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# Mutations That Affect Activity of the *Rhizobium meliloti trpE(G)* Promoter in *Rhizobium meliloti* and *Escherichia coli*

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**The cloned *Rhizobium meliloti trpE(G)* gene is not expressed in *Escherichia coli*. Oligonucleotide-directed mutagenesis was used to introduce base substitution mutations in the promoter region of this gene. Three separate mutations that increased homology of the putative −10 region of this promoter with the *E. coli* −10 promoter consensus sequence by 1 bp converted this promoter to an active promoter in *E. coli*. A deletion extending to position −43 from the 5′ side had a minor effect on transcription in *R. meliloti*. However, transcription was nearly eliminated when a deletion extended to position −33, indicating that the crucial domain of the *R. meliloti trpE(G)* promoter begins in the region downstream of position −43. The *R. meliloti trpE(G)* promoter has two regions that show homology with the *E. coli* −35 and −10 promoter consensus sequences. Mutations in these putative −35 and −10 regions, but not in the spacer region, affected promoter strength in *R. meliloti*. By comparing four known *R. meliloti* promoter sequences, we identified a highly conserved trimer near position −35 (5′-TTG-3′) but no noticeably conserved sequence near position −10.**

Cloning and sequencing of the *Rhizobium meliloti trpE(G)* gene were previously reported (3). Although the cloned DNA fragment contained the entire *trpE(G)* coding sequence and its regulatory region, it failed to complement a *trpE* mutation in *Escherichia coli* (3). Many other *R. meliloti* genes have been cloned and found to be unable to complement equivalent mutations in *E. coli* (6, 10, 18, 21), although some *R. meliloti* genes can complement equivalent *E. coli* mutations (5, 19). When the cloned *R. meliloti trpE(G)* gene was expressed in *E. coli* with the *E. coli lac* promoter and the *R. meliloti trpE(G)* Shine-Dalgarno sequence and AUG initiation codon, it complemented an *E. coli trpE* mutation (1). This result indicates that the inability of the *R. meliloti trpE(G)* gene to complement in *E. coli* is not due to lack of translation or a nonfunctional polypeptide but due to the lack of transcription.

The promoter is the site where RNA polymerase binds and initiates transcription (24, 35). For *E. coli*, hundreds of promoters with defined transcriptional start sites have been reported and a compilation and comparison of the promoter sequences revealed two highly conserved sequences, the −35 (5′-TTGACA-3′) and −10 (5′-TATAAT-3′) regions (13, 14). Although these two hexamers are not the only sequence determinants required for maximum promoter strength, their importance has been supported by the phenotypes of numerous promoter mutations (14). The *R. meliloti trpE(G)* promoter shows some sequence homology with the *E. coli* −35 and −10 consensus regions (three matching base pairs in each region) (Fig. 1).

The promoters of *R. meliloti* genes involved in nodulation, nitrogen fixation and flagellum biosynthesis have been well studied. The *nod* genes have the so-called "*nod* box" upstream of the transcription initiation sites, where the NodD protein binds and activates transcription (11, 22). The *nif*, *fix*, and *dct* genes have *ntr*-type promoters (22). The product of the *ntrA* gene is a sigma factor ($\sigma^{54}$) which is responsible for recognizing these promoters (22). The *fla* genes are probably transcribed by RNA polymerase associated with another σ factor (27), as is the case for *E. coli* and *Salmonella typhimurium* (15). However, the promoters of the *R. meliloti* genes presumably recognized by RNA polymerase associated with the predominant sigma factor are poorly characterized. By comparing several *R. meliloti* promoters which are presumably transcribed by RNA polymerase associated with the predominant sigma factor and whose transcription start sites were determined, we identified a highly conserved sequence near the −35 position (5′-TTG-3′) which is identical to a part of the *E. coli* −35 consensus sequence. However, we were not able to find conserved sequences around the −10 position. Nevertheless, mutational analysis suggests that two distinct regions are important for transcription initiation in *R. meliloti*, one near the −35 region and one near the −10 region.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** *R. meliloti* YB41 (2), *E. coli* JM109 (36) and S17-1 (30), and plasmids pEG220 (3), pYB49 (4), pYB76 (4), pMC1403 (8), and pUC19 (34) were previously described.

**Construction of deletions by nuclease *Bal*31.** The plasmid pYB50 was constructed by inserting a 204-bp *Bcl*I-*Msp*I (filled-in) fragment of pEG220 into the *Bam*HI-*Sal*I (filled-in) site of pUC19. The *Bcl*I site is at position −107 and the *Msp*I site is at position +88 relative to the *trpE(G)* transcription start site. pYB50 DNA (25 μg) was then cleaved with *Eco*RI, which cuts in the vector DNA at position −128 relative to the *trpE(G)* transcription initiation site. After phenol and chloroform extraction, the linearized DNA was ethanol precipitated, vacuum dried, and suspended in deionized water. The volume of the reaction mixture for nuclease *Bal*31 digestion was 50 μl, and the reaction mixture contained 600 mM NaCl, 12 mM $CaCl_2$, 12 mM $MgCl_2$, 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1 U of *Bal*31, and 25 μg of linearized DNA. Aliquots (12 μl) taken after 5, 10, 15, and 20 min of incubation at 30°C were mixed with 88 μl of an EDTA solution to make the final volume 100 μl and the final EDTA

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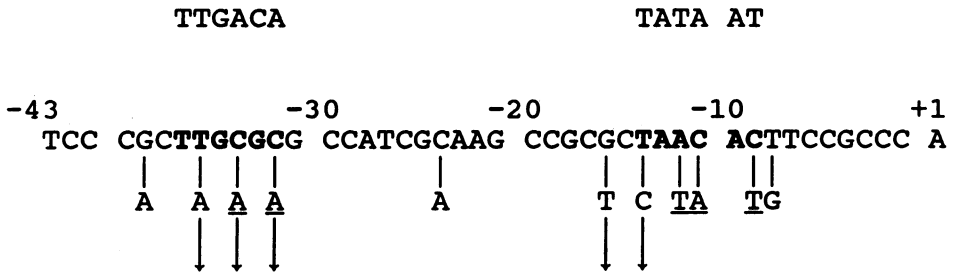


FIG. 1. Mutations introduced in the *trpE(G)* promoter. Base pair changes that increase homology of the *R. meliloti trpE(G)* promoter with the *E. coli* promoter consensus sequences are underlined. The downward arrows indicate mutations that decrease the promoter activity by more than twofold in *R. meliloti* (see Table 3). The putative −35 and −10 regions are in boldface. The *E. coli* −35 and −10 consensus sequences are shown above the *R. meliloti trpE(G)* promoter sequence.

concentration 10 mM. This solution was extracted with phenol and chloroform and ethanol precipitated. The extent of deletion was measured by polyacrylamide gel electrophoresis, and the aliquot taken at 10 min (approximately 20- to 120-bp deletions) was ligated with *Eco*RI linkers. After *Eco*RI digestion, the plasmid DNA was separated from the linkers by a low-melting-temperature agarose gel and self-ligated. The DNA was used to transform *E. coli* JM109, and the transformed cells were plated on Luria-Bertani (25) agar plates containing ampicillin (100 μg/ml). Twenty-four Amp$^r$ colonies were then picked, and plasmid DNA was prepared. The extent of deletion of each plasmid was determined on a polyacrylamide gel. The plasmids with approximately 20- to 120-bp deletions were isolated, and the extent of each deletion was precisely determined by DNA sequencing by the method of Sanger et al. (29). Each plasmid was cleaved with *Nco*I, the ends were filled in, the plasmids were then cleaved again with *Eco*RI, and the fragment carrying the deletion was ligated with plasmid pMC1403 (8) cleaved with *Eco*RI and *Sma*I. This plasmid was then cleaved with *Nco*I, filled in, and cleaved with *Pst*I, which cuts upstream of the *Eco*RI site. Finally, this *Pst*I-*Nco*I (filled-in) fragment was inserted into the *Pst*I-*Bam*HI sites of pYB49 by joining the *Nco*I site to the *Bam*HI site after filling in both the *Nco*I and *Bam*HI sites. Since the *Nco*I site overlaps the AUG initiation codon of the *trpL* sequence, this resulted in an in-frame fusion of the AUG initiation codon of the *trpL* sequence with the 10th codon of *lacZ* (GTC, encoding valine), with a connecting sequence of GATCCC. Deletions extending to positions −97, −65, −43, −33, and −3 were constructed as described above.

Deletions extending to positions −580 and −154 were constructed as follows. The *Eco*RI-*Nco*I fragment of pEG220 (approximately 580 bp) was inserted into the *Pst*I-*Bam*HI sites of pYB49 as described above and used as the deletion extending to position −580. The *Msp*I-*Nco*I fragment of pEG220 was inserted into the *Eco*RI-*Sma*I sites of pMC1403 after ligating an *Eco*RI linker to the *Msp*I site and inserted into pYB49 as described above. The *Msp*I site is located 154 bp upstream of the *Nco*I site and was used as the deletion extending to position −157. All deletions have the same sequence 5′ to the deletion endpoint.

**Site-directed mutagenesis.** Site-directed mutagenesis with synthetic oligonucleotides was performed by the method of Bae and Stauffer, which will be published elsewhere. The mutations were confirmed by DNA sequencing by the method of Sanger et al. (29).

**DNA manipulations.** Subcloning experiments were done by using the low-melting-temperature agarose method of Struhl (31) as modified by Bae et al. (3). Transformation of *E. coli* strains was by the $CaCl_2$ method (28) or by the dimethyl sulfoxide method (9).

**Introduction of plasmids into *Rhizobium* strains.** Plasmids were transferred into *Rhizobium* strains from *E. coli* by filter mating as previously described (3).

**β-Galactosidase assay.** One loopful of frozen *E. coli* cells was used to inoculate 5 ml of Luria broth containing 15 μg of tetracycline per ml, and the culture was grown for 6 h at 37°C with shaking and used for β-galactosidase assays by the method of Miller (25).

*Rhizobium* cells were grown in TY medium (5 g of tryptone, 3 g of yeast extract, and 1.3 g of $CaCl_2$ per liter) supplemented with 800 μg of streptomycin per ml, 5 μg of tetracycline per ml, and 25 μg of cycloheximide per ml at 30°C with shaking for 36 h. Aliquots (5 to 10 μl) of these cultures were used to inoculate 5 ml of M9 minimal medium containing 0.2% glucose, 1 μg of thiamine per ml, 0.15% acid-hydrolyzed casein, 20 μg of L-tryptophan per ml, and 5 μg of tetracycline per ml. After 36 h of incubation, the cultures were used for β-galactosidase assays as described previously (2). All results are the averages of two separate assays.

## RESULTS

**Mutations that convert the *R. meliloti trpE(G)* promoter to an active promoter in *E. coli*.** It was previously reported that the cloned *R. meliloti trpE(G)* gene failed to complement a *trpE* mutation in *E. coli*, although the cloned DNA fragment contained the promoter, attenuator, and the entire *trpE(G)* coding sequence (3). DNA sequencing and S1 nuclease mapping were used to determine the transcription start site (3). The promoter region contained sequences similar to the *E. coli* promoter consensus sequences. The putative −35 region (5′-TTGCGC-3′) has three base pairs that match the *E. coli* −35 consensus sequence (5′-TTGACA-3′), and the putative −10 region (5′-TAACAC-3′) also has three base pairs that match the *E. coli* −10 consensus sequence (5′-TATAAT-3′).

Subsequently, a mutant was isolated in which expression of the *R. meliloti trpE(G)* gene occurred in *E. coli* (3). DNA sequence analysis showed a C → T change at position −9 in the *trpE(G)* promoter, increasing the homology of the putative −10 region with the *E. coli* −10 consensus sequence by 1 bp (3). To extend this study and further define the promoter region of the *R. meliloti trpE(G)* gene, we introduced point mutations in the promoter region by oligonucleotide-directed site-specific mutagenesis. We first cloned a 380-bp *Pst*I-

TABLE 1. Effects of mutations on transcription of the *R. meliloti trpE(G)* gene in *E. coli*

| Mutation | Base pair change | Position | β-Galactosidase activity (Miller units) |
|---|---|---|---|
| Control[a] | | | 0.1 |
| Wild type | | | 0.2 |
| P9T | C→A | −9 | 143 |
| P11A | C→A | −11 | 3.3 |
| P12T | A→T | −12 | 0.4 |
| P32A | C→A | −32 | 0.1 |
| P34A | C→A | −34 | 0.1 |

[a] *E. coli* JM109 carrying pYB76 was used as a control. pYB76 carries the *E. coli lac* operon without a promoter.

TABLE 2. Effects of 5′ deletions on transcription of the *trpE(G)* gene in *R. meliloti*

| Extent of deletion | β-Galactosidase activity (Miller units) | Relative activity (%)[a] |
|---|---|---|
| Control[b] | 1.6 | |
| −580 | 152 | 100 |
| −157 | 149 | 98 |
| −97 | 87 | 56 |
| −65 | 98 | 64 |
| −43 | 67 | 43 |
| −33 | 1.7 | 0.1 |
| −3 | 1.7 | 0.1 |

[a] Relative activities are calculated after subtraction of the activity obtained for the control.

[b] *R. meliloti* YB41 carrying pYB76 was used as a control. pYB76 carries the *E. coli lac* operon without a promoter.

*Bam*HI fragment of pYB49 (4) containing the *R. meliloti* promoter and the AUG initiation codon of *trpL* into the *Pst*I-*Bam*HI sites of phage M13mp19. After oligonucleotide-directed mutagenesis, the mutated *Pst*I-*Bam*HI fragments were cloned back into the *Pst*I-*Bam*HI sites of plasmid pYB49. This results in in-frame fusions of the *trpL* AUG initiation codon of the mutated DNA fragments to the 10th codon of the *E. coli lacZ* gene. We previously showed that the *trpL* and *trpE(G)* coding sequences are transcribed into a single mRNA from a single promoter [*trpE(G)* promoter] (2, 3). Transcription of the *trpL* coding sequence is not regulated, whereas transcription of the *trpE(G)* gene is regulated by attenuation in response to tryptophan (1, 2, 4). Therefore, we constructed and used the *trpL′-′lacZ* fusion in this study to exclude the effect of attenuation on β-galactosidase levels. After transformation of *E. coli* JM109 with the pYB49 or the plasmids carrying each of the mutations, the cells were grown in Luria-Bertani medium (25), and promoter activity was determined by measuring β-galactosidase levels (Table 1).

Mutations P32A and P34A each increased agreement of the putative −35 region with the *E. coli* −35 consensus sequence by 1 bp (Fig. 1) but did not convert the promoter from inert to active in *E. coli* (Table 1). Mutations P9T, P11A, and P12T each increased agreement of the putative −10 region with the *E. coli* −10 consensus sequence by 1 bp (Fig. 1). All three changes converted the promoter from inert to active in *E. coli* with different promoter strengths, indicating that mutations in the putative −10 region are necessary for the promoter to be recognized by *E. coli* RNA polymerase (Table 1). The P9T mutation caused the biggest increase in promoter activity. This strain also formed dark blue colonies on Luria-Bertani agar plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), while strains carrying the P11A or P12T mutations formed pale blue colonies (data not shown). These data suggest that the lack of expression of the *R. meliloti trpE(G)* gene in *E. coli* is due in part to the inability of *E. coli* RNA polymerase to transcribe the *R. meliloti trpE(G)* gene because of differences in the −10 region.

**Effect of deletions on promoter function in *R. meliloti*.** In order to define the functional limits of the *R. meliloti trpE(G)* promoter, deletions from the 5′ side of the *trpE(G)* promoter region were constructed (see Materials and Methods). The DNA fragments carrying these deletions were inserted into plasmid pYB49 to generate in-frame *trpL′-′lacZ* fusions, and the plasmids were introduced into *R. meliloti* YB41 as described previously (2). The levels of expression of these gene fusions are shown in Table 2. A deletion extending to nucleotide −157 had essentially no effect on *trpL′-′lacZ* expression. However, deletion of the region between −157 and −97 reduced transcription about twofold. Deletions that extend to −43 had no additional effect on promoter activity, but a deletion that extends to position −33 completely abolished promoter activity. These results indicate that there are two regions necessary for full function of the *R. meliloti trpE(G)* promoter. One region lies between −157 and −97, and the other region lies between −43 and +3 (+3 was selected as the functional limit from the 3′ side because the downstream DNA from position +3 of the fusion is from the *E. coli lacZ* gene).

**Effects of base pair substitutions on the *trpE(G)* promoter in *R. meliloti*.** In addition to the mutations described above, several additional mutations were constructed and used to examine their effects on promoter function in *R. meliloti* (Fig. 1 and Table 3). Mutations P14C, P16T, P32A, P34A, and P36A reduced *trpL′-′lacZ* expression significantly (at least twofold), indicating that these positions are important for function of the *trpE(G)* promoter. The P14C mutation changed the putative −10 region (5′-TAACAC-3′) to 5′-CAACAC-3′, thereby decreasing agreement of this region with the *E. coli* −10 consensus sequence (5′-TATAAT-3′), and reduced promoter activity about 200-fold, indicating that this position is crucial for promoter function. The P16T mutation is not in the putative −10 region but reduced

TABLE 3. Effects of mutations on transcription of the *trpE(G)* gene in *R. meliloti*

| Mutation | Base pair change | Position | β-Galactosidase activity[a] (Miller units) (%) |
|---|---|---|---|
| Control[b] | | | 1.9 |
| Wild type | | | 2,500 (100) |
| P8G | T→G | −8 | 1,530 (61) |
| P9T | C→T | −9 | 2,730 (110) |
| P11A | C→A | −11 | 3,120 (125) |
| P12T | A→T | −12 | 3,640 (146) |
| P14C | T→C | −14 | 14 (0.5) |
| P16T | G→T | −16 | 441 (18) |
| P24A | C→A | −24 | 2,300 (92) |
| P32A | C→A | −32 | 1,160 (46) |
| P34A | C→A | −34 | 394 (16) |
| P36A | T→A | −36 | 167 (7) |
| P39A | G→A | −39 | 3,320 (133) |

[a] Relative activities are calculated after subtraction of the activity obtained for the control and are shown in parentheses.

[b] *R. meliloti* YB41 carrying pYB76 was used as a control. pYB76 carries the *E. coli lac* operon without a promoter.

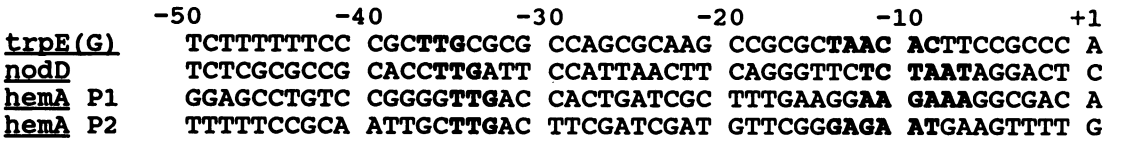


FIG. 2. Comparison of promoter sequences of *R. meliloti*. Only genes whose transcription initiation sites were determined by S1 mapping or primer extension mapping are shown. The highly conserved sequence near position −35 (5′-TTG-3′) is in boldface. Sequences near position −10 with the best match with the *E. coli* −10 consensus sequence are also shown in boldface.

transcription about sixfold. In *E. coli*, several positions outside the consensus hexamers are weakly conserved, and mutations at these positions also affect promoter strength. In *E. coli*, position −14 is one of these weakly conserved base pairs, since 37% of *E. coli* promoters analyzed have G at this position (13). Susskind and coworkers reported that a mutation at this position severely affected the phage P22 *ant* promoter (12). This position also seems to be important in *R. meliloti*, since the P16T mutation, which is equivalent to position −14 of *E. coli* promoters, has a significant effect on promoter activity in *R. meliloti*.

The P32A, P34A, and P36A mutations reduced promoter activity about twofold, sixfold, and 15-fold, respectively, indicating that these positions are important for promoter activity in *R. meliloti*. The P24A mutation, located between the two consensus regions, had little effect on promoter activity, indicating that this position is part of the spacer region. Mutations P8G, P9T, P11A, P12T, and P39A had only slight effects on promoter activity (Table 3).

**Comparison of *R. meliloti* promoter sequences.** We compared the DNA sequences of the *R. meliloti* promoters presumably transcribed by RNA polymerase associated with the predominant sigma factor (10, 26) in an effort to find conserved bases (Fig. 2). Although there are more *R. meliloti* genes available whose DNA sequences were reported than those shown in Fig. 2, we compared only the genes whose promoter locations were determined by biochemical analyses. As shown in Fig. 2, there is a highly conserved triplet (5′-TTG-3′) near position −35. There are no significantly conserved bases near position −10, although the bases flanking the −10 position are slightly A-T rich. Since we compared only four *R. meliloti* promoters, we will have to wait until more *R. meliloti* promoters with well-characterized transcription initiation sites are reported to determine the consensus −35 and −10 regions.

## DISCUSSION

With a few exceptions (5, 19), cloned *R. meliloti* genes are usually not transcribed in *E. coli* (3, 6, 10, 18, 21). Nielsen and Brown (26) reported copurification of a polypeptide with the *R. meliloti* RNA polymerase holoenzyme. However, it is unlikely that the reason why *R. meliloti* genes are not transcribed in *E. coli* is because of the presence of an additional subunit in the *R. meliloti* RNA polymerase holoenzyme. *R. meliloti* RNA polymerase holoenzyme purified by Fisher et al. (10) lacked the copurified polypeptide observed by Nielsen and Brown (26), and this RNA polymerase transcribed both the *E. coli trp* promoter and the *R. meliloti nodD* promoter in vitro.

The promoter region of the *R. meliloti trpE(G)* gene contains several base pairs homologous with the *E. coli* consensus sequences. The spacer region between these two hexamers is 17 bp, which is identical to that of the *E. coli* consensus promoter. Since these features were not sufficient to be recognized and transcribed by *E. coli* RNA polymerase, we introduced mutations in the promoter region to determine what is limiting transcription of the *R. meliloti trpE(G)* promoter in *E. coli*.

In *E. coli*, the frequency of occurrence of each nucleotide in the −35 and −10 regions is different. In the −35 region, each of the first three base pairs (5′-TTG-3′) is present in at least 68% of *E. coli* promoters, while the rest are present at best in 58% of the promoters (13). In the *R. meliloti trpE(G)* promoter, C occurs at positions −34 and −32 (Fig. 1). The equivalent bases in the *E. coli* −35 consensus sequence are As (13, 14). The P32A and P34A mutations increase the homology of the putative *R. meliloti trpE(G)* −35 region with the *E. coli* −35 consensus hexamer by 1 bp each (Fig. 1). However, the P34A and P32A mutations reduced transcription in *R. meliloti* by about sixfold and twofold, respectively, indicating that A may not be preferred relative to C at positions −34 and −32 in *R. meliloti*. Thus, the −35 consensus sequence for *R. meliloti* may be different from that of *E. coli*.

In the *E. coli* −10 consensus hexamer, the first, second, and sixth positions are present in at least 82% of *E. coli* promoters, while the rest are present at best in 59% of the promoters (13). The putative *R. meliloti trpE(G)* −10 region (5′-TAACAC-3′) matches the *E. coli* −10 consensus hexamer (5′-TATAAT-3′) at the first, second, and fifth positions. The P9T, P11A, and P12T mutations increase the homology of the putative −10 region of the *R. meliloti trpE(G)* promoter by 1 bp (Fig. 1), and all activate the *R. meliloti* promoter in *E. coli* (Table 1). The P9T mutation increased the homology with the *E. coli* −10 consensus hexamer at the sixth position, which is the most conserved base pair in the *E. coli* promoter and which has the greatest effect. Although the P11A or P12T mutations converted the inactive promoter to an active one in *E. coli*, transcription from the promoters containing these mutations was much lower than that from the P9T mutant promoter, suggesting that the contribution of each of these three base pairs to promoter strength is different, at least in the context of the *R. meliloti trpE(G)* promoter. The P11A mutation creates a stretch of four As, and this could cause DNA bending (20). Although DNA bending could stimulate transcription, it is unlikely that the 32-fold increase in promoter activity observed in *E. coli* caused by the P11A mutation (Table 1) is due to DNA bending, since the 380-bp *Pst*I-*Bam*HI fragment carrying either the P11A mutation or the wild-type sequence showed identical mobilities during 5 and 10% polyacrylamide gel electrophoreses (data not shown). Since all of the mutations that convert the *R. meliloti trpE(G)* promoter to an active promoter in *E. coli* are found only in the putative −10 region (Table 1), the results suggest that the lack of transcription in *E. coli* is due to differences in the −10 region.

The data shown in Table 2 indicate that the core domain of the *R. meliloti trpE(G)* promoter is confined in a rather small region between positions −43 and +3, which is typical of

bacterial promoters. An upstream segment between −157 and −97 appears to be necessary for full activity of the promoter. This region might contain a DNA sequence necessary for formation of bent DNA (7, 20), or an enhancer sequence such as the NtrC-binding site of the *E. coli glnA* promoter (32). These possibilities need to be studied further.

It has been suggested that the consensus hexamers are not the only DNA sequences necessary for the function of promoters in *E. coli* (12, 17) and that the context of the sequence in the promoter region plays an important role in promoter activity (12, 16, 17, 23, 33). The fact that *R. meliloti* promoters do not have conspicuously conserved base pairs around the −10 region suggests a possibility of the involvement of a strong context effect on promoter activity. In *R. meliloti*, as well as in *E. coli*, certain yet-to-be-identified base pair(s), or several different combinations of base pairs outside of the consensus hexamers, could play roles in promoter strength and in the determination of the transcription start site.

Another possibility is that the *R. meliloti* RNA polymerase associated with the predominant sigma factor is more flexible in recognizing the signal for transcription initiation. Fisher et al. (10) reported that purified *R. meliloti* RNA polymerase started transcription in vitro on both the *R. meliloti nodD* promoter and the *E. coli trp* promoter precisely at the start sites used in vivo. However, *E. coli* RNA polymerase did not transcribe the *R. meliloti nodD* gene in vitro. The fact that the P9T, P11A, and P12T mutations, which increase the homology of the *R. meliloti* promoter with the *E. coli* consensus sequence by 1 bp, are promoter-up mutations in *E. coli* indicates that the *R. meliloti* −10 consensus region might be similar to the *E. coli* −10 consensus region, but that individual *R. meliloti* promoters do not have to be as highly conserved in the −10 region because of the flexibility of RNA polymerase in recognizing these sequences. The highly conserved −35 triplet would then be more strictly required in *R. meliloti* than the −35 hexamer in *E. coli* in order to prevent RNA polymerase from starting transcription at many spurious promoterlike sequences. In support of this hypothesis is the observation that other suspected *R. meliloti* promoters whose transcription initiation sites have not been determined experimentally also have the conserved triplet 5′-TTG-3′ near the −35 position (data not shown).

Although we found that the *R. meliloti trpE(G)* promoter is not transcribed in *E. coli* because of differences in the −10 region, we could not determine a consensus sequence for *R. meliloti* promoters, except the conserved triplet near −35 (5′-TTG-3′). A comparison of more *R. meliloti* promoter sequences will be necessary to determine the consensus promoter sequence clearly.

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