Autoregulated expression of the gene coding for the leucine-responsive protein, Lrp, a global regulator in Salmonella enterica serovar Typhimurium

Kirsty A. McFarland† and Charles J. Dorman

Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Ireland

In this study, the lrp gene encoding the leucine-responsive regulatory protein (Lrp) in Salmonella enterica serovar Typhimurium was found to be negatively autoregulated. Its transcription start site was determined by primer extension analysis, showing that the lrp promoter is located at a different site to that inferred previously from the S. Typhimurium genome sequence. Chromosomal DNA fragments that include the promoter region were bound by purified Lrp protein in vitro, producing up to four distinct protein–DNA complexes. DNase I footprinting identified regions that were protected by the protein in vitro as well as bases that became hypersensitive to DNase I treatment following Lrp binding. A clear pattern of periodic hypersensitivity was detected between positions −130 and +15 that was consistent with wrapping of the DNA around Lrp in a nucleoprotein complex that includes the putative promoter region. Lrp–DNA interaction in this region was fully consistent with the observed repression of lrp transcription by this protein. Leucine was found to modulate Lrp-mediated autorepression by remodelling the Lrp–DNA nucleoprotein complex.

INTRODUCTION

The Lrp protein is an important global regulator in prokaryotes (Brinkman et al., 2003) and the archaebacteria (Bell & Jackson, 2000; Brinkman et al., 2002). In Salmonella and Escherichia coli, Lrp is a DNA-binding protein with a molecular mass of 18.8 kDa whose activity can be modulated by l-leucine. This amino acid influences the oligomeric state of Lrp, with octamer formation being favoured in its presence (Chen et al., 2001, 2005; Chen & Calvo, 2002; De los Rios & Perona, 2007; Peterson et al., 2007). Lrp can activate or repress gene expression, and depending on the system subject to Lrp regulation, leucine can play a stimulatory, inhibitory or neutral role (Brinkman et al., 2003; Calvo & Matthews, 1994). Lrp activity is not restricted to transcriptional control: it can affect other DNA transactions, such as site-specific recombination (Kelly et al., 2006; Roesch & Blomfield, 1998).

Lrp has roles in Salmonella that are distinct from its contributions in E. coli. These include regulating the expression of the spv virulence genes on the Salmonella virulence plasmid (Marshall et al., 1999), which are important for the establishment of a systemic infection in the host (Libby et al., 2000; Rotger & Casadesús, 1999; Paesold et al., 2002). Lrp is also a regulator of the pef fimbrial genes on the same plasmid (Nicholson & Low, 2000). The mechanism by which Lrp regulates pef genes involves competition with the DNA adenine methylase for access to key 5′-GATC-3′ sites in the pef regulatory region that determine the expression state of the fimbrial operon (Nicholson & Low, 2000). This mechanism is similar to the one that controls expression of the pap fimbrial genes in uropathogenic strains of E. coli (Braaten et al., 1992; Hernday et al., 2002; van der Woude et al., 1992). In addition, Lrp controls the expression of the virulence plasmid tral gene (Camacho & Casadesús, 2005), allowing Lrp to influence the conjugal transfer of this episome (Camacho & Casadesús, 2002). On the Salmonella chromosome, the Lrp protein regulates type 1 fimbrial gene expression through a mechanism that is completely distinct from that used to control type 1 fimbrial genes in E. coli (Kelly et al., 2006; McCusker et al., 2008; McFarland et al., 2008; Roesch & Blomfield, 1998). Instead of influencing the efficiency of a site-specific recombination process that moves the E. coli fim genes between ON and OFF expression states, Lrp in Salmonella enterica controls fim gene expression by governing the supply of a regulatory protein, FimZ (McFarland et al., 2008).
Despite its involvement in many important regulatory processes, the *S. enterica lrp* gene has not been previously characterized. Bioinformatic analysis of the *S. enterica* LT2 genome sequence has suggested locations for the *lrp* transcription start signals; however, the inferred binding sites for RNA polymerase differ from the consensus sequences for promoters used by RNA polymerase holoenzyme containing sigma-70 or any other *Salmonella* sigma factor (McClelland *et al.*, 2001). In this study we cloned the *lrp* gene from the *S. enterica* serovar Typhimurium mouse-virulent strain SL1344 and characterized its regulation at the level of transcription using both in vivo and in vitro methods. The transcription control elements were found to be in a different location from that suggested by the available bioinformatic information. The *S. enterica lrp* gene was found to share several regulatory elements with its well-characterized counterpart in *E. coli*.

**METHODS**

**Growth conditions.** Bacteria were cultured at 37 °C with shaking at 250 r.p.m., either in LB broth or in MOPS minimal broth (0.4 %, w/v, glucose, 0.5 mM histidine) which was supplemented with 10 mM L-leucine when required. Overnight cultures of bacteria in 3 ml LB broth were either inoculated by 1:100 dilution into 25 ml fresh LB broth, or washed twice with warmed MOPS minimal broth before inoculation (1:50) to 25 ml MOPS minimal broth. Optical densities of bacterial suspensions were measured at 600 nm and equal numbers of bacteria were used for inoculation.

**Strains, plasmids and oligonucleotides.** The strains and plasmids are listed in Table 1 and the oligonucleotides used in this study are described in Table 2. Plasmid pZEP*lp* was produced by PCR amplification of a 612 bp fragment, including 61 bp of the *lp* ORF and 533 bp of the *lrp* regulatory region of SL1344, using primer pair *lp*-gfp-F and *lp*-gfp-R (Table 1). This fragment and the parental vector pZEP08 were each digested with *Smal* and *XbaI*, purified and ligated. The plasmid pBSK-*lp* was used for DNase I footprinting and associated DNA sequencing reactions. It was generated by PCR of SL1344 DNA with primer pair *lp*-F.*BamHI* and *lrp*-R.*EcoRI*, followed by cloning into the multi-cloning site of PBS *EcoRI* (−). Plasmid pKMC101 was used to determine the *lp* transcription start site by primer extension analysis. This plasmid was produced by PCR amplification of the *lrp* gene and its flanking regions using primer pair Fwd1.3-*EcoRI* and Rev1.3-*PstI* followed by restriction enzyme digestion and ligation of the resulting DNA fragment into the *EcoRI* and *PstI* sites in pCL1921.

**Flow cytometry.** Bacterial samples were fixed by dilution to approximately 10⁶ cells ml⁻¹ in 300 μl PBS containing 3 % (v/v) formaldehyde. The samples were examined for GFP expression using the EPICS-XL flow cytometer (Beckman Coulter). Assays were performed in triplicate and mean values are reported.

**Electrophoretic mobility shift assay (EMSA).** DNA probes were amplified using biotinylated primers to the region of interest (Table 2) and were subsequently purified by gel electrophoresis. Approximately 100 pg biotinylated DNA probe was incubated with binding buffer for 5 min at room temperature as described by Camacho & Casadesús (2002). Increasing concentrations of Lrp protein were added, followed by incubation for 20 min at room temperature. Protein–DNA complexes were formed in a reaction volume of 20 μl, including leucine where applicable. A 10 μl aliquot of the protein–DNA mixture, plus loading dye, was subjected to electrophoresis at 100 V on Novex 6 % DNA Retardation gels (Invitrogen) at room temperature. Gels were transferred onto membrane using the Novex XCell II blot module (Invitrogen), UV-cross-linked, and developed using the Chemiluminescent Nucleic Acid Detection Module (Pierce).

**DNase I footprinting.** DNase I footprinting was carried out as previously described (McFarland *et al.*, 2008). The probes used for DNase I footprinting were generated by PCR using primer pairs *lrp*-F.*BamHI* and *lp*-R.*EcoRI* (Table 2) with pBSK-*lp* as the DNA template. The sequences complementary to the T7 oligonucleotides are located externally to the multi-cloning site of PBS *EcoRI* (−). T4 polynucleotide kinase was used to label the DNA probes at both ends with [γ-³²P]ATP, which were then digested to remove the label from one end, depending on the strand to be analysed. Lrp protein–DNA binding reactions used for DNase I footprinting were identical to those used for EMSA analysis, except that these reactions were performed in 50 μl volumes. DNA sequencing reactions using dideoxy chain-terminators were performed as previously described.

Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Source</th>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL1344</td>
<td>rpsL, hisG</td>
<td>Hoiseth &amp; Stocker (1981)</td>
</tr>
<tr>
<td>CJD3130</td>
<td>SL1344 <em>lp::kan</em></td>
<td>McFarland <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>CJD3132</td>
<td>SL1344 pZEP<em>lp</em></td>
<td>This study</td>
</tr>
<tr>
<td>CJD3133</td>
<td>CJD3130 pZEP<em>lp</em></td>
<td>This study</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
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<tr>
<td>pBluescript II SK (−)</td>
<td>Cloning vector, Amp⁸</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pBSK-<em>lp</em></td>
<td>418 bp <em>lrp</em> promoter in pBluescript II SK</td>
<td>This study</td>
</tr>
<tr>
<td>pZEP<em>lp</em></td>
<td>612 bp <em>lrp</em> promoter region upstream of promoterless <em>gfp</em> in pZEP08</td>
<td>This study</td>
</tr>
<tr>
<td>pCL1921</td>
<td>pSC101 replicon, Spc⁸ Str⁸</td>
<td>Lerner &amp; Inouye (1990)</td>
</tr>
<tr>
<td>pKMC101</td>
<td>1.3 kb <em>lrp</em> gene and flanking regions cloned into pCL1921</td>
<td>This study</td>
</tr>
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**Table 2. Oligonucleotides**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’–3’)</th>
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<tr>
<td>Fwd1.3-EcoRI</td>
<td>ATAGAATTCCGTGTGTTGCGGACACAATGAGACAGAATTGTAGG</td>
</tr>
<tr>
<td>Rev1.3-PstI</td>
<td>ATACCTGGGAGTGGTGGCCGAGAACATG</td>
</tr>
<tr>
<td>lrp-gfp-F</td>
<td>CGTCTGATAGAATGTTACGATCG</td>
</tr>
<tr>
<td>lrp-gfp-R</td>
<td>GTTCATCGGTTCATGCTATTAC</td>
</tr>
<tr>
<td>PE-lrp</td>
<td>ATCAGATGTTTTGCTTTGAC</td>
</tr>
<tr>
<td>lrp-EMSA-F</td>
<td>ATCGATGTTTTGCTTTGAC</td>
</tr>
<tr>
<td>lrp-EMSA-F3</td>
<td>CCCGGG,</td>
</tr>
<tr>
<td>lrp-EMSA-F4</td>
<td>ATCGATGTTTTGCTTTGAC</td>
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<tr>
<td>lrp-EMSA-F5</td>
<td>GACAGCCACGTATCATCAC</td>
</tr>
<tr>
<td>lrp-R.Bio</td>
<td>GCCCTGTCATGCTGTGAGAAG</td>
</tr>
<tr>
<td>lrp-EMSA-R3</td>
<td>GTTCTACCGTTACATGCTATTAC</td>
</tr>
<tr>
<td>lrp-EMSA-R4</td>
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</tr>
<tr>
<td>lrp-EMSA-R5Bio</td>
<td>TACATGCGATTATGAGC</td>
</tr>
</tbody>
</table>

*Restriction enzyme sequences: GAATTC, EcoRI; CTGCAG, PstI; CCCGGG, SnaI; TCTAGA, XbaI; GGATCC, BamHI.

(McFarland et al., 2008), using the T4 DNA sequencing kit (USB) and appropriate sequencing primers. The products of the DNase I footprinting and the DNA sequencing reactions were resolved by electrophoresis through a 6% urea-polyacrylamide gel.

**Primer extension analysis.** RNA was extracted from SL1344 that had been grown to OD<sub>600</sub> 3.5 in LB broth (approx. 5.5 h growth), and to OD<sub>600</sub> 0.5 in MOPS minimal broth, using the SV Total RNA isolation kit (Promega). The oligonucleotide PE-lrp (Table 2) is complementary to 32 bp of the <i>lrp</i> mRNA, 58 nt into the ORF. PE-lrp was labelled with [32P]ATP using T4 phosphonucleotide kinase (New England Biolabs) according to the manufacturer’s instructions. The SuperScript III Reverse Transcriptase kit (Invitrogen) was used to perform the primer extension reaction; the product was then purified by ethanol precipitation, resuspended in formamide loading dye, and heated to 95 °C for 10 min. Samples were analysed by electrophoresis on denaturing 6% polyacrylamide gels alongside sequencing reactions that were generated using primer PE-lrp and pKMC101 as template.

**RESULTS**

**Loss of Lrp expression has a modest negative effect on the growth of strain SL1344**

The creation of the <i>lrp</i> knockout mutant of <i>S. Typhimurium</i> strain SL1344 has been described previously (McFarland et al., 2008). It was important to assess the impact of the <i>lrp</i> mutation on the physiology of the bacterium. For this reason, the growth of the <i>lrp</i> knockout mutant was compared with the wild-type in the complex medium LB and in MOPS minimal medium (with and without leucine) over a 24 h period. In LB, the growth curves of the wild-type and mutant were essentially superimposable, although the wild-type had a slightly shorter doubling time (28 min) than the <i>lrp</i> mutant (30 min). In MOPS minimal medium, the mutant had a doubling time that was ~30% longer than that of the wild-type during the exponential phase of growth; the two strains achieved approximately similar culture densities in stationary phase. This pattern was mildly affected by the addition of leucine; there was some reduction in the growth of the wild-type in exponential phase and the <i>lrp</i> mutant had a shortened lag phase. These data implied an important role for Lrp in the physiology of <i>S. Typhimurium</i> growing in minimal medium. They also showed that, in contrast to the situation in <i>E. coli</i> (Boulou et al., 1992), leucine did not abolish the growth of the bacteria in minimal medium. The reason for this is not known.

**Negative autoregulation of <i>lrp</i> transcription**

The putative <i>lrp</i> transcription signals were cloned into the plasmid pZEP08 to create an <i>lrp-gfp</i> transcriptional fusion (Methods). This plasmid, pZEP<sub>lrp</sub>, allowed the green fluorescent protein to be used as a reporter of <i>lrp</i> promoter activity. The plasmid was introduced into the SL1344 wild-type and the SL1344 <i>lrp</i> knockout mutant. This allowed the effect of the <i>lrp</i> mutation on the activity of the <i>lrp</i> promoter to be measured. Strains SL1344 and SL1344 <i>lrp</i> grew with similar growth curves in LB when harbouring the pZEP<sub>lrp</sub> plasmid (Fig. 1a). In MOPS minimal medium, the <i>lrp</i> mutant grew more slowly than the wild-type and the presence of leucine had a minor negative influence on growth (Fig. 2a).

The absence of the Lrp protein resulted in increased <i>lrp</i> promoter activity in cultures growing in LB (Fig. 1b) or in minimal MOPS (Fig. 2b). When leucine was added to the MOPS culture, the level of <i>lrp-gfp</i> expression increased by between 1.5- and 2-fold, provided the Lrp protein was present; leucine did not have this effect in the <i>lrp</i> knockout mutant (Fig. 2b). These data showed that Lrp repressed the transcription of the <i>lrp</i> gene and that this negative effect was partially alleviated by the presence of leucine.
Identifying the \( lrp \) transcription start site

It was necessary to identify the transcription start site of the \( S. \) Typhimurium \( lrp \) gene by molecular methods. Previous estimates based on bioinformatic techniques suggested that the \( lrp \) promoter had several non-standard features, in particular very poor matches to the \(-35 \) and \(-10 \) hexanucleotide motifs that are bound by the RpoD sigma factor of RNA polymerase (McClelland et al., 2001). If these were confirmed, they would have to be taken into account in assembling a model of \( lrp \) gene regulation.

The transcription start site was determined using a standard primer extension procedure (Methods) and mapped to a cluster of A bases located 257 bp upstream from the translation start codon of the \( lrp \) ORF (Fig. 3). The start site was 10 bases further downstream of the position described previously for the \( lrp \) gene in \( E. \) coli K-12 (Wang et al., 1994). Moreover, the likely binding sites for RNA polymerase were poor matches to the standard features associated with sigma-70 promoters, in keeping with the weak nature of the promoter. The \(-35 \) hexamer matched the consensus sequence in three out of six positions and the \(-10 \) had only two matches out of six to the consensus (Fig. 3). These data showed that the \( S. \) Typhimurium \( lrp \) transcription start site determined previously using \textit{in silico} methods was likely to be incorrect.

The primer extension experiment was performed using mRNA extracted from two SL1344 cultures, one grown in LB and one grown in MOPS minimal medium. The intensity of the extension product showed that the gene was transcribed to a higher level in the culture growing in the minimal medium (Fig. 3), in agreement with the data obtained using the \( lrp-gfp \) reporter fusion (Figs 2b and 3b).

Purified Lrp binds to the \( lrp \) promoter region

Purified Lrp protein (McFarland et al., 2008) was used in a series of EMSAs to assess Lrp interaction with the transcriptional regulatory region of the SL1344 \( lrp \) gene (Fig. 4). Experiments were performed initially with a DNA fragment that extended from 115 bp upstream of the translation initiation codon to 418 bp further upstream, spanning from \(-275 \) to \(+143 \) with respect to the transcription start site (Fig. 4a). The labelled DNA fragment was incubated with Lrp protein at a range of concentrations, and parallel incubations were performed in the presence of leucine or glycine. Two Lrp–DNA complexes were formed in all three cases (Fig. 4b). Leucine encouraged the formation of the slowest-migrating Lrp–DNA complex at the expense of the faster-migrating one; glycine did not have this effect. These results showed that Lrp could bind to the \( lrp \) regulatory region in the absence of amino acids and that leucine could modulate this process.

The 418 bp DNA fragment was subdivided into six shorter sections that were numbered according to length. F probes had a common end that was distal to the \( lrp \) open reading frame while R probes had a common end that was proximal to it; the fragments were numbered according to their lengths in bp (Fig. 4a). The six DNA fragments, F102, F200, F311, R111, R202 and R310, were used in EMSAs with purified Lrp protein. Only F311 and R310 formed complexes with the protein (Fig. 4c). These two fragments
shared a common 203 bp stretch of DNA that included the \( lrp \) promoter. This region of DNA was amplified and used in a further EMSA with Lrp. Three Lrp–DNA complexes were detected, with a fourth being visible at the highest concentration of protein (Fig. 4d). The formation of the slowest-migrating Lrp–DNA complexes was unaffected by the presence of leucine or glycine.

Taken together, the data from the EMSAs showed that Lrp binds to DNA sequences located between positions –167 and +36, numbered with respect to the transcription start site (+1) of the \( S. \) Typhimurium \( lrp \) gene. DNase I footprinting was used to examine Lrp–DNA interaction in this region in more detail. The 418 bp DNA fragment (Fig. 4a, b) was labelled at one end with \( {}^{32} \)P, incubated with increasing concentrations of Lrp protein, and then treated with DNase I. The samples were then separated by electrophoresis on a DNA sequencing gel (Fig. 5). Increasing concentrations of Lrp protein were accompanied by a pattern of protection from, and periodic hypersensitivity to, digestion by DNase I on both the coding (Fig. 5a) and the non-coding DNA strands (Fig. 5b) over a region that extended from –135 to +15. The region of protection included the \( lrp \) promoter, a finding that was consistent with its repression by the Lrp protein. There was an abrupt transition between DNase I sensitivity and protection and vice versa between 50 and 100 nM Lrp. The pattern of protection and hypersensitivity to DNase I was subtly altered by the presence and absence of 15 mM leucine. Addition of the amino acid was accompanied by hypersensitivity to DNase I in the promoter region (–40 to +1) on the coding and non-coding strands, a region that was protected by Lrp in the absence of leucine (Fig. 5a, b). Leucine also reversed the sensitivity of bases to DNase I in the regions –90 to –100 and –120 to –130 (Fig. 5a). Several matches to the degenerate consensus sequence for Lrp binding sites (Wang et al., 1994) were identified in the region between –110 and +15; these are summarized in Fig. 6. In several cases, these sequences were flanked by ‘T’ residues that had shown hypersensitivity to DNase I digestion in the presence of Lrp.

**DISCUSSION**

The knockout mutation in the \( lrp \) gene of \( S. \) Typhimurium SL1344 altered the growth rate of the bacterium in MOPS minimal medium. The wild-type had a doubling time of 58 min while the \( lrp \) mutant doubled every 76 min. These growth rates were very similar to those reported previously for \( E. \) coli (Tuan et al., 1990).

The \( lrp \) promoter in \( E. \) coli has been described as leucine-sensitive (Borst et al., 1996) and leucine-insensitive (Lin et al., 1992; Wang et al., 1994). In our study, the \( lrp \) promoter in \( S. \) Typhimurium consistently displayed between a 1.5- and 2-fold higher activity in the presence of leucine in bacteria growing in minimal MOPS medium (Fig. 1). This modest positive effect of leucine on \( lrp \) transcription was contingent on the presence of the Lrp protein; the \( lrp \) knockout mutant expressed the \( lrp-gfp \) fusion to the same level in the presence or the absence of leucine (Fig. 1). We noted a dip in \( lrp \) transcription at the end of exponential growth (Fig. 1b). While we cannot explain this in mechanistic terms, a similar effect was noted previously in the case of the \( E. \) coli \( lrp \) gene (Landgraf et al., 1996).

EMSAs showed that Lrp binds to a 418 bp DNA fragment and that this binding is not abrogated by the addition of leucine (Fig. 4). It has been shown previously that leucine does not alter the binding of Lrp to the regulatory region of the \( E. \) coli \( lrp \) gene (Wang et al., 1994). However, in the case of the \( S. \) Typhimurium gene, leucine (but not glycine)
alters the relative abundances of the different Lrp–DNA complexes in favour of the slowest-migrating complex.

This suggests that leucine changes the nature of the Lrp–DNA complex without displacing the protein. The details of this alteration to the nucleoprotein complex are unknown but are likely to be subtle given the very modest effect of leucine on lrp promoter activity.

The 418 bp fragment encompasses a 203 bp subfragment that binds Lrp, forming up to four Lrp–DNA complexes in EMSAs (Fig. 4). The number and relative abundances of these complexes do not respond to leucine or glycine. This shows that the subtle leucine-sensitivity of the Lrp–DNA complexes formed with the 418 bp fragment involves DNA sequences not found in this smaller piece of DNA, perhaps...
due to a requirement for long-range protein–DNA interactions that can only occur on the longest DNA fragments used.

DNase I footprinting reveals Lrp-mediated protection and distortion of the DNA structure between approximately positions –130 and +15 (Fig. 5). Protection is seen most clearly in the absence of leucine on the coding (Fig. 5a, lanes 1 to 5) and non-coding strands (Fig. 5b, lanes 1 to 5). Adding leucine has a subtle effect on the patterns of protection and hypersensitivity. It reduces the overall degree of protection afforded by Lrp and it modifies the pattern of DNase I hypersensitivity. In particular, the protection seen between bases –40 and +1 on the coding strand is replaced by a pattern of periodic hypersensitivity (Fig. 5a). This pattern is also detectable on the non-coding strand (Fig. 5b). New regions of hypersensitivity are also seen further upstream on the coding strand, between bases –95 and –130 (Fig. 5a).

The markedly periodic pattern of hypersensitivity to DNase I digestion is consistent with wrapping of the DNA around the Lrp protein, resulting in enhanced exposure of certain DNA sequences that bind Lrp (McFarland et al., 2008; Wagner & Calvo, 1993; Wiese et al., 1997). The 3D structure of the E. coli Lrp protein has been solved (de los Rios & Perona, 2007). This protein, which is almost identical to Lrp in S.
Typhimurium, can form octamers capable of wrapping approximately 120 bp of DNA, a stretch that is similar in length to that shown here to be affected by Lrp. In the Lrp octamer, the eight helix–turn–helix (H-T-H) DNA-binding motifs located in the amino-terminal domains of each monomer are arranged around the circumference of the octameric structure. This allows the protein to dock with Lrp binding sites distributed along the DNA with a spacing of approximately three helical turns, or 30 bp. The fit of each H-T-H motif in the DNA requires adjustment to the Lrp protein structure, suggesting that an induced-fit mechanism operates. It is possible that the binding of leucine to the Regulation of Amino acid Metabolism (RAM) domain in the carboxyl terminus of each Lrp monomer may provide the structural adjustment required to strengthen or to weaken Lrp–DNA interaction, depending on the gene regulatory sequence.

Data from the present study show that Lrp can form up to four complexes with the S. Typhimurium lrp promoter region, with one being located downstream of the transcription start site (Fig. 4). DNase I protection data suggest that Lrp binding occurs as far downstream as base +15, and a leucine-sensitive and DNase-I-hypersensitive base is located at position +35. This pattern of protein–DNA interaction is consistent with repression of the lrp promoter. Possible matches to the (degenerate) consensus sequence for Lrp-binding sites are underlined by hatched boxes. The coordinates of bases in the sequence are numbered with respect to the lrp transcription start site (+1). The T residues that showed hypersensitivity to DNase I digestion in the presence of Lrp are in bold type. Downward arrowheads show the bases that were hypersensitive on the coding strand; upward arrowheads show the bases that were hypersensitive on the non-coding strand. Possible −10 and −35 hexameric promoter motifs are boxed.

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REFERENCES


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