Characterization of the Opposing Roles of H-NS and TraJ in Transcriptional Regulation of the F-Plasmid \textit{tra} Operon

William R. Will and Laura S. Frost*

Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

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The transfer (\textit{tra}) operon of the conjugative F plasmid of \textit{Escherichia coli} is a polycistronic 33-kb operon which encodes most of the proteins necessary for F-plasmid transfer. Here, we report that transcription from \textit{P}_{\text{PY}}\text{, the \textit{tra} operon promoter, is repressed by the host nucleoid-associated protein, H-NS. Electrophoretic mobility shift assays indicate that H-NS binds preferentially to the \textit{tra} promoter region, while Northern blot and transcriptional fusion analyses indicate that transcription of \textit{traY}, the first gene in the \textit{tra} operon, is derepressed in an \textit{hns} mutant throughout growth. The plasmid-encoded regulatory protein TraJ is essential for transcription of the \textit{tra} operon in wild-type \textit{Escherichia coli} ; however, TraJ is not necessary for plasmid transfer or \textit{traY} operon transcription in an \textit{hns} mutant. This indicates that H-NS represses transcription from \textit{P}_{\text{PY}}, directly and not indirectly via its effects on TraJ levels. These results suggest that TraJ functions to disrupt H-NS silencing at \textit{P}_{\text{PY}}\text{, allowing transcription of the \textit{tra} operon.}

Bacterial conjugation allows the transfer of DNA from a donor to a recipient cell by way of a complex conjugative apparatus composed of an F-type IV secretion system and auxiliary proteins for DNA metabolism and regulation (29). In the case of the conjugative F plasmid of \textit{Escherichia coli}, the expression of genes encoding components of this apparatus is extremely growth phase dependent, dropping to undetectable levels as donor cultures enter stationary phase (20, 56). This was recently shown to be due, at least in part, to growth phase-dependent transcriptional silencing by the host nucleoid-associated protein, H-NS (56). H-NS is uniquely suited to regulating transcription in a wide array of mobile genetic elements, since it shows a preference for intrinsically curved, AT-rich DNA, which is a common feature of many promoters (14, 40, 42). H-NS binds preferentially to these curved sequences and nucleates outwards, silencing local promoters (4, 58). Whereas cellular H-NS levels are relatively static throughout growth, at approximately 20,000 molecules per cell, the regions of curvature to which H-NS binds are dynamic, responding to both environmental and nutritional cues via fluctuations in chromosomal supercoiling and protein binding (17, 58). This property allows it to repress many genes, including those acquired via mobile elements in a dynamic, physiologically responsive manner. As a result, the cell may be capable of avoiding nonessential gene expression when under environmentally or nutritionally limiting conditions.

F-plasmid transfer gene expression is regulated by three plasmid-encoded regulators: TraJ, TraY, and TraM (Fig. 1A) (19). TraJ is the primary activator, expressed from its own monocistronic operon. It is required for transcription of the 33-kb transfer (\textit{tra}) operon, which encodes all of the components of the transfer apparatus, from the \textit{P}_{\text{PY}} promoter (46, 57). In most F-like plasmids, \textit{traJ} is subject to posttranscriptional regulation via the FinOP antisense RNA system (55). However, the gene encoding the RNA chaperone, \textit{finO}, is disrupted in the F plasmid by the insertion of an IS3 element, which results in the subsequent derepression of TraJ synthesis (7). The first gene in the \textit{tra} operon, \textit{traY}, encodes the secondary regulator of \textit{P}_{\text{PY}}, TraY. Early results suggested that TraY activates \textit{tra} operon expression, although more recent studies suggest that TraY can also act as a repressor (45, 52). TraY also activates expression of \textit{traM}, a monocistronic operon upstream of \textit{traJ} which encodes the autorepressor TraM (41). In addition to its regulatory role, TraY is essential for mating, since it is a critical component of the relaxosome, a nucleosomal complex that forms at the plasmid origin of transfer and is responsible for nicking and unwinding the plasmid DNA in preparation for transfer (26, 36). TraM is essential for transfer and is part of the mature relaxosome (15). It binds the coupling protein, TraD, thereby linking the relaxosome to the transferosome, a protein complex that spans the cell envelope and forms the channel for DNA transport during conjugation (12, 30).

The mechanism for controlling transcription from \textit{P}_{\text{PY}}, the \textit{tra} operon promoter, remains undefined. Earlier studies hypothesized that a nucleosomal complex forms upstream of \textit{P}_{\text{PY}}, altering local supercoiling and repressing transcription (22). While the exact mechanism of TraJ activity has not been elucidated, it has been suggested that TraJ opposes the formation of this complex to allow the initiation of transcription from \textit{P}_{\text{PY}} (22). In this study, we present data suggesting that H-NS forms the repressor complex previously hypothesized, repressing the \textit{tra} operon promoter, \textit{P}_{\text{PY}}, in a growth-phase-dependent manner, as previously observed for the F-plasmid promoters, \textit{P}_{\text{PY}} and \textit{P}_{\text{tr}} which are responsible for driving the transcriptions of \textit{traM} and \textit{traJ}, respectively (56). Furthermore, we present evidence suggesting that the primary \textit{tra} activator, TraJ, serves at least in part to counter the repressive effects of H-NS at the \textit{P}_{\text{PY}} promoter.

MATERIALS AND METHODS

Strains and growth conditions. All strains used in this study are described in Table 1. In the case of PD32, the \textit{hns} mutant strain, the mutation was freshly made via P1 phage transduction, or the strain was grown from glycerol stocks that had been frozen and stored at −80°C immediately following transduction.
Unless otherwise indicated, all cultures were grown on Luria-Bertani (LB) broth or LB agar plates at 37°C. Antibiotics were added as indicated in selective media at the following concentrations: ampicillin, 25 μg/ml; chloramphenicol, 20 μg/ml; spectinomycin, 100 μg/ml; streptomycin, 200 μg/ml; and tetracycline, 10 μg/ml.

Plasmid construction. All plasmids and oligonucleotides used in this study are described in Table 1. Transcriptional fusions were constructed by cloning the promoter fragments into the RK2/RP4 replicon-based pJLac101, a lacZ transcriptional fusion vector (56). pRWPY101 was generated by cloning a fragment amplified by PCR from pOX38-Tc using the primers RWI59 and RWI63 into the

FIG. 1. (A) The F-plasmid regulatory circuit. A positive effect on transcription is indicated by dashed lines ending in arrowheads, whereas a negative effect is indicated by solid lines ending in bars. Conflicting evidence suggests that TraY can act as both an activator and a repressor at the tra operon promoter, P, and this is indicated by a dashed line ending in both an arrowhead and a bar. The traY, traJ, and finP promoters are indicated by P, P, and P, respectively. Transcription of traM is driven by two promoters, indicated by P and P. The traM, traJ, and finP transcriptional terminators are indicated by T, T, and T, respectively. TraM binding sites are labeled as sbmA, B, and C, whereas TraY binding sites are labeled as sbyA, B, and C. Other regulatory factors not directly relevant to this study have been omitted. (B) The effect of H-NS on tra operon promoter activity was examined using lacZ transcriptional fusion constructs. The coordinates of the promoter fragment termini relative to the transcriptional start site at P ( +1) are indicated. The sizes and positions of the promoter fragments relative to the genetic map above are indicated by solid lines. Standing overnight cultures of MC4100 and PD32 containing each construct were diluted into fresh LB broth and incubated with shaking at 37°C. Samples were taken at 3 and 7 h of growth, and β-galactosidase activity assays were then performed. The results are given in Miller units (32) and indicate the averages and standard deviations from three separate experiments.

<table>
<thead>
<tr>
<th>Strain, plasmid, or oligonucleotide</th>
<th>Genotype, description, or sequence</th>
<th>Reference or source</th>
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<tbody>
<tr>
<td>Strains</td>
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<tr>
<td>ED24</td>
<td>F- Lac- Spc'</td>
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<tr>
<td>MC4100</td>
<td>F- araD139 Δ(argF-lac)U169 rpsL150(Str') relA1fb5301 deoC1 ptsF25 rbsR</td>
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<td>Plasmids</td>
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<td>Flac</td>
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<td>Flac traJ90</td>
<td>traJ, lac' F derivative</td>
<td>1</td>
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<td>pBR322</td>
<td>General cloning vector</td>
<td>New England Biolabs</td>
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<tr>
<td>pJLac101</td>
<td>pPR9TT-1-derived transcriptional fusion-based promoter assessment plasmid</td>
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<tr>
<td>pRWPY101</td>
<td>pJLac101 with F P (including the traY ORF and part of traJ) fused to lacZ</td>
<td>This work</td>
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<td>A2428</td>
<td>5'CGCTTCTGTTAATTCCTGCTTAC'</td>
<td></td>
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<tr>
<td>RWI79</td>
<td>5'GACCCCGAATCACTTCATAATGCT3'</td>
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BglII and KpnI sites of pLac101, pRW102 and pRW103 were similarly constructed using the primer pairs RW158-RW163 and RW159-RW162, respectively.

**DNA curvature prediction.** Intrinsic DNA curvature and flexibility predictions were performed using the BEND-IT server (http://www.igebr.org/dna/bend_it.html), which uses the BEND algorithm to predict intrinsic curvature (24, 35). Predictions were performed using both DNAse I and consensus-based parameters, with a window of 31 bp, and the results were compared. Regions with a predicted curvature of greater than 5° per helical turn (10.5 bp) as determined by use of the BEND algorithm are considered to have significant intrinsic curvature.

**Competitive electrophoretic mobility shift assays.** To determine whether or not H-NS bound P_\text{y}, preferentially, competitive electrophoretic mobility shift assays were performed. For a target, a 386-bp fragment of the P_\text{y} region, stretching from the -260 position to the +129 position relative to P_\text{y} and containing the predicted bends, was amplified via PCR using oligonucleotides RW179 and A2428. Approximately 100 ng of the target fragment was mixed in an equimolar ratio with pBR322 digested with TcI and SpI and then incubated with increasing concentrations of pure native H-NS (kindly provided by S. Rimsky, Université Paris XI). Complexes were incubated and resolved as previously described (56).

**Northern blot analysis.** All cultures were diluted 200-fold from standing overnight cultures grown at 37°C into fresh LB broth containing streptomycin for the MC4100 host strain cultures and ampicillin for the PD32 host strain cultures. These cultures were incubated on a shaker at 37°C, and at the appropriate time points, cell samples with an optical density at 600 nm (OD_{600}) of 1.0 were removed and immediately snap-frozen in a bath of dry ice and ethanol. Total cellular RNA was extracted from these samples using the hot-phenol method (27). The RNA was purified with an RNase-free Qiagen column. Approximately 100 ng of the target fragment was digested in 15% agarose gel containing 5% formaldehyde in MOPS (morpholinepropanesulfonic acid) buffer, and the gel was then transferred to a Zeta-Probe membrane (Bio-Rad) and the membrane was dried and cross-linked as previously described (56). Transfer and loading were checked by staining the membrane with Blot Stain Blue (Sigma-Aldrich). Blots were prehybridized at 55°C for 4 h in hybridization solution (2.5x SSC [1x SSC is 0.15 M NaCl plus 0.015 M Na-citrate]). Denatured DNA (1% [wt/vol] sodium dodecyl sulfate [SDS], 90 mM Tris-HCl [pH 7.5], 0.9 M NaCl, 6 mM EDTA, 200 μg·mL^{-1} E. coli strain W trnRNA type XX [Sigma], and 200 μg·mL^{-1} sonicated, boiled calf thymus DNA [Sigma]). RW178, an oligonucleotide specific to trnY, was then 5' end labeled with γ-32P ATP using polynucleotide kinase (Roche), and the unincorporated label was removed using Quick Spin oligonucleotide columns (Roche). The blot was then probed overnight in fresh hybridization buffer containing approximately 20 pmol of end-labeled RW178 at 55°C. The blot was washed as previously described, except that the final wash was performed at 55°C (43). After being washed, the blots were dried and exposed on a phosphor storage screen (GE Healthcare).

**β-Galactosidase assays.** β-Galactosidase assays to determine the activity of P_\text{y}-lacZ transcriptional fusions were performed as described by Miller (32). Standing overnight cultures containing the fusion constructs were diluted into fresh LB broth containing chloramphenicol, and samples were assayed at the indicated time points. MC4100 cultures were diluted 1:200, whereas PD32 cultures were diluted only 1:100 so as to compensate for their slower emergence from stationary phase. Results were corrected for basal vector activity in the absence of a foreign promoter and represent the averages and standard deviations from at least three independent experiments.

**Mating assays.** MC4100 and PD32 donor cultures containing pOX38-Tc, Flac, or Flac trn90, along with ED24 recipient cell cultures, were diluted 200-fold from standing overnight cultures grown at 37°C into fresh LB broth and grown to an OD_{600} of approximately 0.6. Male donor cells were mixed with 0.1% trp2 lacD+ (pBR322) recipient cultures and grown to a final density of 1.0 mL. The reaction volumes were incubated at 37°C for 1 h, vortexed vigorously to disrupt mating pairs, and then placed on ice for 5 minutes to halt cell division. The reaction volumes were then serially diluted in SSC buffer (150 mM NaCl, 15 mM Na-citrate) and plated on the appropriate media to select for diploids from mating pairs. Mating efficiency was expressed as the number of transconjugants per donor cell.

**Immunoblot analysis.** Cultures were diluted 200-fold from standing overnight cultures into fresh LB broth containing streptomycin and grown to an OD_{600} of approximately 0.6. Samples (OD_{600} 0.1) were then collected and pelleted. Cell pellets were boiled in SDS sample buffer for 5 min and then electrophoresed in a 15% SDS-polyacrylamide gel electrophoresis gel. Samples were then transferred to an Immobilon-P membrane (Millipore) in Towbin buffer (53). Membranes were incubated at 4°C overnight in blocking solution containing 10% powdered skim milk (Dilco) dissolved in TBST (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, and 0.1% Tween 20). The blocked membranes were then incubated in fresh blocking solution containing either polyclonal anti-TraY antiserum diluted 1:2,000 or polyclonal anti-TraM antiserum diluted 1:10,000, for 1 h at room temperature. Membranes were then washed four times for 10 min each in TBST. After being washed, the membranes were then incubated in fresh blocking solution containing diluted secondary antibody (1:10,000-diluted horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin G [Amersham Biosciences]) for 1 h at room temperature and then washed as described above. The membranes were developed using Western Lightning Chemiluminescence Reagent Plus (Perkin-Elmer) and exposed with X-OMAT AR film (Kodak).

**RESULTS**

**H-NS binds to intrinsically curved DNA at P_\text{y}**. Since H-NS binds preferentially to regions of intrinsic curvature (40), we examined the P_\text{y} region for predicted intrinsic curves with the BEND-IT curvature prediction program (http://www.igebr.org/dna/bend_it.html). BEND-IT identified approximately five regions with curvature of approximately 6° or greater per helical turn near P_\text{y} (Fig. 2). The bend center of Y1 is located at position −152 relative to P_\text{y}, upstream of the traM stop codon. The bend center of Y2, located at position −74, overlaps the traM stop codon, and Y3 is immediately downstream, centered at position −67. Y2 and Y3 also overlap the binding site for the positive activator, ArcA (31, 51), as well as an inverted repeat which has been identified as a binding site for TraI in the F-like plasmid R100 (52). The final two regions of significant curvature, Y4 and Y5, are centered at +108 and +154, respectively, within the traY gene.

To determine whether or not H-NS preferentially binds to this region, a competitive electrophoretic mobility shift assay was performed. Equimolar amounts of a PCR-amplified fragment containing the P_\text{y} target region with the five predicted bends and competitor DNA were mixed, incubated with increasing concentrations of H-NS, and separated on a polyacrylamide gel (Fig. 3). pBR322 digested with TcI and SpI was used as a competitor and a positive control, as one of the digested fragments contains the bla promoter, which is known to display significant curvature and be bound preferentially by H-NS (59). H-NS bound the P_\text{y} target at lower concentrations than the bla positive control fragment, suggesting that H-NS specifically binds this region.

**H-NS down-regulates transcription from P_\text{y}**. Although previous studies have shown that TraY levels are increased in hns donor cultures as they enter stationary phase, this might reflect secondary effects due to an increase in cellular levels of the tra activator TraI (56). However, since H-NS preferentially bound the P_\text{y} region, H-NS might also directly repress transcription of the tra operon from P_\text{y}. The effect of H-NS on tra operon transcription was determined by isolating total cellular RNA at regular intervals throughout the growth curves from wild-type (MC4100) and hns (PD32) strains containing pOX38-Tc, a derivative of the F plasmid. The RNA was then used in Northern blot analysis with the end-labeled oligonucleotide RW178, a traY-specific probe. Transcript levels in wild-type donor cells decreased rapidly as the cells progressed through the growth cycle, reaching undetectable levels after 9 h (Fig. 4). In the hns
H-NS appears to limit transcriptional domains by binding and sequestering their respective promoter regions. Whereas traY transcript levels are merely prolonged in the hns donor cells, the longer transcripts appear to be increased in the hns mutant host, accumulating at later time points. This suggests that H-NS merely shuts off transcription from P_Y as cultures enter stationary phase, whereas transcription from upstream promoters, resulting in the larger transcripts, is actively repressed by H-NS, even during exponential growth.

To address whether H-NS acts directly upon P_Y or acts through traD, a series of P_Y-lacZ transcriptional fusions were constructed. Activity was assayed for both minimal and extended promoter regions, with and without a complete traY open reading frame (ORF) (Fig. 1B). pRWPY101 contains an extended promoter region that includes the 3’ half of the traD gene, all of the predicted curves from Y1 to Y5, and a functional traY gene to supply TraY. pRWPY102 contains a minimal promoter region, which lacks almost all of traD and Y1 and part of Y2 but contains a complete traY gene. pRWPY103 contains the extended promoter region of pRWPY101 but lacks a complete traY gene. A construct containing a minimal promoter without a functional traY gene was unstable, suggesting that the absence of either TraY protein or sequences within traY destabilized this construct. The β-galactosidase activity of each fusion was assayed in MC4100 and PD32 cells in exponential phase after 3 hours of growth and in early stationary phase after 7 hours of growth. The test strains did not contain the F plasmid, in order to prevent possible secondary effects from altered TraD levels in PD32. Mutation of hns in pRWPY101 resulted in a derepression of two of the three constructs, pRWPY101 and pRWPY103 (Fig. 1B). pRWPY102 exhibited no detectable activity in either strain. Whereas TraY is generally thought to be an activator of P_Y operon expression, (45), these transcriptional fusion results suggested an additional role as an autorepressor.

The P_Y region contains significant intrinsic curvature. The BEND-IT computer program (http://www.icgeb.org/dna/bend_it.html) was used to identify sequences of DNA with significant intrinsic curvature (greater than 6° per helical turn). Five significant bends were identified in the region examined and were designated Y1 through Y5. The sequence is numbered relative to the position of a BglII site at the start of the transfer region, and all other features are indicated as previously described (19).

FIG. 2. The P_Y region contains significant intrinsic curvature. The BEND-IT computer program (http://www.icgeb.org/dna/bend_it.html) was used to identify sequences of DNA with significant intrinsic curvature (greater than 6° per helical turn). Five significant bends were identified in the region examined and were designated Y1 through Y5. The sequence is numbered relative to the position of a BglII site at the start of the transfer region, and all other features are indicated as previously described (19).

FIG. 3. H-NS binds preferentially to the P_Y region. Competitive electrophoretic mobility shift assays were performed by incubating equimolar amounts of P_Y target DNA and TacI-SspI-digested pBR322 in increasing concentrations of H-NS, indicated above the gel, for 20 min at room temperature. The resulting complexes were then resolved by electrophoresis in a 7.5% Tris-borate-EDTA-polyacrylamide gel. Bound DNA forms higher-order complexes located at the top of the gel. The other bands are thought to bind H-NS with low affinity and are used as negative controls.
FIG. 4. H-NS represses traY transcription as donor cells approach stationary phase. (A) Standing overnight cultures of both MC4100 and PD32 containing pOX38-Tc were diluted into fresh LB and incubated at 37°C with shaking. At the indicated time points, culture samples, from which total cellular RNA was purified, were removed. This RNA was used in a Northern blot that was probed for traY-containing transcripts. The relative positions of a molecular weight marker are indicated on the right of the blot. traM/JY indicates a transcript which is detected by traM-, traJ-, and traY-specific probes and is predicted to be a transcriptional read-through product originating from P_M (data not shown). traYJ indicates a transcript which hybridizes with both traY- and traJ-specific probes (data not shown) and is predicted to be a transcriptional read-through product originating from P_J. traY indicates a transcript which is detected only by a traY-specific probe and is predicted to be the mature, processed traY transcript originating from P_Y. (B) 23S rRNA was visualized on membranes prior to hybridization by staining with Blot Stain Blue (Sigma-Aldrich) to check for loading and transfer quality. (C) The OD_{600} of the donor cultures were measured at each time point. Results for MC4100/pOX38-Tc are indicated by diamonds connected by solid lines, whereas data for PD32/pOX38-Tc are indicated by squares connected by dashed lines.

**TABLE 2.** Plasmid transfer of Flac traJ90 is partially restored in an hns host strain

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<th>Donor strain</th>
<th>Mating efficiency (transconjugants/donor)</th>
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<tr>
<td>MC4100/pOX38-Tc</td>
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<tr>
<td>PD32/pOX38-Tc</td>
<td>0.16</td>
</tr>
<tr>
<td>MC4100/Flac</td>
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<td>MC4100/Flac traJ90</td>
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<tr>
<td>PD32/Flac traJ90</td>
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**DISCUSSION**

In addition to its previously described effects on the traM and traJ promoters, H-NS also appears to repress F-plasmid transfer gene expression at the tra operon promoter, P_Y. Furthermore, our findings suggest a specific role for TraJ, the primary activator, in opposing H-NS-mediated repression of P_Y. Whereas TraJ was previously thought to be essential for transfer gene expression and plasmid transfer (57), it is not required for transfer gene expression in an hns mutant. A mechanism for TraJ activity has not been established; however, our results are in keeping with models presented for TraJ.
function. Gaudin and Silverman (22) first suggested that TraJ served to oppose the formation of an undefined nucleosomal complex. The authors were able to correlate the positive effect of TraJ in vivo with a requirement for Py to be supercoiled to drive transcription in vitro. This suggested that an unknown repressor complex served to alter local DNA supercoiling. This alteration was antagonized by TraJ, thereby allowing the Py region to adopt a more transcriptionally active topology. Modulation of supercoiling has previously been suggested as a general mode of action for H-NS-mediated gene repression (33, 54). Thus, H-NS might be involved in this nucleosomal complex. However, this does not eliminate other possible mechanisms for H-NS-mediated repression at Py. The arrangement of the five predicted curves located both upstream and downstream of Py might facilitate the trapping of RNA polymerase, as previously described for the rntB P1 promoter region (10). This mechanism proposes that the promoter DNA wraps around the RNA polymerase, promoting open complex formation during transcription initiation (8) and forming a DNA loop which brings H-NS bound at both the upstream and downstream sites into close proximity. This promotes strand bridging by H-NS and traps the RNA polymerase bound at the promoter (8, 9). These two mechanisms are not mutually exclusive, and both might be involved in H-NS-mediated repression of Py.

Northern blot analysis suggests that the levels of the traY transcript are unchanged between the wild-type strain and the hns mutant strain at the earlier time points and that traY transcript levels are prolonged as the Py region approaches stationary phase, thereby allowing transcription to be initiated. This is in keeping with results from previous studies, although repression of the upstream promoters in exponential phase as well, which is in keeping with results from previous studies, although repression at the upstream promoters also appears to increase as the donor cultures enter stationary phase. The larger traMJY and traY transcripts appear to be upregulated in an hns mutant, even at the earliest time points, suggesting that whereas H-NS represses Py only as the donor culture enters stationary phase, it represses the upstream promoters in exponential phase as well, which is in keeping with results from previous studies, although repression at the upstream promoters also appears to increase as the donor cultures enter stationary phase (56).

The sequences of known TraJ proteins of the F-like plasmids are exceptionally dissimilar and bear little homology to those of other known proteins (19). Although the data presented here suggest that transcription from Py is lower in an hns traJ mutant strain than in an hns mutant strain, this does not necessarily mean that TraJ has an activa-
tional role at $P_r$, in addition to one opposing H-NS. Reduced $tra$ operon expression may be due to StpA, an H-NS paralog, which is normally repressed by H-NS but is overexpressed in $hns$ mutant strains (18, 48). Whereas the mutation of $stoA$ alone has little effect on F-plasmid transfer or host cell growth, $F^-$ cells containing an $hns$ $stoA$ double mutation grow very slowly, much more so than either $F^+ hns$ or $F^+ hns$ $stoA$ cultures, suggesting partial repression by StpA in $hns$ mutant host cells (Will and Frost, unpublished results). Although TraJ also might be capable of antagonizing increased StpA-mediated repression in $hns$ mutant donors, $P_r$ might not be fully derepressed in an $hns$ $tra$ mutant donor cell.

Another possibility is that TraY, in conjunction with TraJ, also acts to antagonize H-NS repression at the $P_r$ promoter. The role of TraY in regulating $P_r$ as well as in stimulating expression of $traM$ remains unclear. Studies suggesting a role for TraY as a positive activator have been made with large promoter fragments (45) or with the F plasmid or its derivatives, such as pOX38 (Will and Frost, unpublished results). Studies suggesting that TraY is a negative regulator (52; this work), used smaller promoter fragments in transcriptional fusion constructs which lacked the upstream promoters, $P_M$ and $P_r$. TraY-mediated activation of $P_r$ does not appear to be due solely to activation of $P_M$ and subsequent transcriptional read-through of $traM$ and $traD$, as suggested in previous studies of the F-like plasmid R100 (50), since activation has been demonstrated in constructs lacking $P_M$ (45). Thus, TraY could act either as an activator or as a repressor of $P_r$, depending on the DNA context within the promoter region on F plasmid. This context would be influenced by the nature of the repressor complex at $P_r$ and by the degrees of read-through transcription for the upstream promoters $P_M$ and $P_r$, as well as by the superhelical density at $P_r$, which is known to be responsive to supercoiling (22). TraY could also act as an activator that aids TraJ in relieving H-NS repression and initiating transcription from $P_r$. Alternatively, higher intracellular levels of TraY, present at later times in the growth cycle, or when supplied in trans or in cis during promoter assessment assays, could cause repression of the $P_r$ promoter either directly or else by helping to establish the H-NS-based repressor complex. Several studies have demonstrated that all three transfer promoters, $P_M$, $P_r$, and $P_y$, require relatively large segments of the flanking DNA for normal regulation (22, 47, 56). One possible explanation for this is a requirement for transcriptionally generated supercoiling at each of the promoters to provide the appropriate context for regulation. Another possibility is the formation of large gene loops whereby the three main transfer region promoters are linked together through DNA-protein interactions and strand bridging (9). Since H-NS can bind to each of the transfer promoters, it might have a role in connecting these promoters into a single regulatory complex. This would allow for coordinate, cooperative regulation of all three promoters and would explain why the examination of the transfer promoters in isolation results in puzzling or contradictory models for H-NS and TraY action (45, 52, 56).

The results reported here highlight the importance of H-NS, which, along with other host nucleoid-associated proteins, controls the expression of nonessential genes acquired by horizontal transfer. Because of its promiscuous binding activity, H-NS is able to repress a wide assortment of promoters, including those associated with mobile genetic elements (14, 25). These elements appear to encode a number of proteins that can interact directly with H-NS and modulate or inhibit its activity. The F-like plasmids R100 and pRK100 have acquired homologs to Hha, which are known to interact with H-NS (37, 49). In the case of R100, the Hha homolog, RmoA, has been shown to act as a positive regulator of transfer (39). Several IncH plasmids have been shown to carry both H-NS and Hha homologs (5, 23), which appear to play a role in regulating plasmid transfer gene expression (16). We are currently investigating the mechanism of TraJ antagonism of H-NS to determine whether it occurs through binding DNA to disrupt a repressor complex or by binding H-NS directly, thereby affecting its activity.

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