Cloning of the Gene for Phosphoribulokinase Activity from *Rhodobacter sphaeroides* and Its Expression in *Escherichia coli*

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A 3.4-kilobase *EcoRI* restriction endonuclease fragment has been cloned from the facultatively photoheterotrophic bacterium *Rhodobacter sphaeroides* and shown to contain the structural gene (*prkA*) for phosphoribulokinase (PRK) activity. The PRK activity was characterized in *Escherichia coli*, and the product of the reaction was identified. The *prkA* gene was localized to a 1,565-base-pair *EcoRI-PstI* restriction endonuclease fragment and gave rise to a 33-kilodalton polypeptide both in vivo and in vitro. The gene product produced in *E. coli* was shown to be identical to the gene product produced in *R. sphaeroides*. The amino acid sequence for the amino-terminal region deduced from the DNA sequence confirmed that derived for partially purified PRK derived from both *E. coli* and *R. sphaeroides*. In addition, the 3.4-kilobase *EcoRI* restriction endonuclease fragment coded for a 37-kilodalton polypeptide of unknown function, and preliminary evidence indicates that this DNA fragment is linked to genes coding for other activities significant in photosynthetic carbon assimilation. The genetic organization and proposed operon structure of this DNA fragment are discussed.

Rhodobacter sphaeroides is a gram-negative nonsulfur purple facultative photoheterotrophic bacterium. This bacterium, when grown photoheterotrophically on reduced carbon substrates such as succinate, photoautotrophically on CO₂-H₂, or chemolithotrophically on CO₂-H₂-O₂, utilizes an operational reductive Calvin pentose phosphate cycle (22, 51). When cells are grown under these conditions, the synthesis of at least two enzymes unique to the Calvin cycle is derepressed: phosphoribulokinase (PRK) (ATP:D-ribulose 5-phosphate-1-phosphotransferase; EC 2.7.1.19) and ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBPCase) (3-phospho-D-glycerate carboxylase [dimerizing]; EC 4.1.1.39) (46). PRK uses ATP to phosphorylate ribulose 5-phosphate (RuMP) to produce ribulose 1,5-bisphosphate (RuBP), which together with CO₂ can be used by RuBPCase to yield 3-phosphoglycerate, which can then represent either a net increase in cellular carbon or be utilized to regenerate the CO₂ acceptor (RuBP).

Because operation of this pathway requires the utilization of a substantial amount of ATP and reducing power, synthesis of these enzymes is under tight metabolic control. PRK and RuBPCase are not found in cells cultured chemoheterotrophically, and only low levels of these activities are found in cells grown photoheterotrophically on oxidized organic acids. The synthesis of RuBPCase appears to be regulated by the level of CO₂ and the reduced state of the carbon substrate in a host of facultative autotrophic bacteria, including *R. sphaeroides* (6, 10, 11, 13, 46). However, *R. sphaeroides* has two forms of RuBPCase, form I, composed of both large and small subunits which resembles the plant-type enzyme as well as the enzyme found in most autotrophs, and form II, composed of only large subunits, which is similar to the enzyme found in *Rhodospirillum rubrum* (48). The ratio of form I to form II enzyme can change dramatically under different conditions (17, 40).

Although much is known about the factors affecting the activity and, to some extent, the synthesis of RuBPCase, much less is known about PRK. Several reports have at least

suggested that the specific activities of PRK and RuBPCase are coordinately regulated in *R. sphaeroides* (46), *Rhodobacter capsulatus* (L. Ruiz and J. M. Shively, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, K24, p. 197), and in the hydrogen-oxidizing bacterium *Alcaligenes eutrophus* (6, 20). PRK isolated from the related organism *Rhodopseudomonas acidophila* shows similarities in subunit *M_r* and metabolic properties to the PRK isolated from *A. eutrophus* (32). Both enzymes are activated by NADH and inhibited by phosphoenolpyruvate (PEP) (1, 19, 32, 41, 42). Interestingly, a reduced metabolic intermediate and PEP have been suggested as positive and negative effectors of RuBPCase formation in *A. eutrophus* (6, 16, 20).

To begin to address these important considerations, Muller et al. (27) and Quivey et al. (30) cloned the form II RuBPCase gene (*rbcR*) from *R. sphaeroides*. Subsequent studies by Zhu et al. (53) showed that the synthesis of form II RuBPCase is under transcriptional control, with the synthesis of mRNA for this gene regulated by light, oxygen, and reducing power. Similar studies with the form I RuBPCase gene cluster (*rbcL* and *rbcS*) and the *prkA* gene were not possible since the genes from *R. sphaeroides* had not been cloned. However, Muller et al. (27) reported that a 3.0-kilobase (kb) *BamHI* restriction endonuclease fragment containing the form II *rbcR* gene hybridized to a unique 1.6-kb *BamHI* restriction endonuclease fragment from chromosomal restriction endonuclease digests of bulk *R. sphaeroides* DNA, presumably the form I gene.

We report here the cloning of this 1.6-kb *BamHI* restriction endonuclease fragment from *R. sphaeroides* (27). We have shown that there is homology between a region of DNA upstream of the form II *rbcR* gene residing on the 3.0-kb *BamHI* restriction endonuclease fragment and the 1.6-kb *BamHI* restriction endonuclease fragment. Furthermore we have demonstrated that the 1.6-kb *BamHI* restriction endonuclease fragment includes part of the *prkA* gene and that larger DNA fragments which contain the 1.6-kb *BamHI* restriction endonuclease fragment within them express PRK activity in *Escherichia coli*. In addition, we have expressed a 33-kilodalton (kDa) protein, corresponding to the *prkA*

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structural gene product, in both *E. coli* and an *R. sphaeroides* in vitro transcription-translation system. The 5' end of the form I gene cluster, however, was found to be 1 kb from the approximate 3' end of the structural gene for *prkA*.

(A preliminary report of this work has been presented [P. L. Hallenbeck and S. Kaplan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, K92, p. 208].)

MATERIALS AND METHODS

Bacteria, bacteriophage, and plasmids. *E. coli* strains JM83, JM103 (50, 52), and F⁻M15A (33) were used as host strains for plasmid pUC19 (52) and its derivatives. *E. coli* SF8 was used as a host for constructions involving plasmid vector pBR322 (5). Lambda L47.1 and the construction of the *R. sphaeroides* genomic lambda bank have been described previously (27), and *E. coli* LE392 was the host strain for recombinant lambda phage. M13mp18 and M13mp19 were propagated in host JM103 (25, 26, 52). *R. sphaeroides* 2.4.1 was used throughout this study (49).

Media and culture conditions. *E. coli* strains were grown at 37°C in LB for the isolation and analysis of recombinant phage or plasmids (23). Lambda phage and its derivatives were plated on MH plates with 0.7% agar or agarose overlay (27). When appropriate, LB was supplemented with ampicillin (50 µg/ml) or tetracycline (15 µg/ml). Recombinant DNA constructions involving phage M13 and its derivatives as well as pUC19 were screened with XGal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) at 40 µg/ml in the presence of 40 µM IPTG (isopropyl-β-D-thiogalactoside) to detect insertional inactivation of β-galactosidase (25, 26, 52).

DNA manipulations and plasmid constructions. Large-scale preparations of *R. sphaeroides* total DNA (chromosomal and plasmid) were performed as described by Saito et al. (34). Plasmid DNA and replicative form DNA from M13 phage were purified by the protocol of Maquat and Reznikoff (24), whereas small amounts of these DNAs were prepared by the rapid method of Birnboim and Doly (4). DNA from lambda as well as recombinant lambda phage was isolated as described previously (27). Single-stranded DNA from recombinant M13 phage was isolated as in Yanisch-Perron et al. (52). Restriction enzyme digestions were done by the methods of Maniatis et al. (23) or as specified by the manufacturer. Separation and isolation of DNA restriction endonuclease fragments on 5.0% polyacrylamide gels has been previously described (9). DNA restriction endonuclease fragments were electrophoresed on agarose gels and purified by electroelution into dialysis bags (23) or onto DE52 paper (9). Some DNA restriction endonuclease fragments with protruding 5' ends were made blunt-ended for cloning by use of the Klenow fragment of *E. coli* DNA polymerase I (23).

Plaque hybridizations and Southern blots. Plasmid DNA and DNA restriction endonuclease fragments were labeled with [α-³²P]dCTP by nick translation to a specific activity between 10⁷ and 10⁸ cpm/µg of DNA. DNA transfer from agarose gels to nitrocellulose paper and conditions for Southern blot hybridization analysis were as described by Muller et al. (27) and Southern (44). Plaque hybridizations with the *R. sphaeroides* lambda bank have been described previously (27).

In vitro transcription-translation. L-[³⁵S]methionine was used to label plasmid-directed polypeptides synthesized in an *R. sphaeroides* in vitro transcription-translation system, and the products of the synthesis were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis as

described (7). Approximately 1 µg of purified covalently closed circular plasmid DNA was used to program the S30 system.

Expression of PRK and enzyme assays. *E. coli* strains bearing recombinant plasmids were analyzed for the presence of PRK activity as follows. Overnight cultures of JM103(pPRK12b) or JM103(pPKP1565b) and appropriate F⁻M15A strains were grown in LB containing ampicillin (50 µg/ml) and used to inoculate 200 ml of the same medium to an OD₆₀₀ of 0.2. Growth in the presence or absence of 1 mM IPTG was allowed to continue for 4 h at 37°C with shaking prior to harvesting. Cell pellets were washed with 0.025 M Tris buffer, pH 7.9, and suspended in 3 to 5 ml of the same buffer. Cells were broken by passage through the French pressure cell at 10,000 lb/in², and unbroken cells and cell debris were removed following low-speed centrifugation. The membranes were pelleted by centrifugation at 100,000 × g for 1 h, and the resulting supernatants were dialyzed extensively against the same buffer before analysis. Protein concentrations were determined as described by Lowry et al. (21). PRK assays were performed by preincubating for 10 min approximately 4.7 µg of protein from the above extracts with the following components: 0.6 mM NADH, 10 mM dithiothreitol, 4.3 mM RuMP, 10 mM MgCl₂, 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.9), 20 mM NaHCO₃, and 2 µCi of NaH¹⁴CO₃ per assay, all in 250 µl. Also added was either 250 µg of spinach RuBPCase (0.04 U/mg of solid) or 47 µg of *R. rubrum* RuBPCase (2 U/mg of protein) as a coupling enzyme. The reaction was initiated by the addition of ATP to a final concentration of 6 mM. After 6 min the reaction was stopped by addition of 6 N HCl to a final concentration of 1 N HCl. The reaction mixtures were dried, and acid-stable radioactivity was determined with Tritosol as the scintillant (45, 47).

Partial purification of PRK from *R. sphaeroides* and *E. coli* JM103(pPKP1565b). *R. sphaeroides* was grown anaerobically at an incident light intensity of 10 W/m² in Sistrum medium A lacking succinate and supplemented with 0.4% butyrate and 0.1% bicarbonate (43) in 165-ml milk dilution bottles until the late log phase. *E. coli* JM103(pPKP1565b) was grown as described above. Crude extracts from both sources were prepared by French pressure cell disruption as described above. Approximately 3.5 ml of crude extract from each source was layered on the surface of a 39-ml 10 to 60% linear sucrose gradient and fractionated by sedimentation velocity centrifugation in a VTi50 rotor for 14 h at 30,000 rpm (40). Each gradient was fractionated into 42 1-ml portions and assayed for PRK activity as described above. Active fractions were pooled and subjected to native gel electrophoresis as described by Gibson et al. (12). Following elution from native gel slices, fractions showing PRK activity were concentrated by ultrafiltration.

DNA and amino acid sequencing. Amino acid sequence data from the amino-terminal regions of PRK preparations derived from either *R. sphaeroides* or *E. coli* were obtained by automated Edman degradation with a gas phase sequencer at Oak Ridge National Laboratories, kindly provided by F. C. Hartman. Subclones of the 503-base-pair (bp) *Bgl*II-*Eco*RI fragment from pPRK12b containing the coding region for the amino-terminal end of *prkA* were constructed in mp18 and mp19. DNA sequencing was determined by the dideoxy chain termination method of Sanger et al. (35, 36) with the use of 7-deazaguanosine to lessen problems of secondary structure as described (3).

Materials. Restriction enzymes, T4 DNA ligase, agarose, and nick-translation kits were purchased from Bethesda

TABLE 1. Recombinant DNA plasmid constructions

Plasmid	Source of fragment	Cloning vehicle/restriction site	Fragment (kb)
pUI200	<i>R. sphaeroides</i> chromosomal DNA	pBR322/ <i>Bam</i> HI	<i>Bam</i> HI- <i>Bam</i> HI (1.600)
pPRK12b	λ 6A3 ^a	pUC19/ <i>Eco</i> RI	<i>Eco</i> RI- <i>Eco</i> RI (3.400)
pPRK12a ^b	λ 6A3	pUC19/ <i>Eco</i> RI	<i>Eco</i> RI- <i>Eco</i> RI (3.400)
pKP1565b	pPRK12b	pUC19/ <i>Pst</i> I	<i>Pst</i> I- <i>Pst</i> I (1.565)
pKSm1280b	pPRK12b	pUC19/ <i>Sma</i> I	<i>Sma</i> I- <i>Sma</i> I (1.280)
pKSp1050b	pPRK12b	pUC19/ <i>Sph</i> I	<i>Sph</i> I- <i>Sph</i> I (1.050)
pKB950b	pPRK12b	pUC19/ <i>Bam</i> HI	<i>Bam</i> HI- <i>Bam</i> HI (0.950)
pPRK12bXKn	pUC4K ^c	pPRK12b/ <i>Bgl</i> II	<i>Bam</i> HI- <i>Bam</i> HI (1.400) ^d
pPRK12b Δ P550	pPRK12b	pPRK12b/ <i>Pst</i> I	Δ <i>Pst</i> I- <i>Pst</i> I (0.550) ^e
pB1600a	pUI200	pUC19/ <i>Bam</i> HI	<i>Bam</i> HI (1.600)

^a λ 6A3 is a recombinant λ phage isolated from a genomic *R. sphaeroides* λ library.

^b Identical to pPRK12b, but the DNA insert is in the opposite orientation with respect to the *lac* promoter of pUC19.

^c pUC4K is a derivative of pUC19 which carries the kanamycin resistance gene cartridge flanked by polylinkers (50).

^d DNA restriction fragment encoding the kanamycin resistance gene inserted into the *Bgl*II restriction endonuclease site of pPRK12b.

^e This fragment was deleted from pPRK12b.

Research Laboratories or New England Biolabs, Inc. (Beverly, Mass.). A DNA sequencing Deaza-Nucleotide reagent kit was purchased from American Bionetics, Inc., Emeryville, Calif. DNA polymerase I Klenow fragment was from Boehringer Mannheim Chemicals, Indianapolis, Ind. [α -³²P]dCTP (800 Ci/mmol), [¹⁴C]sodium bicarbonate (53.0 mCi/mmol), and L-[³⁵S]methionine (1,100 Ci/mmol) were purchased from Amersham, Chicago, Ill. En³Hance was obtained from New England Nuclear Corp., Boston, Mass. Nitrocellulose and plaque screen were purchased from Schleicher and Schuell, Inc., Keene, N.H. Reagents for PRK assays were obtained from Sigma Chemical Co., St. Louis, Mo. *R. rubrum* RuBPCase was a generous gift from F. C. Hartman, Oak Ridge National Laboratories. All other reagents were of reagent grade except for phenol, which was redistilled before use.

RESULTS

Cloning of the 1.6-kb *Bam*HI fragment. When pLI10, the plasmid carrying a 3.0-kb *Bam*HI restriction endonuclease fragment from *R. sphaeroides* containing the *rbcR* gene, was nick-translated and hybridized against *Bam*HI endonuclease-restricted total DNA from *R. sphaeroides*, several weaker hybridization signals were observed in addition to the 3.0-kb *Bam*HI fragment. Under moderate stringency conditions (50% formamide, 5 $\times$ SSPE, 42°C) pLI10 showed strong homology to a 1.6-kb *Bam*HI restriction endonuclease fragment based on the intensity of hybridization signals (27). This specific 1.6-kb *Bam*HI restriction endonuclease fragment was cloned into pBR322 by using DNA derived from the 1.6-kb region of bulk *Bam*HI restriction endonuclease-digested *R. sphaeroides* DNA by standard techniques and identified following hybridization with the 3.0-kb *Bam*HI restriction endonuclease insert purified from pLI10.

This plasmid (designated pUI200) was then used as a hybridization probe against a lambda bank of *R. sphaeroides* total DNA (27). Out of approximately 10,000 phage plaques screened, 6 phage plaques showed significant homology to pUI200 and were purified further. DNA was isolated from each phage and treated with restriction endonuclease *Bam*HI to check for the presence of the 1.6-kb *Bam*HI restriction endonuclease fragment. Southern hybridization analysis confirmed that five of the six contained the 1.6-kb *Bam*HI restriction endonuclease fragment as in pUI200. One of these phage (λ 6A3) was chosen for further characterization. DNA from λ 6A3 was digested with *Eco*RI, and DNA

restriction endonuclease fragments were isolated and subcloned into pUC19 as described in Materials and Methods and Table 1. One subclone (pPRK12b) was found to contain a 3.4-kb *Eco*RI restriction endonuclease insert which contained within it the 1.6-kb *Bam*HI restriction endonuclease fragment from pUI200 as confirmed by Southern hybridization analysis. It was also determined that the 1.6-kb *Bam*HI restriction endonuclease fragment was flanked by 1,150 and 650 bp of DNA to compose the total 3.4-kb *Eco*RI restriction endonuclease fragment (Fig. 1).

We determined the precise region of homology by Southern hybridization analysis between the 3.0-kb *Bam*HI restriction endonuclease insert in pLI10 and the 1.6-kb *Bam*HI restriction endonuclease fragment in pPRK12b. We hybridized isolated restriction endonuclease fragments from pLI10 to pPRK12b cleaved with a variety of restriction endonucleases. Interestingly, one *Eco*RI-*Pst*I DNA restriction endonuclease fragment from pLI10, which spanned, approximately, from 200 to 365 bp upstream of the initiation methionine codon for the form II *rbcR* structural gene, hybridized strongly to pPRK12b. These hybridization experiments localized the region of homology to a 540-bp *Pst*I-*Bam*HI restriction endonuclease fragment within the 1.6-kb *Bam*HI restriction endonuclease fragment, as shown in Fig. 1. Two DNA restriction endonuclease fragments isolated from the coding region of *rbcR* showed only weak homology to the most distal *Bam*HI-*Eco*RI restriction endonuclease fragment from pPRK12b. This region (directly to the right of the hatch marks in Fig. 1) is now known to contain a portion of the form I *rbcL*, *S* gene cluster (14; J. Shively, P. Hallenbeck, and S. Kaplan, manuscript in preparation).

In vitro transcription-translation analysis of pPRK12b and subclones. We chose to subclone the DNA insert from pPRK12b into pUC19 and analyze each cloned restriction endonuclease fragment in an *R. sphaeroides* in vitro transcription-translation system in order to localize possible polypeptide coding regions within the 3.4-kb *Eco*RI restriction endonuclease fragment. Figure 1 shows each of the subclones generated from pPRK12b that were used to program the *R. sphaeroides* in vitro system. The description of each of the subclones is outlined in Table 1. Both pPRK12b and pPRK12a (which has the 3.4-kb *Eco*RI restriction endonuclease fragment inserted in pUC19 in the opposite orientation of that in pPRK12b) encode at least two polypeptides of 37 and 33 kDa (Fig. 2, lanes 4 and 3, respectively). We have also concluded that upstream of either one or both of these polypeptide coding regions must be an *R. sphaeroides*

FIG. 1. Physical and restriction endonuclease map of the DNA insert in pPRK12b and its derivatives. Shown above the restriction map of pPRK12b are the approximate locations encoding the 33-kDa polypeptide (PRK) and the 37-kDa polypeptide (function unknown) as determined by *in vivo* and *in vitro* analysis as described in the text. Subclones were constructed as described in Table 1. The arrow indicates the direction of transcription relative to the *lac* promoter, which is located to the left of the 33-kDa protein. The hatch marks denote the area of homology to a region of DNA upstream of the form II *rbcR* gene. Kn^R , Kanamycin resistance cassette.

promoter since *in vitro* expression was directed from the DNA insert in both orientations relative to the *lac* promoter of pUC19. When pBI600a (Fig. 1, Table 1) was used as the template, only the 37-kDa polypeptide was produced, suggesting that this gene product must be encoded completely on the 1.6-kb *Bam*HI restriction endonuclease fragment (Fig. 2, lane 6). Furthermore, deletion of a 550-bp *Pst*I restriction endonuclease fragment internal to the 1.6-kb *Bam*HI restriction endonuclease fragment in pPRK12b (pPRK12bΔP550, Fig. 1) abolished expression of the 37-kDa polypeptide but not the 33-kDa polypeptide (Fig. 2, lane 5). However, when a subclone containing a kanamycin resistance gene cartridge inserted into the *Bgl*II restriction endonuclease site of pPRK12b (pPRK12bXKn; Fig. 1, Table 1) was analyzed, we found that expression of only the 33-kDa polypeptide was abolished (Fig. 2, lane 11). This indicated that the gene for the 33-kDa product was located upstream (orientation relative to the *lac* promoter) of that for the 37-kDa polypeptide and should be localized to the 1,565-bp *Eco*RI-*Pst*I restriction endonuclease fragment in pKPI565b (Fig. 1). These data also tentatively suggested that each gene product was encoded on a separate operon since each gene product could be expressed *in vitro* independently of the other.

Further delineation of the region coding for the 33-kDa polypeptide was accomplished by employing subclones pKSm1280b, pKSp1050b, and pKB950b, which represent deletions of the cloned DNA extending towards the *lac* promoter (Fig. 1, Table 1). As expected, the 33-kDa polypeptide but not the 37-kDa polypeptide was expressed when pKPI565b was used as a template (Fig. 2, lane 7). It is also clear that the 33-kDa polypeptide was expressed from

pKSm1280b (Fig. 2, lane 8). The most likely explanation for the failure to detect polypeptide gene products synthesized from both pKSp1050b and pKB950b (Fig. 2, lanes 9 and 10, respectively) was that the deletions extended into the structural gene for the 33-kDa polypeptide. However, we can conclude that the majority of the coding region for the 33-kDa polypeptide is located on a 1,280-bp *Eco*RI-*Sma*I restriction endonuclease fragment. The relative positions of each of the genes coding for the 33-kDa and 37-kDa polypeptides are shown above the restriction map of pPRK12b in Fig. 1.

***In vivo* expression of the 33-kDa polypeptide.** In addition to the expression *in vitro* of the cloned DNA fragments, we looked for the expression of these polypeptides in *E. coli* F⁻M15A harboring the subclones shown in Fig. 1. Analysis of protein patterns in one-dimensional SDS-polyacrylamide gels revealed that a 33-kDa polypeptide was expressed from pPRK12b, pPRK12bΔP550, pKPI565b, and possibly pKSm1280b (Fig. 3, lanes 12, 10, 8, and 7, respectively) but not from pKSp1050b, pKB950b, or pPRK12bXKn (Fig. 3, lanes 6, 5, and 4, respectively). These results were comparable to those determined for expression of these plasmids in the *R. sphaeroides* *in vitro* transcription-translation system as discussed previously. The polypeptide synthesized in *E. coli* was shown to comigrate with the 33-kDa polypeptide expressed in the *in vitro* transcription-translation system (data not shown).

Of major importance, we demonstrated that the expression in *E. coli* of the 33-kDa polypeptide was orientation dependent relative to the *lac* promoter and was therefore not observed from pPRK12a (Fig. 3, lane 9), suggesting that expression was being driven from the *lac* promoter and not

an *R. sphaeroides* promoter. This conclusion is not unreasonable given that the *R. sphaeroides* promoter-containing DNA fragments so far examined do not function in *E. coli*. However, when IPTG was omitted from the culture medium, *E. coli* F⁻M15A(pPRK12b) expressed equivalent amounts of the 33-kDa-polypeptide as it did when IPTG was present in the culture medium, as judged by Coomassie blue staining (Fig. 3, lanes 11 and 12, respectively). However, this observation could be the result of titration of the *lac* repressor since *E. coli* F⁻M15A does not have a *lacI^r* mutation and does carry multiple copies of the pUC19 plasmid derivatives.

Expression of PRK activity in *E. coli*. Our reason for looking for PRK activity in *E. coli* strains bearing the above plasmid constructions was based on several assumptions. First, it has been shown that total PRK and RuBPCase activity increase when cells are grown photoheterotrophically on butyrate rather than malate (46). The increased level of RuBPCase is at least partly due to the increased level of mRNA for the form II RuBPCase gene (53). We therefore hypothesized that *prkA* and *rbcR* may share similar upstream DNA sequences. Second, the PRK isolated from the highly related organisms *R. capsulatus*, *Rhodopseudomonas acidophila*, and the hydrogen bacterium *A. eutrophus* were shown to have PRK subunits of 36, 32, and 33 kDa, respectively (32, 42, 45), which are similar to the polypeptides we demonstrated in vitro i.e., 37 and 33 kDa. We therefore assayed for PRK activity in *E. coli* strains harboring the plasmids shown in Fig. 1. These results (Table 2) demonstrated that the presence of PRK activity in *E. coli* correlated with the expression of the 33-kDa polypeptide (Fig. 3). Plasmids pPRK12b, pPRK12bΔP550, and

FIG. 2. Fluorograph of ³⁵S-labeled polypeptides expressed in an *R. sphaeroides* in vitro transcription-translation system with pPRK12b and its derivatives (Fig. 1) as templates. Shown is a fluorograph of plasmid-encoded polypeptides separated on a 12% SDS-polyacrylamide gel. The arrows to the right indicate *R. sphaeroides* DNA-specific polypeptides. Each lane was programmed with the following template DNA. Lanes: 1, no DNA; 2, pUC19; 3, pPRK12a; 4, pPRK12b; 5, pPRK12bΔP550; 6, pKB1600a; 7, pKP1565b; 8, pKSm1280b; 9, pKSp1050b; 10, pKB950b; 11, pPRK12bXXKn.

FIG. 3. Coomassie blue-stained 12% SDS-polyacrylamide gel of crude extracts from various *E. coli* F⁻M15A strains harboring the plasmids described in Fig. 2. Growth of cultures and preparation of crude cell-free extracts were done as described in Materials and Methods. Each lane contains 50 μg of crude cell protein from the following sources. Lanes: 1, partially purified PRK from *E. coli* JM103(pKP1565b); 2, F⁻M15A; 3, F⁻M15A(pUC4K); 4, F⁻M15A(pPRK12bXXKn); 5, F⁻M15A(pKB950b); 6, F⁻M15A(pKSp1050b); 7, F⁻M15A(pKSm1280b); 8, F⁻M15A(pKP1565b); 9, F⁻M15A(pPRK12a); 10, F⁻M15A(pPRK12bΔP550); 11, F⁻M15A(pPRK12b); 12, F⁻M15A(pPRK12b); 13, F⁻M15A(pUC19); 14, partially purified PRK from *R. sphaeroides*. The sample in lane 11 was grown in the absence of IPTG; all others were grown in the presence of IPTG.

pKP1565b directed the expression of PRK activity in *E. coli*, but plasmids pKSp1050b, pKB950b, pPRK12bXXKn, and pPRK12a in *E. coli* showed no PRK activity in crude extracts. These results strongly indicated that expression of the 33-kDa polypeptide was responsible for PRK activity and defined the coding region to a 1,565-bp *EcoRI*-*PstI* restriction endonuclease fragment on pKP1565b.

Since crude extracts from F⁻M15A(pPRK12b) grown in the presence or absence of 1 mmol of IPTG showed no difference in PRK activity, we therefore measured PRK activity in JM103(pPRK12b) because JM103 carries an F factor containing the *lacI^r* mutation. Under these conditions there was 3.5-fold-higher PRK activity when cells were grown in the presence of IPTG (Table 2). This further confirmed that PRK activity was being expressed from the *lac* promoter on pUC19. Therefore, we tentatively concluded that in plasmids pKSp1050b, pKB950b, and possibly pKSm1280b the deletions extend into the 3' end of *prkA*, eliminating or reducing PRK activity (Table 2).

Characterization of PRK activity. Since we used a coupled assay for measurement of PRK activity produced in *E. coli*, we wished to make certain that the activity we observed in extracts prepared from the *E. coli* strains carrying recombinant plasmids was authentic PRK. We demonstrated that the activity found in JM83(pPRK12b) was dependent on the

FIG. 4. Purification of PRK activity from *R. sphaeroides* and *E. coli* JM103(pKP1565b). (A) Comparison of the mobilities of PRK activity from crude soluble extracts from each bacterium fractionated on linear 10 to 60% sucrose gradients by sedimentation velocity centrifugation. Fractions from the sucrose gradients were collected and assayed for PRK activity. Growth of cultures, preparation of

TABLE 2. Effects of deletion analysis, DNA fragment orientation, and IPTG on PRK activity in *E. coli*

Source of extract	IPTG present	PRK activity (nmol/mg of protein per min)
F ⁻ M15A	+	<1.0
F ⁻ M15A(pUC4K)	+	<1.0
F ⁻ M15A(pPRK12bXKn) ^a	+	<1.0
F ⁻ M15A(pKB950b)	+	<1.0
F ⁻ M15A(pKSp1050b)	+	<1.0
F ⁻ M15A(pKSm1280b)	+	20.6
F ⁻ M15A(pKPI1565b)	+	252.0
F ⁻ M15A(pPRK12a)	+	1.5
F ⁻ M15A(pPRK12bΔP550)	+	154.0
F ⁻ M15A(pPRK12b)	—	199.0
F ⁻ M15A(pPRK12b)	+	215.0
F ⁻ M15A(pUC19)	+	<1.0
JM103(pPRK12b)	+	1,288.0
JM103(pPRK12b)	—	371.0

^a Insertion of 1.4-kb *Bam*HI restriction endonuclease fragment containing the kanamycin resistance gene into the *Bgl*II restriction endonuclease site of pPRK12b (see Table 1 and Fig. 1).

presence of ATP and RuMP, the known substrates of PRK. The product was identified as RuBP by high-pressure liquid chromatography (data not shown). Furthermore, the activity was dependent on the coupling enzyme spinach carboxylase. The activity decreased 95.4% by omission of NADH since this compound has been shown to be a positive activator of PRK from both the purple non-sulfur photosynthetic bacteria (31, 45) and many chemolithotrophic bacteria (6, 42). On the other hand, AMP and PEP were found to be inhibitors of enzyme activity, giving 61.5 and 95% lower activities, respectively, when added to the assay mixture. Leadbeater et al. found similar results with the enzyme from *A. eutrophus* (19) and Rindt et al. with the PRK from *R. sphaeroides* (31).

Amino acid and DNA sequence analysis of PRK. To further define and characterize the *prkA* gene, we sought to purify the active enzyme from *R. sphaeroides* and *E. coli* JM103(pKP1565b) to directly compare the two gene products. Figure 4A shows the profile of a sedimentation velocity sucrose gradient centrifugation of crude extracts from *R. sphaeroides* grown photosynthetically on 0.4% butyrate or 0.1% bicarbonate in Sistrom medium (43) and *E. coli* JM103(pKP1565b) grown as described in Materials and Methods. It appeared that the two samples had the same migration rate for PRK activity as determined from measurements of the activity in each fraction derived from the gradient. Interestingly, some PRK activity from *E. coli* JM103(pKP1565b) appeared to have a much slower migration rate and remained near the top of the gradient. Whether this activity was derived from PRK subunits that were not being assembled or were only incompletely assembled re-

extracts, and enzyme assays are described in Materials and Methods. Symbols: Δ , refractive index (identical for both samples); $\circ$, PRK activity from *E. coli* JM103(pKP1565b); $\bullet$, PRK activity from *R. sphaeroides*. (B) Silver-stained SDS-polyacrylamide gel of purified PRK. Active fractions from the sucrose gradient were pooled and subjected to electrophoresis on a native gel. The gel was then divided into fractions, and protein was eluted and assayed for PRK activity as described in Materials and Methods. Portions were then electrophoresed on a 12% SDS-polyacrylamide gel and silver stained. Lanes: 1, fraction 17 from native gel of *R. sphaeroides* extract; 2, peak activity fraction (fraction 17) from native gel of *E. coli* JM103(pKP1565b); 3, activity fraction (fraction 12) from native gel of *R. sphaeroides* extract.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
<u>R. SPHAEROIDES</u> DNA SEQUENCE:	<u>GGAGC</u> TGAATATCC	ATG	AGC	AAG	AAG	CAT	CCC	ATC	ATC	TCG	GTC	ACG	GGC	TCG	TCC	GGC	GCG	
DEDUCED AA SEQUENCE:		MET	SER	LYS	LYS	HIS	PRO	ILE	ILE	SER	VAL	THR	GLY	SER	SER	GLY	ALA	
<u>E. COLI</u> AA SEQUENCE ¹ :			SER	LYS	LYS	...	PRO	ILE	ILE	SER	VAL	THR	GLY	SER	SER	GLY	ALA	
<u>R. SPHAEROIDES</u> AA SEQUENCE ² :			SER	LYS	LYS	...	PRO	ILE	ILE	SER	VAL	THR	GLY					
<u>A. EUTROPHUS</u> AA SEQUENCE ³ :			X	GLU	ARG	TYR	PRO	ILE	ILE	ALA	ILE	THR	GLY	SER	SER	GLY	ALA	
SPINACH AA SEQUENCE ⁴ :			SER	GLN	GLN	GLN	THR	ILE	VAL	ILE	GLY	LEU	ALA	ALA	ASP	SER	GLY	CYS

	17	18	19	20	21	22	23	24	25	26	27	28
<u>R. SPHAEROIDES</u> DNA SEQUENCE:	GGC	ACC	TCG	ACG	GTG	AAG	CAC	ACG	TTC	GAC	CAG	ATC
DEDUCED AA SEQUENCE:	GLY	THR	SER	THR	VAL	LYS	HIS	THR	PHE	ASP	GLN	ILE
SPINACH AA SEQUENCE ⁴ :	GLY	LYS	SER	THR	PHE	MET	ARG					

FIG. 5. Partial nucleotide sequence of *prkA* from *R. sphaeroides* and the deduced amino acid (AA) sequence compared with the derived amino acid sequences for PRK from: 1, *E. coli* JM103(pKP1565b); 2, *R. sphaeroides* 2.4.1; 3, *A. eutrophus* (18); and 4, spinach (28). Undetermined amino acid residues are indicated with ellipses.

mains unknown. Those fractions containing the slower-migrating PRK were not further purified. Active fractions from both samples were pooled and subjected to native gel electrophoresis as described by Gibson et al. (12). After slicing the gel into a series of equal fractions and eluting the protein, measurements of PRK activity were performed. The *E. coli* PRK activity appeared to have electrophoresed as a single peak (data not shown). There was significant PRK activity from the *R. sphaeroides* sample which comigrated precisely with the *E. coli* sample on the native gel. Interestingly, however, there were several other peaks of activity with slower mobility on the native gel from the *R. sphaeroides* sample. To investigate the possibility that these peaks may have been due to aggregation of the native enzyme, we subjected two different *R. sphaeroides* fractions and the one *E. coli* fraction to SDS-polyacrylamide gel electrophoresis. The two *R. sphaeroides* fractions (one fraction that comigrated with the *E. coli* sample and one that did not) and the *E. coli* fraction contained the 33-kDa polypeptide. This supported our hypothesis that the *R. sphaeroides* PRK was aggregating with other proteins not found in *E. coli*. Determination of the specific activity of these fractions was difficult due to both aggregation and loss of activity following native gel electrophoresis. Nonetheless, these polypeptides showed identical mobilities on an SDS-polyacrylamide gel (Fig. 4B). Furthermore, these polypeptides comigrated with the 33-kDa polypeptide identified as being expressed from all of the subclones shown in Fig. 3, lanes 1 and 14.

In collaboration with F. C. Hartman, the amino-terminal amino acid sequence was determined for both of the activities isolated above. Furthermore, the DNA sequence between the *EcoRI* and *BglIII* restriction endonuclease sites of pPRK12b (Fig. 1) was also determined for both strands. Alignment of the deduced amino acid sequence with that of the amino acid sequence determined for the *R. sphaeroides*-derived PRK positioned the start codon for the *prkA* gene 85 bp upstream of the *BglIII* restriction endonuclease site (Fig. 5). A putative ribosome-binding site was located 10 bp upstream of the ATG start codon. Comparison of the amino acid sequence of the *prkA* gene product synthesized in *E. coli* with that isolated from *R. sphaeroides* demonstrated that the gene product expressed in *E. coli* was not a fusion protein and was being transcribed and translated from the

EcoRI restriction endonuclease site toward the *PstI* site in pPRK12b, and at the level examined was identical to the *R. sphaeroides* gene product produced in vivo. The absence of an unambiguously defined amino acid residue, noted for position 5, was undoubtedly due to impurities in the samples, which take on some prominence when coupled with the difficulty in detecting histidine. However, all other amino acid residues were identical regardless of the source of the enzyme and aligned perfectly with the sequence derived from the DNA sequence.

When compared with the published amino-terminal amino acid sequence of the PRK from *A. eutrophus*, we observed 60% identity at the amino acid level. This is in agreement with the DNA sequence homology since a probe containing the *prk* gene from *A. eutrophus*, kindly provided recently by Dr. Bowien, hybridized to the *prkA* gene from *R. sphaeroides* (data not shown). Although there was only 30% homology between the deduced amino acid sequence of the *prkA* gene of *R. sphaeroides* and the amino acid sequence determined for spinach PRK (only the first 23 amino acids were compared), there was greater than 50% homology when functionally related amino acid residues were compared.

DISCUSSION

A 3.4-kb *EcoRI* restriction endonuclease fragment derived from *R. sphaeroides* was found to contain the *prkA* gene, a gene coding for a 37-kDa polypeptide, and a presumptive portion of the form I *rbcL*, *S* gene cluster as determined by hybridization studies with a portion of the *rbcR* structural gene as a probe and DNA sequencing (unpublished results). We have also determined that there is significant homology between a region of DNA upstream of the form II *rbcR* gene and a region believed to be immediately upstream of the form I *rbcL*, *S* genes.

The structural gene product for *prkA* has been identified as a 33-kDa polypeptide. Its entire coding region was localized on this 3.4-kb *EcoRI* restriction endonuclease fragment to a 1,565-bp *PstI*-*EcoRI* restriction endonuclease fragment by in vitro and in vivo analyses. SDS-polyacrylamide gel electrophoresis of either crude extracts of *E. coli* F⁻M15A (pKP1565b) or the products derived from an in vitro tran-

scription-translation analysis employing this and other related plasmids revealed the presence of a unique 33-kDa polypeptide. Plasmid pKSp1050b, which contains a deletion of 515 bp from the *Pst*I to the *Sph*I restriction endonuclease sites in pKP1565b, did not express the 33-kDa polypeptide in *E. coli* strains harboring the plasmid or in the *R. sphaeroides* in vitro transcription-translation system. Likewise, pPRK12bXKN, which contains a kanamycin resistance gene cartridge inserted into the *Bgl*II restriction endonuclease site of pPRK12b, did not express the 33-kDa polypeptide in vivo or in vitro. Furthermore, high levels of PRK activity were detected in *E. coli* F⁻M15A(pKP1565b) but not in *E. coli* F⁻M15A(pKSp1050b) or *E. coli* F⁻M15A(pPRK12bXKN). Although *E. coli* F⁻M15A(pKSm1280b) showed only low levels of PRK activity in vivo (and barely detectable amounts of 33-kDa polypeptide), the plasmid gave rise to substantial amounts of gene product in vitro. This would appear to be the only discrepancy between the results obtained in vivo and in vitro. However, if this deletion removed only a few of the carboxy-terminal amino acids from the 33-kDa polypeptide, no change in migration might be noted but the stability and, hence, the activity of the protein in vivo could be severely affected.

We have characterized the PRK activity expressed in vivo and verified its authenticity. PRK activity in *E. coli* JM83(pPRK12b) is dependent on the known substrates of PRK, namely ATP and RuMP. Furthermore, the product of the restriction (RuBP) was detected in both enzyme assays and by high-pressure liquid chromatography against known RuBP as a standard.

Activation of the PRK expressed in *E. coli* JM83 (pPRK12b) by NADH was virtually identical to the reported NADH activation of the PRK from *R. sphaeroides* (45). Also observed was the inhibition of PRK activity by AMP and PEP which has been observed for a variety of bacterial groups, including *R. sphaeroides* (2, 19, 31). This form of "metabolic signaling" by metabolic intermediates which reflect the energetic and redox states of the cell (in this case NADH and AMP) is similar for PRKs among both prokaryotes and eucaryotes. In fact, PRK isolated from plants and oxygenic photosynthetic bacteria (cyanobacteria) also show regulation by the redox state of the cell by the ferredoxin-thioredoxin system (8, 29). Furthermore, the PEP inhibition of PRK observed here as well as the NADH activation of PRK was very similar to that observed for the PRK from *A. eutrophus*. Since the subunit M_r of PRK from *R. sphaeroides* is 33,000, it is very similar to that found for the *A. eutrophus* enzyme of 33 kDa (42) and the *R. acidophila* enzyme of 32 kDa (32). Although no attempt was made to accurately size the oligomeric state of the PRK, sedimentation velocity centrifugation of crude extracts from both *E. coli* JM103(pKP1565b) and *R. sphaeroides* revealed that the sedimentation rates for PRK activity from both preparations were nearly identical. Furthermore, the sedimentation rate was near that found for the form II RuBPCase. Therefore, the possibility exists that the PRK from *R. sphaeroides* is an octomer of identical subunits, as is the PRK from *A. eutrophus* (42).

We showed directly that expression of the 33-kDa polypeptide in *E. coli* was responsible for PRK activity in vivo by a comparison of partially purified PRK from *R. sphaeroides* and *E. coli* JM103(pKP1565b). The determination of the amino-terminal amino acid sequence of the enzyme isolated from both sources unambiguously confirmed their identity. Although the amino acid at position 5 was undeterminable, probably because of difficulties in extracting histidine, the

probability that the other 11 amino acids were identical is virtually nonexistent. Alignment of the deduced amino acid sequence derived from the DNA sequence with the determined amino-terminal amino acid sequence from either the *E. coli* or *R. sphaeroides* preparations positioned the start of the *prkA* gene precisely 85 bp upstream of the *Bgl*II restriction endonuclease site in pPRK12b with the end of the structural gene located approximately 285 bp upstream of the *Pst*I site, as shown in Fig. 1.

Comparing the deduced amino acid sequence with the published amino acid sequence of *A. eutrophus* showed greater than 60% homology, supporting our other observations. Furthermore, when the amino acid differences between the *R. sphaeroides* and *A. eutrophus* enzymes are considered, these are virtually homologous for the first 16 amino acids. Regions of similarity between PRK from *R. sphaeroides* and spinach PRK (28; F. C. Hartman, personal communication) can be compared when these amino acid sequences are compared with the amino acid sequences from several ATP-binding proteins (15). With the exception of amino acid 18, the amino acid sequence for both the spinach and the *R. sphaeroides* PRK at positions 12, 14 to 15, and 17 to 20 show remarkable similarity to the proposed phosphate-binding consensus sequence. Other similarities are observed for the amino acid sequences derived for a variety of ATP-binding proteins, especially those which are peripherally associated with the cytoplasmic membrane. For example, alignment of the amino acid sequence of the *hisP* product, which has been reported to be peripherally associated with the cytoplasmic membrane, between positions 35 and 50 and of *R. sphaeroides* PRK between positions 8 and 23 shows 9 of 15 identical residues (15). Interestingly, PRK from *R. capsulatus* has been postulated to be peripherally associated with the cytoplasmic membrane (45). It seems likely that further comparisons will not only reveal extensive conservations of amino acid sequence at the ATP binding site but also shed light on the structure-function relationship of PRK.

The identification and location of the gene coding for the 37-kDa polypeptide are based on the fact that the 3.4-kb *Eco*RI restriction endonuclease fragment directed the synthesis of a 37-kDa polypeptide in both orientations when cloned into pUC19. Deletion and insertion analysis placed the 37-kDa polypeptide coding region 3' of *prkA* and residing entirely on the 1.6-kb *Bam*HI restriction endonuclease fragment, as shown in Fig. 1. We also infer, from still fragmentary evidence, that this gene is transcribed opposite to the *prkA* gene. We have not detected its expression in *E. coli*, primarily because in that region of the gel the background is high, nor do we know its function. However, a DNA fragment internal to the 37-kDa polypeptide coding region shows strong homology to an approximately 165-bp DNA restriction endonuclease fragment which lies upstream of the form II *rbcR* gene. Whether this represents a portion of another copy of the 37-kDa polypeptide has yet to be determined. Finally, the 37-kDa polypeptide expressed in vitro is always accompanied by a smaller polypeptide differing by approximately 1.5 to 1.8 kDa. Although several explanations for these observations are possible, further experimentation is required.

It is also possible that these regions of DNA homology could represent regulatory regions for both the form II *rbcR* and form I *rbcL*, *S* genes since the form I genes were found to lie near the proposed 5' end of the 37-kDa polypeptide. Preliminary evidence for this gene organization was obtained with both the form I *rbcL*, *S* gene cluster from *Anacystis*

nidulans and portions of the structural gene for *rbcR* as hybridization probes (Shively et al., manuscript in preparation) and by a comparison of the restriction endonuclease-generated map of this genetic region with the restriction map recently published for the form I *rbcL* and *rbcS* gene cluster (14, 37–39). Furthermore, comparison of the first 51 deduced amino acids of the form I *rbcL* gene product from *R. sphaeroides* showed greater than 45% homology to the deduced sequence of the first 46 amino acids of the *A. nidulans* form I *rbcL* gene (data not shown) (39). Because this 3.4-kb DNA fragment contained on one end the *prkA* gene and on the other the *rbcL*, *S* genes, we envision that the 37-kDa polypeptide whose coding region lies between these two represents an activity which is important in the operation or regulation of the reductive Calvin pathway. The evidence accumulated to date suggests that *prkA*, the 37-kDa polypeptide, and *rbcL*, *S* are organized in separate transcriptional units. Furthermore, it appears that both *prkA* and *rbcL*, *S* are transcribed in the same direction, with the 37-kDa polypeptide transcribed in the opposite direction. DNA regions on either side of the 3.4-kb *EcoRI* restriction endonuclease fragment are presently available.

Interestingly, there appears to be a region of DNA located between the form I *rbcL* and *rbcS* gene cluster and the *prkA* gene of *A. eutrophus*. However, there are two copies of each gene cluster in *A. eutrophus*, one copy on a plasmid and one copy on the chromosome (18). Although a restriction endonuclease fragment containing at least a portion of the *prkA* gene from the plasmid copy of *A. eutrophus* hybridized to a second *EcoRI* fragment, we have no direct evidence that this represents a second copy of the *prkA* gene, although this possibility cannot be dismissed.

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LITERATURE CITED

- Abdelal, A. T. H., and H. G. Schlegel. 1974. Purification and regulatory properties of phosphoribulokinase from *Hydrogenomonas eutropha* H16. *Biochem. J.* **139**:481–489.
- Ballard, R. W., and R. D. MacElroy. 1971. Phosphoenolpyruvate, a new inhibitor of phosphoribulokinase in *Pseudomonas facilis*. *Biochem. Biophys. Res. Commun.* **44**:614–618.
- Barr, P. J., R. M. Thayer, P. Taybourn, R. C. Najarian, F. Seela, and D. R. Tolan. 1986. 7-Deaza-2'-deoxyguanosine-5'-triphosphate enhanced resolution in M13 dideoxy sequencing. *Biotechniques* **4**:428–432.
- Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**:1513–1523.
- Bolivar, F., R. Rodriguez, P. J. Greene, M. Betlack, H. L. Hegekec, H. W. Boyer, J. Rosa, and S. Falkow. 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* **2**:95–113.
- Bowien, B., and L. Leadbeater. 1984. Molecular and cellular regulation of carbon dioxide assimilation in bacteria, p. 9–13. In R. L. Crawford and R. S. Hanson (ed.), *Microbial growth on C₁ compounds*. American Society for Microbiology, Washington, D.C.
- Chory, J., and S. Kaplan. 1982. The *in vitro* transcription-translation of DNA and RNA templates by extracts of *Rhodospseudomonas sphaeroides*. *J. Biol. Chem.* **257**:15110–15121.
- Crawford, N. A., C. W. Sutton, B. C. Yee, T. C. Johnson, D. C. Carlson, and B. B. Buchanan. 1984. Contrasting modes of photosynthetic enzyme regulation in oxygenic and anoxygenic prokaryotes. *Arch. Microbiol.* **139**:124–129.
- Dretzen, G., M. Bellard, P. Sassone-Corsi, and P. Chambon. 1981. A reliable method for recovery of DNA fragments from agarose or acrylamide gels. *Anal. Biochem.* **172**:295–298.
- Friedrich, C. G., B. Bowien, and B. Friedrich. 1979. Formate and oxalate metabolism in *Alcaligenes eutrophus*. *J. Gen. Microbiol.* **115**:185–192.
- Friedrich, C. G., B. Friedrich, and B. Bowien. 1981. Formation of enzymes of autotrophic metabolism during heterotrophic growth of *Alcaligenes eutrophus*. *J. Gen. Microbiol.* **122**:69–78.
- Gibson, J. L., and F. R. Tabita. 1977. Different molecular forms of D-ribulose 1,5-bisphosphate carboxylase from *Rhodospseudomonas sphaeroides*. *J. Biol. Chem.* **252**:943–949.
- Gibson, J. L., and F. R. Tabita. 1977. Isolation and preliminary characterization of two forms of ribulose 1,5-bisphosphate carboxylase from *Rhodospseudomonas capsulata*. *J. Bacteriol.* **132**:818–823.
- Gibson, J. L., and F. R. Tabita. 1986. Isolation of the *Rhodospseudomonas sphaeroides* from I ribulose 1,5-bisphosphate carboxylase/oxygenase large and small subunit genes and expression of active hexadecameric enzyme in *Escherichia coli*. *Gene* **44**:271–278.
- Higgins, C. F., I. D. Hiles, G. P. C. Salmond, D. R. Gill, J. A. Downie, I. J. Evans, I. B. Holland, L. Gray, S. D. Buckel, A. W. Bell, and M. A. Hermodson. 1986. A family of related ATP binding subunits coupled to many distinct biological processes in bacteria. *Nature (London)* **323**:448–450.
- Im, D. S., and C. G. Friedrich. 1983. Fluoride, hydrogen, and formate activate ribulose bisphosphate carboxylase formation in *Alcaligenes eutrophus*. *J. Bacteriol.* **154**:803–808.
- Jouanneau, Y., and F. R. Tabita. 1986. Independent regulation of synthesis of form I and form II ribulose bisphosphate carboxylase-oxygenase on *Rhodospseudomonas sphaeroides*. *J. Bacteriol.* **165**:620–624.
- Klntworth, R., M. Husemann, J. Salnikow, and B. Bowien. 1985. Chromosomal and plasmid locations for phosphoribulokinase genes in *Alcaligenes eutrophus*. *J. Bacteriol.* **164**:954–956.
- Leadbeater, L., and B. Bowien. 1984. Control of autotrophic carbon assimilation in *Alcaligenes eutrophus* by the activation and reactivation of phosphoribulokinase. *J. Bacteriol.* **157**:95–99.
- Leadbeater, L., K. Siebert, P. Schobert, and B. Bowien. 1982. Relationship between activities and protein levels of ribulose-bisphosphate carboxylase and phosphoribulokinase in *Alcaligenes eutrophus*. *FEMS Microbiol. Lett.* **14**:263–266.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275.
- Madigan, M. T., and H. Gest. 1979. Growth of the photosynthetic bacterium *Rhodospseudomonas capsulata* chemoautotrophically in darkness with H₂ as the energy source. *J. Bacteriol.* **127**:530–542.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Maquat, L. E., and W. S. Reznikoff. 1978. *In vitro* analysis of the *Escherichia coli* RNA polymerase interaction with wild-type and mutant lactose promoters. *J. Mol. Biol.* **125**:467–490.
- Messing, J. 1983. New M13 vectors for cloning. *Methods Enzymol.* **101**:20–77.
- Messing, J., and J. Vieira. 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. *Gene* **19**:269–276.

27. Muller, E. D., J. Chory, and S. Kaplan. 1984. Cloning and characterization of the gene product of the form II ribulose-1,5-bisphosphate carboxylase gene of *Rhodopseudomonas sphaeroides*. J. Bacteriol. **161**:469-472.
28. Porter, M. A., and F. C. Hartman. 1986. Commonality of catalytic and regulatory sites of spinach phosphoribulokinase: characterization of a tryptic peptide that contains an essential cysteinyl residue. Biochemistry **25**:7314-7318.
29. Porter, M. A., S. Milanez, C. D. Stringer, and F. C. Hartman. 1986. Purification and characterization of ribulose-5-phosphate kinase from spinach. Arch. Biochem. Biophys. **245**:14-23.
30. Quivey, R. J., Jr., and F. R. Tabita. 1984. Cloning and expression in *Escherichia coli* of the form II ribulose 1,5-bisphosphate carboxylase/oxygenase gene from *Rhodopseudomonas sphaeroides*. Gene **31**:91-101.
31. Rindt, K. P., and E. Ohmann. 1969. NADH and AMP as allosteric effectors of ribulose-5-phosphate kinase in *Rhodopseudomonas sphaeroides*. Biochem. Biophys. Res. Commun. **36**:357-364.
32. Rippel, S., and B. Bowien. 1984. Phosphoribulokinase from *Rhodopseudomonas acidophila*. Arch. Microbiol. **139**:207-212.
33. Ruther, V., M. Koenen, K. Otto, and B. Muller-Hill. 1981. pUR222, a vector for cloning and rapid chemical sequencing of DNA. Nucleic Acids Res. **9**:4087-4098.
34. Saito, H., and K. Miura. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim. Biophys. Acta. **72**:619-629.
35. Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe. 1980. Cloning in single stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161-178.
36. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463-5467.
37. Shinozaki, K., and M. Sugiura. 1983. The gene for the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase is located close to the gene for the large subunit in the cyanobacterium *Anacystis nidulans* 6301. Nucleic Acids Res. **11**:6957-6967.
38. Shinozaki, K., and M. Sugiura. 1985. Genes for the large and small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase constitute a single operon on a cyanobacterium *Anacystis nidulans* 6301. Mol. Gen. Genet. **200**:27-32.
39. Shinozaki, K., C. Yamada, N. Takakata, and M. Sugiura. 1983. Molecular cloning and sequence analysis of the cyanobacterial gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. Proc. Natl. Acad. Sci. USA **80**:4050-4054.
40. Shively, J. M., E. Davidson, and B. L. Marrs. 1984. Derepression of the synthesis of the intermediate and large forms of ribulose-1,5-bisphosphate carboxylase/oxygenase on *Rhodopseudomonas capsulata*. Arch. Microbiol. **138**:233-236.
41. Siebert, K., and B. Bowien. 1984. Evidence for an octameric structure of phosphoribulokinase from *Alcaligenes eutrophus*. Biochim. Biophys. Acta **787**:208-214.
42. Siebert, K., P. Schobert, and B. Bowien. 1981. Purification, some catalytic and molecular properties of phosphoribulokinase from *Alcaligenes eutrophus*. Biochim. Biophys. Acta **658**:35-44.
43. Siström, W. R. 1962. The kinetics of the synthesis of photopigments in *Rhodopseudomonas sphaeroides*. J. Gen. Microbiol. **28**:607-616.
44. Southern, E. 1979. Gel electrophoresis of restriction fragments. Methods Enzymol. **68**:152-176.
45. Tabita, F. R. 1980. Pyridine nucleotide control and subunit structure of phosphoribulokinase from photosynthetic bacteria. J. Bacteriol. **143**:1275-1280.
46. Tabita, F. R. 1981. Molecular regulation of carbon dioxide assimilation in autotrophic microorganisms, p. 70-81. In H. Dalton (ed.), Microbial growth on C₁ compounds. Heydon and Sons, London.
47. Tabita, F. R., P. Caruso, and W. Whitman. 1978. Facile assay of enzymes unique to the Calvin cycle, with special reference to ribulose 1,5-bisphosphate carboxylase. Anal. Biochem. **84**:462-472.
48. Tabita, F. R., and B. A. McFadden. 1974. D-Ribulose 1,5-diphosphate carboxylase from *Rhodospirillum rubrum*. I. Levels, purification and effects of metabolic ions. J. Biol. Chem. **249**:3453-3458.
49. Van Neil, L. B. 1944. The culture, general physiology, morphology, and classification of the nonsulfur purple and brown bacteria. Bacteriol. Rev. **8**:1-118.
50. Vieira, J., and J. Messing. 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259-268.
51. Weaver, K. E., and F. R. Tabita. 1983. Isolation and partial characterization of *Rhodopseudomonas sphaeroides* mutants defective in the regulation of ribulose bisphosphate carboxylase/oxygenase. J. Bacteriol. **156**:507-515.
52. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103-119.
53. Zhu, Y. S., and S. Kaplan. 1985. Effects of light, oxygen, and substrates on steady-state levels of mRNA coding for ribulose 1,5-bisphosphate carboxylase and light-harvesting and reaction center polypeptides on *Rhodopseudomonas sphaeroides*. J. Bacteriol. **162**:925-932.