H-NS Is a Repressor of the *Proteus mirabilis* Urease Transcriptional Activator Gene ureR

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Expression of *Proteus mirabilis* urease is governed by UreR, an AraC-like positive transcriptional activator. A poly(A) tract nucleotide sequence, consisting of A₆T₆C₅A₂TGGTA₂G₆A₂TG₆A₂, is located 16 bp upstream of the ω₇₀-like ureR promoter P2. Since poly(A) tracts of DNA serve as binding sites for the gene repressor histone-like nucleoid structuring protein (H-NS), we measured β-galactosidase activity of wild-type *Escherichia coli* MC4100 (H-NS⁺) and its isogenic derivative ATM121 (hns::Tn10) (H-NS⁻) harboring a ureR-lacZ operon fusion plasmid (pLC9801). β-Galactosidase activity in the H-NS⁻ host strain was constitutive and sevenfold greater (P < 0.0001) than that in the H-NS⁺ host. A recombinant plasmid containing cloned *P. mirabilis* hns was able to complement and restore repression of the ureR promoter in the H-NS⁻ host when provided in trans. Deletion of the poly(A) tract nucleotide sequence from pLC9801 resulted in an increase in β-galactosidase activity in the H-NS⁺ host to nearly the same levels as that observed for wild-type pLC9801 harbored by the H-NS⁻ host. Urease activity in strains harboring the recombinant plasmid pMD1010 (encoding the entire urease gene cluster of *P. mirabilis*) was equivalent in both the H-NS⁻ background and the H-NS⁺ background in the presence of urea but was eightfold greater (P = 0.0001) in the H-NS⁻ background in the absence of urea. We conclude that H-NS represses ureR expression in the absence of urea induction.

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*Proteus mirabilis* causes acute and chronic urinary tract infections including pyelonephritis (2, 7), particularly in patients with long-term indwelling urinary catheters or structural abnormalities of the urinary tract (23). A complication of infections including pyelonephritis (2, 7), particularly in patients 655 W. Baltimore St., Baltimore, MD 21201. Phone: (410) 706-0466. Fax: (410) 706-6751. E-mail: hmobley@umaryland.edu.
ureR promoter expression is derepressed in the absence of hns. There is a poly(A) tract DNA sequence consisting of A$_6$TA$_2$CA$_2$TGGTA$_5$GA$_6$TGA$_5$ preceding the P2 promoter (measured as a function of β-galactosidase activity [21]) was repressed sevenfold (P < 0.0001) in MC4100(pLC9801) compared to ATM121 (pLC9801) (Fig. 2A).

P. mirabilis hns was PCR amplified from chromosomal DNA using primers MOB998 and MOB999 and Vent DNA polymerase. The plasmid vector pKHKS303 was constructed in our laboratory by PCR amplifying pBCKS using primers B1830 and A1161 and Taq DNA polymerase as previously described (36). The resulting linear pBCKS DNA fragment lacking the ColE1 ori was ligated to a T4 DNA polymerase-treated 888-bp XmnI-HindIII DNA fragment from pACYC184, which encodes the p15A ori. The final recombinant plasmid is compatible with plasmids bearing the ColE1 ori that are not Cmr. PCR amplified hns was ligated to Smal-digested pKHKS303 to form plasmid pCC037, which was verified by restriction enzyme digestion analysis and subjected to DNA sequencing of the insert fragment in both directions using pBluescriptSK and -KS primers by dyeoxy chain termination (30) at the Biopolymer Core Facility at University of Maryland, Baltimore, with an Applied Biosystems model 373A automated DNA sequencer using the Big Dye Terminator Cycle Sequencing Kit.

The derepression of ureR promoter activity was overcome

**TABLE 1.** Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain, plasmid or oligonucleotide</th>
<th>Genotype or relevant characteristics</th>
<th>Reference or source</th>
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</thead>
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<tr>
<td><strong>E. coli</strong></td>
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<td>DH5α</td>
<td>F$^{-}$d80lacZAM15 $\Delta$(lacZYA-argF)]U169 endA1 recA1 hsdR17($^{mK_}$ $^{mC_}$) deoR thi-1 supE44 $\lambda$ $^{gy}$A96 relA</td>
<td>Gibco-BRL</td>
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<td>MC4100</td>
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<td>ATM121</td>
<td>MC4100 hns::Tn10 Tc$^r$</td>
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<td><strong>Plasmids</strong></td>
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<td>pACYC184</td>
<td>Cloning vector, ori p15A Cmr Tc$^r$</td>
<td>New England Biobals</td>
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<td>Cloning vector, ori ColE1 Cmr$^+$</td>
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<td>pBluescriptKS and -SK</td>
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<td>pKHKS303</td>
<td>Cloning vector, ori p15A Cmr$^+$</td>
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<td>pLC9801</td>
<td>pMLB1034-based ureR-lacZ protein fusion construct, ori ColE1 Ap$^+$</td>
<td>This work</td>
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<td>pLX2106</td>
<td>lacZ operon fusion vector, ori p15A Cmr$^+$</td>
<td>This work</td>
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<td>pCC002</td>
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<td>This work</td>
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<td>pBluescriptSK$^+$ containing the ureR-ureD IR, ori ColE1 Ap$^+$</td>
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<td>pCC037</td>
<td>pKHKS303 containing PCR-amplified hns from P. mirabilis under T7 promoter control, ori p15A Cmr$^+$</td>
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<td>pCC050</td>
<td>pLX2106 containing PCR-amplified ureR P2 promoter including preceding poly(A) tract DNA, ori p15A Cmr$^+$</td>
<td>This work</td>
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<td>pCC051</td>
<td>pLX2106 containing PCR-amplified ureR P2 promoter lacking poly(A) tract DNA, ori p15A Cmr$^+$</td>
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<td>MOB1069</td>
<td>5$'${GAATTTCTGTTGGTAGTGGTTGTAATA$3'_3'}; primer anneals to the IR of the ureD start codon</td>
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$^a$The BamHI restriction site is underlined.

$^b$The EcoRI restriction site is underlined.
by in trans complementation with cloned *P. mirabilis* hns on pCC037 but not by a vector control plasmid. Full repression, compared to MC4100(pLC9801), however, was not observed (Fig. 2A). *P. mirabilis* hns is predicted to encode a polypeptide product that differs from *P. vulgaris* H-NS at only one amino acid residue (leucine 115 in *P. vulgaris* and proline 115 in *P. mirabilis*) (17). The predicted amino acid sequence is 71% identical to H-NS encoded by *E. coli* (17). Interestingly, the proline residue at position 115 in *P. mirabilis* H-NS is conserved in other H-NS polypeptides within the *Enterobacteriaceae* (1, 17).

A plasmid expressing ureR (pCC002) that is compatible with pLC9801 was constructed in two steps. pAR10 ureD-lacZ (containing intact ureR [11]) was digested with PstI and AluI to release an ~1.1-kb DNA fragment which was gel purified and ligated to PstI- and EcoRV-digested pBluescriptSK to form pCC001. After digestion of pCC001 with BamHI and HindIII the ~1.1-kb DNA fragment encoding ureR was ligated directionally to BamHI-HindIII-digested pACYC184 to form pCC002. In this construct the promoter and regulatory elements of ureR have been deleted and ureR is under control of the promoter for the Te' determinant of pACYC184.

When ureR was provided in trans on plasmid pCC002, ureR promoter expression was inducible by urea in both H-NS+ and H-NS− host backgrounds as expected; however, overall activity was increased in the H-NS− background compared to the H-NS+ background host (Fig. 2B). Interestingly, ureR promoter expression was not significantly different (*P = 0.072*) in the H-NS+ background when induced by urea compared to expression in the H-NS− background without urea induction (Fig. 2B). Unaccountable factors such as plasmid DNA supercoiling, or other repressors such as the hns analogue stpA (1, 38), could play a role in ureR repression. An *hns* knockout in *P. mirabilis* will be valuable in assessing the role of hns in ureR expression; however, attempts to create an *hns* knockout in *P. mirabilis* have not been successful. This approach has been hampered due to the nominal size of the open reading frame that encodes hns (405 bp) and the lack of availability of DNA sequences flanking hns.

The poly(A) tract DNA sequence is responsible for ureR repression in an hns-dependent manner. We hypothesized that H-NS binds to the poly(A) tract DNA sequence preceding the ureR P2 promoter and prevents transcription of ureR. According to this model, we predicted that ureR promoter repression would be relieved in an H-NS+ host if the poly(A) tract DNA was removed.

Operon fusion constructs consisting of the ureR P2 promoter region, either containing or lacking the poly(A) tract DNA sequence preceding the P2 promoter, were constructed in multiple steps. An approximately 0.6-kb DNA fragment consisting of a 492-bp IR flanked by the divergently oriented ureR and ureD promoters was isolated from pAR10ureD-lacZ (11) on a BamHI-NruI restriction digest DNA fragment and ligated to BamHI-EcoRV restriction-digested pBluescriptKS+ to form pCC007 (Fig. 1). PCR products were amplified from pCC007 using *Pfu* polymerase and primer pairs MOB905/KS and MOB907/KS. The PCR product amplified with the MOB905/KS primers consisted of a 359-bp fragment that included the P2 promoter region preceded by 7 bp, while the PCR product amplified using the MOB907/KS primer pair consisted of a 312-bp fragment that lacked the poly(A) tract region and 6 bp downstream. These products were ligated to the operon fusion vector pHZ2106 that was constructed in our laboratory by digesting pRS415 (33) with SacI and *EagI* to release an ~8.0-kb DNA fragment that was gel purified and ligated to *EagI* and EcoRV-digested pACYC184. This fusion vector contains a transcriptional terminator upstream from the multiple cloning site used for insertion of exogenous DNA promoter sequences, is CmR, and is compatible with ColE1 replication. The resulting recombinant plasmids pCC050 and pCC051 were sequenced to verify the orientation of the insert DNA and to confirm that lacZ would be under ureP2 promoter control. Recombinant plasmid pCC050 is an operon fusion construct that contains 7 nucleotides upstream from the poly(A) tract DNA and the ureR P2 promoter sequences fused to the β-galactosidase gene (Fig. 1). pCC051 is an isogenic plasmid that lacks the poly(A) tract DNA (Fig. 1).

As shown in Fig. 2C, ureR promoter expression in MC4100 (pCC050) was about fourfold (*P < 0.0001*) less than that in ATM121(pCC050). This pattern of ureR expression was similar to that seen when pLC9801 was used as the reporter construct plasmid in these host strains. In contrast, MC4100 and ATM121 harboring pCC051 (which lacks the poly(A) tract DNA) had no difference in expression from MC4100 with pLC9801 (Fig. 2D). This is consistent with the model that the poly(A) tract DNA sequence is responsible for ureR repression and that H-NS binds to this tract DNA sequence.

**FIG. 1.** Cloning strategy used to generate ureR-lacZ fusion constructs. Cloning strategies are described in the text. Thin arrows denote the direction of transcription from ureR and ureD promoter regions; thick arrows represent primers used in PCR amplification procedures (see Table 1). lacZ reporter genes. Restriction endonuclease sites, poly(A) tracts of DNA, and the −10 and −35 regions comprising the P2 promoter are boldfaced.
ureR-lacZ operon fusion recombinants either containing or lacking a poly(A) tract DNA sequence. MC4100 (hns) were representative of at least three experiments. Error bars represent 2 standard deviations for triplicate samples. The data are representative of at least three experiments. (C) ureR expression from E. coli MC4100 and its hns::Tn10 isogenic derivative strain ATM121 harboring a ureR-lacZ protein fusion construct. (A) Bacterial cultures were used to measure β-galactosidase activity (Miller units on y-axis) (21), after the following E. coli strains were grown to mid-log phase at 37°C: MC4100(pLC9801), ATM121(pLC9801), ATM121(pKHKS303) (pLC9801), and ATM121(pLC9801)pCC038). pLC9801 encodes a ureR-lacZ operon fusion. pKHKS303 is the plasmid vector used to clone P. mirabilis hns. Error bars represent 2 standard deviations for triplicate samples. The data are representative of at least three experiments. (B) ureR expression from E. coli MC4100 and its hns::Tn10 isogenic derivative strain ATM121 harboring a ureR-lacZ protein fusion recombinant in the presence of urea. ureR expression from E. coli MC4100 (pCC050) and ATM121(pCC050) were grown as the strains in panel A but in the presence (+) or absence (−) of 100 mM urea. pCC002 encodes ureR, and pLC9801 encodes a ureR-lacZ operon fusion. Error bars represent 2 standard deviations for triplicate samples. The data are representative of at least three experiments. (C) ureR expression from E. coli MC4100 and its hns::Tn10 isogenic derivative strain ATM121 harboring ureR-lacZ operon fusion recombinants either containing or lacking a poly(A) tract DNA sequence. MC4100 (hns+) harboring either pCC050, which contains the poly(A) sequence, or pCC051, which lacks the poly(A) sequence (see Fig. 1 for details) and ATM121 (hns mutant) harboring either pCC050 or pCC051 were grown as the strains in panel A. Error bars represent 2 standard deviations for triplicate samples. The data are representative of six experiments.

DNA sequence present in pCC050] exhibited similar ureR promoter expression (Fig. 2C).

Poly(A) tracts occurring in DNA have been shown to result in DNA bending which can dampen gene expression (16, 18, 27) and are also known to be binding sites for the histone-like nucleoid structuring protein H-NS (37). H-NS is responsible for repression of virulence gene promoters at the level of transcription in many bacterial genera including Escherichia, Shigella, and Salmonella spp. (1). Importantly, the presence or absence of the poly(A) tracts makes no difference for ureR expression in an H-NS− background. The ureR promoter is derepressed in an H-NS-dependent manner, and this repression is contingent on the presence of a poly(A) tract of DNA upstream from the ureR P2 promoter.

Expression of P. mirabilis urease is derepressed in an hns mutant of E. coli. Since UreR is required for transcriptional activation of the P. mirabilis urease gene cluster, we postulated that urease expression would be elevated in an hns mutant host. MC4100 and ATM121 harboring recombinant plasmid pMID1010 (encoding the wild-type P. mirabilis urease gene cluster) produced equivalent amounts of urease (10, 24) when induced with urea (Fig. 3A). In contrast, in the absence of urea, urease activity was significantly greater (8.4-fold; P = 0.0001) in ATM121(pMID1010) compared to the isogenic wild-type strain MC4100(pMID1010), although full urease expression (relative to urea-induced urease expression) was not achieved (Fig. 3B).

This result emphasizes the fact that full urease expression requires both urea and UreR even in an hns-negative background. Since ureR is significantly expressed in the hns-deficient background in the presence of urea and absence of urea (Fig. 2A), we propose that the increased urease activity from ATM121(pMID1010) in the absence of urea is due to binding of UreR in a nonspecific manner to the ureD promoter region and spontaneous low-level activation of the urease gene cluster. In the presence of urea, the ureD promoter region is most likely saturated with UreR, present in its transcription activation state; thus, urease activities in H-NS+ and H-NS− backgrounds are comparable. Furthermore, H-NS gene repression can be fully overcome in the presence of an inducer specific for H-NS-repressed genes. Examples include H-NS repression of

FIG. 2. ureR expression from E. coli MC4100 and its hns::Tn10 isogenic derivative strain ATM121 harboring a ureR-lacZ protein fusion construct. (A) Bacterial cultures were used to measure β-galactosidase activity (Miller units on y-axis) (21), after the following E. coli strains were grown to mid-log phase at 37°C: MC4100(pLC9801), ATM121(pLC9801), ATM121(pKHKS303) (pLC9801), and ATM121(pLC9801)pCC038). pLC9801 encodes a ureR-lacZ operon fusion. pKHKS303 is the plasmid vector used to clone P. mirabilis hns. Error bars represent 2 standard deviations for triplicate samples. The data are representative of at least three experiments. (B) ureR expression from E. coli MC4100 and its hns::Tn10 isogenic derivative strain ATM121 harboring a ureR-lacZ protein fusion recombinant in the presence of urea. ureR expression from E. coli MC4100 (pCC050) and ATM121(pCC050) were grown as the strains in panel A but in the presence (+) or absence (−) of 100 mM urea. pCC002 encodes ureR, and pLC9801 encodes a ureR-lacZ operon fusion. Error bars represent 2 standard deviations for triplicate samples. The data are representative of at least three experiments. (C) ureR expression from E. coli MC4100 and its hns::Tn10 isogenic derivative strain ATM121 harboring ureR-lacZ operon fusion recombinants either containing or lacking a poly(A) tract DNA sequence. MC4100 (hns+) harboring either pCC050, which contains the poly(A) sequence, or pCC051, which lacks the poly(A) sequence (see Fig. 1 for details) and ATM121 (hns mutant) harboring either pCC050 or pCC051 were grown as the strains in panel A. Error bars represent 2 standard deviations for triplicate samples. The data are representative of six experiments.

FIG. 3. Urease expression from E. coli MC4100(pMID1010) and ATM121 (pMID1010) cultured in the presence or absence of urea. E. coli MC4100 and ATM121 harboring pMID1010 (encoding the P. mirabilis urease gene cluster) were grown to late exponential phase in the presence of 50 mM urea (A) or absence of urea (B). Soluble protein (1.0 mg) from bacterial French press lysates was used to measure urease activity in the phenol red spectrophotometric assay. Error bars represent 2 standard deviations for triplicate samples. The data are representative of three experiments. Note different scales in the two panels.
genes encoding the CFA/I pilus of E. coli overcome by the positive activator CfaD (15), repression of CS-1 pili by Rns (25), pap gene repression overcome by PapB (6), and VirF activation of the Shigella virB locus which is repressed by H-NS (35). Interestingly, in H-NS-negative backgrounds, some genes still require the presence of their cognate activator and/or inducer for full expression (P. mirabilis ureR and ureD [this study], Shigella virB [35], and E. coli CS-1 pilus genes [25]) whereas for other genes (E. coli pap [6] and cfa [15] genes) their cognate activator proteins are not required for full expression in the absence of hns.

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REFERENCES