

## A Homolog of the CtrA Cell Cycle Regulator Is Present and Essential in *Sinorhizobium meliloti*

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**During development of the symbiotic soil bacterium *Sinorhizobium meliloti* into nitrogen-fixing bacteroids, DNA replication and cell division cease and the cells undergo profound metabolic and morphological changes. Regulatory genes controlling the early stages of this process have not been identified. As a first step in the search for regulators of these events, we report the isolation and characterization of a *ctrA* gene from *S. meliloti*. We show that the *S. meliloti* CtrA belongs to the CtrA-like family of response regulators found in several  $\alpha$ -proteobacteria. In *Caulobacter crescentus*, CtrA is essential and is a global regulator of multiple cell cycle functions. *ctrA* is also an essential gene in *S. meliloti*, and it is expressed similarly to the autoregulated *C. crescentus ctrA* in that both genes have complex promoter regions which bind phosphorylated CtrA.**

The  $\alpha$ -proteobacterium *Sinorhizobium meliloti* forms nitrogen-fixing nodules on the roots of certain legumes of the genera *Medicago*, *Melilotus*, and *Trigonella*. The bacterium and plant exchange chemical signals during initiation of this process (7, 24). Bacteria attach to root hairs and invade plant cells via an infection thread which encases the bacterial colony as the infection thread grows into the root; bacteria are released from the infection thread into the cytoplasm of plant cells (reviewed in references 4 and 15). After release, one or more rounds of bacterial cell division occur before the bacteria differentiate into nitrogen-fixing bacteroids. These bacteroids are four to seven times longer than vegetative bacteria and are often Y shaped. Bacteroids remain in the host cytoplasm, each one enclosed in a peribacteroid membrane, a structure formed from both bacterial and plant components (4). They continue to fix nitrogen until senescence of the plant cell. Nitrogen-fixing bacteroids are thought to represent a terminally differentiated state; however, it is possible that nondifferentiated bacteria are present in the plant cell and can multiply upon release (40, 44).

While much is known about the genetics and biochemistry of nitrogen fixation, little is known about bacterial release and differentiation. Specific regulatory circuits must exist to coordinate the cessation of DNA replication and cell division with the other processes of bacteroid differentiation (reviewed in reference 30). Some *S. meliloti* genes involved in cell division have been characterized, but neither the mechanisms of control of these genes nor their role in the differentiation process is known (25–27).

In contrast, control of the cell cycle in *Caulobacter crescentus*, a closely related member of the  $\alpha$ -proteobacteria, is better understood. Several regulatory proteins that play essential roles in cell cycle progression have been identified in this organism (17). These include members of the two-component family of signal transduction proteins, the CtrA response reg-

ulator (32), the CckA histidine kinase that phosphorylates CtrA (18), and the DivK histidine kinase (43), as well as a DNA adenine methyltransferase, CcrM, that methylates newly replicated DNA at the end of S phase (39). CtrA is a global regulator that plays a pivotal role in orchestrating the cell cycle. It is involved in the control of a quarter of the cell cycle-regulated genes (23) including genes required for DNA replication (32, 33), DNA methylation (32, 34), cell division (20), and biogenesis of flagella and pili (32, 38). CtrA binds to the chromosomal origin of replication and inhibits DNA replication initiation; it must be cleared from the cell before it enters S phase (9, 33). Transcription of *ctrA* is activated after initiation of DNA replication and reaches threshold levels by an autoregulated positive feedback loop. *ctrA* gene is transcribed from two promoters: P1, a promoter that is active in early S phase and is negatively controlled by CtrA; and a stronger promoter, P2, which is active later in S phase and is positively controlled by CtrA (10). The level of intracellular CtrA is controlled not only by autoregulated transcription but also by specific degradation of this protein when the cells enter S phase and by cell cycle-controlled phosphorylation which activates the protein (9, 18).

Here we report the isolation and characterization of *ctrA* from *S. meliloti*. As a first step in dissecting the regulatory circuit controlling the cell cycle and bacteroid differentiation in *S. meliloti*, we show that *ctrA* is essential for viability of *S. meliloti*. The promoter region was identified and shown to contain multiple CtrA binding sites, suggesting that the *S. meliloti* gene may be autoregulated, as is the case in *C. crescentus*.

### MATERIALS AND METHODS

**Bacterial genetic techniques.** Strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strains were grown in either Luria broth (LB) or 2XYT medium at 37°C. *S. meliloti* strains were grown on LB agar plates or in liquid TY medium at 30°C. Antibiotic concentrations were for *S. meliloti*, neomycin and spectinomycin, 200  $\mu$ g/ml; gentamicin, 25  $\mu$ g/ml; tetracycline, 10  $\mu$ g/ml; streptomycin, 500  $\mu$ g/ml; and trimethoprim, 600  $\mu$ g/ml; for *E. coli*, gentamicin, 5  $\mu$ g/ml; kanamycin, 25  $\mu$ g/ml; tetracycline, 10  $\mu$ g/ml; ampicillin, 50 or 100  $\mu$ g/ml; chloramphenicol, 50  $\mu$ g/ml; and spectinomycin, 40  $\mu$ g/ml. Triparental

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TABLE 1. Strains, plasmids, and primers used

Strain, plasmid or primer	Description <sup>a</sup>	Reference or source
<b>Strains</b>		
DH5 $\alpha$	<i>endA1 hsdR17 supE44 thi-1 recA1 gyrA relA1 Δ(lacZYA-argF) U169 deoR</i>	BRL Corp.
GV310(pMPg90)	<i>Agrobacterium tumefaciens</i>	39
LS2195	NA1000 <i>ctrA401</i> (Ts)	32
MT616	MM294 pRK600, Cm <sup>r</sup>	12
MB492X	Rm1021, single crossover of pMB492 into <i>ctrA</i>	This study
MB506	Rm1021, <i>ctrA</i> mutant with pMB467	This study
MB507	Rm1021, <i>ctrA</i> mutant with pSAL290	This study
Rm1021	<i>S. meliloti</i> RCR2011 Sm <sup>r</sup>	F. M. Ausubel
S2308	<i>Brucella abortus</i>	R. M. Roop
<b>Plasmids</b>		
pBBR1MCS	Cloning vector, pBBR origin	K. M. Peterson
pDYH218	1.6-kb <i>XhoI</i> - <i>RsaI</i> fragment containing <i>S. meliloti ctrA</i> in pMR10	This study
pJQ200	Cloning vector, Gm <sup>r</sup> , ColE1, <i>oriT</i> , <i>sacB</i>	31
pMB393	Cloning vector, Sp <sup>r</sup> Cm <sup>r</sup> version of pBBR1MCS	M. Barnett
pMB453	4.5-kb <i>SalI</i> fragment containing <i>S. meliloti ctrA</i> in pBS SK(-)	This study
pMB454	Same as pMB453, opposite orientation	This study
pMB464	3-kb <i>PstI</i> fragment containing <i>S. meliloti ctrA</i> in pBS SK(-)	This study
pMB467	3-kb <i>PstI</i> fragment from pMB453 in pMB393	This study
pMB492	Mutated <i>S. meliloti ctrA</i> in pJQ200	This study
pMR10	Cloning vector, Km <sup>r</sup> , RK2, <i>oriT</i>	C. Mohr, R. Roberts
pRK290	Cloning vector, IncP	D. Helinski
pSAL290	<i>C. crescentus ctrA</i> in pRK290	32
<b>Primers</b>		
PCR amplification of <i>C. crescentus ctrA</i> probe	5'-ATGCGCGTACTGTTGATCGA 5'-TCAGGCGGCGTTAACCTGC	
<b>Primer extension</b>		
PE-1	5'-CGCGCTGTGCTCTTCGATCAGTAG	
PE-2	5'-GAGCTCGATGCTCTGAGCCGTCGCGC	
PE-3	5'-GGGAATGCCGTTAACTGACTCTTCCGAC	
<b>DNase I protection</b>		
P1-for	5'-GAAGGTGGGATAGGCGAAAT-3'	
P1-rev	5'-ATCTGAAATTGCGCTCCTCA-3'	
P2-for	5'-GCCGGTTCGCCCGGAATTCCCGTGA-3'	
P2-rev	5'-TCCCCTTTTCCGCCGCCCAAGG-3'	

<sup>a</sup> Ap<sup>r</sup>, Cm<sup>r</sup>, Gm<sup>r</sup>, Km<sup>r</sup>, Sp<sup>r</sup>, Sm<sup>r</sup>, Tc<sup>r</sup>, and Tp<sup>r</sup> denote resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, spectinomycin, tetracycline, and trimethoprim, respectively. pBS, pBluescript.

conjugations were performed using MT616 as the helper plasmid strain (12). *C. crescentus* was grown in PYE broth as previously described (11). Kanamycin (20 μg/ml) was used for *C. crescentus*. *Agrobacterium tumefaciens* was grown in LB medium.

**Southern hybridization.** DNA from *S. meliloti*, *C. crescentus*, and *A. tumefaciens* was purified using the PureGene kit (Gentra systems), and *Brucella abortus* strain 2308 DNA was kindly provided by R. M. Roop, Louisiana State University. Genomic DNA was digested with various restriction enzymes and electrophoresed on a 1% agarose gel. The DNA was transferred to Hybond N+ (Amersham) as described previously (37). Hybridization was performed by the Church and Gilbert method (5); a random-primed *EcoRI*-*SalI* restriction fragment from pSALF1, containing *C. crescentus ctrA*, was the probe (32). The blot was hybridized for 4 h at 65°C and then washed once in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 1 h, twice in 1× SSC (20 min each time), and finally once in 0.1× SSC for 20 min.

**Isolation of the *S. meliloti ctrA* clone.** An *S. meliloti* partial *Sau3A* genomic library, constructed in a λ FixII vector (1), was screened using Church and Gilbert hybridization (5). The probe was a random-primed 695-bp PCR product containing *C. crescentus ctrA*; the primers used to amplify this DNA are shown in Table 1. Following hybridization, filters were washed three times in 2X SSC–0.1% sodium dodecyl sulfate and then once in 0.5X SSC–0.1% sodium dodecyl sulfate. Positive plaques were purified and rescreened. DNA was isolated from putative *ctrA*-containing phage, mapped, subcloned, and sequenced. The University of Wisconsin Genetics Computer Group (8) and Sequencher (Gene Codes Corp.) software were used for sequence assembly and analysis.

**Construction and complementation of *S. meliloti ctrA* mutant.** To introduce an insertion mutation into the *S. meliloti ctrA* coding sequence we used pJQ200, which contains the levan sucrose (*sacB*) gene, which confers lethality when strains are grown on sucrose (31). This vector has been previously used to create null mutations in *S. meliloti ccrM* (42). A derivative of pJQ200 lacking polylinker sites from *Ecl136* to *SmaI* was created. A 3-kb *PstI*-*SalI* fragment from pMB453 was cloned into this derivative to form pMB491. A blunted, *BamHI*-*HindIII* fragment containing a neomycin resistance (Nm<sup>r</sup>) cassette was ligated into pMB491 that had been digested with *BamHI*, thereby removing the 5' half of *ctrA*, and blunted with Klenow to form pMB492. In pMB492, the Nm<sup>r</sup> cassette is oriented such that the neomycin promoter reads in the opposite direction from the *ctrA* promoter. 1.35 and 0.95 kb of *S. meliloti* DNA are present upstream and downstream, respectively, of the Nm<sup>r</sup> cassette. After conjugation into *S. meliloti* strain Rm1021, colonies were selected on LB agar plates containing gentamicin, neomycin, and streptomycin; these are the putative single recombinants containing both an intact and a disrupted copy of *ctrA*. Either a *ctrA* plasmid (pMB467 or pSAL290) or the vector only (pMB393 or pRK290) was introduced into this strain by conjugation. The resulting strains were selected on plates lacking gentamicin and containing 5% sucrose. Colonies viable on these plates were screened for gentamicin sensitivity (Gm<sup>s</sup>) in the presence of sucrose (Gm<sup>r</sup> sucrose-resistant mutants likely contain mutations in the *sacB* gene or in host genes that suppress the *sacB* phenotype). Double recombinants, containing a single mutated *ctrA*, are expected to be Gm<sup>s</sup>, Nm<sup>r</sup>, and sucrose resistant. The frequency of apparent double recombinants was calculated for a representative experiment

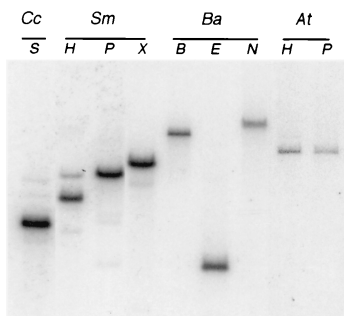


FIG. 1. *ctrA* is present in several members of the  $\alpha$ -proteobacteria. The Southern blot shows hybridization of the *C. crescentus ctrA* probe to chromosomal DNA from *C. crescentus* (Cc), *S. meliloti* (Sm), *B. abortus* (Ba), and *A. tumefaciens* (At). Restriction enzymes used are *Bam*HI (B), *Eco*RI (E), *Hind*III (H), *Not*I (N), *Pst*I (P), *Sal*I (S), and *Xho*I (X).

by dividing the number of Gm<sup>s</sup> Nm<sup>r</sup> sucrose-resistant colonies by the total number of Nm<sup>r</sup> sucrose-resistant CFU.

**Complementation tests of *C. crescentus ctrA* mutants with *S. meliloti ctrA*.** To determine if the *S. meliloti ctrA* gene could replace the *C. crescentus ctrA*, the *S. meliloti ctrA* promoter and gene were ligated into the low-copy-number vector pMR10, generating pDYH218. Both pDYH218 and pMR10 were mated into strains NA1000 (wild-type *C. crescentus*) and LS2195 (NA1000 *ctrA401*, a temperature-sensitive allele of *ctrA*). Logarithmic-phase cultures were diluted serially, plated in duplicate on PYE agar containing kanamycin (20  $\mu$ g/ml), and incubated at either 30 or 37°C. Viable cell number at the permissive (30°C) and restrictive (37°C) temperatures was measured as CFU per milliliter.

**Primer extension analysis.** *S. meliloti* total RNA was purified from LB-grown cells, using Trizol reagent (BRL Corp.), as previously described (3). Primer extension reactions were performed as before (3), using an initial annealing temperature of 65°C for 1 h and then cooling to 40°C over 1.5 h. Extensions were performed at 49°C. The nucleotide sequences of PE-1, PE-2, and PE-3 used for primer extension are shown in Table 1. Extension products were analyzed on a urea-polyacrylamide sequencing gel as described elsewhere (13). Sequencing ladders using each of the three primers and pMB464 as template were used to determine the transcription start site.

**DNase I protection experiments.** DNase I footprinting experiments were performed with purified *C. crescentus* CtrA that was phosphorylated using a maltose-binding protein (MBP)-EnvZ fusion protein as previously described (34). Native *C. crescentus* CtrA was overexpressed in *E. coli* using the pET expression system (Novagen), solubilized from inclusion bodies with 4 M guanidine-HCl, and purified by Q-Sepharose chromatography (K. Ryan and L. Shapiro, unpublished data). Template DNA for footprinting the P1 and P2 promoters was generated by PCR using the oligonucleotides in Table 1. Both reverse primers (P1-rev and P2-rev) were end labeled with [ $\gamma$ -<sup>32</sup>P]ATP and T4 DNA polynucleotide kinase prior to PCR amplification of the template.

## RESULTS

**CtrA is conserved among  $\alpha$ -proteobacteria.** To determine the extent of conservation of the gene encoding the CtrA response regulator among the  $\alpha$ -proteobacterial family, we generated a probe containing the entire *C. crescentus ctrA* coding region and part of the 5' and 3' flanking sequences (32). We hybridized this *Sal*I-*Eco*RI DNA fragment to digested genomic DNA from *S. meliloti*, *B. abortus*, and *A. tumefaciens* (Fig. 1) using fairly stringent conditions. The *C. crescentus ctrA* probe hybridized to at least one band in genomic digests of all these organisms.

To isolate the putative *ctrA* homolog from *S. meliloti*, we screened a genomic library with a probe internal to the coding region of *C. crescentus ctrA*. Positive plaques were purified, and phage DNA was subcloned. Sequence analysis of this DNA

revealed an open reading frame with an excellent pattern of *S. meliloti* codon usage and a high degree of similarity to *C. crescentus ctrA* (80% nucleotide sequence identity; 82% amino acid sequence identity) (Fig. 2). *S. meliloti ctrA* encodes a 26-kDa protein. Genes encoding CtrA-like proteins are also found in three other  $\alpha$ -proteobacteria. Of these, *S. meliloti* CtrA is most similar to CtrA from *B. abortus* (86% amino acid identity) and less similar to the CtrA of *Rhodobacter capsulatus* and CzcR of *Rickettsia prowazekii* (77 and 59% identity, respectively.) Thus, CtrA homologs are present in at least six members of the  $\alpha$ -proteobacteria, suggesting that CtrA is conserved in this group of bacteria.

CtrA is a member of the OmpR family of winged helix-turn-helix response regulators (32). OmpR-type regulators contain an N-terminal regulatory domain and a C-terminal DNA binding domain. The five CtrA-like proteins shown in Fig. 2 form a distinct subgroup within the OmpR family, thus far unique to the  $\alpha$ -proteobacteria. No other winged helix regulators show greater than 35% amino acid sequence identity to CtrA (data not shown). *E. coli* OmpR is only 31% identical to *S. meliloti* CtrA (Fig. 2). The activity of *C. crescentus* CtrA, like OmpR and other response regulators (41), is dependent on phosphorylation at a conserved aspartate residue (Fig. 2) (9). Additional conserved residues are hypothesized to lie within the acidic pocket active site, the phosphorylation site and acidic pocket residues are conserved in all five CtrA proteins (Fig. 2). The three dimensional structure of the C-terminal region of OmpR, including the DNA binding domain, has been determined by X-ray crystallography (21, 28). The OmpR amino acid sequence was used to predict the secondary structures in *S. meliloti* CtrA (Fig. 2). Profile network prediction of secondary structure (PHDsec [35, 36]), with the *S. meliloti* amino acid sequence as input, gave similar results (data not shown). OmpR contains three  $\alpha$  helices and two antiparallel  $\beta$  strands. The  $\alpha$ 3 helix is hypothesized to recognize the major groove, and the two wings may be important for recognition of the minor groove (16). These are nearly identical in the CtrA subfamily (Fig. 2). The  $\alpha$  loop interacts with the  $\alpha$  subunit of RNA polymerase in other OmpR homologs and is well conserved in the CtrA subfamily, even though it is one of the least conserved regions in the OmpR family (29). The least conserved region in the CtrA subfamily is the C terminus of the protein. The amino acid sequence after the  $\beta$ 7 strand shows little conservation.

***S. meliloti ctrA* encodes an essential function.** To determine if *ctrA* is essential for viability in *S. meliloti*, as is the case in *C. crescentus* (32), we disrupted the *ctrA* gene by deleting a 390-bp *Bam*HI fragment by single-crossover, Campbell recombination. This deletion removes the first 112 amino acids of CtrA. Counterselection with the single-crossover strain MB492X was

TABLE 2. Complementation of an *S. meliloti ctrA* mutant

Origin of <i>ctrA</i>	Frequency of Gm <sup>s</sup> colonies	
	MB492X + <i>ctrA</i>	MB492X + vector only
<i>S. meliloti</i>	0.35 ( $n^a = 145$ )	0 ( $n = 162$ )
<i>C. crescentus</i>	0.19 ( $n = 94$ )	0 ( $n = 97$ )

<sup>a</sup>  $n$  is the total number of Nm<sup>r</sup>, a sucrose-resistant colonies screened.





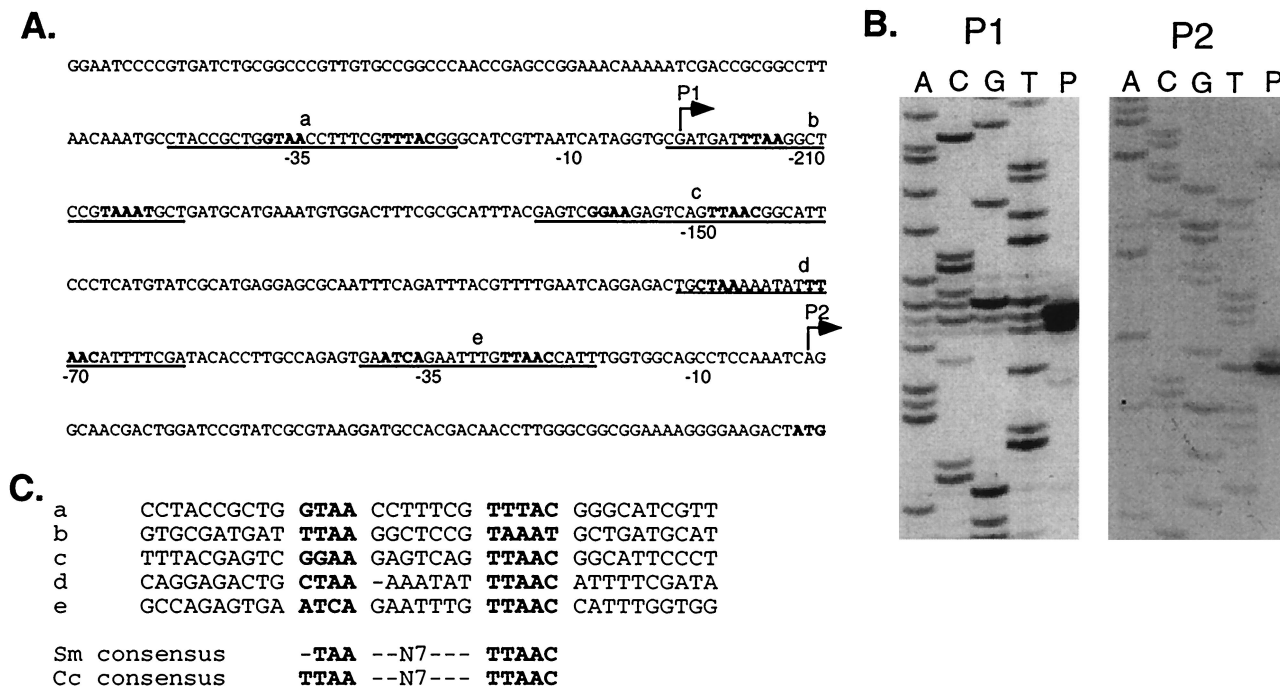


FIG. 3. Identification of the *ctrA* transcription start sites. (A) Sequence upstream of *ctrA*. Locations of the P1 and P2 start sites are shown by bent arrows. The -10 and -35 regions of the two promoters are marked below the sequence. The sequence protected from DNase I digestion by CtrA~P is underlined (see Fig. 4), and the CtrA recognition motifs are in bold. The final ATG is the presumed translational start codon. (B) Autoradiograms of primer extension products (P) and sequencing reactions (labeled A, C, G, and T) for the P1 and P2 start sites of the *S. meliloti ctrA* promoter. The P1 start site was mapped using primer PE-3 (left); the P2 promoter was mapped using primers PE-1 and PE-2. Results obtained with PE-2 are shown (right). (C) Alignment of the five *S. meliloti* CtrA binding sites in the *S. meliloti ctrA* promoter with the *S. meliloti* and *C. crescentus* CtrA consensus sequences (32).

## DISCUSSION

The CcrM methyltransferase is conserved in  $\alpha$ -proteobacteria (42). Here we report the conservation of a second regulatory protein, the CtrA response regulator, in this group of bacteria. We isolated and characterized CtrA, a global regulator of cell cycle progression in *C. crescentus* (32), from *S. meliloti*. A *ctrA* homologue appears to be present in another member of the *Rhizobiaceae*, *A. tumefaciens*. Our data show that by sequence similarity CtrA belongs to a unique subfamily of the OmpR family of helix-turn-helix transcriptional regulators. Thus far, all members of this subfamily belong to the  $\alpha$ -proteobacteria, which are characterized by specialized mechanisms to adapt to unique environments, including mechanisms for pathogenicity and symbiosis (2). Some, particularly the *Rhizobiaceae*, possess large, multipartite genomes (2, 19).

The primary sequence of *S. meliloti* CtrA is very similar to *C. crescentus* CtrA. In addition, antibodies to *C. crescentus* CtrA cross-react with *S. meliloti* CtrA (A. Reisenauer, unpublished data). The sequence conservation among CtrA-like proteins is especially strong for the C-terminal DNA binding domain, suggesting that the DNA target site recognized by these proteins is also conserved. In fact, the promoter region for *S. meliloti ctrA* has five CtrA binding motifs that are nearly identical to the *C. crescentus* consensus motif.

The *S. meliloti* and *C. crescentus ctrA* promoters are strikingly similar. In both species there are two transcriptional start sites, and each promoter (called P1 and P2) contains at least

one CtrA binding motif. In addition, purified and phosphorylated *C. crescentus* CtrA binds the consensus CtrA motifs in vitro in each of the *ctrA* promoters, suggesting that *ctrA* transcription is autoregulated in both species. In fact, CtrA has been shown to repress transcription from P1 and activate transcription from P2 in vivo in *C. crescentus* (10). However, the spacing between P1 and P2 and the number of CtrA binding sites in the *S. meliloti* and *C. crescentus ctrA* promoters are distinct (Fig. 4A). P1 and P2 are separated by 222 bp in *S. meliloti* but only 57 bp in *C. crescentus*. The most distal promoter in *S. meliloti* (P1) is far upstream from the ATG translational start site; however, it is not unusual for *S. meliloti* genes to have long mRNA leader sequences (3, 13).

In the *S. meliloti ctrA* promoter, CtrA~P protects five distinct sites from DNase I digestion; two overlap the -35 regions of P1 and P2, respectively, and there are three additional sites between P1 and P2. Moreover, the P1 transcriptional start site itself is protected from DNase I digestion by CtrA~P. In contrast, CtrA protects only two regions of the *C. crescentus ctrA* promoter, one overlapping the -10 region of P1 and the second overlapping the -35 region of P2 (10). It is tempting to speculate that CtrA molecules bound to the different recognition motifs in each of these promoters act cooperatively to regulate *ctrA* transcription. The CtrA binding sites in the *S. meliloti ctrA* promoter are very similar to the *C. crescentus* CtrA consensus motif. Given the similarity of the C-terminal DNA binding domains and the fact that *C. crescentus* CtrA can sub-

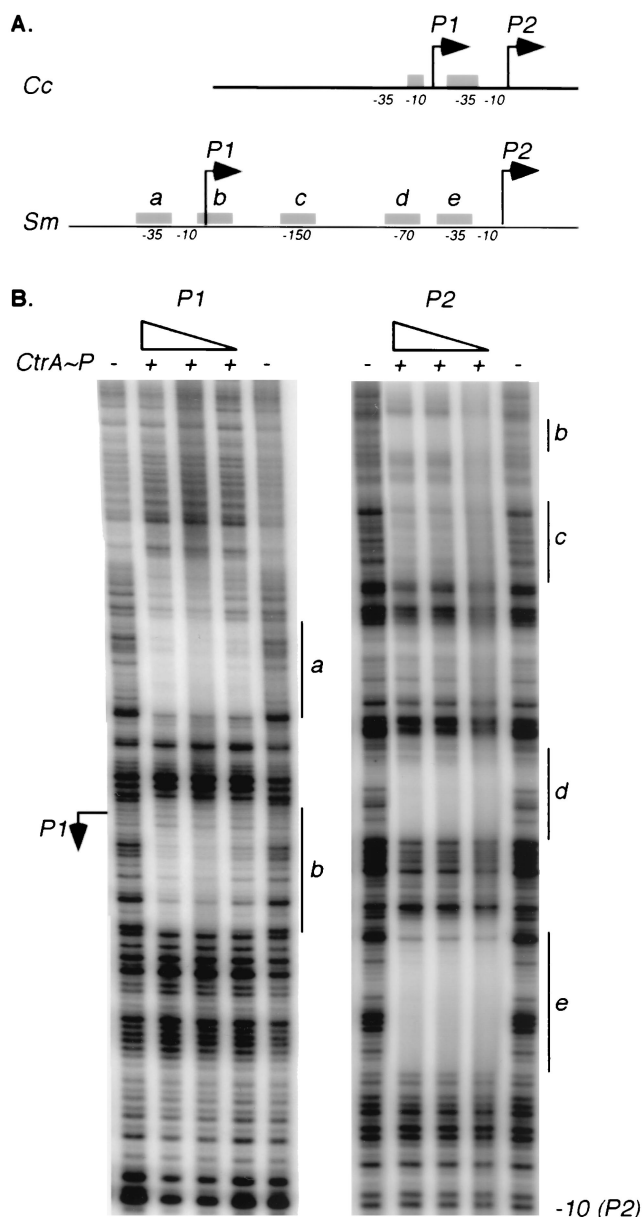


FIG. 4. P CtrA~P binds to five sites in the *ctrA* promoter. (A) Diagram of the *ctrA* promoter regions in *C. crescentus* (top) and *S. meliloti*. Locations of the *ctrA* P1 and P2 transcription start sites are marked by arrows, and the regions protected from DNase I digestion by CtrA~P are shown as gray boxes (labeled a to e to match the footprinted regions in panel B). (B) DNase I protection of the *ctrA* P1 and P2 promoters. CtrA purified from *C. crescentus* was phosphorylated with MBP-EnvZ and used in the footprinting reactions (0.5 to 1.7  $\mu$ M). CtrA~P was omitted from the reactions in the lanes marked with a minus sign. The vertical lines labeled a to e mark the protected regions.

stitute for *S. meliloti* CtrA in vivo, it is likely that CtrA recognizes these same binding sites in both species.

As in *C. crescentus*, *S. meliloti* *ctrA* is essential for viability. In contrast, *ctrA* is not essential in *R. capsulatus* where insertion mutations in *ctrA* were obtained (22). CtrA in *R. capsulatus* is responsible for expression of the gene transfer agent structural

genes. The gene transfer agent is a small phage-like particle that can transfer genes between *R. capsulatus* cells. Possibly, *ctrA* is present in multiple copies in *R. capsulatus* or is not involved in critical cell cycle functions. It is not known if *ctrA* is an essential gene in *B. abortus* or *R. prowazekii*.

Based on sequence similarity, we thought it likely that *C. crescentus* *ctrA* could substitute for *S. meliloti* *ctrA*, as is the case for the conserved *ccrM* in these organisms (42). *C. crescentus* *ctrA*, expressed from its own promoter, complements a *S. meliloti* null mutant for viability. Thus, *S. meliloti* contains the cellular machinery necessary for transcription and phosphorylation of *C. crescentus* CtrA, at least to an extent that supports viability.

Little is known of cell division control in *S. meliloti*, but examination of symbiotic behavior suggests the likelihood of specific controls related to invasion and differentiation. We and others have suggested that bacterial cell division is coordinately controlled to match the expansion rate of the infection thread, in order to keep the infection thread filled but not overrun with bacteria (14, 15). After invasion is complete and bacteria are released into the plant cell cytoplasm, it appears that one or several rounds of bacterial cell division likely occur. Circuitry must exist to halt DNA replication and cell division after this step, as the bacteria differentiate. The essential functions of *S. meliloti* CtrA are not known, but it is an attractive candidate to study in regard to these inferred regulatory steps. In *C. crescentus*, CtrA is known to directly prevent DNA synthesis in the swarmer cell by binding to the origin of replication (33). It will be interesting to determine if perturbation of *ctrA* circuiting in free-living *S. meliloti* cells results in disruption of the invasion process.

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