JOURNAL OF BACTERIOLOGY, Aug. 2011, p. 3794–3803 Vol. 193, No. 15
0021-9193/11/$12.00 doi:10.1128/JB.00191-11

Recognition of DNA by the Helix-Turn-Helix Global Regulatory Protein Lrp Is Modulated by the Amino Terminus▽†

Benjamin R. Hart,[1] Pankaj K. Mishra,[1]‡ Robert E. Lintner,[1]§ Jennifer M. Hinerman,[2] Andrew B. Herr,[2] and Robert M. Blumenthal[1,3]*

Department of Medical Microbiology and Immunology and Program in Infection, Immunity, and Transplantation, University of Toledo College of Medicine, Toledo, Ohio 48104[1]; Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267[2]; and Program in Bioinformatics and Proteomics/Genomics, University of Toledo, Toledo, Ohio 48104[3]

Received 10 February 2011/Accepted 19 May 2011

The AsnC/Lrp family of regulatory proteins links bacterial and archaeal transcription patterns to metabolism. In *Escherichia coli*, Lrp regulates approximately 400 genes, over 200 of them directly. In earlier studies, *lrp* genes from *Vibrio cholerae*, *Proteus mirabilis*, and *E. coli* were introduced into the same *E. coli* background and yielded overlapping but significantly different regulons. These differences were seen despite amino acid sequence identities of 92% (*Vibrio*) and 98% (*Proteus*) to *E. coli* Lrp, including complete conservation of the helix-turn-helix motifs. The N-terminal region contains many of the sequence differences among these Lrp orthologs, which led us to investigate its role in Lrp function. Through the generation of hybrid proteins, we found that the N-terminal diversity is responsible for some of the differences between orthologs in terms of DNA binding (as revealed by mobility shift assays) and multimerization (as revealed by gel filtration, dynamic light scattering, and analytical ultracentrifugation). These observations indicate that the N-terminal tail plays a significant role in modulating Lrp function, similar to what is seen for a number of other regulatory proteins.

A key question in bioinformatics and molecular biology is the extent to which the degree of sequence conservation between proteins predicts conservation of their function. This is a highly nonlinear relationship, as some residues play key structural or functional roles while others matter little or not at all. As an example, two proteins that both act as *S*-adenosyl-L-methionine-dependent methyltransferases can have less than 10% identity between equivalent positions (28), while it is possible for two engineered proteins to be 88% identical yet have distinct structures and ligand specificities (1).

With the flood of bacterial genome sequences becoming available, including some from metagenomic analyses of bacteria that cannot yet be grown in the laboratory (15, 24), there is great interest in predicting the regulatory architecture of these organisms from their DNA sequences. This is also relevant to the emerging field of synthetic biology, where whole bacterial genomes are being designed (29), because regulatory networks add complexity and increased robustness to a genome (43). Predicting regulation generally involves inference from well-studied bacteria, based on the assumption that a conserved transcription factor, a conserved target gene for the regulator, and a predicted binding site for the regulator upstream of the target gene together indicate conserved regulation (27, 41, 54). However, transcription factors appear more likely than most proteins to exhibit significant functional diversification, even over short evolutionary distances (53), perhaps reflecting the need of bacteria for rapid adaptation of regulatory architecture to new niches (40, 43). It is important to understand the basis, as well as the implications, of this flexibility.

The global regulator Lrp (leucine-responsive regulatory protein) directly controls the expression of over 200 *Escherichia coli* genes and affects the expression of many more indirectly (8, 10, 17, 20, 46, 63). Lrp almost certainly binds regulatory DNA via a helix-turn-helix (HTH) motif (Fig. 1A), as alterations to this motif strongly influence Lrp-DNA binding (21, 38, 51). The results from X-ray diffraction of Lrp, crystallized in the presence of target DNA, are consistent with a central role for its HTH in DNA sequence recognition (21). Lrp orthologs from genera across the *Enterobacteriaceae* and *Vibrionaceae* bear a perfectly conserved HTH motif (Fig. 1A). We were therefore surprised to find, in a previous study, that Lrp proteins from *Proteus mirabilis* (98% identical to *E. coli* Lrp) and *Vibrio cholerae* (92%) have significantly different regulatory effects when introduced into the same *E. coli* background (39).

Several lines of evidence led us to suspect that the N-terminal region of Lrp (Fig. 1A) might be responsible for some of the functional differences we observed. First, of the four amino acid differences between *P. mirabilis* Lrp and *E. coli* Lrp, two are located within the N-terminal 13 amino acids (in the case of *V. cholerae* Lrp, this was 3 out of 13 total differences). The logo in Fig. 1A shows that, while the N-terminal 13 residues include strongly conserved positions, there is also substantial

* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, University of Toledo College of Medicine, 3000 Arlington Avenue, Toledo, OH 43614-1021. Phone: (419) 383-5422. Fax: (419) 383-3002. E-mail: Robert.Blumenthal@utoledo.edu.

‡ Present address: Department of Medicine, Center for Immunity and Inflammation, UMDNJ-New Jersey Medical School, Newark, NJ 07101.

§ Present address: Broad Institute of MIT and Harvard University, Cambridge, MA 021423.

† Supplemental material for this article may be found at http://jb.asm.org/.

▽ Published ahead of print on 3 June 2011.

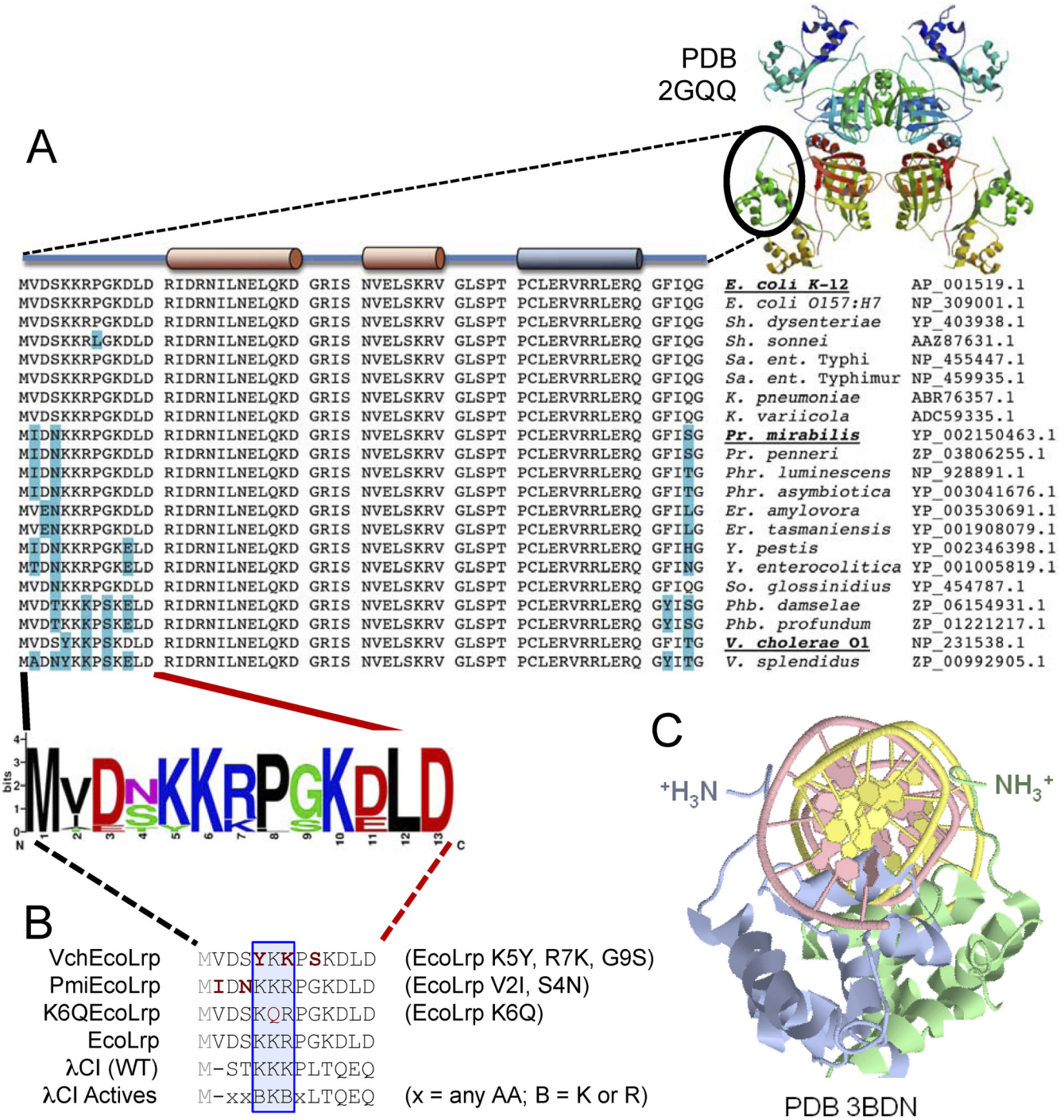

FIG. 1. Amino-terminal region of Lrp. (A) Structure of the EcoLrp octamer. The structure (21) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). The open conformation of the octameric ring presented is seen when the protein is crystallized in the presence of DNA. One of the DNA binding HTH motifs is circled, along with an N-terminal tail (unstructured; shown as straight line). The sequence alignments below show the N-terminal half of the protein, with secondary structure indicated above the sequence. The recognition helix of the HTH motif is highlighted in blue. The variable N-terminal region is expanded into a sequence logo (lower left). GenBank accession numbers are given for each sequence. (B) Amino acid sequence alignment of the N termini of the Lrp alleles used in this study. The alignment also shows the similarity to the N terminus of the CI activator/repressor from λ phage. The "actives" are substitutions in this region of λ CI that retained the ability to maintain lysogeny (18, 23, 36). Letters in red are Lrp residues differing from EcoLrp. The blue rectangle highlights a triplet of basic amino acids shared among λ CI and many Lrp proteins. (C) Crystal structure of the λ CI activator/repressor bound to DNA, showing its N-terminal tails making major groove contacts (also from Protein Data Bank) (18).

sequence variation. Second, when the 10 most N-terminal codons were deleted from the gene for *E. coli* Lrp, DNA binding activity was very substantially reduced (21). Finally the N-terminal portion of Lrp has striking sequence similarity to that of the phage λ repressor/activator CI (Fig. 1B). The N-terminal 6-amino-acid arm of CI is required for proper DNA binding by this HTH protein; deleting the arm reduces operator binding by ~8,000-fold, and there is structural evidence for its role (Fig. 1C) (5, 23, 36, 47).

We report here that, as previously demonstrated for phage λ CI, the N-terminal arm of Lrp also affects its specificity and function. We discuss the implications of these observations for global regulation and for sequence-based regulatory prediction.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Most bacterial strains used in this study were based on the *lrp35*::Tn*10 E. coli* BE10.2 background (42), with pCC1-BAC-based plasmids carrying genes for EcoLrp (*E. coli* Lrp), PmiLrp (*P. mirabilis* Lrp), or VchLrp (*V. cholerae* Lrp) (39). We also prepared and tested hybrid Lrp proteins, where the substitutions changed the N-terminal tail of EcoLrp to that of PmiLrp or VchLrp. In addition, we generated a K6QLrp mutant, with Lys replaced by Gln at the sixth position (Fig. 1B). The *lacZ* transcriptional reporter fusions to Lrp-responsive promoters were in some cases chromosomal (P*gltB*, *E. coli* strain BE3779 [26]) and in others were carried on derivatives of pBH403 (37), a plasmid based on pKK232-8 (9): P*gltB* (pPM2005 [39]), P*livK* (pRHLiv2 [33]), P*lrp* (pPM3001 [39]), and P*ompT* (pRH104 [this work]).

In all cases, cells were grown in baffled flasks shaken at 37°C. For LacZ assays, cells were grown on LB plates and then transferred to M9-glucose plates before being moved to morpholinopropane sulfonic acid (MOPS) glucose minimal medium (Teknova, Hollister, CA), as previously described (39). For P*lrp-lacZ* analyses, additional amino acids and supplements were used at the following final concentrations (where listed): 10 mM L-alanine, 10 mM L-leucine, 0.4 mM L-isoleucine, 0.4 mM L-valine, and 0.01 mM thiamine. (For comparison, LB medium contains 5.8 mM Ala, 8.8 mM Leu, 5.4 mM Ile, and 7.0 mM Val [56].) Antibiotics were used, where indicated, as follows: 100 μg ampicillin/ml, 15 μg chloramphenicol/ml, 100 μg kanamycin/ml, and 10 μg tetracycline/ml. Cells were maintained in log phase for at least 10 generations before being diluted 1:50 for experimental cultures. For protein purification, cells were grown in STG medium (42).

β-Galactosidase assays. Strains were grown to exponential phase in glucose minimal MOPS medium, supplemented as indicated. The assays were carried out as previously described (39, 52). Briefly, overnight cultures were diluted in fresh medium. Experimental cultures were then grown with shaking at 37°C. The optical density at 600 nm (OD_{600}) was measured every 25 min, with samples collected at each interval when the OD_{600} was in the 0.1 to 0.8 range. The samples were lysed by vortex mixing for 30 s in the presence of 50 μl chloroform and 25 μl 10% (wt/vol) SDS and kept on ice until all samples had been taken. Levels of β-galactosidase were determined by *o*-nitrophenyl-D-galactoside (ONPG) hydrolysis. The rate of ONPG hydrolysis was plotted against the culture OD and fitted by linear regression. The resulting slope yields the β-galactosidase activity. When plotting against culture absorbance, this term is excluded from the calculation of β-galactosidase activity, which is thus expressed in "modified Miller units." For the original unit description, see reference 44.

Generation of hybrid Lrp. Lrp hybrids, and the K6Q mutant, were generated using the Quick-Change method (Epicentre, Madison, WI). All altered *lrp* genes were sequence confirmed over their full lengths.

Purification of Lrp. Native Lrp protein was purified essentially as previously reported (42). For EcoLrp, JWD3-1 cells were used (26); for PmiLrp, we used BE10.2 cells containing the gene for PmiLrp inserted into expression vector pTRC99a (2), and the gene for PmiEcoLrp was inserted into expression vector pBAD24 (30) and transformed into BE10.2 cells. Cultures were grown at 37°C in STG medium to an OD_{600} of between 1.0 and 1.5, at which point the cells were induced for 2 h with either 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside) or 0.2% arabinose (for PmiEcoLrp). Cells were pelleted and frozen at −80°C until purification. For purification, the cells were sonicated in TG_{10}ED buffer (10 mM Tris, pH 8.0, 10% glycerol, 0.1 mM EDTA, 0.2 M NaCl, and 0.1 mM dithiothreitol [DTT]) with 100 μl 0.2 g/ml phenylmethylsulfonyl fluoride (PMSF) added per 500 ml cell suspension. The cells were sonicated in a cup horn probe (Ultrasonics, Plainview, NY) at maximum power for five rounds of 1 min, separated by 2 min on ice.

The lysates were centrifuged for 30 min at 15,000 × *g*, and the resulting supernatant was loaded onto a 1- by 12-cm BioRex70 cation-exchange column (Bio-Rad, Hercules, CA) equilibrated with TG_{10}ED. We could not purify VchLrp, VchEcoLrp, or K6Q-EcoLrp via this approach, as we worked only with untagged proteins, and these three did not bind to the cation-exchange column. All three nonbinders contain, relative to EcoLrp, a substitution for a Lys near the N terminus (Fig. 1B). Proteins were eluted with a 0.2 to 1.0 M NaCl gradient, and fractions were analyzed by examining Coomassie-stained SDS-polyacrylamide gels for the 18.9 kDa Lrp band. Fractions containing Lrp were pooled and concentrated with VivaSpin concentrators with a 10-kDa molecular mass cutoff (Sartorius Stedim, Dusseldorf, Germany). The concentrated Lrp fractions were then loaded onto a 1- by 28-cm Superose12 column (GE Healthcare, Uppsala, Sweden) equilibrated with TG_{10}ED buffer. Fractions containing highly purified Lrp were concentrated and dialyzed into MES buffer (10 mM *N*-morpholinoethane sulfonate, pH 6.25, 0.1 mM EDTA, and 0.2 M KCl). In some cases, for analytical ultracentrifugation (AUC) (see below), MES buffer lacking KCl was used.

Dynamic light scattering. Dynamic light scattering was performed using a Dynapro Titan instrument (Wyatt Technology, Santa Barbara, CA) with Dynamic V6 software. The 30-μl samples were spun at 15,000 rpm for 15 min at 4°C (to sediment any particulates) prior to transferring a sample from the top 16-μl to the 12-μl three-window cuvette. Readings were collected 30 times for 30 s each.

Mobility shift assays. Various amounts of purified Lrp were mixed with 23 nM P*lrp* DNA (375-bp fragment) in a solution containing 40 mM Tris, pH 7.4, 60 mM KCl, 0.1 mM EDTA, 5% glycerol, 80 mM NaCl, and 1 mM DTT. The samples were incubated at 23°C for 20 min prior to the addition of 1 μl Novex high-density Tris-borate-EDTA (TBE) sample buffer (Invitrogen, Carlsbad, CA). These samples were immediately loaded onto a 1.5-mm 4% acrylamide TBE gel in an Xcell apparatus (Invitrogen). For the samples with Leu, 30 mM L-leucine was added to the binding and loading buffers and cast into the gel. This leucine concentration was chosen based on Fig. 4 of reference 26 and Fig. 1 of reference 14. Samples were electrophoresed at 110 V until they entered the gel and then resolved at 80 V at room temperature. The gel was stained with 0.5 μg ethidium bromide/ml and visualized with an UltraLum Imager (Omega, Claremont, CA). Densitometry was performed with ImageJ (http://rsb.info.nih.gov/ij/docs/index.html).

Analytical ultracentrifugation. Sedimentation velocity analysis was used to determine the size distribution for each Lrp sample (EcoLrp, PmiLrp, and PmiEcoLrp). AUC experiments were carried out at 25°C in an XL-I analytical ultracentrifuge (Beckman Instruments, Brea, CA) at 30 krpm, with data collection at 230 nm and 238 nm using an absorbance optics system. The data were deconvoluted to determine sedimentation coefficient distributions using the c(s) analysis routine in the program SEDFIT (55).

RESULTS

Comparative effects of an N-terminal tail substitution in Lrp and λ CI. To explore the possible functional activity of the Lrp N-terminal region, we first generated a mutant in which the well-conserved Lys at position 6 (Fig. 1A) was replaced in EcoLrp (Fig. 1B). This substitution was chosen on the basis of the apparent similarity between the N termini of Lrp and the CI repressor-activator of lambda phage (Fig. 1B). In the low-temperature crystal structure of λ CI bound to its DNA target (Fig. 1C), a Lys (K4, where the numbering does not include the removed fMet), forms H bonds in the major groove to the O6 positions of two consecutive Gs in the operator DNA. Any substitutions at K4 (e.g., with Gln, yielding K4Q in λ CI) greatly reduce binding despite the fact that the HTH motif is unchanged (5, 18). To test whether the N-terminal region of Lrp has a functional role similar to that of N-terminal λ CI, we generated the corresponding K6Q substitution in EcoLrp (Fig. 1B).

One test promoter was P*gltB*, which is activated 20- to 30-

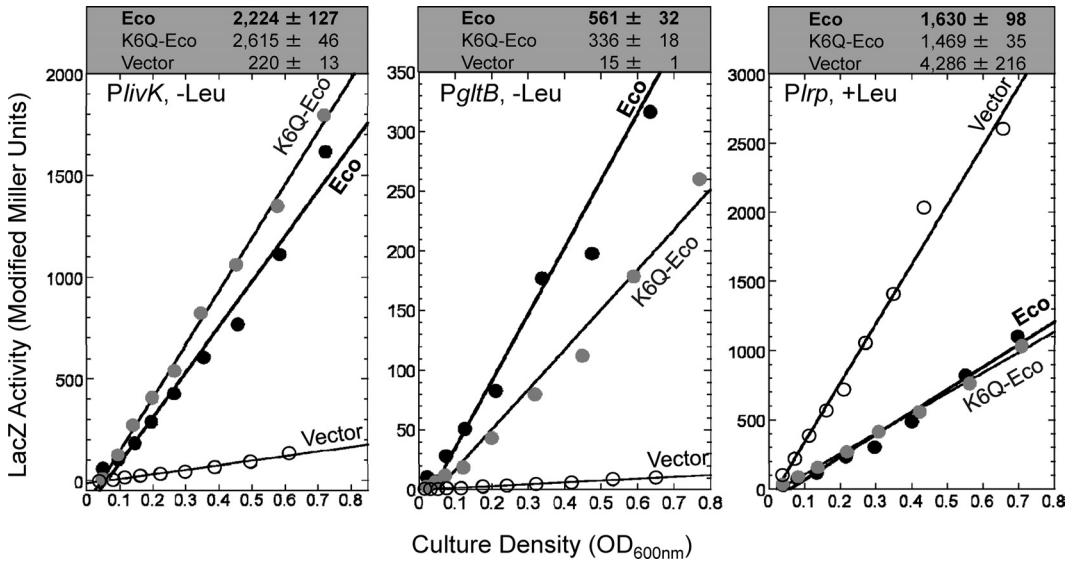

FIG. 2. Effects of K6Q mutation on EcoLrp. LacZ activity is plotted versus culture density, and the slopes indicate relative activity. Three Lrp-responsive promoters were fused to the *lacZ* reporter gene; for P*gltB*, the chromosomal fusion was used. The vector control (no Lrp) is shown as open circles, while WT EcoLrp is represented by filled circles and K6QEcoLrp is shown as gray circles. For all of the least-squares fits, R was $\geq$0.99. The actual slopes and associated standard errors are shown in the gray boxes.

fold by wild-type (WT) Lrp (7, 25, 48, 49, 62). We used a chromosomal P*gltB-lacZ* fusion, with either WT or K6Q EcoLrp supplied from a plasmid. In these experiments, samples are taken throughout the exponential phase of growth. Plotting LacZ activity versus culture density gives good linear fits if the cultures are in balanced growth, and the slopes of these lines provide very accurate measures of relative gene expression. As expected, WT EcoLrp gave nearly 30-fold activation (relative to the Lrp$^-$ vector control) (center panel of Fig. 2). The K6Q mutant gave ~60% of the WT EcoLrp activation level of P*gltB*. The effect of K6Q substitution on Lrp activation of P*gltB* is significant, though substantially smaller than the effect of the equivalent substitution on CI activation of the λ P_{RM} promoter. It is possible that this difference in degree reflects Lrp binding as an octamer or hexadecamer (12, 14, 21), while λ CI binds as a dimer (58).

Two other tested promoters showed essentially no difference between the WT and K6Q Lrp proteins (Fig. 2). One, P*livK*, is activated by Lrp in the absence of coregulator leucine (6, 17, 31, 59) and yielded the expected activation by both Lrp proteins. Another tested promoter was P*lrp* from *E. coli*. P*lrp* is autogenously repressed about 3-fold by Lrp (61). Compared to the vector control, WT EcoLrp repressed P*lrp-lacZ* expression nearly 3-fold (Fig. 2), and the K6Q substitution had no significant effect. This result suggests that, while the N-terminal tail may modulate Lrp activity, it does not have a uniform effect at all of the target promoters.

Differences in the N-terminal tail contribute to some of the regulatory differences among Lrp orthologs. The K6Q EcoLrp results indicate that the relative responsiveness of Lrp-sensitive promoters can be changed by amino acid substitution outside the known HTH DNA recognition motif. While the K6Q effects were modest, that substitution tests the role of just one position in the N-terminal arm as opposed to the multiple, naturally occurring substitutions among Lrp orthologs. We made substitutions in *E. coli lrp* that yielded the N-terminal 13-amino-acid (aa) sequences from *P. mirabilis* (PmiEcoLrp), or from *V. cholerae* (VchEcoLrp) (Fig. 1B), with the remainder of the EcoLrp sequence unchanged. We also used the WT proteins from the three species (EcoLrp, PmiLrp, and VchLrp) (Fig. 1).

We performed β-galactosidase assays using *lacZ* fusions to two promoters that were described above: P*gltB* and P*lrp*. (P*livK* showed no differences between EcoLrp and either PmiLrp or VchLrp [not shown], so the hybrids were not tested.) The P*gltB-lacZ* fusion yielded the expected strong activation by EcoLrp, with modestly decreased activation by PmiLrp and VchLrp (Fig. 3). The hybrid Lrp proteins gave intermediate levels of activation (gray symbols in Fig. 3), though the differences were limited in scale.

For P*lrp-lacZ*, VchLrp produced less repression than EcoLrp, with the VchEcoLrp hybrid behaving like EcoLrp. However, PmiLrp exhibited 2- to 3-fold-greater repression than EcoLrp (as we saw previously [39]). Strikingly, the PmiEcoLrp hybrid behaved exactly like PmiLrp (Fig. 3). These results indicate that the two N-terminal amino acid differences between PmiLrp and EcoLrp are responsible for their P*lrp* regulatory difference.

A third promoter we tested was for the export-processing protease OmpT. P*ompT* was chosen for studies of the role of the Lrp N terminus based on our previously reported microarray analysis of an *E. coli* K-12 *lrp*-Tn*10* strain producing either EcoLrp or PmiLrp (39). Those data revealed that P*ompT* exhibited one of the strongest differential effects of these two *lrp* alleles. We have now confirmed this result using a P*ompT-lacZ* fusion. In the absence of the coregulator leucine, we saw significant and essentially equivalent activation with all tested *lrp* alleles (Fig. 4A). However, in the presence of Leu and Ala, consistent with the earlier microarray results, EcoLrp was associated with repression, while PmiLrp still yielded activation

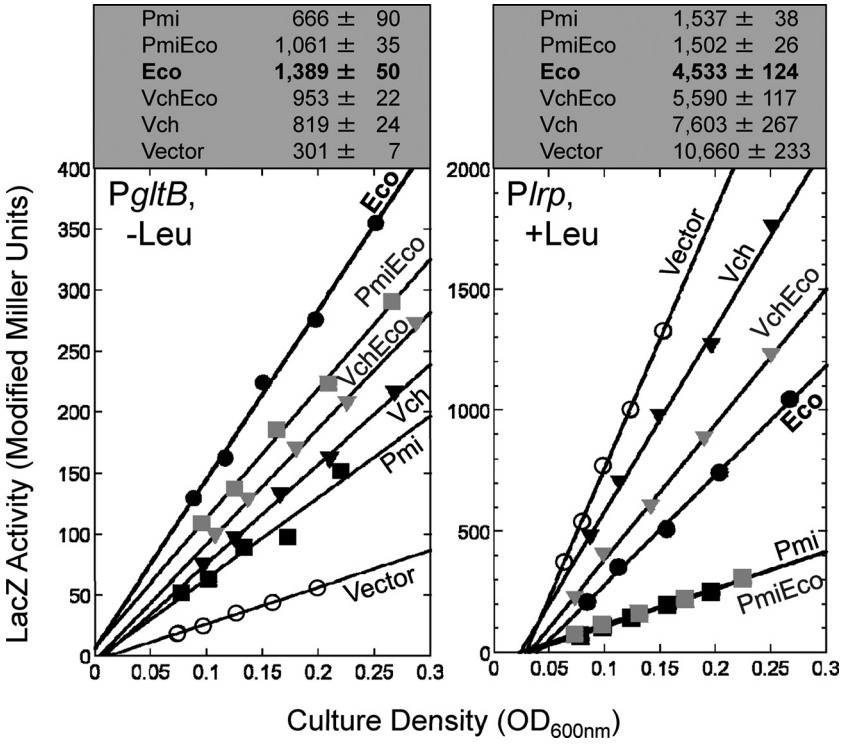

FIG. 3. Effects of Lrp orthologs and hybrids on two promoters. The *gltB* and *lrp* promoters were fused to *lacZ*, and LacZ activities were plotted versus culture density. Only those fusions and conditions yielding significant differences between Lrp orthologs are shown. For all of the least-squares fits, R was ≥ 0.97. The actual slopes and associated standard errors are shown in the gray boxes.

(Fig. 4B). We next tested the hybrid PmiEcoLrp and found that it had an intermediate effect compared to PmiLrp and EcoLrp (no effect compared to the Lrp^- vector control) (Fig. 4B). This is truly an intermediate effect, as opposed to inactivity of the hybrid, as PmiEcoLrp is active *in vivo* (Fig. 3 and 4A) and *in vitro* (see below).

Published and unpublished chromatin immunoprecipitation data indicate that EcoLrp binds to P*ompT in vivo* (17; A. Khodursky, personal communication). We confirmed *in vitro* binding via mobility shift analysis: both PmiLrp and EcoLrp bind P*ompT* DNA in the presence and absence of 10 mM Leu (Fig. 5C and D). For comparison, even 750 μM EcoLrp failed to shift a control fragment (326 bp from the Lrp-responsive promoter P*livK*, but missing part of the Lrp binding site [reference 33 and data not shown]). As with P*lrp*, these *in vivo* and *in vitro* results with P*ompT* indicate that the two N-terminal-arm differences between EcoLrp and PmiLrp have significant regulatory effects.

The Lrp N-terminal tail affects DNA binding. To determine if the stronger P*lrp* repression by PmiLrp than by EcoLrp was due to differential DNA binding, we performed mobility shift assays using P*lrp* DNA and the native EcoLrp and PmiLrp proteins. The results indicated, surprisingly, that PmiLrp bound P*lrp* with lower affinity than did EcoLrp, whether or not the coregulator leucine was present (see Fig. S1 in the supplemental material). As noted above, EcoLrp did not detectibly shift a 326-bp specificity control DNA that partially overlaps P*livK* (reference 33 and data not shown). Possible explanations for this result are raised in Discussion. but for the purposes of the present study, the key question is simply whether or not the N terminus was involved in this *in vitro* affinity difference. Accordingly, we assayed the PmiEcoLrp hybrid. A representative titration from triplicate electrophoretic mobility shift assay (EMSA) gels is shown in Fig. 5A, and the combined results are consistent with the N-terminal tail being responsible for the observed differences in affinity for P*lrp* (Fig. 5B).

Lrp multimerization is affected by the N-terminal tail. Lrp forms octamers and hexadecamers, and the shift between these states may be responsible for regulatory changes in response to the coregulator leucine (12, 14, 21). Presumably the other amino acids that act as Lrp coregulators (33) also modulate Lrp multimerization, though this has not been determined. We tested whether differences at the N terminus affect the Lrp multimeric state.

In purifying the native Lrp proteins, we noticed salt-dependent effects on the multimerization states in the final gel filtration step (not shown). Octamers and monomers were inferred to be the predominant peaks in 0.2 M NaCl, eluting at 25 and 38 min, respectively (slightly faster than the 16.7-kDa myoglobin standard at 40 min). In the case of EcoLrp, the predominant gel filtration peak was at 38 min in the presence of 0.2 M NaCl but shifted to 25 min in 1 M NaCl. Accordingly, subsequent studies were carried out in, at most, 0.2 M salt.

We next used dynamic light scattering (DLS) (32) to assess the Lrp multimerization status in 0.2 M KCl. When leucine was present, EcoLrp, PmiLrp, and PmiEcoLrp formed complexes consistent with octamers (filled symbols in Fig. 6), in agreement with previous findings (12, 14). In the absence of leucine,

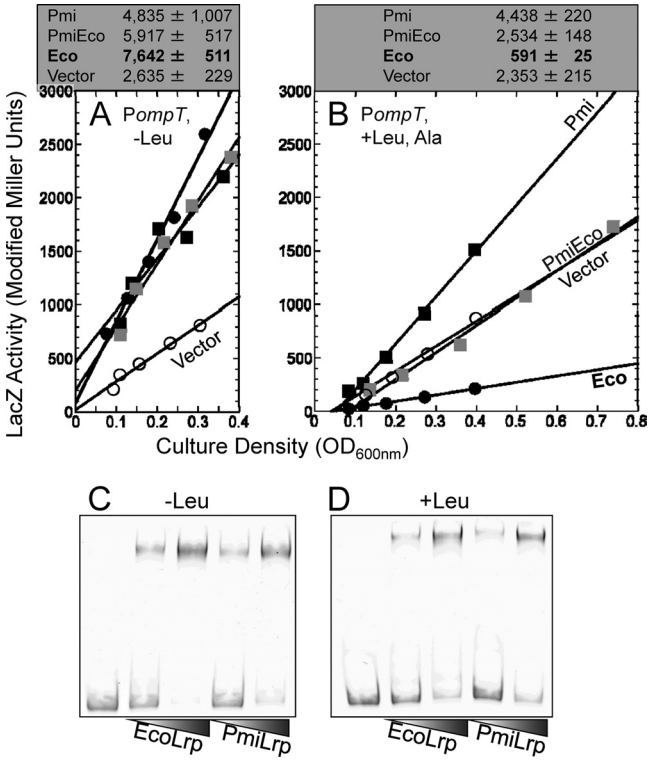

FIG. 4. Effects of EcoLrp and PmiLrp on P*ompT*. A plasmid bearing a P*ompT-lacZ* fusion was transformed into BE10.2 cells that contained plasmids specifying EcoLrp, PmiLrp, or PmiEcoLrp. LacZ activity was plotted versus culture density. (A) Cells were grown in medium containing Ile, Val, and thiamine but no Ala or Leu. For all of the least-squares fits, R was $\geq$0.94. (B) Cells were grown in the same medium as for panel A but with added Ala and Leu. For all of the least-squares fits, R was $\geq$0.99. For both panels A and B, the actual slopes and associated standard errors are shown in the gray boxes. (C) EMSA of 23 nM P*ompT* DNA in the absence of Leu. Lane 1 (from the left) has no Lrp; lanes 2 and 3 have 250 and 500 nM EcoLrp, respectively; and lanes 4 and 5 have 250 and 500 nM PmiLrp, respectively. (D) EMSA of P*ompT* as in panel C, but 10 mM Leu was added to the binding and loading buffers and polymerized into the gel.

under our conditions, we still did not see apparent hexadecamers formed by EcoLrp (open symbols in Fig. 6A). In contrast, PmiLrp yielded forms consistent with hexadecamers, doing so in a concentration-dependent manner above a threshold of ~10 μM (Fig. 6B). We repeated the PmiLrp DLS experiments at several additional concentrations in the absence of leucine, and above the 10 μM threshold, we consistently found that the apparent PmiLrp mass was significantly larger (1.3- to 2.4-fold) than in the presence of leucine. Others were able to detect EcoLrp hexadecamers via DLS under somewhat similar conditions (12, 14), as were we via a different method (see below). However, for the purposes of this study, the key question is whether PmiEcoLrp behaves like PmiLrp or like EcoLrp under our conditions. As shown in Fig. 6C, the hybrid behaved like PmiLrp, yielding apparent hexadecamers in the absence of leucine. These results suggest that the N-terminal arm affects the multimerization behavior of Lrp.

We sought to test our DLS results using an independent method: AUC (57). In the absence of leucine and KCl and at ~20 μM subunit concentrations, both EcoLrp and PmiLrp yielded apparent monomers, tetramers, octamers, and hexadecamers (Fig. 7A to C, gray lines). In 0.2 M KCl, in contrast, neither of these proteins yielded 16-mers (Fig. 7A to C, black lines). The salt sensitivity of 16-mer appearance suggests that the 16-mer results predominantly from electrostatic interactions. Multimers must be stable for hours to be detected by AUC compared to seconds for DLS, and this may explain (at least in part) why PmiLrp yielded apparent 16-mers in 0.2 M KCl that were detectible by DLS but not by AUC.

Once again, the key question for the AUC analysis was whether the PmiEcoLrp hybrid would behave like EcoLrp, like PmiLrp (as in the DLS experiments), or like neither. The last (neither) appears to be the case. Surprisingly, PmiEcoLrp appeared to yield hexadecamers in the presence of 0.2 M KCl (Fig. 7C), but not in its absence. An overlay of AUC results from the three Lrp proteins in 0.2 M KCl is shown in Fig. S2 in the supplemental material. One possible explanation for this difference between the hybrid and the two native Lrp proteins is that PmiEcoLrp has a tendency to aggregate in low concentrations of KCl (not shown). We cannot rule out the possibility

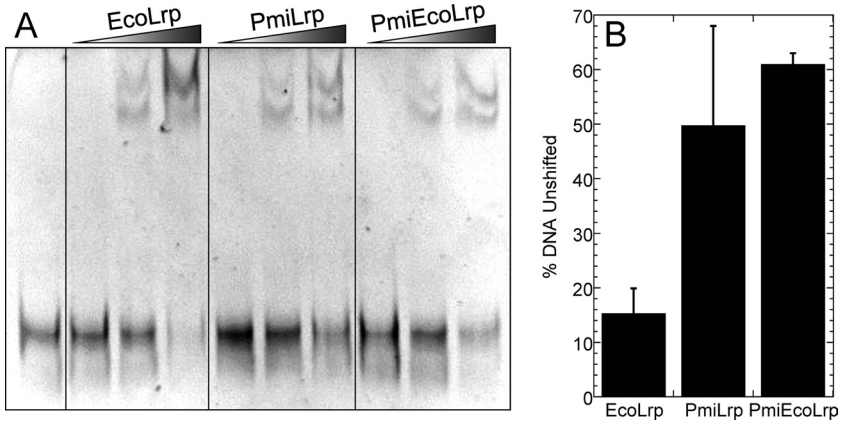

FIG. 5. Single-gel mobility shift assay of P*lrp* DNA. (A) We carried out side-by-side EMSAs with three Lrp variants on one gel, with Lrp concentrations of 30, 225, and 400 nM. (B) Densitometric quantitation of results with 400 nM Lrp (plus the standard error [SE]) from the gel in panel A and two other replicate gels not shown.

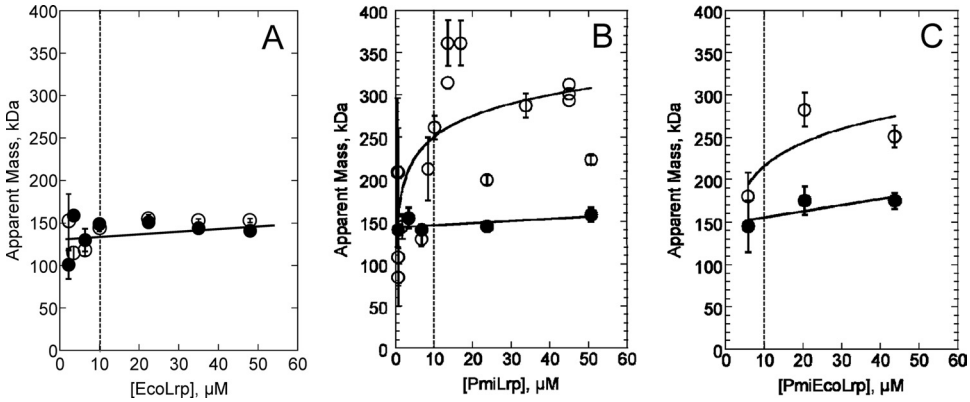

FIG. 6. Multimerization of Lrp as revealed by dynamic light scattering. Lrp was in MES buffer, which contains 0.2 M KCl. Given the shape of multimeric Lrp (Fig. 1), the apparent mass is not expected to vary linearly with the multimeric state; however, for comparison, the expected masses of a Lrp tetramer, octamer, and hexadecamer are approximately 75, 150, and 300 kDa, respectively. The open circles represent samples in the absence of Leu, and the closed circles represent samples in the presence of 10 mM Leu. (A) EcoLrp. (B) PmiLrp. (C) PmiEcoLrp. The symbols and error bars indicate the means and SE from 30 data points collected by the instrument at each concentration. The vertical dotted lines indicate the 10 μM Lrp concentration.

that the protein does form 16-mers under low-salt conditions but that they are undetectible in our assays due to aggregation having decreased the effective protein concentration during centrifugation (consistent with lower levels of the smaller multimers seen in Fig. 7C [see Fig. S2 in the supplemental material]).

DISCUSSION

Role of amino-terminal tails in DNA binding by HTH proteins. A variety of proteins that use helix-turn-helix motifs for sequence-specific DNA binding also rely on flexible amino-terminal tails (sometimes referred to as "arms") for DNA binding (3). These tails generally contain basic amino acids, and examples are λ CI (Fig. 1B and C) (47), the widespread bacterial nickel-responsive regulator NikR (4), and homeodomain proteins in the *Animalia* (22). The disordered tails can act in a number of ways, including provision of additional DNA contacts, increasing the protein's DNA capture radius, and improving the dynamics of sliding or hopping on the DNA during the search for binding sites (60). The effect of these tails can be profound, such as the ~8,000-fold-reduced binding when it is deleted from λ CI (23) or ~1,000-fold when deleted from EcoLrp (21). Further, exchanging N-terminal tails between two homeodomain proteins had a more profound effect on DNA binding than did exchanging their recognition helix sequences (19). However, many other HTH proteins do not rely on amino-terminal tails for DNA binding, and compensatory HTH mutations can in some cases restore binding to tail mutants (5). One possible interpretation is that adding to HTH-DNA interactions by establishing dependence on an amino tail allows greater evolutionary flexibility in fine-tuning the relative affinities for different binding sites. This strategy would be particularly important for global regulatory proteins, such as Lrp, that directly control hundreds of genes and is consistent with the elevated rate of phylogenetic variation within the Lrp N-terminal arm (Fig. 1A).

Effects of the amino-terminal tail on Lrp-promoter interactions. The amino-terminal tails of Lrp proteins appear to play significant roles in promoter regulation, DNA binding, and multimerization. This interpretation is consistent with the profound effects on DNA binding of deleting the first 10 aa of EcoLrp (21), though not surprisingly, the effects of 1 to 3 substitutions reported here are more limited than those of complete tail removal. The pattern of sequence divergence among *Enterobacteriaceae* and *Vibrionaceae* is striking, with Lrp orthologs having >90% overall identity and completely conserved HTH motifs but with substantial variation in their N-terminal tails (Fig. 1A). The effects of sequence variation in the N-terminal tails is presumably amplified by the fact that Lrp is active as an octameric ring, with DNA wrapping around the outside edge (12, 14, 21), consistent with the extended DNase I footprints generated by Lrp on target binding sites (34, 62, 64). Thus, a given contiguous segment of Lrp-bound DNA could theoretically involve contacts with 16 tails.

Table 1 compares the normalized effects on transcriptional fusions of just EcoLrp and its derivatives (K6Q-EcoLrp, PmiEcoLrp, and VchEcoLrp) (Fig. 1B) to eliminate effects of the substitutions outside the amino-terminal tail (in PmiLrp and VchLrp). The results are consistent with the hypothesis that the Lrp tail plays a promoter-specific fine-tuning role. For example, relative to EcoLrp, PmiEcoLrp reduces expression 3-fold from P*lrp* and increases it 4-fold from P*ompT* but causes just a 25% reduction in expression from P*gltB*. Significantly, EMSA revealed that PmiLrp binds P*lrp* DNA with lower affinity than EcoLrp and that this difference involves the N-terminal tail, since the hybrid PmiEcoLrp behaved, in this respect, like PmiLrp (Fig. 5; see Fig. S1 in the supplemental material).

The effects of the amino-terminal tail on *in vivo* and *in vitro* behavior of EcoLrp appear to be significant, and some of the implications of this are discussed below. Nevertheless, it is not yet obvious how the *in vivo* and *in vitro* effects are related to one another. Comparing just EcoLrp and PmiEcoLrp and their interactions with P*lrp*, EcoLrp represses to a lesser extent *in vivo* (Fig. 3) yet has higher affinity for the promoter DNA *in vitro* (Fig. 5; see Fig. S1 in the supplemental material). One possible explanation is that, while the total concentrations of

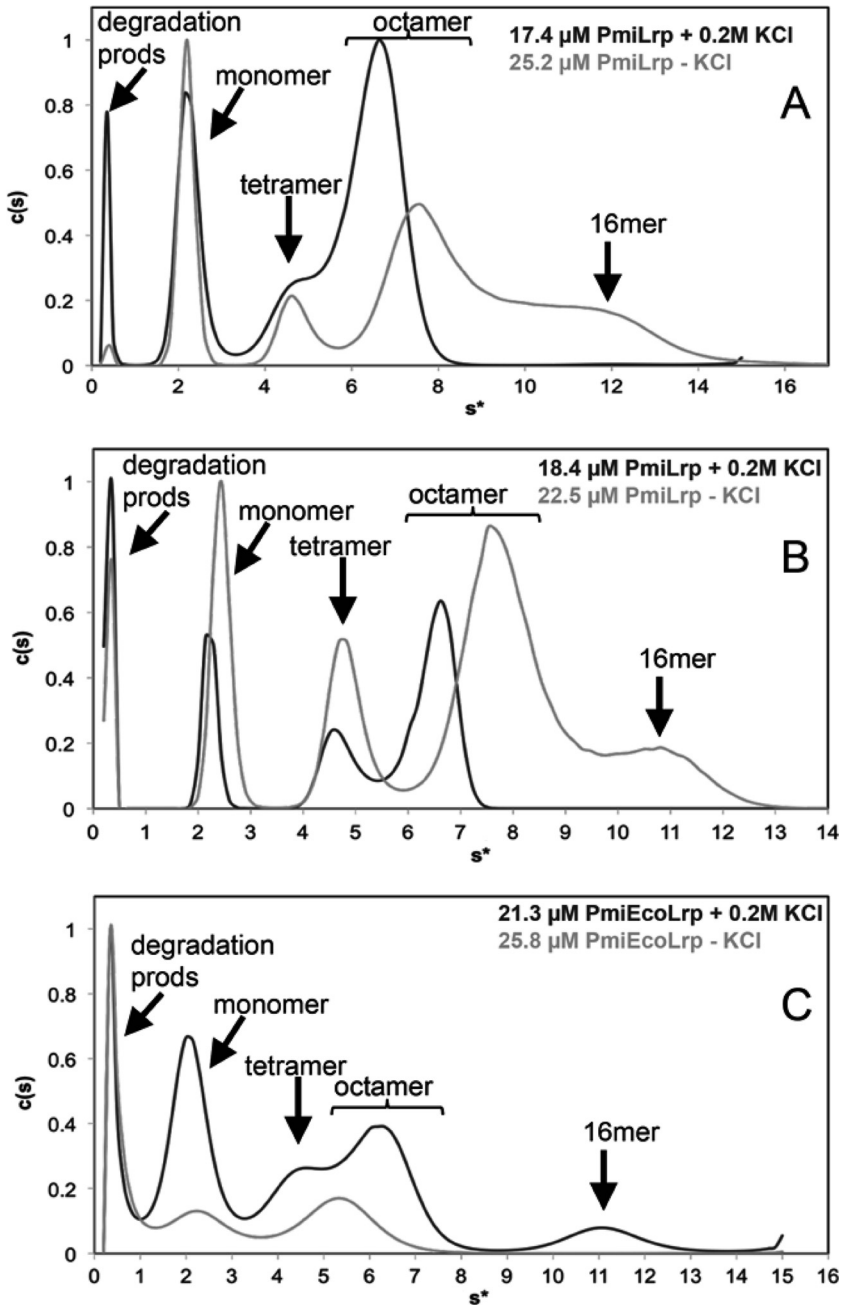

FIG. 7. Velocity sedimentation of Lrp. (A) EcoLrp at ~20 mM with 0.2 M KCl (black lines) and no salt (dialyzed against MES buffer lacking KCl) (gray lines). The figures are plotted with the sedimentation coefficient (s^*) versus the sedimentation coefficient distribution [$C(S)$]. The peaks are consistent with the forms as labeled: s^*12, 16-mer; s^*7, octamer; s^*5, tetramer; and s^*2, monomer. The peak centered around 0.25 is likely to be degradation products. (B) PmiLrp at ~20 mM with 0.2 M (black) or no (gray) KCl. (C) PmiEcoLrp hybrid at ~20 mM with 0.2 M (black) or no (gray) KCl.

Lrp protein are the same, the concentration of free EcoLrp in the cell could be lower than that of PmiEcoLrp, perhaps due to differing affinities for nonspecific DNA (13, 50). Given the variability and high content of charged amino acids in the amino-terminal tail (Fig. 1A), differences in Lrp sequestration on nonspecific DNA seem likely. Furthermore, by analogy with the charged amino-terminal tails of histones (16, 45), it is even possible that binding of proteins such as Lrp is modulated by posttranslational modifications. Finally, the intrinsic affinity of Lrp for DNA is almost certainly affected by its multimeric state, and both may be affected by the amino-terminal tails; our experiments do not allow us to deconvolute these effects.

Possible effects of the amino-terminal tail on Lrp multimerization. Our results suggest that the Lrp amino-terminal tails affect multimerization, as well as DNA binding. DLS revealed a greater propensity for PmiLrp than EcoLrp to form multimers larger than octamers, and the hybrid PmiEcoLrp behaved like PmiLrp (Fig. 6). This is reminiscent of the plasmid-parti-

TABLE 1. Relative effects of EcoLrp and its derivatives on transcriptional fusions

Lrp used	Effect on promoter fusion[a]			
	Without Leu		With Leu	
	P*livK-lacZ*	P*gltB-lacZ*	P*lrp-lacZ*	P*ompT-lacZ*
WT (Eco)	(1.00)	(1.00)	(1.00)	(1.00)
K6Q	1.18	0.60	0.90	ND[b]
V2I, S4N (PmiEco)	ND	0.76	0.33	4.29
K5Y, R7K, G9S (VchEco)	ND	0.69	0.96	ND

[a] Ratio of slopes from the plots of β-galactosidase activity vs. culture density, mutant/WT. Parentheses indicate normalization to the WT value. ND, not determined.

tioning protein ParG, where the N-terminal tail mediates formation of higher-order multimers (11). It is worth noting that the carboxyl-terminal 10 aa of Lrp also appear to play a role in multimerization (14, 21), though EcoLrp and PmiLrp have identical sequences there.

The AUC data suggest that hexadecamers are likely a result of electrostatic interactions (Fig. 7). The 13 N-terminal Lrp residues in the *Enterobacteriaceae* and *Vibrionaceae* have seven highly conserved positions (four basic and three acidic), together forming over half of the tail (see the sequence logo in Fig. 1A). However, neither of the two changes that appear to make PmiEcoLrp 16-mer formation more salt resistant than that of EcoLrp (Fig. 6A and C) involves a charged residue (Fig. 1B).

Implications for bioinformatics prediction of regulation. Predicting transcriptional-regulatory interactions based on a conserved transcription factor, conserved target gene, and conserved binding site for the regulator upstream of the target gene poses significant bioinformatics challenges at each level. Binding site prediction is probably the most difficult of these, but that element of the predictive approach can be obviated for testing purposes by placing different orthologous regulators into the same bacterial background. When this was done with Lrp in *E. coli*, unexpectedly large differences were seen (39). That result suggested that the 4/164 substitutions between PmiLrp and EcoLrp and the 13/164 between VchLrp and EcoLrp had significant functional effects despite the fact that none were within the helix-turn-helix domain. Our results in establishing that the amino-terminal tail of EcoLrp affects regulatory function are consistent with other studies indicating particular evolutionary flexibility of transcription factors (35, 40, 41, 53). This suggests that substantial improvements in the ability to predict regulation from genome sequences are likely to emerge from a deeper understanding of regulator structure and function, particularly in the case of global regulatory proteins.

ACKNOWLEDGMENTS

We thank Ron Viola and Buenafe Arachea for the use of and advice regarding the dynamic-light-scattering instrument, Darren Sledjeski for providing antiserum, Arkady Khodursky for sharing unpublished results, and J. David Dignam for sharing his thoughts and for advice and use of equipment during Lrp purifications. Joseph Calvo, Ivana de la Serna, R. Mark Wooten, Isabel Novella, Sean Corum, and Elise Hondorp provided advice and critiques on the manuscript.

This work was supported by funds from NIH grant R01 AI54716 and a Research Challenge award from the University of Toledo to R.M.B. B.R.H. was also supported, in part, by a graduate fellowship from the University of Toledo Health Science Campus.

REFERENCES

1. **Alexander, P. A., Y. He, Y. Chen, J. Orban, and P. N. Bryan.** 2007. The design and characterization of two proteins with 88% sequence identity but different structure and function. Proc. Natl. Acad. Sci. U. S. A. **104:**11963–11968.
2. **Amann, E., B. Ochs, and K. J. Abel.** 1988. Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in Escherichia coli. Gene **69:**301–315.
3. **Aravind, L., V. Anantharaman, S. Balaji, M. M. Babu, and L. M. Iyer.** 2005. The many faces of the helix-turn-helix domain: transcription regulation and beyond. FEMS Microbiol. Rev. **29:**231–262.
4. **Benanti, E. L., and P. T. Chivers.** 2007. The N-terminal arm of the Helicobacter pylori Ni2+-dependent transcription factor NikR is required for specific DNA binding. J. Biol. Chem. **282:**20365–20375.
5. **Benson, N., C. Adams, and P. Youderian.** 1992. Mutant lambda repressors with increased operator affinities reveal new, specific protein-DNA contacts. Genetics **130:**17–26.
6. **Bhagwat, S. P., M. R. Rice, R. G. Matthews, and R. M. Blumenthal.** 1997. Use of an inducible regulatory protein to identify members of a regulon: application to the regulon controlled by the leucine-responsive regulatory protein (Lrp) in Escherichia coli. J. Bacteriol. **179:**6254–6263.
7. **Borst, D. W., R. M. Blumenthal, and R. G. Matthews.** 1996. Use of an in vivo titration method to study a global regulator: effect of varying Lrp levels on expression of gltBDF in Escherichia coli. J. Bacteriol. **178:**6904–6912.
8. **Brinkman, A. B., T. J. Ettema, W. M. de Vos, and J. van der Oost.** 2003. The Lrp family of transcriptional regulators. Mol. Microbiol. **48:**287–294.
9. **Brosius, J.** 1984. Plasmid vectors for the selection of promoters. Gene **27:**151–160.
10. **Calvo, J. M., and R. G. Matthews.** 1994. The leucine-responsive regulatory protein, a global regulator of metabolism in Escherichia coli. Microbiol. Rev. **58:**466–490.
11. **Carmelo, E., et al.** 2005. The unstructured N-terminal tail of ParG modulates assembly of a quaternary nucleoprotein complex in transcription repression. J. Biol. Chem. **280:**28683–28691.
12. **Chen, S., and J. M. Calvo.** 2002. Leucine-induced dissociation of Escherichia coli Lrp hexadecamers to octamers. J. Mol. Biol. **318:**1031–1042.
13. **Chen, S., Z. Hao, E. Bieniek, and J. M. Calvo.** 2001. Modulation of Lrp action in Escherichia coli by leucine: effects on non-specific binding of Lrp to DNA. J. Mol. Biol. **314:**1067–1075.
14. **Chen, S., M. H. Rosner, and J. M. Calvo.** 2001. Leucine-regulated self-association of leucine-responsive regulatory protein (Lrp) from Escherichia coli. J. Mol. Biol. **312:**625–635.
15. **Chen, T., et al.** 2010. The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. Database (Oxford) 2010:baq013.
16. **Cheng, X., and R. M. Blumenthal.** 2010. Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. Biochemistry **49:**2999–3008.
17. **Cho, B. K., C. L. Barrett, E. M. Knight, Y. S. Park, and B. O. Palsson.** 2008. Genome-scale reconstruction of the Lrp regulatory network in Escherichia coli. Proc. Natl. Acad. Sci. U. S. A. **105:**19462–19467.
18. **Clarke, N. D., L. J. Beamer, H. R. Goldberg, C. Berkower, and C. O. Pabo.** 1991. The DNA binding arm of lambda repressor: critical contacts from a flexible region. Science **254:**267–270.
19. **Damante, G., and R. Di Lauro.** 1991. Several regions of Antennapedia and thyroid transcription factor 1 homeodomains contribute to DNA binding specificity. Proc. Natl. Acad. Sci. U. S. A. **88:**5388–5392.
20. **D'Ari, R., R. T. Lin, and E. B. Newman.** 1993. The leucine-responsive regulatory protein: more than a regulator? Trends Biochem. Sci. **18:**260–263.
21. **de los Rios, S., and J. J. Perona.** 2007. Structure of the Escherichia coli leucine-responsive regulatory protein Lrp reveals a novel octameric assembly. J. Mol. Biol. **366:**1589–1602.
22. **Dragan, A. I., et al.** 2006. Forces driving the binding of homeodomains to DNA. Biochemistry **45:**141–151.
23. **Eliason, J. L., M. A. Weiss, and M. Ptashne.** 1985. NH2-terminal arm of phage lambda repressor contributes energy and specificity to repressor binding and determines the effects of operator mutations. Proc. Natl. Acad. Sci. U. S. A. **82:**2339–2343.
24. **Ellrott, K., L. Jaroszewski, W. Li, J. C. Wooley, and A. Godzik.** 2010. Expansion of the protein repertoire in newly explored environments: human gut microbiome specific protein families. PLoS Comput. Biol. **6:**e1000798.
25. **Ernsting, B. R., M. R. Atkinson, A. J. Ninfa, and R. G. Matthews.** 1992. Characterization of the regulon controlled by the leucine-responsive regulatory protein in Escherichia coli. J. Bacteriol. **174:**1109–1118.
26. **Ernsting, B. R., J. W. Denninger, R. M. Blumenthal, and R. G. Matthews.** 1993. Regulation of the gltBDF operon of Escherichia coli: how is a leucine-

insensitive operon regulated by the leucine-responsive regulatory protein? J. Bacteriol. **175:**7160–7169.
27. **Espinosa, V., A. D. Gonzalez, A. T. Vasconcelos, A. M. Huerta, and J. Collado-Vides.** 2005. Comparative studies of transcriptional regulation mechanisms in a group of eight gamma-proteobacterial genomes. J. Mol. Biol. **354:**184–199.
28. **Fauman, E. B., R. M. Blumenthal, and X. Cheng.** 1999. Structure and evolution of AdoMet-dependent methyltransferases, p. 1–38. *In* X. Cheng and R. M. Blumenthal (ed.), *S*-Adenosylmethionine-dependent methyltransferases: structures and functions. World Scientific, Singapore.
29. **Gibson, D. G., et al.** 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. Science **329:**52–56.
30. **Guzman, L. M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J. Bacteriol. **177:**4121–4130.
31. **Haney, S. A., J. V. Platko, D. L. Oxender, and J. M. Calvo.** 1992. Lrp, a leucine-responsive protein, regulates branched-chain amino acid transport genes in Escherichia coli. J. Bacteriol. **174:**108–115.
32. **Harding, S. E.** 1994. Determination of diffusion coefficients of biological macromolecules by dynamic light scattering. Methods Mol. Biol. **22:**97–108.
33. **Hart, B. R., and R. M. Blumenthal.** 2011. Unexpected coregulator range for the global regulator Lrp of Escherichia coli and Proteus mirabilis. J. Bacteriol. **193:**1054–1064.
34. **Jafri, S., S. Chen, and J. M. Calvo.** 2002. ilvIH operon expression in Escherichia coli requires Lrp binding to two distinct regions of DNA. J. Bacteriol. **184:**5293–5300.
35. **Janga, S. C., and E. Perez-Rueda.** 2009. Plasticity of transcriptional machinery in bacteria is increased by the repertoire of regulatory families. Comput. Biol. Chem. **33:**261–268.
36. **Kim, Y. I., and J. C. Hu.** 1995. Operator binding by lambda repressor heterodimers with one or two N-terminal arms. Proc. Natl. Acad. Sci. U. S. A. **92:**7510–7514.
37. **Knowle, D., R. E. Lintner, Y. M. Touma, and R. M. Blumenthal.** 2005. Nature of the promoter activated by C. PvuII, an unusual regulatory protein conserved among restriction-modification systems. J. Bacteriol. **187:**488–497.
38. **Leonard, P. M., et al.** 2001. Crystal structure of the Lrp-like transcriptional regulator from the archaeon Pyrococcus furiosus. EMBO J. **20:**990–997.
39. **Lintner, R. E., et al.** 2008. Limited functional conservation of a global regulator among related bacterial genera: Lrp in Escherichia, Proteus and Vibrio. BMC Microbiol. **8:**60.
40. **Lozada-Chavez, I., S. C. Janga, and J. Collado-Vides.** 2006. Bacterial regulatory networks are extremely flexible in evolution. Nucleic Acids Res. **34:** 3434–3445.
41. **Madan Babu, M., S. A. Teichmann, and L. Aravind.** 2006. Evolutionary dynamics of prokaryotic transcriptional regulatory networks. J. Mol. Biol. **358:**614–633.
42. **Matthews, R. G., Y. Cui, D. Friedberg, and J. M. Calvo.** 2000. Wild-type and hexahistidine-tagged derivatives of leucine-responsive regulatory protein from Escherichia coli. Methods Enzymol. **324:**322–329.
43. **Mazurie, A., D. Bonchev, B. Schwikowski, and G. A. Buck.** 2010. Evolution of metabolic network organization. BMC Syst. Biol. **4:**59.
44. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Press, Cold Spring Harbor, NY.
45. **Munshi, A., G. Shafi, N. Aliya, and A. Jyothy.** 2009. Histone modifications dictate specific biological readouts. J. Genet. Genomics **36:**75–88.
46. **Newman, E. B., and R. Lin.** 1995. Leucine-responsive regulatory protein: a global regulator of gene expression in E. coli. Annu. Rev. Microbiol. **49:**747–775.
47. **Pabo, C. O., W. Krovatin, A. Jeffrey, and R. T. Sauer.** 1982. The N-terminal arms of lambda repressor wrap around the operator DNA. Nature **298:**441–443.
48. **Paul, L., R. M. Blumenthal, and R. G. Matthews.** 2001. Activation from a distance: roles of Lrp and integration host factor in transcriptional activation of gltBDF. J. Bacteriol. **183:**3910–3918.
49. **Paul, L., P. K. Mishra, R. M. Blumenthal, and R. G. Matthews.** 2007. Integration of regulatory signals through involvement of multiple global regulators: control of the Escherichia coli gltBDF operon by Lrp, IHF, Crp, and ArgR. BMC Microbiol. **7:**2.
50. **Peterson, S. N., F. W. Dahlquist, and N. O. Reich.** 2007. The role of high affinity non-specific DNA binding by Lrp in transcriptional regulation and DNA organization. J. Mol. Biol. **369:**1307–1317.
51. **Platko, J. V., and J. M. Calvo.** 1993. Mutations affecting the ability of Escherichia coli Lrp to bind DNA, activate transcription, or respond to leucine. J. Bacteriol. **175:**1110–1117.
52. **Platko, J. V., D. A. Willins, and J. M. Calvo.** 1990. The ilvIH operon of Escherichia coli is positively regulated. J. Bacteriol. **172:**4563–4570.
53. **Price, M. N., P. S. Dehal, and A. P. Arkin.** 2007. Orthologous transcription factors in bacteria have different functions and regulate different genes. PLoS Comput. Biol. **3:**1739–1750.
54. **Ravcheev, D. A., A. V. Gerasimova, A. A. Mironov, and M. S. Gelfand.** 2007. Comparative genomic analysis of regulation of anaerobic respiration in ten genomes from three families of gamma-proteobacteria (Enterobacteriaceae, Pasteurellaceae, Vibrionaceae). BMC Genomics **8:**54.
55. **Schuck, P.** 2000. Size distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and Lamm equation modeling. Biophys. J. **78:**1606–1619.
56. **Sezonov, G., D. Joseleau-Petit, and R. D'Ari.** 2007. Escherichia coli physiology in Luria-Bertani broth. J. Bacteriol. **189:**8746–8749.
57. **Stafford, W. F., III.** 2009. Protein-protein and ligand-protein interactions studied by analytical ultracentrifugation. Methods Mol. Biol. **490:**83–113.
58. **Stayrook, S., P. Jaru-Ampornpan, J. Ni, A. Hochschild, and M. Lewis.** 2008. Crystal structure of the lambda repressor and a model for pairwise cooperative operator binding. Nature **452:**1022–1025.
59. **Tani, T. H., A. Khodursky, R. M. Blumenthal, P. O. Brown, and R. G. Matthews.** 2002. Adaptation to famine: a family of stationary-phase genes revealed by microarray analysis. Proc. Natl. Acad. Sci. U. S. A. **99:**13471–13476.
60. **Toth-Petroczy, A., I. Simon, M. Fuxreiter, and Y. Levy.** 2009. Disordered tails of homeodomains facilitate DNA recognition by providing a trade-off between folding and specific binding. J. Am. Chem. Soc. **131:**15084–15085.
61. **Wang, Q., J. Wu, D. Friedberg, J. Plakto, and J. M. Calvo.** 1994. Regulation of the Escherichia coli lrp gene. J. Bacteriol. **176:**1831–1839.
62. **Wiese, D. E., II, B. R. Ernsting, R. M. Blumenthal, and R. G. Matthews.** 1997. A nucleoprotein activation complex between the leucine-responsive regulatory protein and DNA upstream of the gltBDF operon in Escherichia coli. J. Mol. Biol. **270:**152–168.
63. **Yokoyama, K., et al.** 2006. Feast/famine regulatory proteins (FFRPs): Escherichia coli Lrp, AsnC and related archaeal transcription factors. FEMS Microbiol. Rev. **30:**89–108.
64. **Zhi, J., E. Mathew, and M. Freundlich.** 1999. Lrp binds to two regions in the dadAX promoter region of Escherichia coli to repress and activate transcription directly. Mol. Microbiol. **32:**29–40.