Mol Gen Genet (1984) 193:214–219

MGG

Cloning and Expression of the *Rhodospirillum rubrum* Ribulosebisphosphate Carboxylase Gene in *E. coli*

Christopher R. Somerville and Shauna C. Somerville
DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

Summary. A genomic library of *Rhodospirillum rubrum* DNA was constructed in the phage replacement vector λ1059. Recombinant phage carrying the gene for ribulose 1,5-bisphosphate (RuBP) carboxylase were identified by radioimmune blotting of plaques. A 6.6 kb *Eco*RI fragment from one of the immunologically positive phage was subcloned into pBR325 and a plasmid which conferred low levels of enzyme activity in *E. coli* was recovered. Expression of the gene was dependent upon the orientation of the restriction fragment in pBR325, suggesting that transcription originated on a plasmid promoter. In order to increase expression, a new plasmid was constructed by replacing the tetracycline resistance determinant of pBR322 with a restriction fragment from phage M13mp7 which encoded part of the *lac* operon. A 2.4 kb restriction fragment containing the RuBP carboxylase gene was then cloned into the unique *Bam*HI site of the *lac* DNA. Induction of *lac*-transcription resulted in a high level of expression of a fully functional RuBP carboxylase enzyme, which was purified to homogeneity. Southern hybridization analysis of the *R. rubrum* genome with a restriction fragment encoding part of the enzyme indicated only one copy of the gene.

Introduction

The genetic reduction of photorespiration in C_3 plant species is a conceptually attractive strategy for improving plant productivity since, in principle, it involves the modification of only one gene (Somerville and Ogren 1982). This gene encodes the catalytic subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO), the enzyme that catalyzes the primary reactions of both photosynthetic CO_2-fixation and photorespiration (Lorimer 1981). As the latter activity is considered deleterious to photosynthetic productivity, we are interested in the possibility of differentially reducing the RuBP oxygenase activity by in vitro genetic manipulation.

In higher plants, algae, and most photosynthetic bacteria, the RubisCO enzyme is a heteromultimer of two non-identical subunits. Genes encoding these subunits have been cloned from several plant and algal species (Coen et al. 1977; Bedbrook et al. 1980; Stiegler et al. 1982), and in several cases the DNA sequence has been determined (McIntosh et al. 1980; Zurawski et al. 1981). Expression of one of the cloned genes has been obtained by placing the *Zea mays* large subunit gene under transcriptional control of a bacteriophage lambda promoter, but the protein was not reported to be catalytically active (Gatenby and Castelton 1982). As this is a prerequisite to any attempt to modify the properties of the enzyme by in vitro mutagenesis, it was considered potentially advantageous, in this context, to clone the RubisCO gene from the photosynthetic bacterium *Rhodospirillum rubrum*. The enzyme from this source, which has been subjected to extensive physical and enzymological analyses (Tabita and McFadden 1974a, b; Schloss et al. 1979; O'Leary et al. 1979; Stringer et al. 1981; Herndon et al. 1982), is unusual in that it is homodimeric. This property of the enzyme makes it uniquely attractive as an object for study by recombinant DNA techniques since the implication is that the enzyme is encoded by a single gene. This obviates concern about problems adjacent to our primary interest such as coordinate expression of two cloned genes, correct processing of precursors, and assembly of subunits into an enzymatically active complex in *E. coli*. Also, because the *R. rubrum* RubisCO appears to be a very divergent form of the enzyme, we are interested in comparing the amino acid sequence, which may be deduced from the nucleotide sequence of the gene, with that of the eukaryotic enzymes.

In this report we describe the cloning of the RubisCO gene, the construction of a plasmid which places the gene under transcriptional control of the *E. coli lac*-promoter, the purification of a fully functional RubisCO enzyme from *E. coli*, and the results of experiments which suggest that there is only one copy of the RubisCO gene in *R. rubrum*.

Materials and Methods

Enzymes and Reagents. Restriction endonuclease *Cla*I and acridine yellow affinity adsorbent were from Boehringer-Mannheim. *Eco*RV was from New England Biolabs. T4 DNA ligase, the Klenow fragment of DNA polymerase I, M13mp8 DNA and primer, λ1059 DNA and all other restriction endonucleases were from BRL. Radioisotopes were from New England Nuclear. All other reagents were from Sigma.

Offprint requests to: C.R. Somerville

Abbreviations: amp ampicillin; tet tetracycline; kb kilobase pairs; IPTG isopropyl-β-D-thiogalactoside; X-gal 5-bromo-4-chloro-indoyl-β-D-galactoside; RubisCO ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP ribulose 1,5-bisphosphate

Bacterial and Phage Strains. *Rhodospirillum rubrum* S1, obtained from the American Type Culture Collection, was grown with incandescent illumination in the butyrate medium described by Tabita and McFadden (1974a). *E. coli* K12 strains HB101 (F⁻ *hsdS proA leu ara galK lacY xyl mtl strA thi supE recA*) and JM103 (*Δ(lac-pro) strA endA sbcB hsdR supE*/F′*lacI*^{q} *ZΔM15 traD pro*$^+$) were provided by F. Nargang. The derivation and growth conditions of phage M13mp7 and host strain JM103 were described by Messing et al. (1981). The construction and properties of phage λ1059 and *E. coli* host strains Q358 and Q359 were described by Karn et al. (1980). L broth (Bolivar and Backman 1979) was used for the growth of all *E. coli* strains, with additions as noted in the text.

Restriction and Ligation Reactions. The conditions for restriction endonuclease digests were those specified by the enzyme supplier. Double digests were performed by averaging the optimal conditions for the two enzymes. The standard ligation reaction contained 0.1 U T4 DNA ligase, 60 mM Tris.Cl (pH 7.6), 7 mM $MgCl_2$, 0.2 mM ATP, 0.1 mM DTT and DNA in concentrations approximating those considered optimal (Dugaiczyk et al. 1975).

Construction of a Genomic Library. Chromosomal DNA was prepared from *R. rubrum* by phenol extraction of lysozyme-treated cells. The DNA was partially digested with *Sau*3A and fragments 15–20 kb in size were recovered following electrophoresis in low-melting agarose gels as described by Karn et al. (1980) except that acridine yellow affinity absorbant was used to purify the DNA from agarose contaminants. Sized DNA was ligated into the *Bam*HI arms of λ1059 DNA and packaged in vitro using the bacterial strains and methods described by Hohn (1979). The resulting phage were titered, then plated at a density of about 2,000 plaques per plate on *E. coli* Q359. Phage from about 20,000 plaques was pooled to form a representative library.

Plasmid Constructions. The expression plasmid pXG21 was constructed in two stages. A mixture of M13mp7 replicative form and pBR322 DNA was digested to completion with *Ava*I and *Cla*I, heated to inactivate the restriction enzymes, and treated with ligase. *E. coli* strain JM103 was transformed (Bolivar and Backman 1979) with the ligation mixture and plated on L broth agar containing Amp (50 μg/ml) IPTG, (8 μg/ml) and X-gal (40 μg/ml) at 37° C. Plasmid DNA was isolated from several blue Amp^r Tet^s transformants, and the composition of the plasmids derived from restriction analysis. Unexpectedly, all of the plasmids had four *Ava*I-*Cla*I fragments and were unsuitable as cloning vectors because of the presence of multiple *Bam*HI sites. One plasmid which carried an *Ava*I-*Cla*I fragment containing the *lac*-promoter and part of the *lacZ* gene in a desirable configuration was digested to completion with *Ava*I then partially digested with *Hha*I to remove a small piece of M13 DNA adjacent to the *Ava*I site. The partial digest was electrophoresed in low-melting agarose and fragments of desired size were eluted from the gel as before. The DNA was ligated and JM103 was transformed as before. Plasmids were isolated from 10 blue Amp^r transformants and checked for the presence of unique or closely-spaced *Bam*HI or *Sal*I sites characteristic of the modified *lacZ* gene present in M13mp7 (Messing et al. 1981). pXG21 was arbitrarily chosen from among several suitable plasmids. Plasmids pRR116 and pRR124 were constructed by digesting a mixture of λRR101 and pBR325 DNA (Bolivar and Backman 1979) to completion with *Eco*RI, ligating, transforming *E. coli* HB101, selecting for Amp^r and scoring for chloramphenicol sensitivity. Among the plasmids recovered in this way, pRR116 and pRR124 were retained for further analysis on the basis of a positive response to radioimmune screening of chloroform-lysed colonies. The first step in the construction of pRR2119 was the preparation of a partial *Sau*3A digest of λRR101 DNA and the recovery of fragments in the range of 1.5–2.5 kb as described above. The DNA was ligated into *Bam*HI restricted pXG21 and used to transform JM103 to Amp^r on L broth agar containing IPTG and X-gal. PRR2119 was identified among the resulting white colonies by radioimmune screening. Plasmid DNA was prepared as described by Bolivar and Backman (1979) except that a VTi65 rotor was used for equilibrium CsCl gradients. DNA was suspended in solution containing 0.95 g CsCl per ml, 12 μl of ethidium bromide (10 mg/ml) per ml was added and the tubes were centrifuged for 10 hours at 50,000 r.p.m.

Radioimmune Screening. Antibody production was induced in a rabbit by two intramuscular injections, two weeks apart, of a total of 600 ug of pure *R. rubrum* RubisCO in complete Freunds adjuvant. Blood was collected two weeks after the final injection and the serum was passed through DEAE cellulose, precipitated with 65% NH_4SO_4, dialyzed and resuspended in the original volume of 10 mM potassium phosphate (pH 7.4), 150 mM NaCl. A sample of serum (300 ug) was radioiodinated to a specific activity of 5×10^6 cpm/ug with $Na^{125}I$ and chloramine-T as described by Broome and Gilbert (1978). Plaques or chloroform-lysed colonies were screened for the presence of RubisCO by coating vinyl discs with unlabelled IgG, blotting the plaques or colonies with the coated vinyl, labelling the bound antigen with ^{125}I-labelled IgG, and autoradiographic localization as described by Broome and Gilbert (1978).

Enzyme Extraction and Purification. Cultures of *E. coli* were grown at 37° C in L-broth containing glycerol as the carbon source, and 50 μg Amp per ml for plasmid-bearing strains. Where indicated, expression of the RubisCO gene was induced by addition of 0.1 mM IPTG to mid-log cultures for 90 min before the cells were harvested by centrifugation at 6,000 g for 10 min. Crude extracts of *R. rubrum* or *E. coli* were prepared by resuspending the cell pellets in 50 mM Tris-Cl (pH 8.0), 50 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 1 mM phenethylmethyl sulfonyl fluoride, sonicating and centrifuging at 30,000 × g for 30 min. The supernatant was quickly brought to 50° C, swirled continuously for 10 min, then cooled on ice and recentrifuged to removed precipitated protein (Tabita and McFadden 1974a). At this stage the enzyme activity was stable at 4° C for at least a week. RubisCO was purified from crude extracts of *R. rubrum* and *E. coli* by column chromatography as described by Schloss et al. (1979). The purity of the preparations was assessed by electrophoresis in SDS-polyacrylamide (10%) slab gels as described by Chua (1980).

Enzyme Assays. RubisCO activity was determined at 30° C as RuBP-dependent $^{14}CO_2$-fixation. The enzyme was activated for 15 min at 22° C in 50 mM Tris-Cl (pH 8.0),

10 mM $MgCl_2$, 20 mM $NaH^{14}CO_3$ (2 uCi/umol), 10 mM 2-mercaptoethanol, and 0.1 mM EDTA. The assay was initiated with RuBP to give a final concentration of 0.4 mM, and terminated after 60 s by the addition of acetic acid to 3 N. The reactions were dried at 85° C, resuspended in 1 N HCl and counted. Protein was determined with a dye-binding assay (Spector 1978).

DNA Filter Hybridization. Restricted chromosomal DNA from *R. rubrum* (5 µg/lane) was electrophoresed in 0.8% agarose, transferred to nitrocellulose and hybridized to ^{32}P-labeled probe as described by Southern (1975). The probe, a promoter-proximal *Pst*I-*Bgl*II fragment from pRR2119 cloned in M13mp8, was labeled by incubating primed single stranded phage DNA with ^{32}P-dATP, the Klenow fragment of DNA polymerase I and unlabeled deoxynucleotides in a reaction mixture analogous to that used for dideoxy DNA sequencing (Messing et al. 1981).

Results and Discussion

Cloning the RubisCO Gene

Because of the apparent lack of amino acid sequence homology between the cysteine-containing peptides of the *R. rubrum* and *Zea mays* or spinach RubisCO (Lorimer 1981), and the observation that antibodies directed against the plant enzyme do not react with the bacterial enzyme (Tabita and McFadden 1974b), it was considered unlikely that the cloned plant genes would be sufficiently specific probes to permit identification and cloning of the *R. rubrum* gene by DNA filter hybridization techniques. The cloning strategy was, therefore, predicated on the naive assumption that the cloned gene would be expressed in *E. coli* at levels detectable by radioimmune blotting of plaques or colonies. The sensitivity of the method employed is reported to be about 10 molecules of antigen per cell (Broome and Gilbert 1978).

A genomic library of *R. rubrum* DNA was constructed in λ1059, a replacement vector which permits selective recovery of recombinant phage (Karn et al. 1980). To ensure unbiased representation of the entire genome, chromosomal DNA was partially digested with *Sau*3A and electrophoretically sized fragments of 15–20 kb were ligated into the *Bam*HI arms of the phage. Following in vitro packaging of the DNA, approximately 20,000 recombinant phage were pooled to form the library. A dilution of the lysate was plated on *E. coli* strain Q359 and a large number of immunologically positive plaques identified by radioimmune screening. Phage from several of the positive plaques was purified and the presence of foreign DNA verified by restriction analysis. One of the phage, which carried an insert of about 20 kb, was designated λRR101 and arbitrarily chosen for further characterization.

Rather than attempt an unequivocal demonstration that the recombinant phage carried the RubisCO gene, phage DNA was subcloned into a plasmid to facilitate enzymological analysis. Aliquots of DNA from phage λRR101 were separately cleaved with *Bam*HI, *Sal*I and *Eco*RI and the resulting fragments were ligated into the corresponding unique restriction sites of pBR325 (Bolivar and Backman 1979). *E. coli* strain HB101 was transformed with the ligation mixtures and the resulting amp^r transformants were screened for the production of antigen by radioimmune blotting. Several positive clones were found among the transformants carrying plasmids with *Eco*RI inserts, and all of these were found to carry the same 6.6 kb *Eco*RI fragment from λRR101. A restriction map of one of these plasmids, designated pRR116, is presented in Fig. 1.

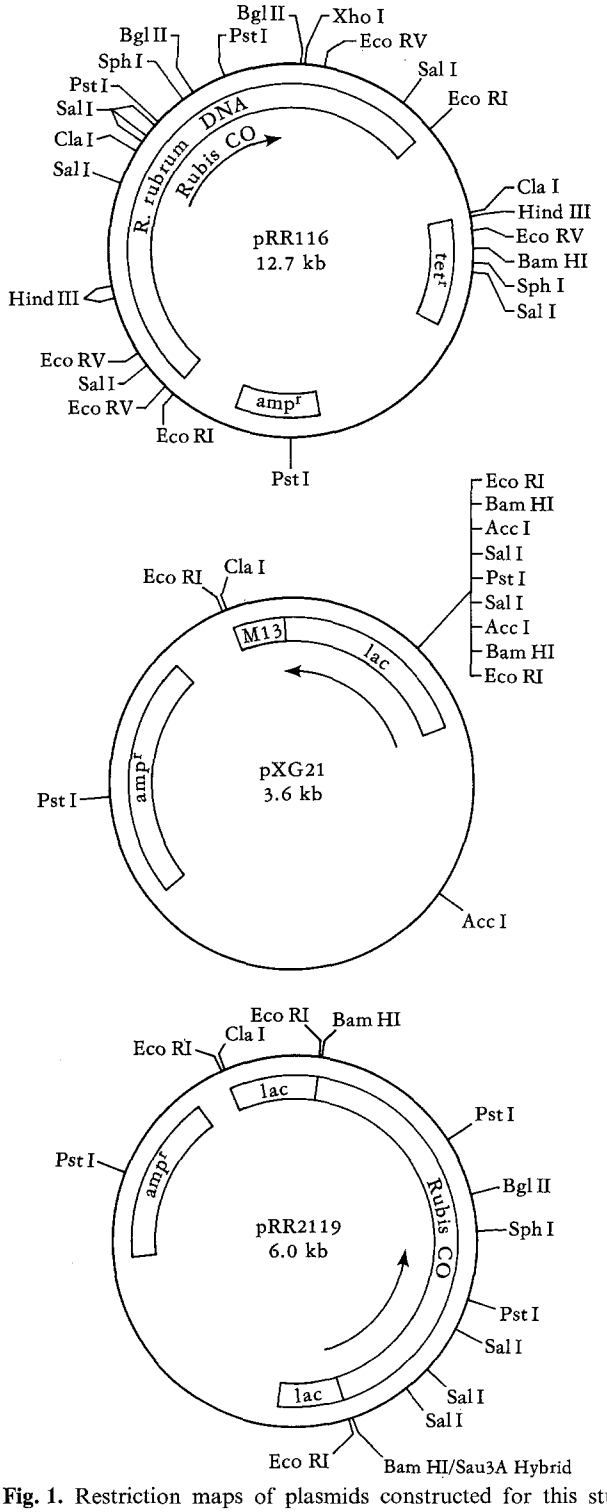

Fig. 1. Restriction maps of plasmids constructed for this study. pRR116 was constructed by insertion of an *Eco*RI fragment into pBR325. pXG21 is a derivative of pBR322 in which the tet region has been replaced with a *lac*-containing fragment from M13mp7. pRR2119 contains a *Sau*3A generated fragment of pRR116 inserted in the *Bam*HI site of pXG21. The arrows indicate the direction of transcription of relevant genes

Direct evidence that pRR116 carries a complete RubisCO gene was obtained by assaying crude extracts of cells carrying the plasmid for RuBP-dependent $^{14}CO_2$-fixation. Crude extracts of HB101 (pRR116) had about 3% as much activity on a protein basis as a similarly prepared extract from *R. rubrum* grown under conditions which induce synthesis of the enzyme (Table 1). In contrast, pRR124, which carries the same restriction fragment as pRR116 but in the opposite orientation, does not confer detectable RubisCO activity on *E. coli* hosts (Table 1). Cells carrying pRR124 were, however, detected as immunologically positive by radioimmune blotting which is several orders of magnitude more sensitive than enzyme assay. These observations suggest that transcription of the RubisCO gene in cells carrying pRR116 is primarily initiated from a plasmid borne promoter – possibly that of the chloramphenicol transacetylase gene in which the RubisCO gene is inserted. Attempts to increase the expression of the RubisCO gene by varying the composition of the medium or the growth conditions were without significant effect. Preliminary attempts to reduce the size of the insert in pRR116 by removing restriction fragments from either end were frustrated by the apparent necessity of maintaining the correct orientation of the insert relative to the unidentified plasmid promoter.

Construction of an Expression Plasmid

Since one of the objectives of this work was to construct a system which would permit facile analysis of the product of a cloned RubisCO gene, the level of expression obtained with pRR116 was considered unacceptable. We, therefore, constructed a derivative of pBR322, designated pXG21, which carrys the *lac* promoter and operator and part of the *lacZ* gene from phage M13mp7 (Fig. 1). In host strains such as JM103, which carry the M15 deletion of the N-terminal region of β-galactosidase, the presence of the plasmid confers a $LacZ^+$ phenotype which is conveniently recognized by the formation of blue colonies on plates containing IPTG and X-gal. The insertion of foreign DNA into the closely spaced *Bam*HI sites which have been introduced into the *lacZ'* gene of M13mp7 (Messing et al. 1981) generally results in loss of ability of the *lacZ'* gene to complement the M15 deletion. The plasmid was constructed largely as a matter of expediency and has several disadvantages as a general purpose cloning vector by comparison with the plasmid of similar design recently constructed by Rüther et al. (1981). The relevant feature in the present context is that the plasmid carrys *Bam*HI cloning sites adjacent to the *lac* promoter in the correct orientation to permit *lac*-promoted transcription of DNA inserted at this site.

In order to obtain expression of a small DNA fragment encoding the RubisCO gene, the high frequency of *Sau*3A sites in most DNA sequences was exploited to generate an essentially randomly cut collection of fragments just large enough to encode a protein the size of RubisCO (56,000 daltons). Sized DNA from a *Sau*3A partial digest of λRR101 was ligated into the *Bam*HI sites of pXG21. *E. coli* strain JM103 was transformed with the plasmids and replicas of $lacZ^-$ transformants were screened for the presence of RubisCO by radioimmune blotting. This resulted in the recovery of a plasmid, designated pRR2119 (Fig. 1), which carrys a 2.4 kb insert in the *lacZ'* gene of pXG21. Crude extracts of JM103 cells carrying the plasmid show high levels of RubisCO activity when grown in the presence of the non-metabolizable *lac*-inducer IPTG, but have very low levels in the absence of the inducer (Table 1). It is, therefore, apparent that the vast majority of the transcription of the RubisCO gene is under *lac* transcriptional control. Comparison of preliminary DNA sequence analysis of pRR2119 and the N-terminal amino acid sequence of RubisCO (Schloss et al. 1979), revealed that the N-terminal f-met codon of RubisCO is located 55 base pairs from the *lac* promoter-proximal *Eco*RI site (Nargang et al. 1984).

Table 1. RubisCO activity of purified enzyme and crude extracts of *R. rubrum* and various strains of *E. coli*

Source of extract	RubisCO activity (nmol mg protein^{-1} min^{-1})
HB101 or JM103	n.d.
HB101 (pRR116)	2.0
HB101 (pRR124)	n.d.
JM103 (pRR2119), not induced	7.1
JM103 (pRR2119), induced	353.2
JM103 (pRR2119), purified enzyme	2723.0
R. rubrum	65.2

n.d. = not detectable

Alignment of the restriction map of pRR2119 with that of pRR 116 permitted approximate demarcation of the coding region on pRR116 (Fig. 1). Since this region of DNA is flanked by several kb of DNA on the presumptive 5'-side of the gene, it seems possible that pRR116 carries the promoter for RubisCO, but that this is ineffective in *E. coli*. In contrast, this region of DNA must be transcriptionally very active in *R. rubrum* since the enzyme has been reported to account for as much as 40% of cellular protein in fully induced cultures (Schloss et al. 1979). The availability of the plasmid should facilitate investigation of the factors responsible for the regulated expression of the gene in *R. rubrum*.

Characterization of the Cloned Gene Product

As a preliminary characterization of the product of the cloned gene, RubisCO was purified to homogeneity from an induced culture of JM103 (pRR2119) by the procedures established for the purification of the enzyme from *R. rubrum* (Schloss et al. 1979). The purified enzyme had a specific activity of 2.7 Units/mg (Table 1), which is comparable to that obtained with enzyme purified from *R. rubrum* (Tabita and McFadden 1974a; Schloss et al. 1979), and was of similar or identical apparent molecular weight on SDS gels (Fig. 2). Thus, available evidence indicates that the enzyme produced in *E. coli* is indistinguishable from that obtained from the native source.

On the basis of activity measurements, RubisCO accounts for about 12% of the protein in crude heat-treated extracts of an IPTG-induced culture of *E. coli* JM103 (pRR2119), and is readily apparent as the most prominent band in SDS gels of crude extracts (Fig. 2). Since the $lacI^q$ mutation in JM103 results in overproduction of the *lac* repressor, it may be possible to obtain higher levels of RubisCO production by transferring the plasmid to a different genetic background. Although this plasmid may produce less than the maximal level of expression that could be obtained by further manipulations (Guarente et al.

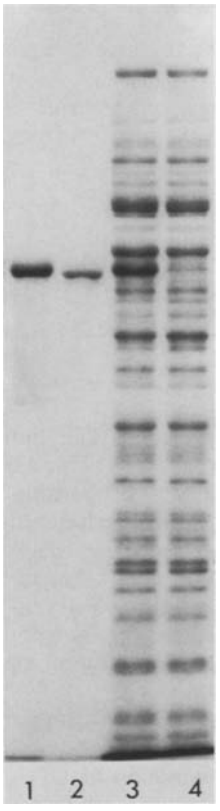

Fig. 2. SDS polyacrylamide gel showing IPTG-induced synthesis of RubisCO by *E. coli* strain JM103 (pRR2119). Lane: (1) RubisCO purified from *R. rubrum*; (2) RubisCO purified from *E. coli* JM103 (pRR2119); (3) Crude extract of IPTG-induced JM103 (pRR2119); (4) Non-induced culture of JM103 (pRR2119)

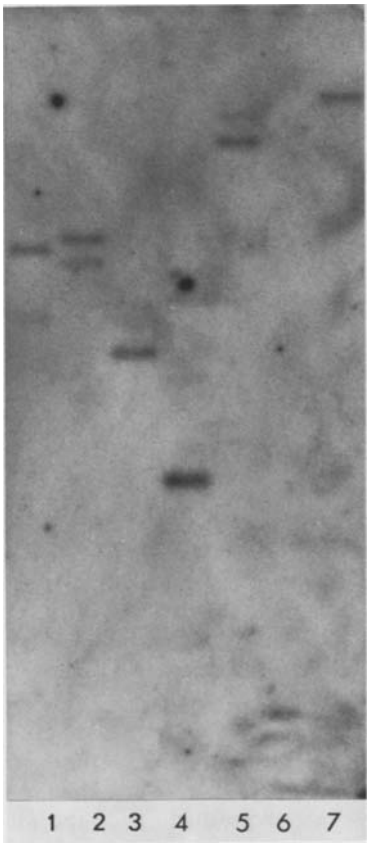

Fig. 3. Autoradiogram of a Southern blot of restricted chromosomal DNA from *R. rubrum* probed with the small *Pst*I-*Bgl*II fragment from pRR2119 cloned in M13mp8. The DNA was cleaved with: (1) *Bgl*II; (2) *Pvu*II; (3) *Eco*RI; (4) *Sal*I; (5) *Cla*I; (6) *Ava*I; (7) *Eco*RV (incomplete digest)

1980), it is considered sufficient to permit use of the cloned gene as an accessible template for enzyme production and DNA sequence modification.

DNA Filter Hybridization

In view of the high level of expression of RubisCO in induced cultures of *R. rubrum* (Schloss et al. 1979), and the observation that the enzyme purified from *R. rubrum* exhibits two bands in isoelectric focusing gels (Johal et al. 1982), we thought it worthwhile to examine the possibility that *R. rubrum* has more than one copy of the RubisCO gene. Restriction digests of chromosomal DNA were blotted by the method of Southern (1975) with the promoter-proximal *Pst*I-*Bgl*II fragment from pRR2119, which was cloned in M13mp8. Preliminary DNA sequence analysis indicated that this fragment is contained entirely within the RubisCO gene.

The results of this experiment (Fig. 3) are consistent with the presence of only one gene for RubisCO in *R. rubrum*. The probe hybridized to only one *Eco*RI, *Sal*I and *Ava*I (faint) fragment of the size expected from the restriction map of pRR116 (Fig. 1). The *Eco*RV digestion was unfortunately incomplete and, therefore, uninformative. *Pvu*II cleavage resulted in two bands of similar intensity which is consistent with the presence of a *Pvu*II site within or very close to the *Pst*I-*Bgl*II fragment (unpublished). Contrary to the expectation, cleavage with *Bgl*II and *Cla*I gave two high molecular weight bands of significantly different intensity. The faint bands may indicate the presence of a sequence which is weakly homologous to RubisCO, or to M13mp8 which was not separated from the *Pst*I-*Bgl*II fragment. Whatever the case, the relatively poor homology of the secondary bands is inconsistent with the presence of a second functional RubisCO of similar amino acid composition. The apparent heterogeneity observed following isoelectric focusing of RubisCO must, therefore, be due to a post-translational modification of the protein.

It is hoped that the construction of the plasmids described here, which are available on request, will facilitate novel approaches to the functional analysis of this important enzyme.

Acknowledgements. We wish to thank F. Nargang, R. Grumbles, K. Cliff and L. Ostafichuk for advice and technical assistance. This work was supported in part by a grant from the Natural Sciences and Engineering Research Council of Canada which was in effect while the authors were members of the Department of Genetics, University of Alberta.

References

Bedbrook JR, Smith S, Ellis JR (1980) Molecular cloning and sequencing of cDNA encoding the precursor to the small subunit of chloroplast ribulose 1,5-bisphosphate carboxylase. Nature 287:692–697

Bolivar F, Backman K (1979) Plasmids of *Escherichia coli* as cloning vectors. In: Wu R (ed) Methods in enzymology, vol 68. Academic Press, New York, p 245–268

Broome S, Gilbert W (1978) Immunological screening methods to detect specific translation products. Proc Natl Acad Sci USA 78:4520–4524

Chua NH (1980) Electrophoretic analysis of chloroplast proteins. In: San Pietro A (ed) Methods in enzymology, vol 69. Academic Press, New York, p 434–446

Coen DM, Bedbrook JR, Bogorad L, Rich A (1977) Maize DNA fragment encoding the large subunit of ribulosebisphosphate carboxylase. Proc Natl Acad Sci USA 74:5487–5491

Dugaiczyk A, Boyer HW, Goodman HM (1975) Ligation of EcoRI endonuclease generated DNA fragments into linear and circular structures. J Mol Biol 96:174–184

Gatenby AA, Castelton JA (1982) Amplification of maize ribulose bisphosphate carboxylase large subunit synthesis in *E. coli* by transcriptional fusion with the lambda N operon. Mol Gen Genet 185:424–429

Guarente L, Lauer G, Roberts T, Ptashne M (1980) Improved methods for maximizing expression of a cloned gene: A bacterium that synthesizes rabbit β-globin. Cell 20:543–553

Herndon CS, Norton IL, Hartman FC (1982) Reexamination of the binding site for pyridoxal 5′-phosphate in ribulosebisphosphate carboxylase/oxygenase from *Rhodospirillum rubrum*. Biochemistry 21:1380–1385

Hohn B (1979) In vitro packaging of λ and cosmid DNA. In: Wu R (ed) Methods in enzymology, vol 68. Academic Press, New York, pp 299–309

Johal S, Bowman LH, Chollet R (1982) Electrofocusing variants of ribulose bisphosphate carboxylase from *Rhodospirillum*. Biochim Biophys Acta 704:374–378

Karn J, Brenner S, Barnett L, Cesareni G (1980) Novel bacteriophage λ cloning vector. Proc Natl Acad Sci USA 77:5172–5176

Lorimer GH (1981) The carboxylation and oxygenation of ribulose 1,5-bisphosphate: The primary events in photosynthesis and photorespiration. Ann Rev Plant Physiol 32:556–560

McIntosh L, Poulsen C, Bogorad L (1980) Chloroplast gene sequence for the large subunit of ribulose bisphosphate carboxylase of maize. Nature 288:556–560

Messing J, Crea R, Seeburg PH (1981) A system for shotgun DNA sequencing. Nucl Acid Res 9:309–322

Nargang F, McIntosh L, Somerville C (1984) Nucleotide sequence of the Ribulosebisphosphate carboxylase gene from *Rhodospirillum rubrum*. Mol Gen Genet 193:220–224

O'Leary MH, Joworski RJ, Hartman FC (1979) ^{13}C nuclear magnetic resonance study of the CO_2 activation of ribulose bisphosphate carboxylase from *Rhodospirillum rubrum*. Proc Natl Acad Sci USA 76:673–675

Rüther U, Koenen M, Otto K, Müller-Hill B (1981) pUR222 a vector for cloning and rapid chemical sequencing of DNA. Nucl Acid Res 9:4087–4097

Schloss JV, Phares EF, Long MV, Norton IL, Stringer CD, Hartmann FC (1979) Isolation, characterization, and crystallization of ribulosebisphosphate carboxylase from autotrophically grown *Rhodospirillum rubrum*. J Bacteriol 137:490–501

Somerville CR, Ogren WL (1982) Genetic modification of photorespiration. Trends Biochem Sci 7:171–174

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Spector T (1978) Refinement of the coomassie blue method of protein quantitation. Anal Biochem 86:142–146

Stiegler GL, Matthews HM, Bingham SE, Hallick RB (1982) The gene for the large subunit of ribulose 1,5-bisphosphate carboxylase in *Euglena gracilis* chloroplast DNA: Location, polarity, cloning and evidence for an intervening sequence. Nucl Acid Res 10:3427–3445

Stringer CD, Norton IL, Hartman FC (1981) Sequences of tryptic peptides containing the five cysteinyl residues of ribulosebisphosphate carboxylase/oxygenase from *Rhodospirillum rubrum*. Arch Biochem Biophys 208:495–501

Tabita FR, McFadden BA (1974a) D-ribulose 1,5-diphosphate carboxylase from *Rhodospirillum rubrum* I: Levels, purification, and effects of metallic ions. J Biol Chem 249:3453–3458

Tabita FR, McFadden BA (1974b) D-ribulose 1,5-diphosphate carboxylase from *Rhodospirillum rubrum* II: Quaternary structure, composition, catalytic and immunological properties. J Biol Chem 249:3459–3464

Zurawski G, Perrot B, Bottomley W, Whitfeld PR (1981) The structure of the gene for the large subunit of ribulose 1,5-bisphosphate carboxylase from spinach chloroplast DNA. Nucl Acid Res 9:3251–3270

Communicated by G.A. O'Donovan

Received September 15, 1983