JOURNAL OF BACTERIOLOGY, May 1988, p. 2153–2158
0021-9193/88/052153-06$02.00/0

Vol. 170, No. 5

Localization and Mapping of CO_2 Fixation Genes within Two Gene Clusters in *Rhodobacter sphaeroides*

JANET L. GIBSON AND F. ROBERT TABITA*

Center for Applied Microbiology and Department of Microbiology, The University of Texas at Austin, Austin, Texas 78712-1095

Received 24 November 1987/Accepted 3 February 1988

Two fructose 1,6-bisphosphatase structural genes (*fbpA* and *fbpB*) have been identified within two unlinked gene clusters that were previously shown to contain the *Rhodobacter sphaeroides* sequences that code for form I and form II ribulose 1,5-bisphosphate carboxylase-oxygenase and phosphoribulokinase. The *fbpA* and *fbpB* genes were localized to a region immediately upstream from the corresponding *prkA* and *prkB* sequences and were found to be transcribed in the same direction as the phosphoribulokinase and ribulose 1,5-bisphosphate carboxylase-oxygenase genes based on inducible expression of fructose 1,6-bisphosphatase activity directed by the *lac* promoter. A recombinant plasmid was constructed that contained the tandem *fbpA* and *prkA* genes inserted downstream from the *lac* promoter in plasmid pUC18. Both gene products were expressed in *Escherichia coli* upon induction of transcription with isopropyl β-D-thiogalactoside, demonstrating that the two genes can be cotranscribed. A *Zymomonas mobilis* glyceraldehyde 3-phosphate-dehydrogenase gene (*gap*) hybridized to a DNA sequence located approximately 1 kilobase upstream from the form II ribulose 1,5-bisphosphate carboxylase-oxygenase gene. Although no corresponding *gap* sequence was found within the form I gene cluster, an additional region of homology was detected immediately upstream from the sequences that encode the form I and form II ribulose 1,5-bisphosphate carboxylase-oxygenases.

Rhodobacter sphaeroides synthesizes isofunctional forms of two key regulatory enzymes of the Calvin cycle; phosphoribulokinase (PRK) and ribulose 1,5-bisphosphate carboxylase-oxygenase (RuBISCO) (9, 11). PRK catalyzes the phosphorylation of ribulose 5-phosphate to yield ribulose 1,5-bisphosphate, the CO_2 acceptor molecule, whereas RuBISCO catalyzes the carboxylative cleavage of ribulose 1,5-bisphosphate to yield two molecules of 3-phosphoglyceric acid. The two forms of RuBISCO are structurally distinct; the form I RuBISCO is comprised of eight large subunits and eight small subunits, whereas the form II RuBISCO is composed exclusively of large subunits (9). The form I and form II RuBISCOs exhibit marked differences in catalytic properties in vitro (9, 14), and physiological studies suggest that in vivo the two enzymes are subject to independent regulation (15, 16). Less is known about the two forms of PRK, except that immunologically the two proteins are indistinguishable, and DNA hybridizations suggest that a high degree of similarity exists between the two genes (11). Therefore, the two kinases appear to be closely related, in contrast to the vast divergence of structure and function of the carboxylases.

We recently showed that the RuBISCO- and PRK-coding sequences are linked in two separate gene clusters within the *R. sphaeroides* genome (11). The *prkA* gene is located a short distance of less than 1 kilobase (kb) upstream from the *rbcL rbcS* genes that encode the form I RuBISCO (11, 12). The *prkB* gene is situated 3.5 kb upstream from *rbpL* (11), the gene encoding the form II RuBISCO large subunit (19, 20). The RuBISCO and PRK genes within each cluster are transcribed in the same direction (11). Although the biological relevance of the isofunctional enzymes is not clear, the occurrence of two distinct forms of RuBISCO in a limited number of bacterial species probably reflects a unique metabolic requirement for this group of bacteria. The purple nonsulfur photosynthetic bacteria are known for metabolic versatility, and the synthesis of isofunctional enzymes of the Calvin cycle with different catalytic and regulatory characteristics may provide a broadened capacity for growth in diverse environments.

To better understand the significance of the two sets of enzymes, we examined more closely the molecular organization of the two gene clusters with respect to other structural genes encoding Calvin cycle enzymes. In this paper we show that, in addition to *prkA* and *prkB*, other genes encoding enzymes involved in CO_2 assimilation are present in duplicate copies and are linked to the PRK and RuBISCO genes.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* JM107 (26) was used for transformation and propagation of pUC derivatives, whereas *E. coli* HB101 (3) was the host strain for the pVK102 derivatives pJG336 and pJG106. The plasmids used in this study are listed in Table 1.

Medium and growth conditions. *E. coli* was routinely grown in Luria broth (LB) at 37°C (7). Ampicillin was used at 50 μg/ml, and tetracycline was used at 25 μg/ml. For detection of inserts in pUC vectors, LB agar plates contained 5-bromo-4-chloro-3-indolyl-β-D-galactoside and isopropyl-β-D-thiogalactoside (IPTG) at final concentrations of 40 μg/ml and 0.1 mM, respectively. For expression studies in liquid cultures, IPTG was added to a final concentration of 1 mM as described previously (10, 11).

DNA manipulations. Most of the procedures used in this study, including transformation, agarose gel electrophoresis, and elution of DNA fragments from agarose gels, were described by Maniatis et al. (17). Nick translations of probes were carried out by the procedure of Rigby et al. (21). Southern blots and DNA hybridizations were performed according to the protocol supplied with Gene-Screen Plus membranes (NEN Research Products, DuPont) as described

* Corresponding author.

TABLE 1. Plasmids used in this study

Plasmid	Relevant characteristics	Source or reference
pUC8	Ap^r	Vieira and Messing (25)
pUC9	Ap^r	Vieira and Messing (25)
pUC18	Ap^r	Yanisch-Perron et al. (26)
pJS33	Ap^r, pBR322 containing *E. coli fbp* gene	Sedivy et al. (22)
pLOI312	Ap^r, pUC8 containing *Z. mobilis gap* gene	Conway et al. (6)
pJG336	Tc^r, pVK102 with 24-kb *Hin*dIII insert of *R. sphaeroides* DNA	Gibson and Tabita (10)
pJG106	Tc^r, pVK102 with 26-kb *Hin*dIII insert of *R. sphaeroides* DNA	Gibson and Tabita (10)
pJG6	Ap^r, pUC8 with 3.4-kb *Eco*RI insert containing *prkA*	Gibson and Tabita (10)
pJG93	Ap^r, pUC9 with 1.8-kb *Xho*I-*Bam*HI insert containing *fbpA*	This study
pJG183	Ap^r, pUC18 with 1.8-kb *Xho*I-*Bam*HI insert of pJG93 in reverse orientation	This study
pJG16	Ap^r, pUC8 with 1.6-kb *Bam*HI insert containing *fbpB*	This study
pJG15	Ap^r, pUC8 with 1.6-kb *Bam*HI insert of pJG16 in reverse orientation	This study
pJG1211	Ap^r, pUC18 with 2.8-kb *Xho*I fragment containing *fbpA prkA*	This study
pJG1244	Ap^r, pUC18 with 2.8 kb *Xho*I fragment of pJG1211 in reverse orientation	This study
pJG549	Ap^r, pUC8 with 2.1-kb *Bam*HI fragment containing *gapB*	This study
pJG5410	Ap^r, pUC8 with 2.1-kb *Bam*HI fragment of pJG549 in reverse orientation	This study

previously (10). Basically, DNA hybridizations were performed in 35% formamide at 39°C, and washes were in 5 × SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 55°C. All enzymes were used as specified by the supplier.

Enzyme assays. Cell extracts for enzyme assays were prepared from sonically disrupted cells as described previously (11). Fructose 1,6-bisphosphatase (FBP) activity was measured spectrophotometrically by using a coupled enzyme assay essentially as described by Springgate and Stachow (23). The reaction mixture contained (in 1.0 ml) 100 mM Tris chloride (pH 8.5), 1.0 mM NADP, 0.1 mM $MnCl_2$, 1.0 mM dithiothreitol, 0.2 mM fructose 1,6-bisphosphate, and 2 U each of phosphoglucose isomerase and glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of enzyme. Any variations in these assay conditions are noted in the text.

Sedoheptulose-1,7-bisphosphatase activity was measured by determination of Pi formed by the method of Ames (2) with the assay conditions described above for FBP, except that 0.2 mM sedoheptulose 1,7-bisphosphate replaced fructose 1,6-bisphosphate, and the coupling enzymes and $NADP^+$ were omitted from the reaction mixture. For comparison of sedoheptulose 1,7-bisphosphatase and FBP activity, both enzyme activities were determined based on formation of Pi.

PRK activity was determined as previously described (24). Glyceraldehyde-3-phosphate dehydrogenase assays were performed as described by Cerff (5). Protein concentrations were determined by the modified Lowry procedure of Markwell et al. (18) with bovine serum albumin as the standard.

RESULTS

In a previous study, we demonstrated the presence of two unlinked sets of PRK and RuBISCO genes within the *R. sphaeroides* chromosome (11). The location of the RuBISCO and PRK genes and the direction of transcription are indicated in the restriction maps of the plasmids pJG336 and pJG106 (Fig. 1). The experiments described in this study were performed to examine further the extent of clustering and duplication of genes coding for enzymes involved in CO_2 assimilation in this organism.

Identification of *fbpA* and *fbpB*. One molecular strategy used to identify functionally related DNA sequences surrounding the PRK and RuBISCO genes has involved Southern hybridizations between the plasmids pJG336 and pJG106 and heterologous probes containing genes coding for other enzymes in the Calvin cycle. The plasmid pJS33 contains a FBP gene isolated from *E. coli* (22). A 1.5-kb *Mlu*I-*Xho*I fragment that contains the FBP sequence exclusively was isolated, nick translated, and used as a probe of *Bam*HI-*Hin*dIII restriction digests of pJG336 and pJG106. Under moderate stringency, hybridization, and wash conditions, the *E. coli fbp* gene hybridized to a single restriction fragment within both plasmids; a 2.9-kb *Bam*HI-*Hin*dIII end fragment of pJG336 and a 1.6-kb *Bam*HI internal fragment of pJG106 (Fig. 2). The *fbp* genes located within pJG336 and pJG106 will be referred to as *fbpA* and *fbpB*, respectively, in accordance with the designations of the *prk* genes.

Further restriction digests and Southern hybridizations more precisely localized the sequences that hybridized to the *fbp* probe (data not shown). In Fig. 1, open boxes denote the location of *fbpA* and *fbpB* derived from Southern blot analyses. In both cases the *fbp* hybridizing fragments correspond to DNA sequences that are closely linked and upstream from the corresponding *prk* genes (Fig. 1).

Subcloning of *fbpA* and *fbpB* and expression of the gene products in *E. coli*. To characterize the gene products of *fbpA* and *fbpB*, subclones of the hybridizing fragments were constructed in pUC vectors to enable expression of the enzymes in *E. coli*. For subcloning of *fbpA*, a 1.8-kb *Xho*I-*Bam*HI fragment of pJG336 was ligated with *Sal*I-*Bam*HI-digested pUC9 and pUC18 to yield pJG93 and pJG183, placing *fbpA* in the opposite orientation with respect to the *lac* promoter (Fig. 1). To subclone *fbpB*, the 1.6-kb *Bam*HI fragment of pJG106 was obtained in both orientations in pUC8, yielding plasmids pJG15 and pJG16 (Fig. 1). To test for production of FBP in *E. coli*, extracts were made from cells harboring each of the plasmid constructs after incubation with IPTG. FBP was measured in the extracts at pH 8.5 in the presence of 0.1 mM Mn^{2+} because these assay conditions were determined to be optimal for FBP assayed in extracts of *R. sphaeroides* (data not shown). The background FBP activity of *E. coli* was not measurable with these reaction conditions. The results of the enzyme assays are shown in Table 2. In both cases, orientation-dependent expression of FBP activity was observed. Cells transformed with pJG93 (*fbpA*) acquired the ability to produce levels of FBP significantly above the negligible background upon induction with IPTG, whereas virtually no FBP activity was detected in extracts of cells containing pJG183 (*fbpA*), where

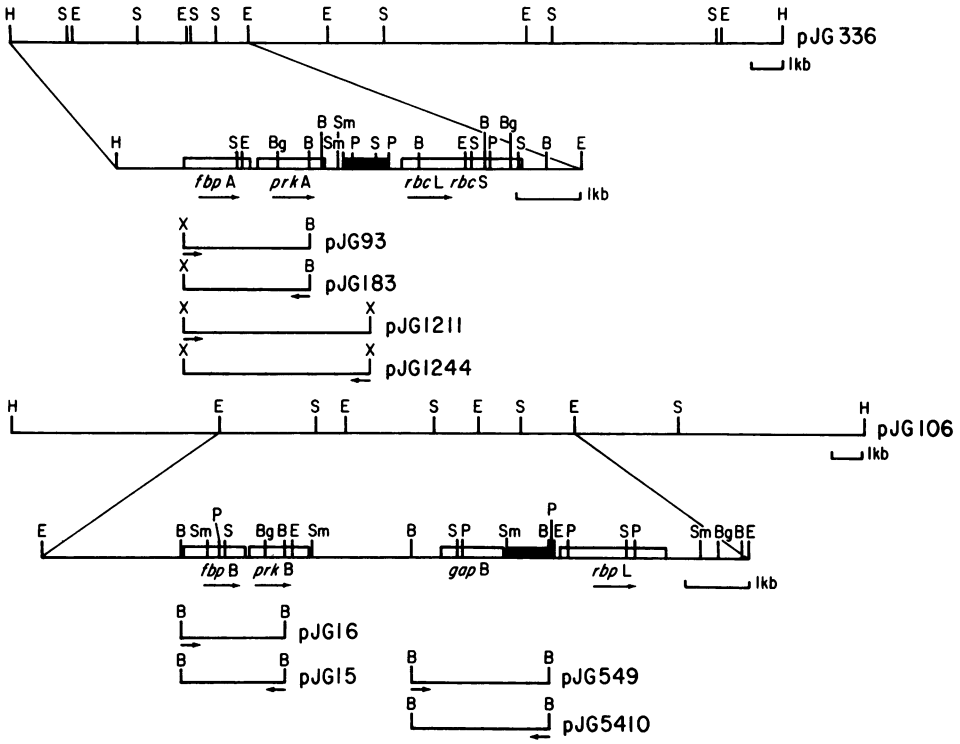

FIG. 1. Restriction endonuclease maps and genetic organization of CO_2 fixation genes within cloned *Hin*dIII inserts of pJG336 and pJG106. The approximate locations of structural genes are indicated by open boxes, and the direction of transcription is shown by the arrow drawn below the gene designation. The solid box within each gene cluster denotes a region of homology of unknown function. Plasmids used for expression analysis in this study are drawn beneath the restriction maps of pJG336 and pJG106. The small arrows beneath each fragment indicate the direction of transcription from the *lac* promoter of the vector. Restriction sites: H, *Hin*dIII; S, *Sal*I; E, *Eco*RI; Bg, *Bgl*II; B, *Bam*HI; Sm, *Sma*I; P, *Pst*I; X, *Xho*I.

the insert is in the reverse orientation (Table 2). Similarly, extracts prepared from IPTG-induced *E. coli* cells harboring pJG16 (*fbpB*) exhibited high rates of fructose 1,6-bisphosphate hydrolysis compared with the level measured in cells containing pJG15 (*fbpB*) (Table 2). However, in this case the orientation dependence was not absolute, since small but reproducible levels of FBP were measured in *E. coli* (pJG15), suggesting that the *R. sphaeroides fbpB* promoter is present within the insert and is recognized by *E. coli* RNA polymerase. The *fbpA* gene product will be referred to as the form I FBP, and the *fbpB* gene product will be called the form II FBP, consistent with the nomenclature used for PRK and RuBISCO in *R. sphaeroides* (11). The FBP activity expressed from pJG93 and pJG16 was compared at pH 7.5 and 8.5 in the presence of 10 mM Mg^{2+} or 0.1 mM Mn^{2+}. Both the form I and form II FBPs exhibited maximum rates of fructose 1,6-bisphosphate hydrolysis at pH 8.5 in the presence of 0.1 mM Mn^{2+} (data not shown). Both FBP activities were greatly stimulated by dithiothreitol, and its presence was absolutely required for enzyme stability in cell extracts. In addition, both enzymes hydrolyzed sedoheptulose 1,7-bisphosphate at approximately 80% of the rate observed for fructose 1,6-bisphosphate hydrolysis (data not shown).

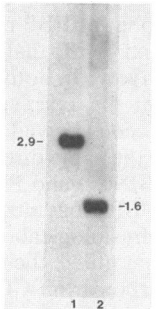

FIG. 2. Southern blot hybridization of *E. coli fbp* to restriction digests of pJG336 and pJG106. The 1.5-kb *Mlu*I-*Xho*I fragment of pJS33 was nick translated, and the ^{32}P-labeled probe was hybridized to *Bam*HI-*Hin*dIII restriction digests of pJG336 (lane 1) and pJG106 (lane 2). Numbers refer to fragment sizes in kilobases.

Cotranscription of *fbpA* and *prkA*. As mentioned previously, Southern hybridizations indicated that the *fbp* and *prk* genes were very closely linked and that expression of the gene products in *E. coli* had demonstrated a common direction of transcription. To investigate the possibility that the

TABLE 2. Enzyme activity in extracts of *E. coli* JM107 harboring different plasmid constructs after induction with IPTG[a]

Plasmid	Sp act (μmol/min per mg of protein)	
	FBP	PRK
pJG93	0.053	<0.001
pJG183	<0.001	<0.001
pJG16	0.192	<0.001
pJG15	0.022	<0.001
pJG1211	0.141	0.044
pJG1244	<0.001	<0.001

[a] Cells were incubated with 1 mM IPTG for 8 h before harvest.

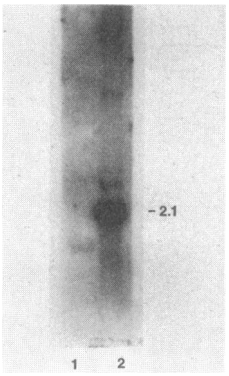

FIG. 3. Southern blot hybridization of *Z. mobilis gap* gene to restriction digests of pJG336 and pJG106. Two internal 0.7-kb *Pst*I-*Hin*dIII fragments of pLOI312 containing glyceraldehyde-3-phosphate-dehydrogenase sequences were isolated, radiolabeled by nick translation, and hybridized to *Bam*HI-*Hin*dIII restriction digests of pJG336 (lane 1) and pJG106 (lane 2). Fragment size is given in kilobases.

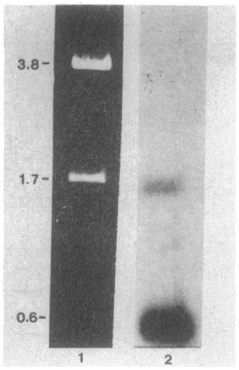

FIG. 4. Southern hybridization analysis of regions upstream from *rbcL*. Lane 1, Ethidium bromide-stained agarose gel showing *Pst*I digest of pJG6; lane 2, corresponding autoradiogram resulting from hybridization to the 0.7-kb *Sma*I-*Bam*HI fragment of pJG549. Fragment sizes shown are in kilobases.

two genes might comprise a single transcriptional unit, a 2.8-kb *Xho*I fragment was inserted into the *Sal*I site of pUC18 in both orientations, and *E. coli* cells transformed with these plasmids were assayed for FBP and PRK activities after incubation with IPTG. FBP and PRK were expressed in cells harboring the plasmid pJG1211, where the genes are in the correct orientation with respect to the *lac* promoter (Table 2). No activity of either enzyme was detected in extracts of cells harboring pJG1244, where the *Xho*I fragment is in the opposite orientation (Table 2). This evidence indicates that in *E. coli* the two genes can be transcribed from a single promoter.

Identification of *gapB*. To further investigate the possibility that other genes involved in CO_2 fixation were duplicated and/or located within either gene cluster, *Bam*HI-*Hin*dIII digests of pJG336 and pJG106 were probed with a *Zymomonas mobilis* gene coding for glyceraldehyde-3-phosphate dehydrogenase. The *gap* gene probe hybridized to a 2.1-kb *Bam*HI fragment of pJG106 but not to sequences within pJG336 (Fig. 3). The *Bam*HI fragment containing the presumptive *gap* sequence (*gapB*) corresponds to a locus adjacent to and upstream from the form II RuBISCO gene (Fig. 1).

The *Bam*HI fragment was subcloned into pUC18 in both orientations to yield plasmids pJG549 and pJG5410 (Fig. 1). Attempts to demonstrate plasmid-encoded GAP activity in IPTG-induced cells containing the two constructs have been hampered by the high background activity in *E. coli*, but the gene appears to be transcribed in the same direction as that determined for the other genes (data not shown).

Homologous regions upstream of the form I and form II RuBISCO genes. Experiments designed to localize a second *gap* gene within pJG336 were based on the assumption that two genes from the same background would be more similar than sequences from heterologous sources. To test this possibility, the *Bam*HI insert of pJG549 containing *gapB* was used to probe pJG336. The DNA sequence between *prkA* and *rbcL* hybridized strongly to this probe (data not shown). However, it seemed unlikely that the strong hybridization signal was due to *gap* homology, because even under conditions of low stringency no hybridization to pLOI312 could be demonstrated. To determine the reason for the anomalous hybridization, the region within pJG549 showing homology to the *Z. mobilis gap* probe was further delineated. The restriction enzyme *Sma*I cuts at a single site within the 2.1-kb *Bam*HI fragment of pJG549. When *Sma*I-*Bam*HI digests of pJG549 were probed with pLOI312, hybridization was observed only to the larger 1.3-kb fragment whereas no hybridization was detected to the 0.7-kb *Sma*I-*Bam*HI fragment that is located just upstream of *rbpL* (data not shown). The two *Sma*I-*Bam*HI fragments of pJG549 were used separately as probes of pJG6, a subclone of pJG336 that contains *prkA*, and most of *rbcL* within a 3.4-kb *Eco*RI fragment (11). No hybridization was detected when the larger 1.3-kb *Sma*I-BamHI fragment was used as a probe (data not shown). However, strong hybridization to a 0.6-kb *Pst*I fragment situated between *prkA* and *rbcL* was detected when pJG6 was probed with the 0.7-kb *Sma*I-*Bam*HI fragment (Fig. 4). A very weak signal corresponding to the 1.7 kb *Pst*I fragment was also observed (Fig. 4). These results suggest that the hybridization does not reflect the presence of an additional *gap* sequence located within the form I gene cluster but rather a region of unassigned function positioned upstream from both RuBISCO genes. The solid boxes in Fig. 1 indicate the corresponding regions of homology within the two gene clusters.

DISCUSSION

Data presented in this paper extend our earlier work demonstrating the presence of duplicate sets of RuBISCO and PRK genes located in separate clusters within the *R. sphaeroides* genome. Examination of the DNA adjacent to the PRK and RuBISCO genes by Southern hybridizations with a heterologous probe for FBP revealed the presence of two *fbp* genes, *fbpA* and *fbpB*, that were closely linked to *prkA* and *prkB*, respectively. In both cases the *fbp* gene was situated upstream from the corresponding *prk* gene, and expression studies in *E. coli* showed that transcription of the *fbp* genes proceeded in the same direction as that previously determined for the RuBISCO and PRK genes (11, 12).

The occurrence of FBP isozymes is widespread, underscoring the important physiological role of this enzyme (1, 8, 27, 28). In organisms that utilize the Calvin cycle, FBP must regulate the flow of carbon from CO_2 assimilation to other metabolic pathways in addition to its gluconeogenic function. In this situation, FBP isozymes with different regulatory properties confer the ability to selectively control activity within both pathways. The FBP isozymes from *R. sphaeroides* appear to be similar in catalytic properties. The

functional similarity, coupled with the genetic context of *fbpA* and *fbpB*, suggests that both enzymes function in a photosynthetic capacity, raising the possibility of yet a third "heterotrophic" FBP in this organism.

The tandem arrangement of the *fbp* and *prk* genes and the common transcriptional orientation suggested that the two genes might be cotranscribed; indeed, cells harboring a construct containing both *fbpA* and *prkA* produced both gene products upon induction of transcription from the *lac* promoter with IPTG. These results at least demonstrate that in *E. coli* the two genes can be transcribed from a single promoter. The possibility of cotranscription of the *fbp* and *prk* genes in *R. sphaeroides* is especially intriguing in light of earlier work by Joint et al. (13), who described a complex of an alkaline FBP and PRK that could be isolated from extracts of *Rhodospirillum rubrum*. If the two genes form an operon, cotranscription could facilitate coordinated expression of FBP and PRK as well as formation of an enzyme complex. The reason for an FBP-PRK complex is not obvious, since the two enzymes do not catalyze consecutive reactions, but the association may be relevant for regulatory purposes.

R. sphaeroides synthesizes drastically different levels of RuBISCO in response to changes in growth conditions, such as the concentration of CO_2 or the nature of the organic electron donor during photoheterotrophic growth (9, 15, 16). Although the biological significance of two enzymes is still unclear, the structural and catalytic properties are so distinct that the two enzymes must play different physiological roles in vivo. Much less is known about the properties of the duplicate PRK and FBP enzymes, but preliminary studies indicate that the isozymes do not exhibit dramatic differences in catalytic properties. Certainly at the level of DNA hybridization the genes are very similar, in contrast to the divergent RuBISCO genes. Therefore, the redundancy of PRK and FBP may represent a mechanism for selective regulation at the level of protein synthesis rather than at the level of activity, and the linkage would provide a means for a coordinate activation in response to a common regulatory signal. The homologous regions adjacent to the form I and form II RuBISCO genes may prove to be important in this regulation. It has been reported that the sequence upstream of *rbcL rbcS* encodes a polypeptide of M_r 37,000 and that the message for this protein is transcribed in the direction opposite to that determined for the RuBISCO genes (12). The close proximity of this sequence to the RuBISCO genes and the orientation of transcription make it tempting to speculate that these sequences represent divergent control regions, whereby a single promoter could bidirectionally direct the synthesis of RuBISCO and a regulatory protein.

In plants, the activities of FBP, PRK, RuBISCO, and glyceraldehyde-3-phosphate dehydrogenase are regulated by light via a complex phytochrome-mediated control system (4). It is interesting that the CO_2 fixation genes that are duplicated and/or located within the form I or form II gene cluster are those subject to light regulation in plants. The regulatory signals involved in control of these enzymes in bacteria have not yet been defined, but knowledge of the molecular organization of the genes provides a good basis for studies concerning the regulation of the Calvin cycle in photosynthetic procaryotes.

ACKNOWLEDGMENTS

We are very grateful to Florence Waddill for excellent technical assistance and to T. Conway, L. O. Ingram, and D. Fraenkel for providing plasmids used as probes in this study.

This work was supported by grants 83-CRCR-1-1344 and 87-CRCR-1-2330 from the U.S. Department of Agriculture and by grant F-691 from the Robert A. Welch Foundation.

LITERATURE CITED

1. **Amachi, T., and B. Bowien.** 1979. Characterization of two fructose bisphosphatase isoenzymes from the hydrogen bacterium *Nocardia opaca* 1b. J. Gen. Microbiol. **113**:347–356.
2. **Ames, B. N.** 1966. Assay of inorganic phosphate. Methods Enzymol. **8**:115–116.
3. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
4. **Buchanan, B. B.** 1980. Role of light in the regulation of chloroplast enzymes. Annu. Rev. Plant Physiol. **31**:341–374.
5. **Cerff, R.** 1982. Separation and purification of NAD- and NADP-linked glyceraldehyde-3-phosphate dehydrogenases from higher plants, p. 683–694. *In* M. Edelman, R. B. Hallick, and N.-H. Chua (ed.), Methods in chloroplast molecular biology. Elsevier Biomedical Press, Amsterdam.
6. **Conway, T., G. W. Sewell, and L. O. Ingram.** 1987. Glyceraldehyde-3-phosphate dehydrogenase gene from *Zymomonas mobilis*: cloning, sequencing, and identification of promoter region. J. Bacteriol. **169**:5653–5662.
7. **Davis, R. W., D. Botstein, and J. R Roth (ed.).** 1980. Advanced bacterial genetics: a manual for genetic engineering. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Gerbling, K.-P., M. Steup, and E. Latzko.** 1984. Electrophoretic and chromatographic separation of two fructose-1,6-bisphosphatase forms from *Synechococcus leopliensis*. Arch. Microbiol. **137**:109–114.
9. **Gibson, J. L., and F. R. Tabita.** 1977. Different molecular forms of D-ribulose-1,5-bisphosphate carboxylase from *Rhodopseudomonas sphaeroides*. J. Biol. Chem. **252**:943–949.
10. **Gibson, J. L., and F. R. Tabita.** 1986. Isolation of the *Rhodopseudomonas sphaeroides* form I ribulose 1,5-bisphosphate carboxylase/oxygenase large and small subunit genes and expression of the active hexadecameric enzyme in *Escherichia coli*. Gene **44**:271–278.
11. **Gibson, J. L., and F. R. Tabita.** 1987. Organization of phosphoribulokinase and ribulose bisphosphate carboxylase/oxygenase genes in *Rhodopseudomonas (Rhodobacter) sphaeroides*. J. Bacteriol. 169:3685–3690.
12. **Hallenbeck, P. L., and S. Kaplan.** 1987. Cloning of the gene for phosphoribulokinase activity from *Rhodobacter sphaeroides* and its expression in *Escherichia coli*. J. Bacteriol. **169**: 3669–3678.
13. **Joint, I. R., I. Morris, and R. C. Fuller.** 1972. Purification of a complex of alkaline fructose 1,6-bisphosphatase and phosphoribulokinase from *Rhodospirillum rubrum*. J. Biol. Chem. **247**:4833–4838.
14. **Jordan, D. B., and W. L. Ogren.** 1981. Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. Nature (London) **291**:513–515.
15. **Jouanneau, Y., and F. R. Tabita.** 1986. Independent regulation of synthesis of form I and form II ribulose bisphosphate carboxylase-oxygenase in *Rhodopseudomonas sphaeroides*. J. Bacteriol. **165**:620–624.
16. **Jouanneau, Y., and F. R. Tabita.** 1987. *In vivo* regulation of form I ribulose 1,5-bisphosphate carboxylase/oxygenase from *Rhodopseudomonas sphaeroides*. Arch. Biochem. Biophys. **254**:290–303.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Markwell, M. A., S. M. Haas, L. L. Bieber, and N. E. Tolbert.** 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. **87**:206–210.
19. **Muller, E. D., J. Chory, and S. Kaplan.** 1985. 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.

20. **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.
21. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
22. **Sedivy, J. M., F. Daldal, and D. G. Fraenkel.** 1984. Fructose bisphosphatase of *Escherichia coli*: Cloning of the structural gene (*fbp*) and preparation of a chromosomal deletion. J. Bacteriol. **158**:1048–1053.
23. **Springgate, C. F., and C. S. Stachow.** 1972. Fructose 1,6-diphosphatase from *Rhodopseudomonas palustris*. I. Purification and properties. Arch. Biochem. Biophys. **152**:1–12.
24. **Tabita, F. R.** 1980. Pyridine nucleotide control and subunit structure of phosphoribulokinase from photosynthetic bacteria. J. Bacteriol. **143**:1275–1280.
25. **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.
26. **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.
27. **Zimmerman, G., G. J. Kelly, and E. Latzko.** 1976. Efficient purification and molecular properties of spinach chloroplast fructose 1,6-bisphosphatase. Eur. J. Biochem. **70**:361–367.
28. **Zimmerman, G., G. J. Kelly, and E. Latzko.** 1978. Purification and properties of spinach leaf cytoplasmic fructose-1,6-bisphosphatase. J. Biol. Chem. **253**:5952–5955.