***Xanthobacter flavus* employs a single triosephosphate isomerase for heterotrophic and autotrophic metabolism**

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The expression of the *cbb* and *gap-pgk* operons of *Xanthobacter flavus* encoding enzymes of the Calvin cycle is regulated by the transcriptional regulator CbbR. In order to identify other genes involved in the regulation of these operons, a mutant was isolated with a lowered activity of a fusion between the promoter of the *cbb* operon and the reporter gene *lacZ*. This mutant was unable to grow autotrophically and had a reduced growth rate on medium supplemented with gluconate or succinate. The regulation of the *gap-pgk* operon in the mutant was indistinguishable from the wild-type strain, but induction of the *cbb* operon upon transition to autotrophic growth conditions was delayed. Complementation of the mutant with a genomic library of *X. flavus* resulted in the isolation of a 1.1 kb *Apal* fragment which restored autotrophic growth of the mutant. One open reading frame (ORF) was present on the *Apal* fragment, which could encode a protein highly similar to triosephosphate isomerase proteins from other bacteria. Cell extracts of the mutant grown under glycolytic or gluconeogenic conditions had severely reduced triosephosphate isomerase activities. The ORF was therefore identified as *tpi*, encoding triosephosphate isomerase. The *tpi* gene is not linked to the previously identified operons encoding Calvin cycle enzymes and therefore represents a third transcriptional unit required for autotrophic metabolism.

Keywords: RuBisCO, triosephosphate isomerase, autotrophy, regulation, *Xanthobacter flavus*

INTRODUCTION

Xanthobacter flavus grows autotrophically by fixing CO₂ via the Calvin cycle using energy obtained from the oxidation of hydrogen, methanol or formate. In addition, heterotrophic growth is supported by a wide variety of organic substrates, e.g. gluconate or succinate. In this case, the fixation of CO₂ is not necessary and the Calvin cycle is not induced (Meijer *et al.*, 1990a; Croes *et al.*, 1991). A superficial inspection of the Calvin cycle would suggest that only two enzymes, phosphoribulokinase and ribulosebiphosphate carboxylase (RuBisCO), need to be synthesized in order to allow CO₂ fixation via this pathway to proceed; the other

activities of the Calvin cycle are also required for gluconeogenesis and the pentose phosphate cycle and are already present during heterotrophic growth (Meijer *et al.*, 1990a; van den Bergh *et al.*, 1996; Meijer *et al.*, 1996).

However, it has become clear that extensive reprogramming of central metabolism is required for the transition from heterotrophic to autotrophic growth (Gibson & Tabita, 1996; Meijer, 1996). In addition to the synthesis of phosphoribulokinase and RuBisCO, a dramatic increase in the activity of the other Calvin cycle enzymes is required in order to support the increased flux of carbon via this pathway (Meijer, 1994). Furthermore, isoenzymes of some of the gluconeogenic or pentose phosphate cycle enzymes with biochemical properties adapted for a role in CO₂ fixation are induced (Meijer *et al.*, 1991, 1996; van den Bergh *et al.*, 1995, 1996).

Abbreviation: RuBisCO, ribulosebiphosphate carboxylase.

The GenBank accession number for the sequence reported in this paper is U77930.

Table 1. Bacteria and plasmids used in this study

Strain/plasmid	Relevant genotype/phenotype	Reference/source
<i>E. coli</i>		
S17-1	<i>thi pro res⁻ mod⁺ Sm^R Tp^R recA</i> , RP4-2 (Tc::Mu; Km::Tn7)	Simon <i>et al.</i> (1983)
DH5 α	<i>supE44 ΔlacU169 (ϕ80lacZΔ M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>	Bethesda Research Laboratories
DH5 α R	Spontaneous rifampicin-resistant DH5 α	Meijer (1994)
<i>X. flavus</i>		
H4-14	Wild-type strain	Lidstrom-O'Conner <i>et al.</i> (1983)
G1	Aut, <i>tpi</i>	This study
Plasmids		
pBluescript KSII	Ap ^R , <i>lacZ'</i> , cloning vector	Stratagene
pRK415	Tc ^R , <i>incP1 mob lacZ'</i> , cloning vector	Keen <i>et al.</i> (1988)
pVK100	Tc ^R , <i>incP1 mob</i> , cloning vector	Knauf & Nester (1982)
pRK2013	Km ^R , <i>tra</i>	Ditta <i>et al.</i> (1980)
pXA1	Tc ^R , <i>incP1 mob cbbR cbbL::lacZ</i>	Meijer <i>et al.</i> (1991)
pYO1	Tc ^R , <i>tpi</i> , 22 kb <i>Hind</i> III fragment in pVK100	This study
pYO8	Tc ^R , <i>tpi</i> , 18 kb <i>Sac</i> I- <i>Hind</i> III fragment in pRK415	This study
pYO9	Tc ^R , <i>tpi</i> , 15 kb <i>Kpn</i> I- <i>Hind</i> III fragment in pRK415	This study
pYO12	Tc ^R , <i>tpi</i> , 11.5 kb <i>Kpn</i> I- <i>Apa</i> I fragment in pRK415	This study
pYO14	Tc ^R , <i>tpi</i> , 3.5 kb <i>Eco</i> RI- <i>Hind</i> III fragment in pRK415	This study
pYO210	Tc ^R , <i>tpi</i> , 1.1 kb <i>Apa</i> I fragment in pRK415	This study
pYO304	Tc ^R , <i>tpi</i> , 1.9 kb <i>Xho</i> I- <i>Eco</i> RI fragment in pRK415	This study
pYO2041	Ap ^R , <i>tpi</i> , 1.1 kb <i>Apa</i> I fragment in pBluescript KSII	This study

The genes encoding enzymes of the Calvin cycle of *X. flavus* identified to date are organized into two operons. The *cbb* operon encodes the unique Calvin cycle enzymes RuBisCO and phosphoribulokinase and in addition isoenzymes of gluconeogenic and pentose phosphate cycle enzymes (Meijer *et al.*, 1990b, 1991; van den Bergh *et al.*, 1996; Meijer, 1996). Since these are only required for CO₂ fixation, the *cbb* operon is only transcribed following a transition to autotrophic growth conditions (Meijer *et al.*, 1991). In contrast, the *gap-pgk* operon, encoding glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase, is constitutively transcribed. The expression of this operon is dramatically increased when the Calvin cycle is induced (Meijer, 1994; Meijer *et al.*, 1996).

The Calvin cycle is maximally induced under carbon-limited growth conditions with an ample supply of hydrogen, methanol or formate. At present it is unclear how the physiological status of the cell is transduced to the transcription apparatus, although it is firmly established that the transcriptional regulator CbbR is involved. This LysR-type transcriptional regulator is required for both the induction and super-induction of, respectively, the *cbb* and *gap-pgk* operon (van den Bergh *et al.*, 1993; Meijer *et al.*, 1996). In general, LysR-type regulators activate transcription upon binding of a ligand; the identity of the ligand binding to CbbR is still unknown.

In order to identify additional components involved in the regulation of the Calvin cycle, mutants were isolated

with an altered regulation of the promoter of the *cbb* operon (Meijer, 1994). Using this approach, we previously identified a *pgk* mutation which caused enhanced repression of the *cbb* promoter by gluconeogenic substrates and prevented autotrophic growth. The triosephosphate isomerase mutant described in this paper displays a similar phenotype to the *pgk* mutant isolated previously. The role of triosephosphate isomerase in heterotrophic and autotrophic metabolism of *X. flavus* and the regulation of the Calvin cycle by glycolytic intermediates will be discussed.

METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1.

Media and growth conditions. *Escherichia coli* strains were grown on Luria-Bertani (LB) medium at 37 °C (Sambrook *et al.*, 1989). *X. flavus* strains were grown in a H₂/CO₂/air mixture, on yeast extract (0.8%, w/v) or in minimal media supplemented with gluconate (10 mM), succinate (10 mM) or methanol (0.5%, v/v) at 30 °C as described previously (Meijer *et al.*, 1990a). *X. flavus* was grown on a mixture of gluconate (5 mM) and formate (20 mM) in a 3 l batch fermenter with automatic titration with formic acid (25%, v/v) to maintain a constant pH. When appropriate, the following supplements were added: ampicillin, 50 µg ml⁻¹; X-Gal, 20 µg ml⁻¹; isopropyl- β -D-thiogalactoside, 0.1 mM; rifampicin, 50 µg ml⁻¹; tetracycline, 12.5 µg ml⁻¹ (*E. coli*) or 7 µg ml⁻¹ (*X. flavus*). Agar was added for solid media (1.5%, w/v).

Mutant isolation. Mutants were isolated following UV-irradiation as described previously (Meijer, 1994).

Curing of plasmid pXA1. *X. flavus* G1 harbouring pXA1 was grown on succinate medium without tetracycline until the late exponential growth phase. This *X. flavus*(pXA1) culture was used to inoculate fresh succinate medium and the process was repeated three times. The culture was subsequently plated on succinate plates and individual colonies were transferred to succinate plates with and without tetracycline. More than 99% of the colonies were tetracycline sensitive, indicating the loss of plasmid pXA1.

Mobilization of plasmids. Mobilization of plasmids (genomic library) to *X. flavus* using *E. coli* S17-1 containing the appropriate plasmids was performed as described by Simon *et al.* (1983). Plasmids were mobilized from *X. flavus* G1 to *E. coli* DH5 α R via a triparental mating. *X. flavus* G1, *E. coli* DH5 α R and *E. coli*(pRK2013) were concentrated via centrifugation, mixed in a 1:1:1 ratio, spotted on a yeast extract plate and incubated at 30 °C for 16 h. The mating mixture was plated on LB plates containing rifampicin and tetracycline to select for *E. coli* DH5 α R containing the plasmid conferring tetracycline resistance.

DNA manipulations. Plasmid DNA was isolated via the alkaline lysis method of Birnboim & Doly (1979). DNA modifying enzymes were obtained from Boehringer Mannheim and were used according to the manufacturer's instructions. Other DNA manipulations were done according to standard protocols (Sambrook *et al.*, 1989).

Nucleotide sequencing. Dideoxy sequencing reactions were done using T7 DNA polymerase, with either 5'-end labelled primers or with unlabelled primers and fluorescein-labelled ATP (Voss *et al.*, 1992; Zimmermann *et al.*, 1990). Nucleotide sequencing was done with the Automated Laser Fluorescent DNA sequencer (Pharmacia). The nucleotide sequence data were compiled and analysed using the programs supplied in the PC/GENE software package (Intelligenetics). Amino acid sequences were aligned with ClustalW (Thompson *et al.*, 1994).

Enzyme assays. Cell extracts were prepared using a French pressure cell as described previously (Meijer *et al.*, 1991). Phosphoglycerate kinase activity was determined as described previously (Meijer, 1994). Triosephosphate isomerase activity was determined by measuring the glyceraldehyde-3-phosphate-dependent oxidation of NADH at 340 nm in an assay mixture containing: 25 mM Tris/HCl (pH 7.9); 15 μ M NADH; 20 μ g glycerol-3-phosphate dehydrogenase; 0.3 mM glyceraldehyde 3-phosphate. RuBisCO activity was determined by measuring the incorporation of 14 CO $_2$ into acid-stable compounds (Gibson & Tabita, 1977). Protein was determined according to Bradford (1976) using bovine serum albumin as standard.

RESULTS

Isolation and characterization of *X. flavus* G1

X. flavus mutants with an altered regulation of the *cbb* operon promoter were isolated by making use of a *cbbL::lacZ* gene fusion present *in trans* on plasmid pXA1 (Meijer, 1994). *X. flavus* harbouring pXA1 forms green colonies when plated on succinate plates containing X-Gal. This is due to a low activity of the *cbb* promoter which results in the synthesis of low amounts of β -galactosidase. Following UV-irradiation to induce mutations, colonies forming either blue or yellow

colonies due to enhanced or lowered activities of the *cbb* promoter were selected and further characterized. *X. flavus* G1 harbouring pXA1 formed yellow colonies under these conditions, indicating a decrease in *cbb* promoter activity.

Following three successive transfers to succinate medium without tetracycline, a plasmid-free derivative of *X. flavus* G1 was isolated. This strain was unable to grow autotrophically (Aut phenotype) on molecular hydrogen or methanol. The growth rates of *X. flavus* G1 on succinate ($\mu_{\max} = 0.20 \pm 0.01 \text{ h}^{-1}$) and gluconate ($\mu_{\max} = 0.12 \pm 0.01 \text{ h}^{-1}$) were 13 and 31% lower than those of the wild-type strain on these substrates (succinate: $\mu_{\max} = 0.23 \pm 0.01 \text{ h}^{-1}$; gluconate: $\mu_{\max} = 0.16 \pm 0.01 \text{ h}^{-1}$). Values are the means of growth rates determined for three cultures.

Regulation of the *cbb* and *gap-pgk* operons

X. flavus G1 was identified by the reduced expression of a *cbbL-lacZ* gene fusion during growth on succinate. We therefore examined whether the induction of the *cbb* and *gap-pgk* operons during a transition from heterotrophic to autotrophic growth conditions was affected in this mutant. To this end, *X. flavus* G1 and the wild-type strain were grown on gluconate (5 mM) and the synthesis of the Calvin cycle was induced by adding formate (20 mM) to the culture (Fig. 1).

The growth rate of the wild-type strain was not altered upon the addition of formate. However, the growth rate of *X. flavus* G1 increased to that of the wild-type ($\mu_{\max} = 0.16 \pm 0.01 \text{ h}^{-1}$), indicating that the Aut phenotype was not caused by an inability to oxidize autotrophic substrates as, for example, formate. The super-induction of phosphoglycerate kinase, indicative of the expression of the *gap-pgk* operon, proceeded in a similar fashion for both wild-type and mutant strains. However, induction of the *cbb* operon, as indicated by the activity of RuBisCO, was delayed in *X. flavus* G1 (Fig. 1).

Complementation of *X. flavus* G1

A genomic library of *X. flavus* constructed in the broad-host-range cosmid pVK100 (Meijer, 1994) was mobilized to *X. flavus* G1. Following plating of the conjugation mixture on methanol plates, colonies were observed that had regained the ability to grow autotrophically. Six colonies resulting from independent complementation experiments were purified on methanol plates. The complementing plasmids were subsequently mobilized to *E. coli* DH5 α R by triparental mating and analysed by restriction mapping. All plasmids contained an identical 22 kb *Hind*III fragment which was completely different from the two previously isolated *Hind*III fragments on which the *cbb* and *gap-pgk* operons are located (Lehmiche & Lidstrom, 1985; Meijer, 1994). One of these plasmids, pYO1, was selected for further analysis. Reintroduction of pYO1 into *X. flavus* G1 restored autotrophic growth. Sub-

Fig. 1. Activities of (a) RuBisCO and (b) phosphoglycerate kinase (PGK) of *X. flavus* wild-type (Δ) and *X. flavus* G1 (*tpi*; ●) following the addition of 20 mM formate to a culture growing on 5 mM gluconate at $t = 0$ h. The pH is kept constant by automatic titration with formic acid (25%, v/v). The graphs representing typical data from two independent experiments are shown. Each point in the graph represents the mean of two measurements. The values differed from the mean by < 10%. Enzyme activities are in $\text{nmol min}^{-1} (\text{mg protein})^{-1}$.

Fig. 2. Restriction map of the 22 kb *Hind*III insert of pYO1 and the results of the complementation analysis using derivatives of pYO1. The position and direction of transcription of the *tpi* gene are indicated by an arrow.

sequent subcloning of pYO1 into pRK415 reduced the complementing fragment to a 1.1 kb *Apal* fragment (Fig. 2).

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GGGCCGGCGGGAAGCGCGCATCATAGGGAGGGGGGCGCTCGCAACGGCGCATGGTGCACCCCTTTCCAGCGG  80
GCGCCCGCTGTGTACGTGAGGCCCTTACGTGAGCCTTGTGCTGTGCAAAACCCGCGGACCGCTATGACGGCGGA  160
      M T A E

ACGCCCGCCCTGATCGCAGCAACTGGAAGATGAACGGCTCGCGGGCGGCAAGTTCGAGCTTGGCGGCGCGGCGG  240
R R P L I A G N W K M N G L R A A K S E L R T V A A

GCCTTGGCGGCTTTGGCGGAAACATGATCTGCCACCTTACCTCATCGCGGCTTTCGCGCGGAGCTCGCGGCGG  320
G L A G L S A R T M I C P P F T L I G A F A A D V A G

ACCCAGCTGATGATCGCGGACAGGACTGCCACCGGCGGAGAGCGGCGCCATACCGGCGACATCTCCGCGGAGATGCT  400
T C L M I G G Q D C H P A E S G A H T G D I S A E M L

GCCGACGCGCTGCGGTGGCGGTGATTCTCGGCCATTCCGAGCGCGCATCGACATCAGGAGGCGGATGCCATCGTCA  480
R D A G A V A V I L G H S E R R I D H Q E G D A I V

AGGCCAAGGTCAAGGCTGCGTGGCGGGCGGCTGCTCCGGTCTGCTGCTGGCGGAGACGCTGGTGGAGCGTGGTGC  560
K A X V K A A W R A G L L P V V C V G E T L V E R D A

GGGAAGCGCGCTGCCGTGGTACGCGCGAGGTGCGCCAGTCCGTGCGCGAGGCGCGGAGCGCGCTGTCATCGC  640
G E A A A V V T R Q V R Q S V P E G A T A V S L V I A

CTACGAGCCATCTGGGCGATCGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG  720
Y E P I W A I G T G R T P T T D D V A E V H G A I R

ACATCTCGCGAGCGCTTCCGCGCGGAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG  800
H I L A E R F G A E A N G I R I L Y G G S V K P D N A

GCTGCCCTGCTGCCACCGCAAGTGGACGCGCGCGTGGTGGCGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG  880
A A L L A T A N V D G A L V G G A S L K A A D F L A I

CGCGCGCGCGGTGGTGGAGCGGATTTCGCCCTTCCCTCGAAGTCGCGACGCGGACTTCGAGGGGAGAGGGGCGTT  960
A R A V G

GCCAGTGTGCGGGGCGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG  1040
GGTGTGGAGCGCGCACAGGGCGCC
  
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Fig. 3. Nucleotide sequence of the 1.1 kb *Apal* fragment restoring autotrophic growth of *X. flavus* strain G1. The potential ribosome-binding site is boxed. The putative terminator structure is indicated by arrows. The translation of the *tpi* gene is presented below the nucleotide sequence using the one-letter amino acid code.

Nucleotide sequence of the *aut* locus

In order to determine the identity of the gene complementing the *Aut* phenotype of *X. flavus* G1, the nucleotide sequence of both strands of the 1.1 kb *Apal* fragment was determined. One open reading frame (ORF), preceded by a potential ribosome-binding site, was identified which could encode a protein of 249 amino acids with a molecular mass of 25 542 Da (Fig. 3). Downstream from the ORF, a 48 bp G + C-rich inverted repeat is present which may form a transcriptional terminator structure (Platt, 1986). A comparison of the hypothetical protein encoded by this ORF with entries in GenBank using BlastP (Altschul *et al.*, 1990) showed that the protein is highly similar (up to 55% identical) to triosephosphate isomerase proteins from other bacteria. The ORF was therefore designated *tpi*, encoding triosephosphate isomerase.

Triosephosphate isomerase in *X. flavus* G1

Triosephosphate isomerase plays an important role in both autotrophic and heterotrophic metabolism. The activity of this enzyme was therefore determined in *X. flavus* G1 and the wild-type strain following growth on gluconate and succinate. High activities of triosephosphate isomerase were present in cell extracts of *X. flavus* following growth on gluconate [$1.4 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] and succinate [$1.9 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]. In sharp contrast, triosephosphate isomerase activities were reduced by 99% in *X. flavus* G1 grown on gluconate [$14 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$] or succinate [$28 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$]. Values are the means of at least two measurements each of two

independent cultures. The values differed from the mean by < 10%.

DISCUSSION

The UV-induced mutation in the *tpi* gene of *X. flavus* G1 causes a dramatic decrease in triosephosphate isomerase activity. As a result, the growth rate of the *tpi* mutant on heterotrophic substrates is reduced and autotrophic growth is no longer possible. It has been estimated that during growth of *E. coli* on succinate, only 5% of the total flux of carbon is via the gluconeogenic pathway, which includes triosephosphate isomerase (Irani & Maitra, 1977). In sharp contrast, all cellular carbon derived from autotrophic CO₂ fixation has to pass via the pool of triosephosphates. The remaining capability of *X. flavus* G1 to generate triosephosphates is sufficient to partially fulfil the biosynthetic needs of the cell during growth on succinate, but cannot support the high rate of CO₂ fixation required for autotrophic growth.

The genes encoding the enzymes of the Calvin cycle of *X. flavus* identified to date are organized into two transcriptional units: the *cbb* operon and the *gap-pgk* operon. Both heterotrophic and autotrophic growth of *X. flavus* G1 are affected, which shows that the *tpi* gene is clearly required for both. Since it is located on a different *Hind*III fragment to the *cbb* and *gap-pgk* operons, it represents a third transcriptional unit required for the operation of the Calvin cycle. The *cbb* gene clusters from other autotrophic bacteria studied to date do not contain a gene encoding triosephosphate isomerase, indicating that, like *X. flavus*, these bacteria may also utilize the same triosephosphate isomerase gene for both heterotrophic and autotrophic metabolism (Paoli *et al.*, 1995; Strecker *et al.*, 1994; Falcone & Tabita, 1993; Chen *et al.*, 1991; Gibson *et al.*, 1991; Schäferjohann *et al.*, 1993, 1995).

The organization of the Calvin cycle genes into different transcriptional units reflects the metabolic role of the enzymes encoded by them. The enzymes encoded by the *cbb* operon are specialized for their role in autotrophic CO₂ fixation: they either catalyse unique reactions, e.g. RuBisCO, or are isoenzymes which possess allosteric or kinetic properties which are tailored for a role in the Calvin cycle (van den Bergh *et al.*, 1995, 1996; Meijer *et al.*, 1991). Consequently, the *cbb* operon is induced only following a transition to autotrophic growth conditions (Meijer *et al.*, 1991; Meijer, 1996). The genes encoding triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase are not located within the *cbb* operon and are required for both heterotrophic and autotrophic metabolism (Meijer *et al.*, 1996; Meijer, 1994). Apparently they have biochemical properties which are suited for both types of metabolism. However, an increased activity of these enzymes is essential to sustain the increased flux of carbon via the Calvin cycle required for autotrophic CO₂ fixation. The *gap-pgk* operon is therefore constitutively expressed but is super-induced during auto-

trophic growth (Meijer *et al.*, 1996; Meijer, 1994). The expression of the *tpi* gene may be regulated in a similar fashion.

The activity of the *cbb* promoter in *X. flavus*, *Alcaligenes eutrophus* and *Pseudomonas oxalaticus* is apparently very sensitive towards mutations affecting the activity of glycolytic enzymes which probably influence the cellular concentration of glycolytic intermediates (Meijer, 1994; Meijer & Dijkhuizen, 1988; Reutz *et al.*, 1982; Im & Friedrich, 1983). The *tpi* mutation described in this paper and the previously isolated *pgk* mutation in *X. flavus* both result in a reduced activity of the promoter of the *cbb* operon during growth on gluconeogenic substrates (Meijer, 1994). Inhibition of enolase or a mutation abolishing phosphoglycerate mutase activity in *A. eutrophus* resulted in an increased expression of the *cbb* operon during growth on fructose (Reutz *et al.*, 1982; Im & Friedrich, 1983). A plausible explanation for these phenomena is a regulatory protein which upon interaction with a glycolytic intermediate down regulates the activity of the *cbb* promoter (Dijkhuizen & Harder, 1984). High intracellular concentrations of this metabolite signal that sufficient carbon is available to the cell and no need exists for the fixation of CO₂ via the Calvin cycle. Interestingly, the regulation of the activity of phosphoribulokinase follows a similar pattern. The activity of most bacterial phosphoribulokinase proteins is stimulated by NADH and inhibited by the glycolytic intermediate phosphoenolpyruvate (Tabita, 1988; Leadbeater & Bowien, 1984). Current studies aim to elucidate whether the protein interacting with a glycolytic intermediate is CbbR, or whether another regulator is involved.

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