The manganese-responsive repressor Mur of *Rhizobium leguminosarum* is a member of the Fur-superfamily that recognizes an unusual operator sequence

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The manganese uptake regulator Mur of *Rhizobium leguminosarum* is a close homologue of the global iron regulatory protein Fur. Mur represses the *sitABCD* operon, which encodes a Mn\(^{2+}\) transport system, specifically in response to Mn\(^{2+}\) but not Fe\(^{2+}\). In previous work the authors mapped the 5′ ends of two *sit* operon transcripts, termed TS1 and TS2, which were co-ordinately regulated by Mn\(^{2+}\)-Mur, but this paper now shows that only TS1 is a primary transcript. DNase I protection analyses showed that purified Mur bound, with similar affinity, to two sites in the regulatory region of *sitABCD*, but only when Mn\(^{2+}\) was present in the reaction buffer. These Mn\(^{2+}\)-Mur-binding sites, termed MRS1 and MRS2 (Mur-responsive sequence), were closely related in sequence to each other and were separated by 16 bp, spanning the transcription initiation site TS1. The extent of the protected DNA was 34 and 31 bp for MRS1 and MRS2, respectively, which is in accord with other members of the Fur family. The DNA sequences recognized by Mn\(^{2+}\)-Mur are wholly different from conventional Fur boxes, but some similarities to a recognition sequence for the Fur regulator from *Bradyrhizobium japonicum* were noted. Transcription analysis of the *R. leguminosarum mur* gene showed its expression to be independent of Mn\(^{2+}\)-Mur.

Thus, Mur is a sequence-specific DNA-binding protein that responds in vitro to manganese, and thus can occlude RNA polymerase access to the *sitABCD* promoter. Moreover, Mur recognizes a DNA sequence atypical for the Fur superfamily and, like Fur from *B. japonicum*, defines a new subclass of Fur-like transcriptional regulators.

**INTRODUCTION**

The rhizobia comprise a group of symbiotic \(\varepsilon\)-proteobacteria that can induce N\(_2\)-fixing nodules in the roots of leguminous plants. Recent studies show that these bacteria regulate gene transcription in response to the availability of iron very differently to the model \(\gamma\)-proteobacteria *Escherichia coli* and *Pseudomonas aeruginosa* (Andrews et al., 2003; Johnston, 2004). In particular, the ferric uptake regulator Fur plays a pivotal role in iron homeostasis, repressing the transcription of >100 genes in both *E. coli* and *P. aeruginosa* (Andrews et al., 2003). In the presence of Fe\(^{2+}\), *E. coli* Fur binds to conserved sequences, known as fur boxes, near the promoters of the genes that it represses (Andrews et al., 2003; Hantke, 2001). Since one of the genes (ryhB) that Fur represses in Fe-replete conditions encodes a small, negatively acting regulatory RNA, this accounts for the fact that Fur can appear to be an activator of some genes in *E. coli* (Massé & Gottesman, 2002). Although the structure of the *P. aeruginosa* Fur protein has been determined (Pohl et al., 2003), there is still uncertainty as to the molecular structures involved when Fur, complexed with Fe\(^{2+}\), binds to the fur boxes.

However, a very different protein, called RirA, appears to be the major Fe-responsive transcriptional repressor in at least two rhizobial species, namely *Rhizobium leguminosarum* (Todd et al., 2002, 2005) and *Sinorhizobium melliloti* (Viguier et al., 2005). These two species both have a protein with amino acid sequence similarity to *bona fide* Fur, and in *R. leguminosarum* this protein can complement an *E. coli fur* mutant and it can bind to Fur boxes (Wexler et al., 2003). Nonetheless, in *Rhizobium* and *Sinorhizobium* this homologue appears to be relegated to a minor role involved in the uptake of manganese, not iron (Chao et al., 2004;
Diaz-Mireles et al., 2004; Platero et al., 2004). We have renamed this Fur-like protein Mur, because it regulates the sitABCD operon, which encodes an ABC-type transporter for Mn\textsuperscript{2+}, repressing its transcription under conditions of Mn\textsuperscript{2+} sufficiency. A similar role for Mur of S. mellioti in the regulation of its sitABCD operon was also demonstrated. Furthermore, as noted previously (Wexler et al., 2003), Mesorhizobium loti has no Fur-like protein in its deduced proteome, nor, interestingly, does it have a sitABCD operon.

It had been shown by gel shifts (Diaz-Mireles et al., 2004) that R. leguminosarum Mur can bind to the cis-acting regulatory region of sitABCD, most probably to two versions of a conserved sequence, termed the MRS motif, which differs significantly in sequence from the putative −10 RNA polymerase recognition sequence of sitABCD operon start 1 (TS1) that was mutated from AATAAGT to AAGCTT (generating a HindIII restriction site) using the Stratagene Ex-Site PCR-based site-directed mutagenesis kit and oligonucleotides TS1-HindF (5'-GGTTGCAAAAGGGTCTGCAAAGCATG-3') and TS1-HindR (5'-TTGTGGAATATTAGTAGTGGC-3'). The nucleotide sequences that constitute the restriction site are underlined. The resulting plasmid was named pBIO1528. Similarly, the predicted −10 RNA polymerase recognition sequence of the putative TS2 promoter was mutated from TTTGACT to ATCGCTT (generating a Clai site) using oligonucleotides TS2-ClaiF (5'-ATGAGTTCTCCGGCGCTTTTGCAATTTGGCT-3') and TS2-ClaiR (5'-CAAGTTGGCGGTTACGAAAGTATTGGC-3'). The nucleotide sequences that constitute the restriction site are underlined. The resulting plasmid was named pBIO1526.

The inserts of all the plasmids formed by cloning PCR products were ratified by DNA sequencing.

**METHODS**

**Strains, media and growth conditions.** The R. leguminosarum wild-type strain 3841, whose genome has been sequenced [http://www.sanger.ac.uk/cgi-bin/blast/submitblast/r_leguminosarum](http://www.sanger.ac.uk/cgi-bin/blast/submitblast/r_leguminosarum), was used as the source of genomic template DNA for PCR amplifications. Strain J251 is the immediate parent of the sitABCD operon, nor, interestingly, does it have a sitABCD operon. Furthermore, as noted previously (Wexler et al., 2003), Mesorhizobium loti has no Fur-like protein in its deduced proteome, nor, interestingly, does it have a sitABCD operon.

Here, we identify the R. leguminosarum Mur-binding site, confirming that Mur requires Mn\textsuperscript{2+} to bind in vitro and that its cognate DNA-binding sequences do not resemble those described for conventional Fur.

The Fur-like protein (Fur\textsubscript{Bj}) of Bradyrhizobium japonicum, which is more distantly related to Sinorhizobium and to Rhizobium, also has some unusual characteristics. Although Fur\textsubscript{Bj} regulates another regulatory gene, irr, in response to Fe availability (Hamza et al., 2000), the sequence to which it binds has little or no similarity to that of a conventional Fur box (Friedman & O'Brian, 2003).

In vivo and in vitro genetic manipulations. Plasmid conjugation, isolation and transformation were as described by Wexler et al. (2001). Primer extension analyses of the mur and the sitABCD transcripts were done as described by Sawers & Böck (1989) using 25 μg total RNA isolated from R. leguminosarum J251 or appropriate derivatives using a Qiagen RNeasy kit. In some cases the strain from which the RNA was isolated contained the corresponding cloned gene, in order to amplify the signal. The primers used were 0-2 pmol32P-labelled oligonucleotide Mur-1 (5’-CATGAGGCTGCTCGGCGTACCGACGGAGCTCGGCGGTCGACGAAG-3’) or sit-PE (Diaz-Mireles et al., 2004) (5’-GGTTGACATTGGCCGAGCGGCGG-3’) as appropriate. The 5' end of the mur transcript was precisely located by running in parallel a DNA sequence ladder generated using plasmid pBIO939 (de Luca et al., 1998) as template and labelled Mur-1 oligonucleotide as the sequencing primer. The DNA sequence ladder used with the sit operon transcript was generated using plasmid pBIO1457 (Diaz-Mireles et al., 2004). DNA sequencing employed a T7 DNA sequencing kit as described by the manufacturer (USB).

**DNase I footprinting.** A 318 bp DNA fragment spanning the transcription start site and regulatory sequences of sitABCD was amplified using oligonucleotides sit-PE (see above) and sit-ds (5’-GGTTGGCTTGGAGGTTGGACGG-3’), with pBIO1552 DNA as template. To demonstrate binding of Mur to the coding and anti-coding strands, 50 pmol of either sit-ds or sit-PE, respectively, was labelled with 50 μCi (1850 kBq) [32P]ATP prior to inclusion in the PCR reaction. The reaction conditions were essentially as described by Tucker et al. (2004). A 0.5 μg aliquot of labelled DNA fragment was mixed with 2.5 μl of 10 × binding buffer (100 mM Tris/HCl, pH 8.0, 300 mM KCl), 10 μg bovine serum albumin, 0.01 unit poly(dI-dC) (Roche), purified Mur protein freshly diluted in 1 × binding buffer (Diaz-Mireles et al., 2004) to a final concentration of between 0 and 10 μM, 1 μl 2.5 mM MnCl\textsubscript{2} (where specified), in a final volume of 25 μl. After incubating the mixture for 30 min at 25 °C, 1 μl (0-2 units freshly diluted in 1 × binding buffer and 50 % w/v glycerol) DNase I (Amersham Biosciences) was added and after 15 s the reaction was stopped by adding 100 μl stop solution (50 μg proteinase K ml\textsuperscript{-1}, 20 mM EDTA, 0.5-5 % w/v, SDS, 0.2 mg glycogen ml\textsuperscript{-1}) and 100 μl 2-propanol. The DNA was precipitated at −20 °C for 30 min and after centrifugation and washing with cold 80 % (v/v) ethanol, the air-dried pellet was resuspended in 8 μl formamide dye. Reaction products were separated on a denaturing sequencing gel of 6 % (w/v) acrylamide and 7 M urea. The locations of the Mur-binding sites were determined using radioactively labelled 32P-labeled DNA digested with HindIII.
Other methods. β-Galactosidase enzyme assays on R. leguminosarum cells, grown in minimal Y medium, were done essentially as described by Rossen et al. (1985). Experiments were repeated three times using independent cultures.

RESULTS AND DISCUSSION

Expression of the R. leguminosarum mur gene is not subject to autoregulation

To determine if R. leguminosarum mur is regulated in response to manganese, the expression of the mur–lacZ derivative pBIO939 was examined in R. leguminosarum wild-type strain J251 containing this plasmid, grown in metal-depleted (DP-treated) medium or in medium supplemented with 50 μM MnCl₂. The β-galactosidase activity in the former was 153 (standard error 3) Miller units, and in the latter, 96 (2) units. Thus, mur is expressed at a low level and is slightly reduced by the presence of Mn²⁺. When this mur–lacZ fusion plasmid was present in strain J325, the results were similar in the wild-type [159 (2) units in metal-depleted and 103 (2) in Mn-replete media], showing that mur expression is not autoregulated.

To locate the mur transcriptional initiation site, total RNA was isolated from strain J251 containing pBIO939 after growth in TY medium. Primer extension analysis revealed one major 5’ end at an adenosine, 74 bp 5’ of the mur translation initiation codon (Fig. 1). The 5’ end of a further, very weak transcript was observed at a cytosine 15 bp 5’ of the major initiation site.

The Mur-regulated sitABCD operon is transcribed from a single promoter

Previously, Díaz-Mireles et al. (2004) mapped the 5’ ends of two sitABCD transcripts, termed TS1 and TS2, separated by 57 bp (Fig. 2a). Both transcripts were co-ordinately regulated in response to Mn²⁺ and a functional Mur regulator. However, it was not established if TS1 and TS2 emanated from two separate promoters or if one was derived from the other, particularly since substantial degradation products were also noted (Díaz-Mireles et al., 2004). To distinguish these possibilities, two new sit–lacZ fusion plasmids were made. One of these, pBIO1480, spanned the TS1 site, and the other, pBIO1479, contained the TS2 site, plus 70 bp of 5’ sequence, which did not extend significantly beyond the TS1 site. A third plasmid, pBIO1552, contained both the TS1 and the TS2 regions (see Fig. 2a). Each of these plasmids was mobilized into wild-type R. leguminosarum (strain 251) and into the Mur²⁻ mutant strain J325. The transconjugants were grown in Mn²⁺-replete and Mn²⁺-depleted medium before assaying β-galactosidase activities. As expected, the strain with pBIO1552 (intact sitABCD regulatory region in the fusion), was expressed at high levels in Mn²⁺-depleted medium but was repressed by the addition of Mn²⁺ in the wild-type background, this repression being lost in the Mur⁻ mutant (Table 1). Similarly, in the strains with pBIO1480, the sitA–lacZ fusion was expressed at high levels in the Mn-deficient medium, in both the J251 and the J325 backgrounds and, in the former, the transcription of the fusion was subject to Mn²⁺-dependent repression. Indeed, for reasons that are not clear, the β-galactosidase activities in strains with pBIO1480 were higher than those with the

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**Fig. 1.** Identification of the mur gene transcription initiation site. (a) Lane 1, RNA isolated from wild-type R. leguminosarum J251 containing pBIO939 was analysed by primer extension as described in Methods. The DNA sequence at the transcription initiation site is shown on the left. The major, gene-proximal transcription initiation site is 74 bp upstream of the translation initiation codon of the mur gene. The locations of the transcription initiation sites in the mur gene regulatory sequence are shown as angled arrows. The adenosine residue of the major transcription start site is identified as position +1. A further weak transcription initiation site is located at a cytosine 15 bp upstream of the major site and is depicted by a dotted, angled arrow. The AUG (ATG) translation initiation codon of the mur gene is underlined and italicized and the horizontal arrow signifies the direction of transcription of the mur gene.

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larger insert, in pBIO1552. Thus, the region spanning the TS2 start is dispensable for normal, Mn$^{2+}$-responsive gene regulation of sitABCD (Table 1). In contrast, the fusion plasmid pBIO1479 yielded no detectable enzyme activity either in the wild-type or the mur mutant (Table 1), further demonstrating the importance of the DNA in the TS1 region for the expression and regulation of sitABCD.

It was still possible that TS2 represents a genuine transcript but that DNA further upstream of the 5' end of that cloned

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**Table 1. Effect of promoter mutations on sit–lacZ expression**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>DP</th>
<th>MnCl$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>J251 (wild-type)</td>
<td>pBIO1552 (native promoter)</td>
<td>7439 (80)</td>
<td>185 (9)</td>
</tr>
<tr>
<td></td>
<td>pBIO1480 (spans TS1)</td>
<td>9812 (158)</td>
<td>106 (18)</td>
</tr>
<tr>
<td></td>
<td>pBIO1479 (spans TS2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>pBIO1526 (mutated in −10 of TS2*)</td>
<td>2836 (53)</td>
<td>181 (6)</td>
</tr>
<tr>
<td></td>
<td>pBIO1528 (mutated in −10 of TS1*)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>J325 (Mur−)</td>
<td>pBIO1552</td>
<td>7775 (133)</td>
<td>5065 (15)</td>
</tr>
<tr>
<td></td>
<td>pBIO1480 (spans TS1)</td>
<td>11890 (37)</td>
<td>4597 (75)</td>
</tr>
<tr>
<td></td>
<td>pBIO1479 (spans TS2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>pBIO1526</td>
<td>3204 (100)</td>
<td>1857 (85)</td>
</tr>
<tr>
<td></td>
<td>pBIO1528</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
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in pBIO1479 is needed for its synthesis. We used site-directed mutagenesis of each of the −10 regions that corresponded to the TS1 and TS2 transcripts (Table 1) to examine this possibility.

When the region 10 bps 5′ of the TS2 putative transcript start was mutated (in plasmid pBIO1526, see Table 1) expression of the sit–lacZ fusion was reduced by little more than twofold, with both Mur- and Mn2+-dependent regulation being maintained. The overall twofold reduction in expression of the mutated plasmid relative to the wild-type construct pBIO1552 is possibly attributable to increased instability of the transcript due to the mutation. In contrast, when the −10 sequence of TS1 was mutated (pBIO1528), no expression was detected. Thus, expression of sitABCD depends upon the presence of a functional TS1 promoter. However, sequences downstream of the TS1 promoter, which were mutated in pBIO1526, may also augment sitABCD expression, perhaps by affecting mRNA stability.

Primer extensions verified that inactivation of the TS1 promoter in pBIO1528 abolished transcription in the fusion derivative, whereas mutating the −10 region of TS2 had only minor effects on the transcription patterns (Fig. 2b).

Taken together, these observations indicate that the smaller TS2 transcript results from processing of the longer TS1 transcript or from reverse transcriptase stalling at the GC-rich sequence in the neighbourhood of TS2 in the primer extension experiments.

The Mn2+-Mur complex interacts specifically with two DNA sites in the sitABCD regulatory sequence

In a previous study (Diaz-Mireles et al., 2004), Mur was shown to bind in gel-shift assays to the 1–222 bp and to the 200–504 bp DNA fragments depicted in Fig. 2(a). The DNA sequences around TS1 and TS2 have two putative heptameric inverted repeats, termed MRS1 and MRS2, which were proposed to be recognized by Mur (Diaz-Mireles et al., 2004). We set out to locate the Mur-binding sites precisely by DNase I footprinting. The target was a 318 bp PCR-amplified DNA fragment that extended from position −108 relative to the TS1 transcription initiation and included the first 99 bp of the sitA structural gene (see Fig. 2). Two distinct sites of interaction were seen both on the anti-coding (Fig. 3a) and the coding strand (Fig. 3b), and these sites overlap the previously identified MRS1 and MRS2 motifs. Binding was saturated with 5 μM Mur protein, since increasing Mur to 10 μM did not affect the protection pattern observed (data not shown). Both the MRS1 and MRS2 sites appeared to have similar affinity for Mur, suggesting that the binding to both sites is unlikely to be cooperative.

It is notable that Mur interacted with the DNA if Mn2+ ions were present; but no specific interaction with the coding strand was noted with up to 10 μM Mur when Mn2+ was omitted from the binding reaction (Fig. 3c). However, a single hypersensitive site between the MRS1 and MRS2 sites was noted in these experiments, suggesting interaction of metal-free Mur with this region of the DNA, but not with either MRS1 or MRS2.

The extent of DNA sequence protected from DNase I digestion by Mn2+-Mur was 34 bp for MRS1 and 31 bp for MRS2, suggesting that the 14 kDa Mur protein might bind as a tetramer, which is generally observed for other Fur family members (Pohl et al., 2003; Friedman & O’Brien, 2003, 2004). However, this must be verified biochemically. MRS1 and MRS2 are separated by 16 bp and the almost central location of the transcription initiation site means that when the sitABCD promoter is bound by Mn2+-Mur, binding of RNA polymerase would be prevented (Fig. 4a).

The MRS1 and MRS2 motifs are very similar to each other, with 21 bp being identical (Fig. 4b). This conserved sequence encompasses a perfect heptameric inverted repeat sequence in the case of MRS1 (TGCAATT-N7-AATTGCA) and a similar, but imperfect repeat (TGCAAAT-N7-AATCGCA) for MRS2, as identified previously (Diaz-Mireles et al. 2004). This motif also occurs in the likely regulatory region of the sitABCD operon of S. meliloti (Chao et al., 2004). Interestingly, though, S. meliloti has only one version of the MRS motif, its location being equivalent to that of MRS1 in R. leguminosarum (Diaz-Mireles et al., 2004). Notably, this motif is not found 5′ of the R. leguminosarum mur gene, consistent with the lack of mur autoregulation.

The Fe2+-dependent Fur protein from B. japonicum (FurBj) binds, in vitro, to a 19 bp recognition sequence in the promoter region of the irr gene of this species (Friedman & O’Brien, 2003). This sequence not only differs from the MRS motifs reported here but also is very different from ‘typical’ Fur recognition sequences (Baichoo & Helmann, 2002; Escolar et al., 1999).

The DNA recognition sequence of conventional Fur, for example from E. coli, P. aeruginosa and Bacillus subtilis, is 19 bp, to which two Fur dimers are thought to bind (Baichoo & Helmann, 2002; Fuanthong & Helmann, 2003). The two Fur dimers are proposed to recognize heptameric inverted repeats with a 7-1-7 organization, two of which, offset by 6 bp, occur in the conventional Fur box (see Fig. 4b). The Zur and PerR regulators are also members of the Fur superfamily and are proposed to bind similar 7-1-7 sequences (Fuanthong & Helmann, 2003). The DNA recognition sequences for Fur, Zur and PerR differ from each other in two key base pairs, allowing selective and exclusive binding by the cognate regulator, suggesting that the DNA-recognition helices of at least some members of the Fur superfamily must be highly discriminatory (Fuanthong & Helmann, 2003).

The Mur-binding sequence has some similarity to the FurBj binding site. This is confined to two direct repeat sequences of 6 bp – TTGCAA/C for the R. leguminosarum Mur
recognition sequence and TTGCA/GT/G for Fur$_{Bj}$. Fur$_{Bj}$ and Mur$_{Rl}$ can both bind to canonical E. coli or P. aeruginosa Fur boxes (Friedman & O’Brian, 2003; Wexler et al., 2003) and the cloned Mur of R. leguminosarum can partially correct the regulatory defect of an E. coli Fur$^{-}$ mutant in response to Fe$^{2+}$ but not to Mn$^{2+}$ availability (Wexler et al., 2003; E.D.-M., unpublished observations). However, their normal functions in R. leguminosarum and B. japonicum are substantially different from that of the conventional Fur Fe-responsive transcriptional regulator. In R. leguminosarum, Mur responds to Mn$^{2+}$ and not to Fe$^{2+}$ in its ability to repress the sit$^{ABCD}$ Mn$^{2+}$-uptake operon, the role of the global Fe-responsive regulator having been taken over by the very different RirA protein (Todd et al., 2002, 2005). Furthermore, as shown here, the only known recognition sequence of Mur in R. leguminosarum itself has essentially no similarity to a canonical Fur box. The sequences of Mur in R. leguminosarum, S. meliloti and its homologues in other closely related genera (Agrobacterium, Bradyrhizobium, Brucella, Bartonella and Caulobacter) are closely related to each other and form a clade that is more closely related in sequence to bona fide Fur of E. coli than to the other members of the Fur superfamily, Irr, PerR and Zur. It appears that the progenitor of the fur genes of R. leguminosarum and S. meliloti evolved to respond to a different metal (manganese) and to recognize a different DNA sequence, and is no longer needed for iron homeostasis. In the case of B. japonicum, its Fur still responds to iron but its role may be more limited in terms of the range of genes that it regulates and, certainly, its recognition site, too, is very different from the conventional Fur box, although it has some similarity to the Mur-binding sites of R. leguminosarum. We cannot exclude the possibility that Mur regulates other promoters in R. leguminosarum, perhaps in response to other divalent cations. However, growth of R. leguminosarum in the presence of 10 $\mu$M Fe$^{3+}$, Zn$^{2+}$, Co$^{2+}$, Cu$^{2+}$ or Ni$^{2+}$ did not affect the levels of expression of the sit–lacZ reporter (E.D.-M., unpublished).

Fig. 3. Interaction of Mur with the sit operon regulatory region. In these DNase I protection experiments, different concentrations of purified Mur were incubated in the presence of 100 $\mu$M MnCl$_2$ with the anti-coding (a) and coding (b) strands of the sit$^{ABCD}$ regulatory region and in the absence of MnCl$_2$ with the coding (c) strand. The regions of Mn$^{2+}$-Mur complex interaction with the DNA are delineated by vertical bars and labelled MRS1 and MRS2 (Díaz-Mireles et al., 2004; and see Fig. 4). The regions protected by Mur were determined from analysis of several independent experiments, a typical one of which is shown. The DNA size markers, generated by digesting φX174 DNA with HinfI, are shown in the left lane of each panel. The sizes of the individual markers, in base pairs, are shown at the left of the panels. The final concentrations of Mur in the reactions shown for (a) and (b) were: lane 1, 0 $\mu$M Mur; lane 2, 0.1 $\mu$M; lane 3, 0.5 $\mu$M; lane 4, 1.0 $\mu$M; lane 5, 5 $\mu$M. In (c), Mur was added at 0 $\mu$M (lane 1), 1 $\mu$M (lane 2), 5 $\mu$M (lane 3) and 10 $\mu$M (lane 4). For reference, in (c) the striped bars indicate the corresponding locations of MRS1 and MRS2.
To date there have been few molecular genetic studies on the Fur-like proteins of other α-proteobacteria (Park et al., 2001). It will be of interest to see the extent to which they resemble those of the rhizobia or other lineages of bacteria in their functions. It will also be intriguing to determine, from a structural point of view (Pohl et al., 2003), how these rhizobial regulatory proteins discriminate such different cis-acting sequences compared to those of the much-studied E. coli Fur and, at least in the case of Mur described here, how they recognize different DNA motifs in response to the availability of different transition metals.

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