***Rhodobacter capsulatus* genes encoding form I ribulose-1,5-bisphosphate carboxylase/oxygenase (*cbbLS*) and neighbouring genes were acquired by a horizontal gene transfer**

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Analysis of the nucleotide sequence of the form I ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) genes (*cbbL* and *cbbS*) of the non-sulfur purple bacterium *Rhodobacter capsulatus* indicated that the deduced amino acid sequence of the large subunit was not closely homologous to the large subunit from related organisms. Indeed, phylogenetic analysis suggested that the large subunit protein (CbbL) more closely resembled the enzyme from α / β / γ purple bacteria and cyanobacteria and is within a 'green-like' radiation of the RubisCO phylogenetic tree, well separated from CbbL of the related organism *Rhodobacter sphaeroides*. A *cbbQ* gene was discovered downstream of *cbbS* in *Rh. capsulatus*, a gene arrangement which also appears to be limited to certain organisms containing a 'green-like' RubisCO. Upstream, and divergently transcribed from *cbbLSQ*, is a gene (*cbbR*) that encodes a LysR-type transcriptional activator. Phylogenetic analysis of the deduced amino acid sequence of CbbR, also suggests that this protein is quite distinct from the *Rh. sphaeroides* CbbR protein, and is even distinct from the previously described CbbR_{II} protein, the gene of which is upstream and divergently transcribed from the *cbb_{II}* operon of *Rh. capsulatus*. Interestingly, *Rh. capsulatus* CbbR_I is more closely related to CbbR from bacteria whose RubisCO falls within the 'green-like' radiation of the CbbL tree. These studies suggest that the *cbbR*–*cbbL*–*cbbS*–*cbbQ* genes were acquired by *Rh. capsulatus* via horizontal gene transfer from a bacterial species containing a 'green-like' RubisCO.

Keywords: RubisCO, *Rhodobacter capsulatus*, phylogeny, *cbbR*, *cbbQ*

INTRODUCTION

Rhodobacter capsulatus, a non-sulfur purple photosynthetic bacterium, assimilates CO₂ via the Calvin–Benson–Bassham (CBB) reductive pentose phosphate pathway. The CBB enzyme responsible for fixing CO₂ is ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), an enzyme which exists in two distinct forms. The form I RubisCO is a large hexadecameric

enzyme (L₈S₈) composed of eight large subunits ($M_r \sim 55\,000$) and eight small subunits ($M_r \sim 15\,000$) which in proteobacteria are encoded by the *cbbL* and *cbbS* genes, respectively. The form II RubisCO, encoded by the *cbbM* gene (Gibson, 1995), is composed of large subunits only (L₈); these are biochemically and genetically distinct from form I large subunits. The non-sulfur purple photosynthetic bacterium *Rhodobacter sphaeroides* was the first organism shown to synthesize both forms of RubisCO (Gibson & Tabita, 1977a) and the organization and regulation of the genes encoding CBB enzymes (*cbb* genes) have been intensely studied in *Rh. sphaeroides* and other autotrophic bacteria (Gibson & Tabita, 1996).

The organization of the *cbb* genes in the closely related

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Abbreviation: RubisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase.

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organism *Rh. capsulatus* was determined recently (Paoli *et al.*, 1995). The *Rh. capsulatus* form II RubisCO gene, *cbbM*, has been sequenced (Larimer *et al.*, 1995) and found to encode a protein that is very similar to the form II RubisCO from *Rh. sphaeroides*. As for the form I enzyme, earlier studies had indicated that the *Rh. capsulatus* form I RubisCO exhibited properties that were distinct from its counterpart in *Rh. sphaeroides*, as noted by differences in its elution behaviour from anion-exchange columns and by differences in antibody cross-reactivity (Gibson & Tabita, 1977b). More recently, recombinant *Rh. capsulatus* form I RubisCO was shown to cross-react with antibodies directed against cyanobacterial RubisCO, with little or no reactivity to anti-*Rh. sphaeroides* form I RubisCO in Western blot experiments (Paoli *et al.*, 1995). In the hope of gaining a better understanding of its unusual properties, the current study was undertaken to determine the primary structure of the *Rh. capsulatus* form I RubisCO as well as upstream and downstream sequences.

The unique *cbb* gene organization and subsequent phylogenetic analyses of the *Rh. capsulatus* CbbL and putative upstream transcriptional activator CbbR₁ indicated that the genes encoding *Rh. capsulatus* form I RubisCO and neighbouring genes were acquired by a horizontal gene transfer.

METHODS

DNA sequencing and analysis. The sequence of the *Rh. capsulatus cbbLSQ* genes from cosmid pRCFI was determined by automated sequencing using an ABI 377a DNA sequencer

with ABI Taq DyeDeoxy Cycle sequencing reagents and a Perkin-Elmer Cetus thermal cycler, as described by Perkin-Elmer. Plasmids pRKFI, pK18FISB, pK18FI5EH, pK18FI6E, pK18FI7EH, pULHSm531 and pULHSm320 (Table 1) were used, and, as necessary, oligonucleotide primer walking was employed to generate complete double-stranded sequence. Synthetic oligonucleotides were purchased from Integrated DNA Technologies. Because of the unusual nature of the *Rh. capsulatus cbbLS* sequence, the identity of the *cbbL* gene was confirmed from partial sequencing of clones derived from two additional sources (pEULA4 and pK1H2E7; Fig. 1, Table 1). The nucleotide sequence of *Rh. capsulatus cbbR₁* and *cbbL*, from plasmids pEULA4 and pK1H2E7, was determined using an ABI Prism 310 Genetic Analyzer. The M13/pUC forward 23 base sequencing primer (5'-CCCAGTCACGACGTTGT-AAAACG-3'), M13 reverse (-48) primer (5'-AGCGGATA-ACAATTTTCACACAGGA-3') and sequence-specific synthetic primers were used to complete the double-stranded sequence. Sequences were analysed using the University of Wisconsin Genetics Computing Group (GCG) software, the EGCG Extension Programs (The Sanger Centre, Hinxton, England) and MacVector Sequence Analysis software (International Biotechnology, Inc., New Haven, CT, USA).

Phylogenetic analysis of *Rh. capsulatus* RubisCO and CbbR protein sequences. Multiple sequence alignments were performed using CLUSTAL W (Thompson *et al.*, 1994) and trees were generated by neighbour-joining and maximum parsimony methods using PHYLIP 3.5c (Felsenstein, 1989) and by maximum likelihood using Puzzle 2.5.1 (Strimmer & von Haeseler, 1996). Accession numbers (SWISS-PROT or GenBank databases) of sequences used for phylogenetic analysis of form I RubisCO large subunit proteins (CbbL or RbcL) are as follows: *Anabaena* sp. PCC 7120, P00879; *Chlamydomonas reinhardtii*, P00877; *Chromatium vinosum*

Table 1. Plasmids used in this study

Plasmid	Relevant characteristics	Source/reference
pK18, pK19	Km ^R ; pUC derivatives	Pridmore (1987)
pRKFI	pRK415 containing the 9 kb <i>Pst</i> I fragment of pRCFI containing the <i>Rh. capsulatus cbbLS</i> genes	Paoli <i>et al.</i> (1995)
pK18FISB	pK18 containing part of the <i>Rh. capsulatus cbbL</i> gene on a 1.8 kb <i>Bam</i> HI– <i>Sal</i> I fragment cloned from pRKFI	This work
pK18FI5EH	pK18 containing part of the <i>Rh. capsulatus cbbL</i> gene on a 0.5 kb <i>Hind</i> III– <i>Eco</i> RI fragment cloned from pK18FISB	This work
pK18FI6E	pK18 containing part of the <i>Rh. capsulatus cbbL</i> gene on a 0.6 kb <i>Eco</i> RI fragment cloned from pK18FISB	This work
pK18FI7EH	pK18 containing part of the <i>Rh. capsulatus cbbL</i> gene on a 0.7 kb <i>Eco</i> RI– <i>Hind</i> III fragment cloned from pK18FISB	This work
pEULA4	pK19 containing part of the <i>Rh. capsulatus cbbL</i> gene and upstream sequence on a 4 kb <i>Eco</i> RI fragment	This work
pULHSm531	pK19 containing part of the <i>Rh. capsulatus cbbL</i> gene and upstream sequence on a 0.5 kb <i>Hind</i> III– <i>Sma</i> I fragment cloned from pEULA4	This work
pULHSm320	pK19 containing part of the <i>Rh. capsulatus cbbR₁</i> sequence on a 0.36 kb <i>Hind</i> III– <i>Sma</i> I fragment cloned from pEULA4	This work
p1H2	A <i>cbbL</i> -hybridizing clone derived from an <i>Rh. capsulatus</i> SB1003 overlapping cosmid library	Fonstein <i>et al.</i> (1993)
pK1H2E7	pK18 containing part of the <i>Rh. capsulatus cbbL</i> gene sequence on a 0.7 kb <i>Eco</i> RI fragment cloned from p1H2	This work

1, P22849; *Chromatium vinosum* 2, P22859; *Cryptomonas* Φ , P14957; *Cyanophora paradoxa*, P24312; *Cuscuta reflexa*, P30401; *Cylindrotheca* sp. N1, P24673; *Euglena gracilis*, P00878; *Hydrogenovibrio marinus* 1, D43621; *Hydrogenovibrio marinus* 2, D43622; *Ipomoea purpurea*, P28260; *Mollugo verticillata*, P25832; *Nicotiana tabacum*, P00876; *Nitrobacter vulgaris*, L22885; *Odontella sinensis*, P49520; *Olisthodiscus luteus*, P14959; *Porphyra purpurea*, P50255; *Prochlorococcus marinus*, D21833; *Prochloron* sp., D21834; *Prochlorothrix hollandica*, P27586; *Pseudomonas hydrognotermophila*, D30764; *Rh. capsulatus*, L8200; *Rh. sphaeroides*, P27997; *Ralstonia eutropha* ATCC 17707 (chromosomal), M17744; *Ralstonia eutropha* ATCC 17699 (chromosomal), P09657; *Ralstonia eutropha* ATCC 17699 (plasmid), U20585; *Spinacia oleracea*, P00875; *Synechococcus* sp. PCC 6301, P00880; *Synechococcus* sp. PCC 7002, D13971; *Synechococcus* sp. WH7803, U46156; *Thiobacillus denitrificans*, L42920; *Thiobacillus ferrooxidans*, P28895; Vent worm (*Alvinococcha*) symbiont (potential *Thiobacillus* sp.), P24672; *Triticum aestivum*, P11383; *Xanthobacter flavus*, P23011.

Accession numbers of sequences used for phylogenetic analysis of form II RubisCO large subunit proteins (CbbM or RbcL) are as follows: *Gonyaulax polyedra*, LL41063; *Hydrogenovibrio marinus*, D28135; *Rh. capsulatus*, P50922; *Rh. sphaeroides*, P29278; *Rhodospirillum rubrum*, P04718; *Symbiodinium* sp., U43532; *T. denitrificans*, L37437.

Accession numbers of sequences used for phylogenetic analysis of CbbR proteins are as follows: *Chr. vinosum* (CbbR), P25544; *Cyanophora paradoxa* (plastid) (YCF30/RbcR), P48271; *Odontella sinensis* (plastid) (YCF30/RbcR), P49518; *Porphyra purpurea* (plastid) (YCF30/RbcR), P51205; *Ra. eutropha* (CbbR), P42722; *Rh. capsulatus* (AmpR), P14145; *Rh. capsulatus* (CbbR_I), U87283; *Rh. capsulatus* (CbbR_{II}), U87282; *Rh. sphaeroides* (CbbR), P52690; *Rs. rubrum* (CbbR), P52595; *Synechocystis* sp. PCC 6803 (RbcR1), CyanoBase ORF ID = SLL0030; *Synechocystis* sp. PCC 6803 (RbcR2), CyanoBase ORF ID = SLL1594; *T. ferrooxidans* (CbbR), Q06610; *X. flavus* (CbbR), P25545.

RESULTS

Organization of the *Rh. capsulatus cbbLS* genes and neighbouring regions

In a previous study, gene-specific probes and a *cbbL*-hybridizing *Rh. capsulatus* library clone indicated that the *Rh. capsulatus cbbLS* genes, encoding form I RubisCO, were not associated with any other known *cbb* structural genes (Paoli *et al.*, 1995). DNA sequence analysis of subclones derived from *cbbLS* containing cosmid pRCFI revealed that the 31 kb chromosomal DNA insert in this library clone contained only 100 bp *Rh. capsulatus* DNA upstream of the *cbbL* gene. A 4 kb *EcoRI* fragment containing part of the *Rh. capsulatus cbbL* gene and upstream DNA was cloned from the *Rh. capsulatus* SB1003 chromosome into pK19 (pEULA4; Fig. 1) to determine if other *cbb* genes were present upstream of the form I RubisCO genes. The organization of *cbbLS* and the neighbouring region is presented in Fig. 1. Nucleotide sequencing revealed an open reading frame (ORF) with a deduced amino acid sequence similar to the CbbR class of LysR-type transcriptional activators upstream and divergently transcribed from *cbbL*; in addition, the previously

identified *anfA* gene (Kutsche *et al.*, 1996) was found to be about 2 kb downstream of *cbbR_I*. The subscript I indicates that the divergently transcribed *cbbR_I* gene is associated with the form I RubisCO genes. A second *cbbR* gene, *cbbR_{II}*, is present upstream and divergently transcribed from a group of *cbb* genes associated with the form II RubisCO gene of *Rh. capsulatus* (Paoli *et al.*, 1995). Additional nucleotide sequencing indicated that no other known *cbb* genes were present in the region between *cbbR_I* and *anfA* (J. L. Gibson, unpublished). Sequencing downstream of *cbbS* revealed an ORF with a predicted amino acid sequence having significant similarity to the *cbbQ* gene product from *Ps. hydrognotermophila* (Yokoyama *et al.*, 1995).

Nucleotide sequence of *cbbR_I*, *cbbL*, *cbbS* and *cbbQ*

The nucleotide sequence for both strands of a 3993 nucleotide region including the form I RubisCO genes and approximately 1 kb downstream (GenBank accession no. L82000) and approximately 1 kb upstream (GenBank accession no. U87283) of *cbbLS* was determined. An ORF of 897 nucleotides, 110 nucleotides upstream of *cbbL* and divergently transcribed from *cbbL*, extends from an ATG start codon at position 948 to a TGA stop codon at position 52. The ORF is preceded by a potential ribosome-binding site, GGA, 11 bp upstream of the putative start codon. The coding region is 298 amino acids long with a deduced *M_r* of 31766. A database search for proteins with amino acid homology revealed that the product of this ORF is most similar to members of the CbbR family of LysR-type transcriptional activators. In a number of autotrophic bacteria (Gibson & Tabita, 1996), *cbbR* is divergently transcribed from the *cbb* genes and in several cases CbbR has been shown to activate transcription of the associated *cbb* genes (Gibson & Tabita, 1993; Kusano & Sugawara, 1993; van den Bergh *et al.*, 1993; Viale *et al.*, 1991; Windhovel & Bowien, 1991). Recently, genome sequencing projects have revealed ORFs whose products have homology to CbbR, including those in the cyanobacterium *Synechocystis* sp. PCC 6803, the plastids of the non-green algae *Odontella sinensis* and *Porphyra purpurea*, and the plastids of the glaucophyte *Cyanophora paradoxa*. In addition, *Rh. capsulatus* CbbR_I shares 51.0% deduced amino acid sequence identity with CbbR from *T. ferrooxidans*, but only 42.7% identity with *Rh. capsulatus* CbbR_{II}, and 37.2% identity with *Rh. sphaeroides* CbbR.

Rh. capsulatus cbbL starts at position 1058 and is preceded by a ribosome-binding site five nucleotides upstream of the ATG start codon. A T-N₁₁-A motif has been identified as a common feature of the binding site for LysR-type transcriptional activators (Goethals *et al.*, 1992); this motif usually has a 3 bp inverted repeat at each end and is often repeated two or three times upstream of the regulated gene or operon. Three such T-N₁₁-A motifs were found in the *cbbR_I*-*cbbL* intergenic region. The *cbbL* gene was shown to be 1422 nucleotides in length and to encode a protein of 473 amino acids

Fig. 1. Genetic and physical map of the *Rh. capsulatus* *cbb*₁ gene cluster. Arrows below the genes indicate the size and direction of potential transcripts. Restriction enzymes sites: E, *Eco*RI; H, *Hind*III; P, *Pst*I; S, *Sal*I; Sm, *Sma*I; X, *Xho*I. The thicker lines represent cosmid DNA that was subcloned along with the *Rh. capsulatus* chromosomal DNA in the original library clones. Only 100 bp *Rh. capsulatus* chromosomal DNA is present upstream of the *cbbL* gene in pRKFIIP. pEULA4 is a 4 kb *Eco*RI fragment cloned directly from *Rh. capsulatus* chromosomal DNA. pK1H2E7 contains a 700 bp *Eco*RI fragment subcloned from cosmid p1H2 (Fonstein *et al.*, 1993). As indicated, the insert in plasmid pK1H2E7 is in part cosmid DNA, because cosmid p1H2 did not contain the entire *cbbL* gene. The *Sau*3A (S3) site at the end of the *Rh. capsulatus* chromosomal DNA insert in p1H2 is shown.

Rcap	MTDPTQPFRIAPAEWYRPADEIALFEAAHAARMPVMLKGTGCGKTRFVEHMAWRLGKPLVTVACNEDMTASDL	75
Phyd	-MDLRNQYLVRSEPYHYAGVDEIERFEAAAYANRIPMLKGTGCGKSRFVEYMAWLGKPLITVACNEDMTAADL	74
Paer	-----MRDATPFYEATGHEIEVFERAWRHGLPVLLKGTGCGKTRFVQYMARRELEPLYSVACHDDLGAADL	67
	* * * * *	
Rcap	VGRFLLDATGTRWQDGLTFAARHGAICYLDEVVEARQDTTIAIHPLTDNRRLVPLEKKGELLRAHPDFQLVVISY	150
Phyd	VGRFLLDKEGTRWQDGLTFAARHGAICYLDEVVEARQDTTIVIHPLTDHRRILPLDKKGEVVEAHPDFQLVVISY	149
Paer	LGRHLIGADGTWQDGLTFAARHGAICYLDEVVEARQDTTIVAIHPLADRRRELYLERTGETLQAPSPFMLVVSY	142
	* * * * *	
Rcap	NPQYQALMKDLKQSTKQRFALDPTWPEHGEVEIVAHETGIDPALAQKLVAIAERARNLKGHGLDEGISTRMLV	225
Phyd	NPQYQAMKDLKSTKQRFALDPTWPEHGEVEIVAHESGVDAATAKKLVEVAIRSRHLKGHGLDEGISTRLLV	224
Paer	NPQYQNLKGLKPSRQRFALDPTWPEHGEVEIVAHESGVDAATAKKLVEVAIRSRHLKGHGLDEGISTRLLI	217
	* * * * *	
Rcap	HAGGLIAQGVAPLAACRMALVRPITDDPMDALDAAVTTF- 267	
Phyd	YAGSLITKGIAPLIACEMALICPITDDPDLRYALRAAAQTLFA 267	
Paer	FAARLIGDGMDPREACRVALAEPLSDPATVAALMDIVDLHVA 260	
	* * * * *	

Fig. 2. CbbQ amino acid sequence alignment. Amino acid alignment of the presumptive CbbQ of *Rh. capsulatus* (Rcap) compared to CbbQ of *Ps. hydrogenothermophila* (Phyd) and *Ps. aeruginosa* NirQ (Paer). The putative P-loop nucleotide-binding motif [(G/A) X₂GK(S/T)] (Walker *et al.*, 1982) is indicated by the dark bar over the alignment.

with a predicted M_r of 53036. The highest sequence identity (86.0%) was found with CbbL of *Ps. hydrogenothermophila*. Significantly less identity (57.8%) was noted when the *Rh. capsulatus* CbbL was compared to the *Rh. sphaeroides* form I RubisCO large subunit. This is particularly interesting in light of earlier reports which indicated that the *Rh. capsulatus* form I RubisCO was immunologically distinct from that of the closely related organism *Rh. sphaeroides* (Gibson & Tabita, 1977b; Paoli *et al.*, 1995). The *cbbL* sequence was obtained from subclones of pRCFI (Table 1, Fig. 1), a cosmid clone from an *Rh. capsulatus* SB1003 chromosomal DNA library (Scolnik & Haselkorn, 1984). Because of the unusual sequence of the *cbbL* gene, the sequence was confirmed by analysing clones from two additional sources. Portions of the *cbbL* gene present on the *Rh. capsulatus* SB1003 chromosomal DNA fragment in plasmid pEULA4 (Table 1, Fig. 1) and a subclone (pK1H2E7; Fig. 1, Table 1) from a cosmid clone obtained from an *Rh. capsulatus* SB1003 overlapping cosmid library (Fonstein *et al.*, 1993) were also sequenced. The sequence derived from these clones was identical to the sequence obtained from the pRCFI subclones.

An ORF encoding the *Rh. capsulatus* form I RubisCO small subunit starts at an ATG codon 22 nucleotides downstream of the *cbbL* stop codon and a potential ribosome-binding site was present six nucleotides upstream of the *cbbS* start codon. The *Rh. capsulatus* CbbS protein was predicted to be 118 amino acids in length, with an M_r of 13548. The highest sequence identity, 67.8%, was to *T. denitrificans* CbbS. The *Ps. hydrogenothermophila* CbbS was 60.7% identical and CbbS from the closely related organism *Rh. sphaeroides* shared only 33.0% identity. Two inverted repeat sequences that might function in transcriptional termination were found downstream of *cbbS*. Further downstream of *cbbS* was an ORF predicted to encode a protein of 267 amino acids with an M_r of 29429. The putative ATG codon at position 2958 was 100 nucleotides downstream of the *cbbS* stop codon and preceded by a plausible ribosome-binding site. The deduced amino acid sequence of the product of this ORF was 72.0% identical to CbbQ of *Ps. hydrogenothermophila* and 52.3% identical to *Pseudomonas aeruginosa* NirQ. Alignment of the predicted amino acid sequence of the *Rh. capsulatus* CbbQ with the *Ps. hydrogenothermophila* CbbQ and *Ps. aeruginosa* NirQ (Fig. 2) revealed

Fig. 4. Multiple sequence alignment of CbbR conserved regions. The large dark bar represents the CbbR polypeptide. The open box indicates the region of the CbbR deduced amino acid multiple sequence alignment used for phylogenetic analysis. The aligned amino acid sequences of the two most conserved regions are shown. The sequences are labelled as follows: 6803 1, *Synechocystis* sp. 6803 RbcR1; 6803 2, *Synechocystis* sp. 6803 RbcR2; Reut, *Ra. eutropha* CbbR; Cpar, *Cyanophora paradoxa* YCF30/RbcR; Cvin, *Chr. vinosum* CbbR; Osin, *Odontella sinensis* YCF30/RbcR; Ppur, *Porphyra purpurea* YCF30/RbcR; Rcap I, *Rh. capsulatus* CbbR_I; Rcap II, *Rh. capsulatus* CbbR_{II}; Rrub, *Rs. rubrum* CbbR; Rrph, *Rh. sphaeroides* CbbR; Tfer, *T. ferrooxidans* CbbR; Xfla, *X. flavus* CbbR.

tree topology. Trees with similar topology were generated by maximum parsimony and maximum likelihood analysis.

Phylogenetic analysis of CbbR transcriptional activators

Like the *Rh. capsulatus* form I RubisCO large subunit, the CbbR_I sequence showed a significantly lower similarity to *Rh. sphaeroides* CbbR than to CbbR from more distantly related organisms. Thus, the possibility that *Rh. capsulatus* CbbR_I has a similar phylogeny to that of the *Rh. capsulatus* form I RubisCO was investigated.

A phylogenetic analysis of 50 LysR-type transcriptional activators revealed that the CbbR class of LysR-type proteins grouped together (not shown). Analysis of the LysR-type proteins was carried out using the N-terminal region of the amino acid sequence which includes the functionally conserved helix–turn–helix DNA-binding domain. The region of the amino acid sequences used for tree building was a 145 residue sequence alignment generated by CLUSTAL W (Fig. 4). Alignment of amino acid sequences of 50 LysR-type proteins revealed no absolutely conserved residues within this region and only four residues that were represented by conservative substitutions (data not shown). A multiple sequence alignment of the CbbR deduced amino acid sequences revealed additional sequence similarity within this class of LysR-type transcriptional activator (Fig. 4). Most of the sequence similarity was limited to the helix–turn–helix motif and a second relatively conserved region of 36 residues corresponding to a previously recognized C-terminal domain (Schell, 1993). The C-terminal domain is thought to be involved in ligand binding or multimerization required for DNA binding (Schell, 1993).

Fig. 5. CbbR phylogenetic tree. A multiple sequence alignment of 145 amino acids [corresponding to the region of the CbbR polypeptide indicated by the open box (Fig. 4), amino acids Q7–G148 of *Rh. capsulatus* CbbR_I] was generated by CLUSTAL W. The CbbR tree and bootstrap values were produced by the methods detailed in the legend to Fig. 3. The tree was rooted using *Rh. capsulatus* AmpR. The relationship of *Chr. vinosum* CbbR to the other CbbR proteins varied when different phylogenetic analyses were used. It was thus excluded from the analysis presented to prevent giving undue weight to a single topology.

The results of a neighbour-joining analysis of CbbR proteins (Fig. 5) shows the phylogenetic relationship between *Rh. capsulatus* CbbR_I and *T. ferrooxidans*

CbbR in addition to the more distant branching of the *Rh. sphaeroides* CbbR, which is itself closely related to *Rh. capsulatus* CbbR_{II}. This tree is based on the CLUSTAL W alignment of the N-terminal region and trees with identical topology were generated from an alignment of the complete CbbR sequences. In addition, maximum parsimony and maximum likelihood analyses generated trees of the same topology. The CbbR phylogenetic tree (Fig. 5) is in good agreement with the RubisCO phylogenetic tree (Fig. 3) in that *Rh. capsulatus* CbbR_I is on a branch with CbbR from the 'green-like' RubisCO-containing bacterium *T. ferrooxidans* and the cyanobacterium *Synechocystis* sp. strain PCC 6803. It should be pointed out that the two CbbR homologues from *Synechocystis* sp. strain PCC 6803, and those encoded by red plastid genomes, are not transcribed divergently from *cbb* genes; thus, their classification as CbbR/RbcR proteins is based solely on the similarity of their deduced amino acid sequences to other CbbR proteins. The deep branching of the *Synechocystis* RbcR proteins (Fig. 5) suggests that they may well be CbbR homologues, despite the fact that neither of the genes which encode these proteins is divergently transcribed from *cbb* genes. In contrast, the red-plastid-encoded putative RbcR proteins form a separate branch in the CbbR tree. In fact, the red plastid RbcR sequences grouped within the CbbR family branch of the neighbour-joining and maximum likelihood LysR trees, but fell outside the CbbR family branch in a maximum parsimony LysR tree (not shown). Thus, the phylogenetic association of the red plastid RbcR proteins with the other CbbR proteins is tentative.

DISCUSSION

The nucleotide sequence of the genes encoding the *Rh. capsulatus* form I RubisCO was determined and additional *cbb* genes were found upstream and downstream from *cbbLS*. The *cbbR_I* gene was found upstream and divergently transcribed from *cbbL*, and is the second *cbbR* gene found in *Rh. capsulatus*. The other *cbbR* gene, now designated *cbbR_{II}*, is upstream and divergently transcribed from the *Rh. capsulatus* *cbb_{II}* operon (Paoli *et al.*, 1995). *T. denitrificans* has two *cbbR* genes (Lorbach & Shively, 1995) and, in this organism, *cbbR_I* and *cbbR_{II}* are directly upstream and divergently transcribed from the *cbbLS* and *cbbM* genes, respectively. More detailed experiments have shown that a single CbbR regulates the expression of at least two *cbb* operons in *Rh. sphaeroides* (Gibson & Tabita, 1993) and the chromosomal and plasmid *cbb* operons of *Alcaligenes eutrophus* (now called *Ralstonia eutropha*) (Windhovel & Bowien, 1991). In *X. flavus*, CbbR is required for the regulated expression of the *cbb* operon (van den Bergh *et al.*, 1993) and at least one other operon (Meijer *et al.*, 1996). The presence of two CbbR proteins in *Rh. capsulatus* could allow independent regulation of the *cbb* operons of this organism. If CbbR_I and CbbR_{II} bind different inducer molecules, these proteins might allow the expression of the two *cbb* operons to be regulated by different environmental or metabolic sig-

nals. Thus, it will be interesting to determine the role of each CbbR in *cbb* gene expression in *Rh. capsulatus*.

A gene encoding an apparent CbbQ protein was found downstream of *cbbS* in *Rh. capsulatus*. Likewise, the *cbbQ* gene is immediately downstream from *cbbS* in both *Ps. hydrogenothermophila* and *Chr. vinosum* (Yokoyama *et al.*, 1995). CbbQ shares considerable amino acid homology with the NirQ protein of *Pseudomonas* spp. (Yokoyama *et al.*, 1995; Fig. 2). The *nirQ* genes of *Ps. aeruginosa* (Arai *et al.*, 1994) and *Pseudomonas stutzeri* (Jungst & Zumft, 1992) are within a cluster of genes encoding proteins required for nitrite respiration. A strain of *Ps. stutzeri* in which *nirQ* had been disrupted lacked nitrite reductase and nitric oxide reductase activity, despite the fact that the proteins were still synthesized, suggesting that NirQ may be involved in post-translational assembly or modification of the *nir* gene products (Jungst & Zumft, 1992). A function has not yet been assigned to CbbQ, but preliminary investigations indicate that it may be involved in the post-translational activation of RubisCO (Igarashi & Kodama, 1996). In this respect, the association of the *cbbQ* and *cbbLS* genes in *Rh. capsulatus* is very interesting. Since it is feasible to construct genetic knock-out strains of *Rh. capsulatus* the function of CbbQ may eventually be discerned.

The assertion that *Rh. capsulatus* form I RubisCO was acquired by horizontal gene transfer is not the first suggestion that such events have taken place. The incongruence of the RubisCO phylogenetic tree with the 16S rRNA phylogenetic tree necessitates at least three (now four) horizontal transfers within the proteobacteria (Delwiche & Palmer, 1996; Watson & Tabita, 1996, 1997). However, the evidence that *Rh. capsulatus* form I RubisCO was acquired by such a mechanism is far more compelling than for any other presumed bacterial RubisCO gene transfer event. *Rh. sphaeroides* and *Rh. capsulatus* have been grouped in the same genus based on classical morphological and chemotaxonomic properties (Imhoff *et al.*, 1984). These two species have been found to be closely related based on phylogenetic analysis of their 16S rRNA (Woese *et al.*, 1984), 5S rRNA (Boulygina *et al.*, 1993), 23S rRNA (Ludwig *et al.*, 1995), cytochrome *c* (Dickerson, 1980; Woese *et al.*, 1980) and reaction centre L and M proteins (Blankenship, 1992). Even the form II RubisCO amino acid sequences of *Rh. capsulatus* and *Rh. sphaeroides* are more than 94% identical and show a close phylogenetic relatedness. Nevertheless, the form I RubisCO proteins from these two organisms are very distantly related (Fig. 3).

The phylogenetic analysis of the *Rh. capsulatus* form I RubisCO is further supported by its unusual immunological properties. The enzyme cross-reacts poorly with antibody raised against *Rh. sphaeroides* form I RubisCO (Gibson & Tabita, 1977b; Paoli *et al.*, 1995) but cross-reacts well with antibody raised against the *Synechococcus* sp. 6301 RubisCO (Paoli *et al.*, 1995). In addition, several years ago, J. L. Gibson, W. B. Whitman & F. R.

Tabita (unpublished results) examined cell extracts from several species of non-sulfur purple photosynthetic bacteria (*Rhodocyclus tenuis*, *Rhodospirillum rubrum*, *Rhodospseudomonas palustris*, *Rhodomicrobium vanielii*, *Rh. sphaeroides* and *Rh. capsulatus*) by Ouchterlony immunodiffusion. They found that antibody raised against the *Rh. sphaeroides* form I RubisCO cross-reacted with extracts from every species except *Rh. capsulatus*. Thus, *Rh. capsulatus* may be unique amongst the non-sulfur purple photosynthetic bacteria in having a 'green-like' form I RubisCO. On the strength of this evidence, it would seem that the *Rh. capsulatus* form I RubisCO was acquired by a horizontal gene transfer from a bacterial species containing a 'green-like' RubisCO. Although the evidence is not as compelling, phylogenetic analysis of the CbbR deduced amino acid sequences (Fig. 5) suggests that *Rh. capsulatus* CbbR_I may also have been acquired from an organism containing a 'green-like' RubisCO. In addition, *Rh. capsulatus* RubisCO is most closely related to the RubisCO of *Ps. hydrogenothermophila*, which also has a *cbbQ* gene directly downstream of its *cbbS* gene (Yokoyama *et al.*, 1995). The only other species known to have a *cbbQ* gene directly downstream of *cbbS* is *Chr. vinosum* (Yokoyama *et al.*, 1995) and the *Chr. vinosum* RubisCO falls within the same 'green-like' radiation as *Rh. capsulatus* and *Ps. hydrogenothermophila*. Thus, it seems likely that the *cbbR*, *cbbLS* and *cbbQ* genes of *Rh. capsulatus* were acquired by a single gene transfer event from some as yet unidentified bacterial species whose RubisCO falls within the bacterial/cyanobacterial 'green-like' RubisCO radiation (Fig. 4).

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