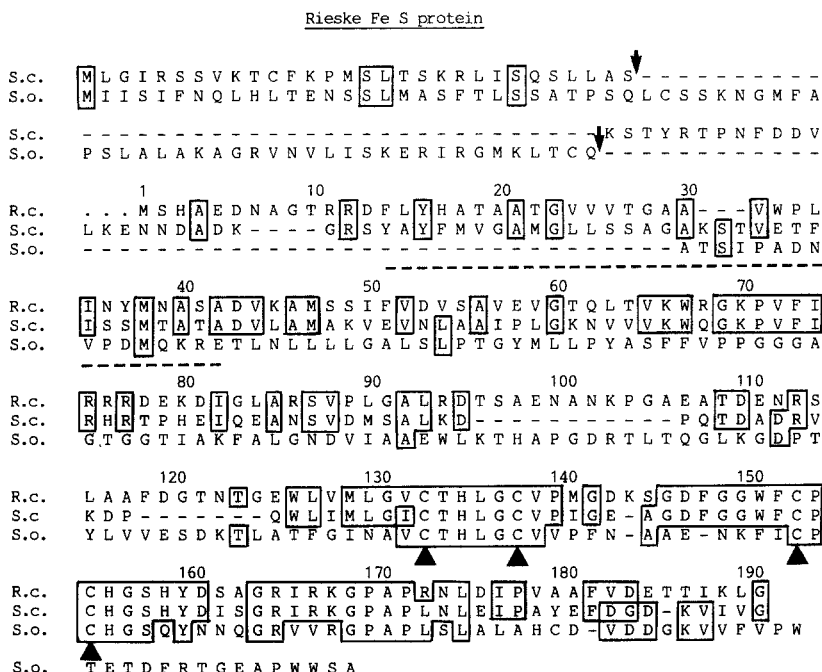
Rieske Fe S protein

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                                                                    ↓
S.c.    M L G I R S S V K T C F K P M S L T S K R L I S Q S L L A S - - - - - - - - - -
S.o.    M I I S I F N Q L H L T E N S S L M A S F T L S S A T P S Q L C S S K N G M F A

S.c.    - - - - - - - - - - - - - - - - - - - - - - - - - K S T Y R T P N F D D V
S.o.    P S L A L A K A G R V N V L I S K E R I R G M K L T C Q - - - - - - - - - - - -

             1              10              20              30
R.c.    . . . M S H A E D N A G T R R D F L Y H A T A A T G V V V T G A A - - - V W P L
S.c.    L K E N N D A D K - - - - G R S Y A Y F M V G A M G L L S S A G A K S T V E T F
S.o.    - - - - - - - - - - - - - - - - - - - - - - - - - A T S I P A D N

               40              50              60              70
R.c.    I N Y M N A S A D V K A M S S I F V D V S A V E V G T Q L T V K W R G K P V F I
S.c.    I S S M T A T A D V L A M A K V E V N L A A I P L G K N V V V K W Q G K P V F I
S.o.    V P D M Q K R E T L N L L L L G A L S L P T G Y M L L P Y A S F F V P P G G G A

               80              90              100             110
R.c.    R R R D E K D I G L A R S V P L G A L R D T S A E N A N K P G A E A T D E N R S
S.c.    R H R T P H E I Q E A N S V D M S A L K D - - - - - - - - - - - P Q T D A D R V
S.o.    G T G G T I A K F A L G N D V I A A E W L K T H A P G D R T L T Q G L K G D P T

               120             130             140             150
R.c.    L A A F D G T N T G E W L V M L G V C T H L G C V P M G D K S G D F G G W F C P
S.c     K D P - - - - - - - Q W L I M L G I C T H L G C V P I G E - A G D F G G W F C P
S.o.    Y L V V E S D K T L A T F G I N A V C T H L G C V V P F N - A A E - N K F I C P
                                                        ▲                          ▲

               160             170             180             190
R.c.    C H G S H Y D S A G R I R K G P A P R N L D I P V A A F V D E T T I K L G
S.c.    C H G S H Y D I S G R I R K G P A P L N L E I P A Y E F D G D - K V I V G
S.o.    C H G S Q Y N Q G R V V R G P A P L S L A L A H C D - V D D G K V V F V P W
         ▲

S.o.    T E T D F R T G E A P W W S A
```

Fig. 6. Alignment of the Rieske FeS protein sequence from *Rhodobacter capsulatus* (R.c.), *Saccharomyces cerevisiae* (S.c.), and *Spinacea oleracea* (S.o.). Identical amino acid residues are placed in boxes. The positions of leader sequence cleavage sites are marked by arrows. The hydrophobic sequence is underlined by broken lines. The four conserved cysteines that bind the FeS cluster are marked by triangles. The numbering refers to the R.c. sequence.

segments VI and VII, might be part of the quinol-oxidizing site, Q_o (Hauska *et al.*, 1987). Interestingly bacterial cytochrome *b* have an insertion immediately after this region. Furthermore it has been proposed that the conserved Tyr-247, and Phe-248 that is replaced with a Tyr in spinach (Fig. 5), could be involved in the quinone reductase site, Q_i, which is inhibited by antimycin A. The insensitivity of cytochrome b_6 to antimycin A could be due to the splitting of cytochrome b_6 and subunit IV in this region (Hauska *et al.*, 1987). The subunit IV of spinach includes an additional sequence at the N-terminus, which is partially shared by the prokaryotic cytochrome *b*. It is possible that the additional sequences contribute to the formation of Q_o and Q_i sites in bacteria, and the insertion at the Q_i sites might be functionally homologous to the Q-binding protein of mitochondria b/c_1 complex (Yu and Yu, 1981).

Rieske FeS Protein

Figure 6 shows the alignment of the Rieske FeS protein from *R. capsulatus* with the corresponding sequences from yeast mitochondria (Beckmann *et al.*,

1987) and spinach chloroplasts (Steppuhn *et al.*, 1987). The sequence from *R. capsulatus* shows 46% homology with the sequence from yeasts and only 14% homology with spinach chloroplasts. The Rieske FeS protein of *Paracoccus* (Kurowski and Ludwig, 1987, not shown) is 69% homologous with that of *Rhodobacter*.

The FeS protein sequences shown in Fig. 6 are not conserved in the vicinity of the N-terminus, and in bacteria the sequence is shorter than in mitochondria, but somewhat larger than in chloroplasts. The general topology of the protein, however, is conserved between these species. The hydropathy profile of the Rieske FeS protein of *Rhodobacter* showed that the polypeptide is largely exposed to the aqueous phase and has a short hydrophobic "anchor" near the N-terminus, indicated in Fig. 6. Such a topology is in agreement with the functional location of the polypeptide on the outer positive side of the cytoplasmic membrane. A similar topology was also deduced from the *Neurospora* FeS protein sequence (Harnisch *et al.*, 1985) and from the sequences from yeasts and spinach. At present it is unclear whether the N-terminal hydrophobic sequence forms a transmembrane span. Biochemical evidence indicates that the FeS protein is loosely bound to the b/c_1 complex, and this subunit could be anchored to the membrane only through protein–protein interactions. The most conserved portion of the polypeptide is located near the C-terminus and includes four cysteines which are the likely ligands for the 2Fe–2S cluster (Stout, 1982). The Cys residues are located in a moderately hydrophobic environment. The involvement of nitrogen in the binding of the iron of the 2Fe–2S cluster has been suggested for a Rieske-type FeS protein isolated from *Thermus thermophilus* (Fee *et al.*, 1984) and recently also for the Rieske protein of the mitochondrial b/c_1 complex (Tesler *et al.*, 1987). Indeed there are two highly conserved His in the C-terminal region of the protein. This moderately hydrophobic region, which carries the FeS cluster, is expected to be in the vicinity of the low redox potential heme b, to form the Q_o site. Many conserved charged residues found in the C-terminal region could be involved in the contact with hydrophilic segments of cytochrome b.

The central hydrophilic domain of the Rieske FeS protein shows considerable conservation between purple bacteria and mitochondria. It includes six conserved positively charged residues that could be responsible for the interaction with the more negative cytochrome c_1 at the reducing site of the FeS protein. The corresponding domain of the spinach FeS protein would interact with the cytochrome f and is not homologous with the b/c_1 type FeS proteins. Indeed the cytochrome reductase site of the b/c_1 complex is functionally different from the plastocyanin reductase site of the b_6/f complex.

Cytochrome c_1

The sequence homology between *R. capsulatus* cytochrome c_1 (Gabellini and Sebald, 1986), yeast mitochondria cytochrome c_1 (Sadler *et al.*, 1984), and spinach chloroplasts cytochrome *f* (Alt and Herrmann 1984) is shown in Fig. 7. The two cytochrome c_1 sequences show 31% identical residues, and the homology between this prokaryotic cytochrome c_1 and the cytochrome *f* is only 12%.

The hydropathy profile of the *Rhodobacter* cytochrome c_1 indicated that the polypeptide is largely hydrophilic. A short strength of amino acids near the C-terminus most probably forms a transmembrane α-helix that binds the polypeptide to the hydrophobic domain. A similar topology was also deduced from the hydropathy profile of mitochondrial cytochrome c_1 and chloroplast cytochrome *f*. The heme is covalently bound with the sulfur atoms of the side chains of residues Cys-55 and Cys-58. Two additional bonds connect the heme iron to a nitrogen atom of His-59 and to the sulfur atom of the conserved Met-205 (Fig. 7). The conservation of sequence and topology of the heme-binding sites between cytochrome c_1 and cytochrome *c* suggests that these two polypeptides are folded in a similar way (Dickerson and Timkovich, 1975). The structure of the heme-binding peptides of cytochrome c_1 have been highly conserved between *Rhodobacter* and mitochondria (Wakabayashi *et al.*, 1980; Sadler *et al.*, 1984). The sequence of *R. capsulatus* cytochrome c_1 is 53% homologous with that of *P. denitrificans* (Kurowski and Ludwig, 1987). The cytochrome c_1 of *Paracoccus* is larger and includes an additional sequence of about 150 residues, located near the N-terminus which is extremely acidic. A comparable structure was not found in the b/c_1 complex of the related *Rhodospirillaceae*. The negatively charged portion of *Paracoccus* cytochrome c_1 could have an analogous function to the hinge protein of mitochondrial b/c_1, mediating cytochrome c_1–c complex formation (Wakabayashi *et al.*, 1982).

Beside the heme-binding site, there is no significant homology between cytochrome c_1 and chloroplast cytochrome *f*, although the general topology of the polypeptides is conserved (Willey *et al.*, 1984; Alt and Herrmann, 1984). In general the subunits of the prokaryotic b/c_1 complex and of the b_6/f complex are larger than those of mitochondria. The cytochrome c_1 from *R. capsulatus* includes one insertion after the Ala 129 which is shared by the sequences from *P. denitrificans* c_1 and from spinach. A second insertion after Phe 180 is even larger in the chloroplast polypeptide (Fig. 7). The extra sequence elements could compensate for some functions that are performed by the numerous additional subunits of the mitochondrial reductase.
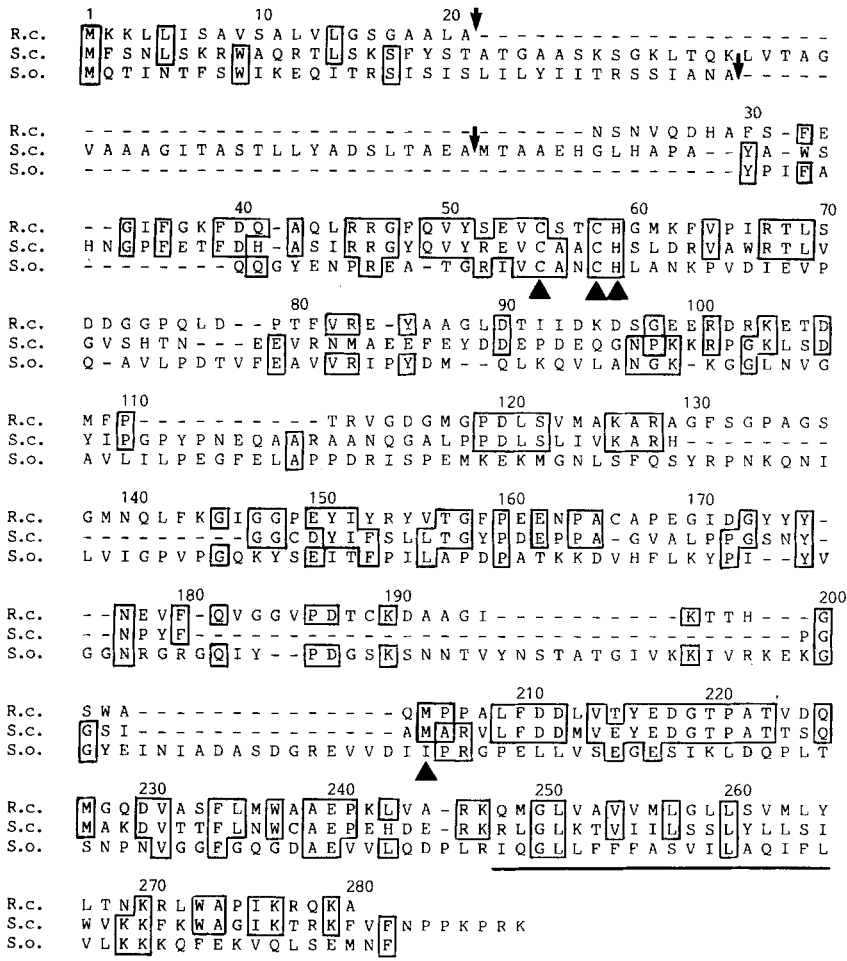
Cytochrome c_1 / f

```
         1                 10                  20 ▼
R.c.  M K K L L I S A V S A L V L G S G A A L A - - - - - - - - - - - - - - - - - -
S.c.  M F S N L S K R W A Q R T L S K S F Y S T A T G A A S K S G K L T Q K L V T A G
S.o.  M Q T I N T F S W I K E Q I T R S I S I S L I L Y I I T R S S I A N A V - - - - -

                                                                          30
R.c.  - - - - - - - - - - - - - - - - - - - - - ↓ - - - - - - N S N V Q D H A F S - F E
S.c.  V A A A G I T A S T L L Y A D S L T A E A M T A A E H G L H A P A - - Y A - W S
S.o.  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Y P I F A

           40                 50                  60                  70
R.c.  - - G I F G K F D Q - A Q L R R G F Q V Y S E V C S T C H G M K F V P I R T L S
S.c.  H N G P F E T F D H - A S I R R G Y Q V Y R E V C A A C H S L D R V A W R T L V
S.o.  - - - - - - - Q Q G Y E N P R E A - T G R I V C A N C H L A N K P V D I E V P
                                                ▲       ▲▲

             80                 90                  100
R.c.  D D G G P Q L D - - P T F V R E - Y A A G L D T I I D K D S G E E R D R K E T D
S.c.  G V S H T N - - - E E V R N M A E E F E Y D D E P D E Q G N P K K R P G K L S D
S.o.  Q - A V L P D T V F E A V V R I P Y D M - - Q L K Q V L A N G K - K G G L N V G

           110                120                 130
R.c.  M F P - - - - - - - - - T R V G D G M G P D L S V M A K A R A G F S G P A G S
S.c.  Y I P G P Y P N E Q A A R A A N Q G A L P P D L S L I V K A R H - - - - - - - -
S.o.  A V L I L P E G F E L A P P D R I S P E M K E K M G N L S F Q S Y R P N K Q N I

           140                150                 160                 170
R.c.  G M N Q L F K G I G G P E Y I Y R Y V T G F P E E N P A C A P E G I D G Y Y Y -
S.c.  - - - - - - - - - G G C D Y I F S L L T G Y P D E P P A - G V A L P G S N Y -
S.o.  L V I G P V P G Q K Y S E I T F P I L A P D P A T K K D V H F L K Y P I - - Y V

               180                190                          200
R.c.  - - N E V F - Q V G G V P D T C K D A A G I - - - - - - - - - K T T H - - - G
S.c.  - - N P Y F - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - P G
S.o.  G G N R G R G Q I Y - - P D G S K S N N T V Y N S T A T G I V K K I V R K E K G

                                              210                 220
R.c.  S W A - - - - - - - - - - - - - Q M P P A L F D D L V T Y E D G T P A T V D Q
S.c.  G S I - - - - - - - - - - - - - A M A R V L F D D M V E Y E D G T P A T T S Q
S.o.  G Y E I N I A D A S D G R E V V D I I P R G P E L L V S E G E S I K L D Q P L T
                                              ▲

           230                240                 250                 260
R.c.  M G Q D V A S F L M W A A E P K L V A - R K Q M G L V A V V M L G L L S V M L Y
S.c.  M A K D V T T F L N W C A E P E H D E - R K R L G L K T V I I L S S L Y L L S I
S.o.  S N P N V G G F G Q G D A E V V L Q D P L R I Q G L L F F F A S V I L A Q I F L

           270                280
R.c.  L T N K R L W A P I K R Q K A
S.c.  W V K K F K W A G I K T R K F V F N P P K P R K
S.o.  V L K K K Q F E K V Q L S E M N F
```

Fig. 7. Alignment of the cytochrome c_1 sequence from *Rhodobacter capsulatus* (R.c.), *Saccharomyces cerevisiae* (S.c.), and the cytochrome *f* sequence from *Spinacea oleracea* (S.o.) Identical residues are placed in boxes, and the cleavage sites of leader peptides are indicated by arrows. The hydrophobic sequence probably forming a trans-membrane span is underlined. The position of the two cysteines and of the histidine that covalently bind the heme is indicated by triangles, as well as the conserved methionine identified as the sixth ligand to the heme iron in the c-type cytochromes. The numbering refers to the R.c. sequence.

Evolutionary Considerations

Analysis of the homologies between the ancestral structure of the prokaryotic b/c_1 complex and the relative eukaryotic structures allows one to propose an evolutionary pathway for this enzyme.

The sequences that have been strictly conserved between bacteria, mitochondria, and chloroplasts are found in the cytochrome b and in the C-terminal region of the FeS protein. These sequences form the catalytic sites for the electrogenic redox reactions with the quinone (Q_o and Q_i) and are the most conserved structures of the oxidoreductase and most probably arose only once during evolution.

The cytochrome reductase site, which consists of cytochrome c_1 and probably of the central hydrophilic domain of the FeS protein, could tolerate more amino acid substitutions than Q_o and Q_i. The cytochrome reductase site is not homologous with the plastocyanin reductase site formed by the cytochrome f and the central domain of the FeS protein of cyanobacteria and chloroplasts. Possibly the reductase site underwent divergent evolution in the two phylogenetic lines of cyanobacteria and purple bacteria, or it arose by convergent evolution of diverse genes. Probably the capacity of aerobic respiration was developed among purple photosynthetic bacteria. In these organisms the b/c_1 segment of the photosynthetic electron transport chain was also used for aerobic respiration. The b/c_1 complex can therefore be considered as one of the most ancient components of respiratory chains.

The evaluation of the homology suggests that the b/c_1 complex of purple photosynthetic bacteria is the ancestor of mitochondrial complex III. This result further supports the hypothesis that mitochondria arose by endosymbiosis of prokaryotic entities related to purple bacteria. During evolution the minimal catalytic structure of the prokaryotic oxidoreductase became more elaborate. Besides the three redox polypeptides, the b/c_1 complex of mitochondria includes six to eight additional subunits whose functional significance is still uncertain. All three subunits of the mitochondrial b/c_1 complex carry insertions that could compensate for the lack of additional subunits.

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