Transcription of the \textit{glnB} and \textit{glnA} genes in the photosynthetic bacterium \textit{Rhodospirillum rubrum}

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The \( \text{P}_\text{II} \) protein, encoded by \textit{glnB}, has a central role in the control of nitrogen metabolism in nitrogen-fixing prokaryotes. The \textit{glnB} gene of \textit{Rhodospirillum rubrum} was isolated and sequenced. The deduced amino acid sequence had very high sequence identity to other \( \text{P}_\text{II} \) proteins. The \textit{glnA} gene, encoding glutamine synthetase, was located 135 bp downstream of \textit{glnB} and was partially sequenced. \textit{glnB} is cotranscribed with \textit{glnA} from a promoter with high similarity to the \( \sigma^{54} \)-dependent promoter consensus sequence. A putative \( \sigma^{70} \) promoter was also identified further upstream of \textit{glnB}. Northern blotting analyses showed that in addition \textit{glnA} is either transcribed from an unidentified promoter or, more likely, that the \textit{glnBA} transcript is processed to give the \textit{glnA} mRNA. The total level of the two transcripts was much higher in nitrogen-fixing cells than in ammonia-grown cells.

\textbf{Keywords:} \( \text{P}_\text{II} \) protein, \textit{glnB}, \textit{glnA}, \textit{Rhodospirillum rubrum}, regulation of nitrogen metabolism

\textbf{INTRODUCTION}

The photosynthetic purple bacterium \textit{Rhodospirillum rubrum} is a free-living nitrogen-fixing organism. The fixed nitrogen is assimilated via the glutamine synthetase-glutamate synthase pathway (Nordlund \textit{et al.}, 1985; Soliman \\& Nordlund, 1989; Carlberg \\& Nordlund, 1991). In enteric bacteria, glutamine synthetase, encoded by \textit{glnA}, is regulated both by feedback control and by covalent modification by adenylylation controlled through a regulatory cascade in which a number of enzymes participate (Rhee \textit{et al.}, 1989).

The \textbf{uridylyltransferase/uridylyl-removing enzyme (UTase), encoded by \textit{glnD}, senses the intracellular ratio of glutamine/2-oxoglutarate and catalyses uridylylation/deuridylylation of the \( \text{P}_\text{II} \) protein (Rhee \textit{et al.}, 1989). \( \text{P}_\text{II} \) is a trimer (Vasudevan \textit{et al.}, 1994) of identical subunits, encoded by \textit{glnB}. Each subunit can be uridylylated on a conserved tyrosine, residue 51 (Son \\& Rhee, 1987). Unmodified \( \text{P}_\text{II} \) stimulates the enzyme adenylyltransferase (\textit{ATase}), encoded by \textit{glnE}, to catalyse inactivation of glutamine synthetase by adenylylation, whereas \( \text{P}_\text{II}-\text{UMP} \) promotes the deadenylylation activity of the enzyme, leading to activation of glutamine synthetase (Rhee \textit{et al.}, 1989).

In addition to regulating glutamine synthetase activity, \( \text{P}_\text{II} \) also has an important function in controlling transcription from NtrC-activated \( \sigma^{54} \)-dependent promoters (Holtel \\& Merrick, 1988; Magasanik, 1989), which are of particular interest in nitrogen-fixing organisms (for a review see Kennedy \textit{et al.}, 1994). These promoters regulate transcription from genes encoding proteins involved in nitrogen metabolism, e.g. \textit{glnA}. Transcription from these promoters is activated by the phosphorylated form of NtrC, NtrC-P, which binds upstream of the promoter and interacts, by a loop on the DNA, with the \( \sigma^{54} \)-dependent RNA polymerase, leading to initiation of transcription (Minchin \textit{et al.}, 1989). Binding of NtrC-P to DNA in some cases also prevents transcription from \( \sigma^{70} \)-dependent promoters. Phosphorylation/dephosphorylation of NtrC is regulated by the status of the \( \text{P}_\text{II} \) protein. Unmodified \( \text{P}_\text{II} \) interacts with NtrB, thereby favouring the phosphatase activity of this bifunctional enzyme, whereas \( \text{P}_\text{II}-\text{UMP} \) cannot interact with NtrB, which then works as a kinase, phosphorylating NtrC. In enteric bacteria \textit{glnA} is localized in the same operon as \textit{ntrB} and \textit{ntrC} (Miranda-Rios \textit{et al.}, 1987). \textit{glnA} is transcribed from a \( \sigma^{70} \) promoter during nitrogen excess, but from a \( \sigma^{54} \) promoter during nitrogen limitation. \textit{glnB}
is localized elsewhere on the chromosome in these organisms (Rhee et al., 1989).

The metabolic regulation of glutamine synthetase in some phototrophic bacteria is similar to that of the Escherichia coli enzyme. Adenylylation of glutamine synthetase has been shown in Rhodopseudomonas palustris (Alef & Zumft, 1981), Rhodobacter sphaeroides (Engelhardt & Klemme, 1982) and Rhodobacter capsulatus (Johansson & Gest, 1977; Michalski & Nicholas, 1984). However, in Rsp. rubrum the situation seems to be more complex, although the enzyme can be adenylated (Nordlund et al., 1985; Woehe et al., 1990). Upon addition of ammonium ions to a nitrogen-fixing culture of Rsp. rubrum both the transference and the biosynthetic activity of glutamine synthetase are substantially decreased and cannot be reactivated by treating the enzyme with snake venom phosphodiesterase (Nordlund et al., 1989). Glutamine synthetase in Rsp. rubrum can also be ADP-ribosylated (Woehe et al., 1990).

The glnB gene in Rh. capsulatus has been identified upstream of glnA. In this organism glnB is cotranscribed with glnA either from a 

\[ \sigma^{32} \]

promoter or from a promoter, 

\[ \sigma^{54} \]

which requires NtrC-P but not the rpoN gene product (Foster-Hartnett & Kranz, 1994). The Rh. sphaeroides glnB gene is also localized upstream of glnA but it is transcribed from a single 

\[ \sigma^{32} \]

promoter (Zichenko et al., 1994). Other organisms in which glnB is localized upstream of glnA are Azospirillum brasilense (de Zamaroczy et al., 1990), Rhizobium leguminosarum (Chiurazzi & Iaccarino, 1990) and Bradyrhizobium japonicum (Martin et al., 1989).

\[ P_{1} \]

in the cyanobacterium Synechococcus sp. PCC 7942 has not been shown to be uridylylated, but phosphorylation on a serine residue has been demonstrated (Forchhammer & Tandeau de Marsac, 1994) and serine 49 has been suggested as the phosphorylation site (Cheah et al., 1994). Regulation of this phosphorylation process seems to be coupled to the redox state in the cell (Tsinoremas et al., 1991).

In this investigation we have sequenced the glnB gene from Rsp. rubrum and shown that it is localized upstream of glnA. We have also studied the transcription of these two genes under different conditions with respect to the nitrogen source supplied.

**METHODS**

**Bacterial strains, plasmids, media and growth conditions.** Strains and plasmids used in this study are listed in Table 1. E. coli strains were grown at 37°C in Luria Broth (LB) supplemented, when needed, with ampicillin at a final concentration of 50 mg ml\(^{-1}\). Rsp. rubrum strain S1 was grown in the minimal medium described by Ormerod et al. (1961), with the omission of glutamate. Rsp. rubrum strain UR 381 (Zhang et al., 1995) was grown in the presence of kanamycin (10 μg ml\(^{-1}\)) and streptomycin (100 μg ml\(^{-1}\)). Cultures were incubated at 30°C in the light. For growth under nitrogen-fixing conditions, referred to as N–, cultures were grown in sterile serum bottles and bubbled with a gas mixture of 95% N\(_2\)/5% CO\(_2\). Ammonia-grown cells, N+, were supplemented with 28 mM NH\(_4\)Cl.

**Cloning and sequencing of glnB.** The Rsp. rubrum λ-DNA library constructed by Falk et al. (1985) was screened by using the glnB gene from Rhizobium leguminosarum as a probe. A 670 bp SalI–SacI fragment (Fig. 1) was isolated and cloned into a pGEM3zf(+) vector (Promega), to construct the plasmid pJO1. A 0.8 kb BamHI–BamHI fragment (Fig. 1) containing the Rsp. rubrum glnB gene was subcloned, to produce plasmid pJO2. Both strands of the 0.8 kb fragment were sequenced by using T7 Sequencing Mixes (Pharmacia). Compressed GC-rich areas were resolved by using the Deaza G/A T7 Sequencing mixes (Pharmacia). M13 universal primers (forward and reverse) and designed oligonucleotides were used as primers. Both strands of the upstream region of glnB were sequenced from pJO1 with designed oligonucleotides as primers.

**RNA isolation.** All solutions used were treated with diethyl pyrocarbonate (1 ml l\(^{-1}\)) over night and autoclaved to remove RNase activity. Rsp. rubrum cultures were grown either with ammonia (N+) or under nitrogen-fixing conditions (N–) to an OD\(_{600}\) of 0.6. Then 50 ml of each culture was transferred to a 250 ml tube filled with ice and centrifuged at 6000 × g for 10 min. The pellets were washed with resuspension buffer (5 M sucrose, 10 mM sodium acetate, pH 4.5), transferred to micro-centrifuge tubes and centrifuged. Each pellet was resuspended in 0.25 ml resuspension buffer, followed by addition of 75 μl 250 mM EDTA to each tube and incubation on ice for 5 min. Then 375 μl lysis buffer (2%, w/v, SDS, 10 mM sodium acetate, pH 4.5) was added to each tube and the mixture incubated at 65°C for 3 min. Hot phenol (700 μl at 65°C) was added and after incubation at 65°C for 3 min, the tubes were placed in an ethanol/dry ice bath for 15 s. The samples were centrifuged at 12000 × g for 5 min. The phenol extraction was repeated twice followed by a phenol/chloroform extraction. RNA was precipitated with 0.3 M sodium acetate and 2.5 vol. ethanol. The pellets were washed with 70% (v/v) ethanol, dried and dissolved in RNA storage buffer (20 mM Na₂HPO₄, 1 mM EDTA, pH 6.5) and stored at –70°C.

**Northern blotting, primer extension and S1 nuclease mapping analyses.** For Northern blotting, 20 μg total RNA, from N+ and N– cultures, was analysed on a gel containing 2% (v/v) formaldehyde and transferred to an Hybond N filter. The filter was baked under vacuum at 80°C, hybridized with part of the Rsp. rubrum glnB gene, and probed with part of glnA (Fig. 1). The ECL direct nucleic acid labelling detection system (Amersham) was used for detection. The amount of transcript was estimated by laser densitometry using a Molecular Dynamics Personal Densitometer.

To identify the start of the mRNA transcripts for glnB and glnA two methods were used: primer extension and S1 nuclease mapping. Primer extension analyses were carried out according to Ausubel et al. (1991) with the modification that the primers (Fig. 2) were incubated with total RNA for 10 min at 65°C and then slowly cooled to 30°C, followed by hybridization overnight at this temperature. Twenty-five micrograms of total RNA purified from N+ and N– cells was used.

The 5′-end-labelled probes (Fig. 1) used for S1 nuclease mapping were obtained by digesting pJO1 and pJO2 (Table 1) with PstI and BamHI, respectively. The fragments generated were isolated and purified, treated with alkaline phosphatase and labelled with [γ-32P]ATP (Amersham) by polynucleotide kinase according to the manufacturer’s instructions (Pharmacia). The fragments were digested with SspI and BspHI respectively and the probes (Fig. 1) were separated and extracted from low-melting-point agarose and purified by Wizard DNA clean up (Promega). S1 nuclease mapping was done according to Berk & Sharp (1977)
**Table 1.** Bacterial strains and plasmids

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<th>Strain or plasmid</th>
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<td><em>E. coli</em> Q358</td>
<td><em>hisR</em>&lt;sub&gt;x&lt;/sub&gt; <em>hisM</em>&lt;sub&gt;x&lt;/sub&gt; <em>supE</em>&lt;sub&gt;460&lt;/sub&gt;</td>
<td>An su host used for growth of phage λ (Karn <em>et al.</em>, 1980)</td>
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<td><em>E. coli</em> JM109</td>
<td>*endA1 recA1 gyrA96 thi hisR17 (r&lt;sub&gt;53&lt;/sub&gt;, m&lt;sub&gt;53&lt;/sub&gt;) rolA1 supE44 Δ(lac–pro)Δ [F'&lt;trD36 proAB lacZΔM15]</td>
<td>Promega</td>
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<td><em>Rsp. rubrum</em> S1</td>
<td>Wild-type</td>
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<td><em>Rsp. rubrum</em> UR 381</td>
<td>Δ*trBC1::kan Sm&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Zhang <em>et al.</em> (1995)</td>
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<td><strong>Plasmids</strong></td>
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<td>pAH3</td>
<td>pUC19 derivative containing 0.4 kb <em>Rhizobium leguminosarum</em> DNA fragment with part of <em>glnB</em> and flanking region</td>
<td>Constructed by A. Holtel, University of Sussex, UK</td>
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<td>pJOM</td>
<td>pGEM3zf(+) derivative containing 6.7 kb <em>Rsp. rubrum</em> DNA fragment with <em>glnB</em>, <em>glnA</em> and flanking regions</td>
<td>This work</td>
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<tr>
<td>pJOB</td>
<td>pGEM3zf(+) derivative containing 0.8 kb <em>Rsp. rubrum</em> DNA fragment with <em>glnB</em> and flanking regions</td>
<td>This work</td>
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<tr>
<td>pJOD</td>
<td>pGEM3zf(+) derivative containing 2.6 kb <em>B. japonicum</em>-<em>Rsp. rubrum</em> DNA fragment with upstream region and part of <em>glnB</em></td>
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![Fig. 1. Map of the 6.7 kb fragment containing glnB and glnA. The location and size of the glnB and glnA probes used for Northern blotting analyses and for S1 nuclease mapping are also shown.](image)

as modified by Weaver & Weissman (1979). Total RNA (50 μg) and probe (0.5 μg) were mixed, precipitated, dried and dissolved in 30 μl hybridization buffer (40 mM Pipes pH 6.4, 80%, v/v, formamide, 0.4 M NaCl and 1 mM EDTA). The samples were denatured for 10 min at 85 °C and hybridized overnight at 42 °C. Then 300 μl S1 nuclease buffer (280 mM NaCl, 5 mM sodium acetate pH 4.6 and 4.5 mM ZnSO<sub>4</sub>) and 300 μl S1 nuclease were added and the samples were incubated for 1 h at 30 °C. The samples were extracted once with phenol/chloroform, ethanol-precipitated with carrier yeast tRNA, resuspended in formamide/dye mixture and analysed on a 4% (w/v) polyacrylamide gel. Sequenced M13 DNA was used as size markers for the trimmed RNA–DNA hybrids.

**Computer analysis.** Sequence analyses were done using the GCG subprogram FASTA (Genetics Computer Group, Madison, WI, USA) to identify similar sequences. The amino acid alignment of the *P<sub>N</sub>* proteins from *Rsp. rubrum*, *A. brasilense*, *Rh. capsulatus*, *Rhizobium leguminosarum*, *B. japonicum*, *Klebsiella pneumoniae*, *E. coli*, *Rb. sphaeroides* and the cyanobacterium *Synechococcus* sp. PCC 7942 was carried out with the program Gene Works (IntelliGenetics).

**RESULTS**

**Nucleotide sequence of the glnB locus**

The 0.8 kb *BamHI–BamHI* fragment cloned into pJOM was sequenced. To facilitate investigation of the transcriptional regulation of *glnB* the sequence was extended by sequencing the pJOM plasmid with two designed oligonucleotides as primers. This generated an additional 202 bp upstream of the *BamHI* site. The total sequence is presented in Fig. 2.

The nucleotide sequence of the ORF shows up to 83% identity to *glnB* genes from other organisms (Son & Rhee, 1987; Holtel & Merrick, 1988; Martin *et al.*, 1989; Chiuarazzi & Iaccarino, 1990; de Zamaroczy *et al.*, 1990; Kranz *et al.*, 1990; Tsionoremas *et al.*, 1991; Zinchenko *et al.*, 1994). A possible ribosome-binding site was identified 12 bp upstream of *glnB*, and a sequence similar to the σ<sup>54</sup>-dependent promoter consensus was identified 63 bp upstream of the gene. In this sequence the C normally
The mRNA start sites identified are indicated by bold and underlining. Putative promoter sequences are bold and a consensus sequence for a NtrC-P binding site. This site overlaps the possible promoter sequence upstream of glnB.

Another ORF that starts 135 bp downstream of glnB shows high similarity to glnA from other organisms. Only 239 bp (approximately 17% of glnA) have so far been sequenced. We have not yet been able to identify any obvious promoter sequences upstream of glnA.

**Features of the predicted gene products**

The polypeptide deduced from glnB shows very high similarity to P11 proteins from other organisms (Fig. 3). The identity with *Rb. capsulatus* and *Rb. sphaeroides* is 77 and 73% respectively, but the highest identity is to *A. brasilense*, 88%. The *Rsp. rubrum* sequence also has a tyrosine at residue 51, which is the uridylation site of the *E. coli* protein. Residue 49, which in *Synechococcus* sp. PCC 7942 is the serine that has been suggested to become phosphorylated (Cheah et al., 1994; Forchhammer & Tandeau de Marsac, 1994), is replaced with an alanine in the *Rsp. rubrum* sequence and in all other known P11 proteins.

The product of the other ORF shows high similarity to the N-terminal part of glutamine synthetase from other organisms. The highest similarity is with glutamine synthetase from *A. brasilense*, with which the identity is 66% (Bozouklian & Elmerich, 1986). The ORF and a sequence of 36 amino acids from the N-terminus of glutamine synthetase from *Rsp. rubrum*, which has previously been sequenced (Woehle, 1992), differ by one amino acid in the region now sequenced; a lysine at position 21 is a histidine in the sequence now reported. We believe these findings are convincing evidence for the gene downstream of glnB being the structural gene for glutamine synthetase.

**Expression of glnB and glnA in R. rubrum**

Northern blotting analyses showed that glnB is expressed as a single mRNA transcript (Fig. 4). The size of the transcript is approximately 20 kb, as estimated by comparison with rRNA standards. glnB is expressed under high-ammonia conditions, but the level of transcript is 4.8 times higher under nitrogen-fixing conditions.

When glnA was used as a probe for Northern blotting analysis, two transcripts, one of 20 kb and one about 400 bp shorter, were detected (Fig. 4). This indicates that glnA is cotranscribed with glnB from the glnB promoter(s). The presence of the shorter transcript could be explained either by transcription from another promoter or by specific processing of the glnBA mRNA. The expression of this shorter transcript was also much higher during nitrogen-fixing conditions.

**Identification of mRNA start sites for glnBA and glnA**

To identify the transcription start sites of the mRNAs demonstrated by Northern blotting, primer extension and S1 nuclease mapping were used with the primers and probes shown in Fig. 2 and Fig. 1, respectively. Both methods clearly identified two start sites upstream of glnB (Fig. 5). The one localized 52 bp from glnB is in agreement with a putative α4-dependent promoter (glnBp2 in Fig. 5) at a location similar to that normally found for such
promoters. As shown in Fig. 5, an mRNA starting 164 bp upstream of glnB can also clearly be identified. This would be in good agreement with glnBp1 (Fig. 5), which we propose to be a σ70-dependent promoter. Fig. 5 also shows the results of S1 nuclease mapping of mRNAs in the ntrBC mutant of Rsp. rubrum (Zhang et al., 1995). Also in this strain two start sites can be identified corresponding to glnBp1 and glnBp2, although the latter is much weaker than in the wild-type. These results verify that NtcC enhances transcription from glnBp2, but also indicate that transcription from this promoter is not strictly dependent on NtcC in Rsp. rubrum.

Both methods were also used to determine the start of the glnA mRNA. Primer extension identified a site within glnB; however, this could not be verified by S1 nuclease mapping. By the latter method two 5' ends in the region between glnB and glnA could be clearly demonstrated.

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**Fig. 3.** Amino acid sequence comparison of the P56 proteins from Rsp. rubrum, Azospirillum brasilense (de Zamaroczy et al., 1990), Escherichia coli (Son & Rhee, 1987), Klebsiella pneumoniae (Holtel & Merrick, 1988), Rhodobacter capsulatus (Kranz et al., 1990), Rhodobacter sphaeroides (Zinchenko et al., 1994), Rhizobium leguminosarum (Chiurazzi & Iaccarino, 1990), Bradyrhizobium japonicum (Martin et al., 1989) and Synechococcus sp. PCC7942 (Tsinoremas et al., 1991). Conserved amino acids (46%) are shaded.

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**Fig. 4.** Northern blotting analyses of glnB and glnA expression from nitrogen-fixing (N-) and ammonia-grown (N+) cells. The total RNA shown (used as size markers) was stained with ethidium bromide and visualized under UV light.

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**Fig. 5.** Localization of the transcription start sites upstream of glnB. (a) S1 nuclease mapping of mRNA from Rsp. rubrum wild-type and the ntrBC mutant using probe A shown in Fig. 1. (b) Primer extension of mRNA from Rsp. rubrum wild-type using primer 1 (Fig. 2). (c) Primer extension of mRNA from Rsp. rubrum wild-type using primer 2 (Fig. 2). (d) Schematic representation of the location of the promoters deduced from (a-c).
The high similarity of the amino acid sequence deduced from the glnB gene of *Rsp. rubrum* can be adenylylated and the identification of an ATase (Woehle et al., 1990; Woehle, 1992). However, whether Pn has also been involved in the ADP-ribosylation of glutamine synthetase (Woehle et al., 1990) remains to be established.

The sequence of glnBp2, the putative $\sigma^{54}$-dependent promoter, TGGCACGN$_2$GTGAT, is 75% similar to the consensus TGGCACGN$_2$TTGCA (Merrick, 1992) but it is localized at $-13/-25$ bp from the transcription start site instead of the usual $-12/-24$. Interestingly, the conserved C normally located at $-12$ is replaced with an A. This has to our knowledge only previously been found in the promoter sequences of *nifH* in *Rhiz. leguminosarum* (Roelvink et al., 1990), *Rhiz. trifolii* (Watson & Schofield, 1985) and *Rhiz. phaseoli* (Quinto et al., 1985) and of *nifN* in *Rhiz. meliloti* (Aguilar et al., 1987). The possible NtrC-P binding site TGGCTATATTTCGGGCA contains all the conserved nucleotides (bold) compared with the extended consensus for the NtrC recognition site TGGACCC(A/T)$_3$TGGTGCA (Foster-Hartnett & Kranz, 1994). These similarities with the Ntr system and the fact that the level of the glnBA transcript seems to be regulated according to the nitrogen status, make it likely that a similar system regulating glnBA transcription is present in *Rsp. rubrum*. Furthermore, Zhang et al. (1995) have recently identified the ntrBC genes in *Rsp. rubrum* and shown that in a mutant in which part of ntrBC was deleted, glutamine synthetase activity and protein level were both approximately 50% of wild-type. Our studies with this mutant are in good agreement with that report.

Taken together these results suggest the operation of a ntr system in *Rsp. rubrum* and the transcription of glnBA from a NtrC-activated promoter but also from one that is NtrC-independent.

In many organisms, e.g. *Azospirillum brasilense*, a $\sigma^{70}$-dependent promoter has been identified upstream of the $\sigma^{34}$-dependent glnB promoter (de Zamaroczy et al., 1990). The sequence GCGCCAN$_2$TATATT, glnBp1, found upstream of glnB in *Rsp. rubrum*, resembles the consensus sequence for $\sigma^{70}$ binding, especially in the $-10$ region. Repeated primer extension and S1 nuclease mapping experiments indicate that a transcription start site is located 164 bp upstream of glnB, giving a promoter and an mRNA start more distal than usually found for $\sigma^{70}$ dependent transcription. The transcript was more abundant relative to the glnBp2 transcript in ammonia-grown wild-type cells than in cells grown under nitrogen-fixing conditions, and under both conditions in the ntrBC mutant. This could be due to blocking of the promoter by NtrC-P in diazotrophically grown cultures, as the possible NtrC-P binding site overlaps the proposed $\sigma^{70}$ promoter sequence.

The Northern blotting experiments showed that the expression of the other glnA transcript in *Rsp. rubrum* also varies according to the nitrogen status. In *Rh. capsulatus* (Foster-Hartnett & Kranz, 1994), *A. brasilense* (de...
Zamaroczy et al., 1993) and Rhizog. leguminosarum (Amar et al., 1994), studies using reporter genes have shown the existence of a specific glnA promoter. Transcription from the promoter in A. brasilien·s is weaker under N− compared to N+ conditions. In Rhizog. leguminosarum the promoter is weak but constantly expressed. The situation is different in Rsp. rubrum, where the glnA mRNA is 70% more abundant than the glnA transcript during N− conditions, indicating a nitrogen-regulated promoter, upstream of the transcription start site of glnA. S1 nuclease mapping and primer extension experiments identify mRNAs starting between glnB and glnA, as well as within the 3′-terminal part of the glnB gene. In the light of these results, and as we have not been able to identify any sequence similar to a known promoter sequence in this region, we suggest that the glnA mRNA is the product of specific processing of the glnA transcript. A similar proposal was recently made for the glnB mRNA in Rhodospirillum rubrum (Borghese & Wall, 1995). Fig. 6(c) shows a possible RNase E cleavage site in the region between glnB and glnA. This enzyme has been suggested by Fritsch et al. (1995) to be involved in the specific cleavage of the polycistronic puf mRNA in Rhodospirillum. These authors also propose a cleavage site in the puf sequence similar to that in the region between glnB and glnA in Rsp. rubrum.

The results presented in this investigation demonstrate for the first time that glnB, encoding the regulatory PII protein, is present in Rsp. rubrum. The similarity of the sequences identified with established promoter and NtrC-binding sites indicates that N-regulated gene expression in Rsp. rubrum has features in common with that in other diazotrophs. To investigate the physiological role(s) of PII in Rsp. rubrum, we have tried to generate glnB mutants, but have so far been unsuccessful. This could be due to such mutations being lethal, or alternatively that it is difficult to get a high recombination frequency in the glnB region, as has been reported for some Rhizobium species (Martin et al., 1989; Amar et al., 1994). Other investigations have however been initiated to obtain information about the involvement of PII not only in the transcriptional regulation of nitrogen fixation and ammonia assimilation, but possibly also in the metabolic regulation operating in Rsp. rubrum (Ludden & Roberts, 1989), which has recently been suggested on the basis of studies of the ntrBC mutant (Zhang et al., 1995).

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