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 A₉T₇A₇C₇TGTTAG₇A₉G₇A₉G₇A₉, is located 16 bp upstream of the α²⁰-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 (hnsΔTn10) (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 a sevenfold 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 pMID1010 (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.

*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 infection with *P. mirabilis* is the formation of kidney and bladder stones due to the rise of pH in the urine caused by the hydrolysis of urea by *P. mirabilis* urease (urea amidohydrolase EC 3.5.1.5) (8, 9).

Urease produced by *P. mirabilis* contributes to virulence in an animal model of ascending urinary tract infection (12). A urease-negative mutant of *P. mirabilis* was unable to persist in the urinary tract and caused less histological damage in a mouse model of infection (12) compared to the isogenic wild-type strain. Urea, present at concentrations of up to 500 mM in human urine (8), induces urease production by the bacterium (29). Cultures of wild-type organisms produce only low levels of urease in vitro in the absence of urea induction (13).

The genetic basis for urease induction has been characterized. Urea serves as a cofactor in the transcriptional activation of the urease gene cluster in *P. mirabilis* (3, 11, 26). In the presence of urea, UreR, an AraC-like positive activator (3, 4, 5, 11, 26), promotes transcription of genes required for the synthesis of urease structural and accessory proteins. These respective polypeptides make up the urease apoenzyme and are responsible for nickel incorporation into the apoenzyme to produce active holoenzyme (22). The direct mechanism of activation of the urease gene cluster is not known; however, it is postulated that UreR changes conformation or forms multimeric complexes upon urea binding and is able to bind avidly to specific DNA sequences in the region of the *ureD* promoter and up-regulate urease gene expression. AraC-like transcriptional activators are hypothesized to act by interacting directly with RNA polymerase, thus promoting transcription (28). The urease gene cluster in *P. mirabilis* is organized such that *ureR* is divergently transcribed relative to the genes encoding urease structural and accessory proteins, the first of which is *ureD*. An intergenic region (IR), consisting of 492 bp of DNA, separates the start codons for *ureR* and *ureD* and has been shown to contain promoter-like sequences for each of these genes (3, 34). Using a gel shift assay, D’Orazio et al. (3) showed that the intergenic region from the homologous plasmid-encoded urease gene cluster found in *Providencia stuartii*, *Escherichia coli*, and *Salmonella* species exhibits decreased mobility in polyacrylamide electrophoresis gels when incubated with whole-cell extracts from *E. coli* expressing a UreR-His-Tag fusion protein, implying that UreR binds directly to DNA sequences in the intergenic region (3, 34). Putative promoters for both *ureR* and *ureD*, based on primer extension studies and analysis of intergenic region deletion constructs, have been assigned according to the results obtained in the aforementioned study. Curiously, the *ureR* P2 promoter exhibits a strong *E. coli* α⁷⁰-like promoter sequence (TTGTTA–17 bp–TATATT; 4 of 6 bp matches for consensus 35 and 35 sequences, respectively), yet *ureD* does not appear to be expressed at significant levels even when present on multicopy plasmids in *E. coli* (unpublished observations).

In this study, we investigated the mechanism of repression of *ureR* and showed that the presence of the histone-like nucleoid structuring protein gene, *hns* (polypeptide product is H-NS), is responsible for repression of the *ureR* promoter in *E. coli*. We also demonstrated that *P. mirabilis* hns is able to restore repression of the *ureR* promoter P2 when provided in trans in an H-NS-deficient host background and that the poly(A) tract nucleotide sequence located upstream of P2 contributes to repression of P2 in an H-NS-dependent manner. All strains, plasmids, and oligonucleotide primers used in this study are described in Table 1, and cloning procedures were performed as described elsewhere (19).

(A preliminary report of this work has appeared previously...
ureR promoter expression is derepressed in the absence of hns. There is a poly(A) tract DNA sequence consisting of A₉T₉A₉C₉T₉G₉T₉A₉G₉A₉T₉G₉A₉ preceding the EcoRV restriction digest site present in the IR (Fig. 1). Immediately downstream (16 bp) of the poly(A) tract sequence is a putative 70-like promoter consisting of the DNA sequence TTGT and an EcoRI restriction site is underlined. 

The derepression of ureR promoter expression (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 ureD start codon 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 SmaI-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 dideoxy 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 by dideoxy 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
by in trans complementation with cloned \textit{P. mirabilis} \textit{hns} on pCC037 but not by a vector control plasmid. Full repression, compared to MC4100(pLC9801), however, was not observed (Fig. 2A). \textit{P. mirabilis} \textit{hns} is predicted to encode a polypeptide product that differs from \textit{P. vulgaris} H-NS at only one amino acid residue (leucine 115 in \textit{P. vulgaris} and proline 115 in \textit{P. mirabilis}) (17). The predicted amino acid sequence is 71\% identical to H-NS encoded by \textit{E. coli} (17). Interestingly, the proline residue at position 115 in \textit{P. mirabilis} H-NS is conserved in other H-NS polypeptides within the \textit{Enterobacteriaceae} (1, 17).

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

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

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

Operon fusion constructs consisting of the \textit{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 \textit{ureR} and \textit{ureD} promoter regions was isolated from pAR10\textit{ureD-lacZ} (11) on a \textit{BamHI}-\textit{NruI} restriction digest DNA fragment and ligated to \textit{BamHI}-\textit{EcoRV} restriction-digested \textit{pBluescriptKS}\textsuperscript{+} to form pCC007 (Fig. 1). PCR products were amplified from pCC007 using \textit{Pfu} polymerase and primer pairs MBO905/KS and MBO907/KS. The PCR product amplified with the MBO905/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 MBO907/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 pL2X2106 that was constructed in our laboratory by digesting pRS415 (33) with \textit{SacI} and \textit{EagI} to release an ~8.0-kb DNA fragment that was gel purified and ligated to \textit{EagI}- and \textit{EcoRV}-digested \textit{pACYC184}. This fusion vector contains a transcriptional terminator upstream from the multiple cloning site used for insertion of exogenous DNA promoter sequences, is Cm\textsuperscript{r}, and is compatible with CoE1 plasmids. The resulting recombinant plasmids pCC050 and pCC051 were sequenced to verify the orientation of the insert DNA and to confirm that \textit{lacZ} would be under \textit{ureR} P2 promoter control. Recombinant plasmid pCC050 is an operon fusion construct that contains 7 nucleotides upstream from the poly(A) tract DNA and the \textit{ureR} P2 promoter sequences fused to the \textit{β}-galactosidase gene (Fig. 1). pCC051 is an isogenic plasmid that lacks the poly(A) tract DNA (Fig. 1).

As shown in Fig. 2C, \textit{ureR} promoter expression in MC4100 (pCC050) was about fourfold ($P < 0.0001$) less than that in ATM121 (pCC050). This pattern of \textit{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) responded much more weakly to induction by urea.
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) tract DNA sequence present in pCC050] 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 urease gene cluster (21), after the following E. coli strains were grown to mid-log phase at 37°C: MC4100(pCC002)(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(pLC9801), and ATM121(pLC9801)(pCC038). pLC9801 encodes a ureR-lacZ operon fusion. pKHKS303 is the plasmid vector used to clone P. mirabilis hns.

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(pCC002)(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. pCC038 encodes 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. 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 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.

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 pil of E. coli overcome by the positive activator CfaD (15), repression of CS-1 pilus overcome 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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