Regulation of Glutamine Synthetase II Activity in Rhizobium meliloti 104A14

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Most rhizobia contain two glutamine synthetase (GS) enzymes: GS1, encoded by glnA, and GS2, encoded by glnII. We have found that WSU414, a Rhizobium meliloti 104A14 glutamine auxotroph derived from a glnA parental strain, is an ntrA mutant. The R. meliloti glnII promoter region contains DNA sequences similar to those found in front of other genes that require ntrA for their transcription. No GSII was found in the glnA ntrA mutant, and when a translational fusion of glnII to the Escherichia coli lacZ gene was introduced into WSU414, no β-galactosidase was expressed. These results indicate that ntrA is required for glnII expression. The ntrA mutation did not prevent the expression of GS1. In free-living culture, the level of GSII and of the glnII-lacZ fusion protein was regulated by altering transcription in response to available nitrogen. No GSII protein was detected in alfalfa, pea, or soybean nodules when anti-GSII-specific antiserum was used.

Most gram-negative bacteria have only one glutamine synthetase (GS), but two distinct GS proteins are found in many bacterial species that are associated with plants, including Rhizobium and Bradyrhizobium species (11, 12), Agrobacterium species (16), and Frankia species (15). GS1 (encoded by glnA) is similar to the GS found in other bacteria (8, 11, 38), but GSII (encoded by glnII) is more closely related to the enzyme found in eucaryotes (7; R. G. Shatters and M. L. Kahn, J. Mol. Evol., in press). Why this second enzyme is uniquely found in plant-associated bacteria is unclear; although very different in size and structure, the two GS enzymes have similar affinities for their substrates and synthesize glutamine at similar rates (11). Inactivation of either Rhizobium gene by mutation does not block nodulation or nitrogen fixation (9, 37; J. Somerville, R. Shatters, and M. Kahn, submitted for publication), and inactivation of either Agrobacterium gene does not affect virulence (36).

The most striking difference between GS1 and GSII is how the enzymes are controlled in response to nitrogen availability. The activity of GS1 is altered posttranslationally by adenylylation, but the level of the protein remains relatively constant (11, 25, 26). In contrast, the level of GSII enzyme activity is different in bacteria grown on different nitrogen sources (6, 11, 20, 29) or at different oxygen concentrations (1, 33); this effect has been ascribed to transcriptional control of glnII.

Regulation of procaryotic GS activity is best characterized in the enteric bacteria that have a single GS-type enzyme (24, 28). This GS is regulated both transcriptionally and by adenylylation of the protein in response to available nitrogen. Transcription is controlled by the nitrogen regulatory system (ntr system), which also regulates the synthesis of various enzymes involved in nitrogen uptake and metabolism (24, 28). Three genes involved in the ntr system are ntrA (pnpN), which encodes an alternate RNA polymerase sigma subunit (18, 19); ntrC, which encodes a regulatory protein (NRI) that can act as both a transcriptional repressor and an activator (18, 19, 30, 34); and ntrB, which encodes a kinase-phosphatase activity that modifies NRI (30). In the enteric bacteria, mutations in any of these genes lead to glutamine auxotrophy (24, 28). In Rhizobium species, the role of the ntr system in the control of ammonia assimilation is not as clear. R. meliloti 1021 ntrA (35) and ntrC (39) mutants have been isolated, but neither mutant is a glutamine auxotroph.

To understand the role GSII might play in rhizobial nitrogen metabolism, we found mutants that were unable to express GSII, by isolating glutamine auxotrophs derived from an R. meliloti glnA mutant (38a). We report here that one of the mutants we isolated is defective in the Rhizobium equivalent of the ntra gene. This observation, together with the recent work of Martin et al. (29) showing that the ntrC gene is involved in regulating GSII in B. japonicum, indicates that GSII is controlled in the rhizobia by a regulatory system similar to that of enteric bacteria. By using a fusion of glnII to the Escherichia coli β-galactosidase gene, we show that the activation of glnII transcription is consistent with regulation by the ntr system. By using antibodies to GSII, we also show that the GSII protein is induced when cells are grown on poor nitrogen sources. These antibodies are unable to detect GSII protein in alfalfa, pea, or soybean nodules.

MATERIALS AND METHODS

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

Media and chemicals. All the media used in this study have been previously described (38). Affi-Gel Blue agarose (Bio-Rad Laboratories, Richmond, Calif.) and DEAE-cellulose DE-52 (Whatman, Inc., Clifton, N.J.) were used in GSII purification. Proteins transferred to nitrocellulose were detected by using goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase (Sigma Chemical Co., St.

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TABLE 1. Genetic materials

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant properties</th>
<th>Reference or source</th>
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<tbody>
<tr>
<td>E. coli ET8051</td>
<td>ET8000 ΔglnA-rapha</td>
<td>26</td>
</tr>
<tr>
<td>R. meliloti</td>
<td></td>
<td></td>
</tr>
<tr>
<td>104A14</td>
<td>Wild type</td>
<td>38</td>
</tr>
<tr>
<td>GLN210</td>
<td>104A14 glnA210</td>
<td>38</td>
</tr>
<tr>
<td>WSU414</td>
<td>104A14 glnA210 ntrA</td>
<td>38a</td>
</tr>
<tr>
<td>WSU660</td>
<td>10A14 glnII</td>
<td>38a</td>
</tr>
<tr>
<td>L. japonicum</td>
<td>Wild type</td>
<td>9</td>
</tr>
<tr>
<td>B. japonicum</td>
<td>Wild type</td>
<td>21</td>
</tr>
<tr>
<td>B. leguminosarum 300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Plasmids

- pMK318: P4 cos, IncP1 replicon 38
- pJS36: R. meliloti glnA in pMK318 38
- pJS73: R. meliloti glnII in pMK318 38a
- pJS87: R. meliloti ntrA in pMK318 38a
- pJS95: ntrA::Tn5 from WSU414 38a
- pRS16: glnI Tet' IncP1 replicon 38a
- pSKS105: lacZYA Amp', pMB9 replicon 10
- pUC18: lacZ Amp', multiple cloning site 32

Recombinant DNA techniques. The recombinant DNA methods used in this study have been described previously (22). Restriction enzymes were purchased from various commercial sources, and digests were carried out as specified by the manufacturers. Restriction fragments were isolated either by cutting the appropriate bands out of an agarose gel and extracting them with phenol (4) or by using low-melting-temperature SeaPlaque agarose for in-gel ligation, as described by the manufacturer.

DNA sequences were determined by using the dideoxy-nucleotide termination method with T7 DNA polymerase supplied in the Sequenase kit from U.S. Biochemicals, [α-35S]dATP from Du Pont, NEN Research Products, Boston, Mass., and single-stranded M13 DNA templates (40). The sequence was determined for both strands and across each cloning site. Sequence analysis was carried out by using the University of Wisconsin Sequence Analysis software package (13) with the assistance of Susan Johns and the Washington State University Visualization, Analysis and Design in the Molecular Sciences center.

Restoring the wild-type glnA gene in R. meliloti WSU414. Plasmid pJS6, which contains the wild-type glnA gene (38), was transduced into R. meliloti WSU414(pPH111) by using the P2 encapsidation procedure described previously (38). We screened for replacement of the mutant glnA210 allele on the chromosome with the wild-type allele by using Southern blotting (38). glnA' recombinants were isolated as streptomycin-resistant, gentamicin-resistant, tetracycline-sensitive colonies.

Construction of the glnII-lacZ translational fusion. The codon for amino acid 28 of glnII was fused to the codon for amino acid 6 of the E. coli lacZ gene (Fig. 1). Plasmid pRS16 contains the entire glnII gene cloned as a 2.8-kilobase-pair (kb) HindIII-BglII fragment (38a). It contains a 555-base-pair (bp) EcoRI fragment within the glnII gene (Shattters and Kahn, in press). This EcoRI fragment was replaced with a 9.8-kb DNA fragment from pRS20-41 that contains the lacZ gene. pRS20-41 was derived from pSKS105 (10) by digestion with BamHI, removing the 4-bp protruding ends with S1 nuclease, and ligating the blunt ends. This deletion puts the EcoRI site into the proper frame for construction of the glnII-lacZ fusion.

Enzyme assays. Glutamine synthetase activity was measured by using the transferase assay (38). β-Galactosidase activity in cells that had been permeabilized with toluene was measured by using the hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG) (23).

GSI1 protein purification. pRS11, a plasmid that expresses the glnI gene at a high level, was constructed by cloning the 1.5-kb HindIII-BamHI fragment from pJS73 that contains glnI (38a) downstream from the lac promoter in pUC18. In ET8051, an E. coli glutamine auxotroph (27), this plasmid expressed high levels of GSI1 with or without isopropyl-β-D-thiogalactopyranoside (IPTG) in the medium. E. coli ET8051(pRS11) (8 liters) was grown to early stationary phase in LB medium and harvested by centrifugation. The pellet was suspended in 2 volumes of 1M buffer (20 mM imidazole hydrochloride, 1 mM MnCl2 [pH 7.0]) and the cells were broken by using a French press. After centrifugation at 25,000 × g for 30 min, streptomycin sulfate was added to the supernatant to give a final concentration of 5 mg/ml. This was stirred on ice for 30 min and centrifuged at 25,000 × g for 20 min; the supernatant was dialyzed against 1M buffer (pH 6.5). Portions of this extract were loaded on a 30-ml DE-52 DEAE-cellulose column equilibrated with 1M buffer (pH 6.5), and GSI1 activity was eluted with a 0 to 175 mM NaCl gradient in 1M buffer (pH 6.5). Fractions containing GSI1 activity were pooled and applied directly to a 30-ml Affi-Gel Blue column. The column was washed with 90 ml of each of the following: 1M buffer (pH 6.0), 1M buffer (pH 6.0) plus 0.5 M NaCl, 1M buffer (pH 6.0), 1M buffer (pH 7.0) plus 10 mM NaCl, and 1M buffer (pH 7.0). GSI1 was eluted with 1M buffer (pH 7.0) plus 10 mM ADP. Fractions containing GSI1 activity were concentrated in a stirred cell (Amicon Corp., Lexington, Mass.) by using a YM10 membrane. This fraction was purified further by electrophoresis on a 7%
nondenaturing polyacrylamide gel, and the GSII band was detected by staining for activity in situ (25). After the mobility of the GSII protein had been determined, preparative gels were stained with Coomassie blue, and the band corresponding to GSII was used to immunize a New Zealand White rabbit (R and R Rabbity, Stanwood, Wash.). The rabbit serum was used directly for the described procedures.

**Western blots.** Crude extracts of nodules were prepared from *Pisum sativum* cv. Wisconsin Perfection inoculated with *R. leguminosarum* 300, *Glycine max* cv. Prize inoculated with *B. japonicum* BJ110, and *Medicago sativa* cv. Ladak inoculated with *R. meliloti* 104A14. Four- to six-week-old nodules of each were crushed with a glass homogenizer in 0.5 M sucrose–25 mM Tris–5 mM dithiothreitol in the presence of 10 mg of polyvinylpyrrolidone per ml and 1 mg of p-aminobenzamidine per ml. The nodule slurry was filtered through two layers of Miracloth and stored at -20°C. crude extracts were prepared from cultures of *R. meliloti* 104A14, *R. leguminosarum* 300, and *B. japonicum* BJ110 by growing 250-ml cultures in YMB broth, harvesting the cells by centrifugation, and suspending them in 0.5 ml of IM buffer (pH 7.4). The cells were broken by sonication, debris were removed by centrifugation, and the supernatants were used as the crude extracts for Western immunoblotting. Protein concentrations were determined by using a modified Bradford assay (Bio-Rad). Total protein (25 to 75 μg) from the sonic extracts was separated on sodium dodecyl sulfate-10% polyacrylamide gels and transferred to nitrocellulose, and the separated proteins were visualized immunologically (5).

**RESULTS**

An *R. meliloti* mutant that does not express GSII has a mutation in *ntra*. WSU414 is a Tn5 mutant of *R. meliloti* GLN210 that was isolated as a glutamine auxotroph (38a). By comparing restriction enzyme digests of pJS95, a plasmid that contains the Tn5 insert, and pJS87, a plasmid that complements the insertion in WSU414, we located the region of pJS87 that surrounds the Tn5 mutation. When we determined the DNA sequence of this region (Fig. 2), we found an open reading frame in which 86.4% of the bases were identical to those in the recently sequenced *R. meliloti* 1021 *ntra* gene (35). Two gaps were placed in the *R. meliloti* 1021 *ntra* sequence to obtain the best alignment, a 3-base gap starting at base 158 and a 1-base gap at base 1533. We believe that the two genes are functionally equivalent, since there are no restriction enzyme bands in digests of *R. meliloti* 104A14 that hybridize to pJS87 except those found in the plasmid (data not shown). WSU414 and its glnlA+ derivative, WSU415 (see below), have growth characteristics similar to those of *R. meliloti* 1021 *ntra* mutants described by Ronson et al. (35). All are Fix+ and are unable to utilize succinate as a carbon source or nitrate as a nitrogen source (data not shown).

One line of evidence that *ntra* acts in controlling *glnII* transcription was obtained by analyzing kanamycin-resistant WSU414 revertants able to grow in the absence of glutamine. When restriction digests of these revertants were examined by Southern hybridization with Tn5 DNA as a probe, approximately half retained the original Tn5 insertion but had a new and unique band (J. Somerville, Ph.D. thesis, Washington State University, Pullman, 1985). Southern hybridization analysis with the *glnII* gene as a probe showed that the new insertion was linked to *glnII* and that only a portion of the Tn5, most probably a single copy of IS50, had inserted into the *glnII* promoter region (data not shown). When total DNA from one of these revertants was digested with *Hind*III and ligated into pUC18, plasmids containing this altered *glnII* gene could be isolated by their ability to complement the *E. coli* glutamine auxotroph ET8051. Since the wild-type *glnII* gene is not expressed in *E. coli* (38a), we believe that the insertion of IS50 activated the expression of the *glnII* gene. This implies that the mutation in WSU414 affected *glnII* transcription and not some posttranscriptional control. Although this class of WSU414 revertants could grow with ammonia as the sole nitrogen source, they could not grow on nitrate as the sole nitrogen source or on succinate as the sole carbon source, and they still formed Fix- nodules on alfalfa. Therefore, they have retained other components of the *ntra* phenotype.

*ntra* is not required for expression of *glnA*. WSU414, the *ntra* mutant we isolated, also contains a *glnA* mutation. To determine whether *ntra* was required for *glnA* expression, we recombined the wild-type *glnA* gene back into the *R. meliloti* WSU414 genome as described in Materials and Methods. The resulting strain, WSU415, was not a glutamine auxotroph and had significant heat-stable GS activity (data not shown).

*ntra* control sequences are present in the *glnII* promoter region. We have sequenced the *R. meliloti* *glnII* gene (Shatters and Kahn, in press). The upstream region (Fig. 3) contains an 18-bp sequence that is very similar to the general *ntr* consensus sequence (2, 14) and, in particular, to the version of this sequence found in the *B. japonicum* *glnII* promoter (9) (Table 2). In this sequence the highly conserved bases were located in two groups centered at -24 and -12 with respect to the transcriptional start site. From this we have inferred the start of *R. meliloti* *glnII* transcription, as indicated in Fig. 3 and Table 2.

An upstream activator sequence (termed the NRI consensus) is also required for NRI-mediated transcriptional activation in members of the family *Enterobacteriaceae* (17, 22, 32). Carlson et al. (9) found a potential NRI consensus sequence between -103 and -119 in the *B. japonicum* *glnII* promoter. The *R. meliloti* *glnII* promoter has a similar potential NRI consensus between -98 and -114 (Fig. 3; Table 2). In *E. coli*, NRI consensus sequences are functional up to 2 kb upstream from the start of transcription (34), so a precise distance between NRI and the start of transcription is not critical for function. GSII activity of free-living *R. meliloti* 104A14 is transcriptionally controlled in response to available nitrogen. A translational fusion of the *glnII* gene to the coding sequence for β-galactosidase was constructed in pRS21-1 (Fig. 1) to investigate how *glnII* transcription is controlled. Minimal mammalian media containing either NH4Cl or glutamate as a nitrogen source were inoculated with either wild-type *R. meliloti* or *R. meliloti* (pRS21-1), and the β-galactosidase activity in cultures in the late stage of logarithmic growth was determined. *R. meliloti* 104A14 (pRS21-1) grown with glutamate as the nitrogen source contained 6,300 U of β-galactosidase activity, compared with 500 U when ammonia was the nitrogen source. A background activity of 2 U was observed for *R. meliloti* 104A14 lacking pRS21-1. WSU414 (pRS21-1) and WSU413 (pRS21-1) had only background levels of β-galactosidase activity when the cells were grown on either glutamate or ammonia (data not shown). This result is consistent with the idea that *ntra* is needed for *glnII* transcription.

Genes regulated by the enteric *ntr* system are typically repressed after ammonia is added (24, 28). To determine the
FIG. 2. Comparison of the *R. meliloti* 104A14 and the *R. meliloti* 1021 *ntrA* DNA sequences. The top line shows the three-letter amino acid code for the *R. meliloti* 104A14 *ntrA* sequence, and the middle line shows the *R. meliloti* 1021 *ntrA* DNA sequence. The bottom line shows the bases in the *R. meliloti* 1021 DNA sequence that differ from those in the *R. meliloti* 104A14 sequence. Dots present in the *R. meliloti* 104A14 sequence represent gaps inserted to give the best alignment. The arrowhead at base 255 indicates the site of Tn5 insertion on pJS95.
effect on glnII transcription of adding ammonia to glutamate-grown cells, we diluted stationary-phase cultures of R. meliloti 104A14 and R. meliloti 104A14(pRS21-1) in minimal mannitol medium containing glutamate. Replicate cultures were grown to the mid-log phase, and NH₄Cl was added to one of the replicates to give a final concentration of 15 mM. The cultures were grown for a further 10 h, and samples were taken at intervals to determine β-galactosidase activity (Fig. 4). After the addition of ammonia, β-galactosidase specific activity decreased steadily, indicating that glnII promoter activity was lower when ammonia was available.

This conclusion is similar to that reached with B. japonicum (11), R. phaseoli (6), and Rhizobium sp. strain ANU289 (20). In all of these studies, GSII transferase activity decreased after ammonia was added to glutamate-grown cultures. We also measured GS transferase activity in our experiments and found that although the pattern of our results was qualitatively similar to that seen with the β-galactosidase assays, the specific activity of GS varied significantly with the concentration of protein in the assay. We were therefore unable to quantitatively confirm the results cited above.

Detection of the GSII protein in free-living cells and bacteroids of R. meliloti. To study the level of GSII protein in free-living cells and bacteroids of R. meliloti, we purified GSII protein and used it to prepare anti-GSII antiserum. When this antiserum was used to stain Western blots, a band at approximately 36 kilodaltons, the size of the GSII monomer (7, 11; Shatters and Kahn, in press), was visible in extracts prepared from glutamate-grown cultures of R. meliloti 104A14 or GLN210 (glnA) but not cultures of WSU660 (glnII) (Fig. 5). When 104A14 and GLN210 were grown on ammonia as the nitrogen source, the intensity of this band decreased substantially (data not shown). These data are consistent with the results obtained with the glnII::lacZ fusion.

The anti-GSII antiserum was also used to probe Western blots of crude extracts from alfalfa, soybean, and pea nodules (Fig. 5). The serum reacts well with the GSII proteins in these other bacteria, as would be expected from the stability of the DNA sequence (Shatters and Kahn, in press). No band corresponding to GSII was detected in any of the nodule extracts. Despite the high concentration of protein and antiserum used in this experiment, no band was seen in the nodule extracts at 36 kilodaltons, although reaction of a minor component of the serum with other bacterial proteins can be seen in both bacterial and nodule lanes. The bands between 60 and 66 kilodaltons also appear in the lane containing the molecular mass standards and are most probably an artifact due to the presence of β-mercaptoethanol (L. Thomashow, personal communication). The lack of a detectable protein band at 36 kilodaltons in the nodule crude extracts agrees with results of previous work with B. japonicum, in which no GSII transferase activity

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<th>Proposed NtrA-binding site (ntr consensus)</th>
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<tr>
<td>Overall consensus&lt;sup&gt;b&lt;/sup&gt;</td>
<td>TGG YAYNNNNNT TGC (A/T)</td>
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<tr>
<td>Rhiobium nif&lt;sup&gt;c&lt;/sup&gt; promoter</td>
<td>Y TGG CAYGNNTT TGC (A/T)</td>
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<tr>
<td>R. meliloti glnII promoter&lt;sup&gt;d&lt;/sup&gt;</td>
<td>T TGG CACGTTTGA TGC TT</td>
<td>This work</td>
</tr>
<tr>
<td>NRI (ntrC product) - binding site</td>
<td>TGCACY NNNNN GGTGCA</td>
<td>3, 17</td>
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<tr>
<td>Overall consensus&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-119 -103</td>
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</tr>
<tr>
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<tr>
<td>R. meliloti glnII promoter</td>
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<td></td>
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<tr>
<td>Rhiobium glnII promoter consensus</td>
<td>AGCGCC ATCCT TGCCCA</td>
<td>This work</td>
</tr>
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</table>

<sup>a</sup> Sequence is numbered starting at the site of initiation of transcription.
<sup>b</sup> This sequence was deduced by comparing the -24 to -12 region of ntr-regulated promoters from Klebsiella pneumoniae, Salmonella typhimurium, two Azotobacter species, Rhizobium nif promoters, and two Pseudomonas species.
<sup>c</sup> The Rhizobium nif consensus was deduced by comparing 13 Rhizobium nif and fix promoters.
<sup>d</sup> The R. meliloti glnII transcriptional start site has not been determined; this placement was assigned by using the consensus sequence for alignment.
<sup>e</sup> This sequence was deduced by comparing ntrC activated promoters in K. pneumoniae, the rhizobia, and E. coli.
could be detected in isolated bacteroids (11), but it is difficult to reconcile with the results of Martin et al. (29), who recently showed that high levels of glnII mRNA are present in B. japonicum bacteroids isolated from soybean nodules. It is possible that glnII is transcribed but not translated or that GSII is not stable in nodules.

DISCUSSION

It has been suggested that the glnII gene is regulated by nitrogen availability by using a regulatory system similar to that of enteric bacteria. However, the most direct evidence that NtrA is required in the expression of genes involved in intermediary nitrogen metabolism is that an R. meliloti ntrA mutant is unable to grow with nitrate as its sole nitrogen source (35). We have shown here that a functional R. meliloti ntrA gene is required for the expression of the R. meliloti GSII protein, that there is a potential ntr consensus sequence and an NRI upstream activator sequence in the glnII promoter region, that the glnII promoter is ntrA dependent, and that it is regulated in response to nitrogen availability. This is strong evidence that ntrA is required for the expression of a gene involved in intermediary nitrogen metabolism in R. meliloti. Since ntrA was not required for the expression of GSII, our ntrA mutant was not a glutamine auxotroph.

The R. meliloti 104A14 ntrA mutant, WSU414, has the same phenotypes attributed to a R. meliloti 1021 ntrA mutant (35). It forms Fix− nodules on alfalfa and is unable to grow with either succinate as a carbon source or nitrate as a nitrogen source. Despite considerable sequence divergence, the gene we have mutated plays the same role as the R. meliloti 1021 ntrA gene. The Fix− phenotype was not caused by the glutamine auxotrophy of this strain, since neither prototrophic revertants of WSU414 nor those of its glnA+ derivative, WSU415, are effective.

Transcription of glnII was affected by the form of nitrogen available. In experiments with anti-GSII antiserum, GSII protein levels were higher when glutamate was substituted for ammonia as the nitrogen source. In cells containing a translational fusion that put E. coli lacZ under the control of the glnII promoter, β-galactosidase activity was high when glutamate was the nitrogen source but low when ammonia was present. After ammonia was added to glutamate-grown cells containing the gene fusion, β-galactosidase specific activity decreased steadily. This decline is similar to that seen when GSII enzymatic activity is assayed in response to ammonia addition (6, 11, 20), but more directly indicates that changes in transcription are responsible for the decrease.

The inability to detect GSII protein in nodules when using antiserum against GSII suggests that GSII activity in the bacteroids is repressed either by low oxygen levels or by the presence of ammonia. This result is consistent with previous results showing low levels of GSII activity in nodules (10) and under microaerobic conditions (33), but, unless there is posttranscriptional regulation, it is not consistent with the recent report (29) that glnII is actively transcribed in nodules.

In our experiments, ntrA was needed for the expression of glnII, a gene apparently repressed in bacteroids. It had previously been shown that ntrA is needed for the expression of dcta, a gene expressed in bacteroids (35). Since each of these genes had an associated transcriptional activator, our results support the idea (31) that NtrA is a component of the transcriptional machinery used to recognize a subclass of
genes involved in various metabolic pathways but does not itself sense the environmental signals.

ACKNOWLEDGMENTS

This work was supported by grants from the U.S. Department of Agriculture Competitive Research Grants Office and from the Washington Technology Center.

We thank Jennifer Kraus for helpful advice, Mike Minnick for help in preparing the antisera, Linda Moore for editorial assistance, and Greg Martin for communicating his results prior to publication.

ADDITION IN PROOF

An expanded version of the work by Rossbach et al. (37) has been recently published by de Bruijn et al. (F. J. de Bruijn, S. Rossbach, M. Schneider, P. Ratet, S. Messmer, W. W. Szeto, F. M. Ausubel, and J. Schell, J. Bacteriol. 171:1673–1682, 1989).

LITERATURE CITED


