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The *Escherichia coli stpA* Gene Is Transiently Expressed during Growth in Rich Medium and Is Induced in Minimal Medium and by Stress Conditions

ANDREW FREE* AND CHARLES J. DORMAN

Department of Microbiology, Trinity College, Dublin 2, Republic of Ireland

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The transcriptional regulation of the *stpA* gene, encoding the *Escherichia coli* H-NS-like protein StpA, has been studied as a function of a variety of environmental conditions, and its response to *trans*-acting factors has been characterized. Chromosomally located *stpA* is expressed primarily from a promoter immediately upstream of the gene which is severely repressed by the homologous nucleoid-associated protein H-NS. However, we show here that even in a strain containing functional H-NS, *stpA* is transiently induced during growth of a batch culture in rich medium. It can also be induced strongly by osmotic shock and, to a lesser extent, by an increase in growth temperature. Moreover, when cells are grown in minimal medium, we observe a more sustained induction of *stpA* which is dependent on the leucine-responsive regulatory protein (Lrp). This enhanced level of *stpA* transcription is virtually abolished in an H-NS-independent manner when the culture undergoes carbon starvation. A sensitivity of the *stpA* promoter to DNA topology may contribute to some of these responses. Results reported here show that cloned fragments of the *stpA* promoter region can confer H-NS and Lrp responsiveness upon a *lacZ* reporter gene and suggest that several hundred base pairs of DNA upstream of the transcriptional start may be required for regulation by these two proteins.

The *stpA* gene of *Escherichia coli* encodes a 15.3-kDa protein which was originally identified as a multicopy suppressor of the splicing defect of a mutant bacteriophage T4 *td* intron (32). At the amino acid level, StpA is 58% identical to the *E. coli* nucleoid protein H-NS, which is a major component of the bacterial nucleoid and has been identified as a pleiotropic regulator of gene expression, recombination, and genome stability (see reference 30 for a review). It has been shown recently that in addition to possessing sequence similarity, the StpA and H-NS proteins also share a number of in vitro and in vivo properties. Like H-NS, StpA can constrain DNA supercoils in vitro and can repress transcription from a synthetic *gal* promoter containing an upstream curved sequence both in vitro and in vivo while protecting similar regions of this promoter in in vitro DNase I footprinting assays (34). Multicopy *stpA* has also been found to complement an *hns* mutant in terms of the pH-dependent regulation of arginine decarboxylase gene expression (28). There are, however, specific differences in the properties of the two proteins; StpA can enhance the splicing of a mutant *td* intron in vivo or the wild-type intron in vitro more efficiently than H-NS, probably due to its greater effectiveness in promoting RNA annealing and strand exchange (33, 34).

The expression of *hns* is subject to negative autoregulation (6, 10, 15, 29), and production of its mRNA is largely confined to the logarithmic phase of growth (11, 15), although certain gene fusion constructs have given conflicting results (6, 29). The abundant DNA-binding protein FIS (factor for inversion stimulation) can also make a positive contribution to transcription from the *hns* promoter both in vitro and in vivo (10, 11). More recently, it has been shown that *hns* and *stpA* exhibit negative cross-autoregulation; that is, each gene product can inhibit both its own gene promoter and that of the other (34). However, the strongest effect seems to be the repression of *stpA* by H-NS; this causes the level of *stpA* expression in a wild-type cell to be very low and has led to the suggestion that StpA might act as a "molecular backup" of H-NS which is expressed only when the latter protein is absent (34). While this is an attractive possibility, it fails to take into account the fact that the induction of *stpA* in an *hns* mutant cannot complement the many phenotypes caused by the *hns* mutation and that there are significant differences in the properties of H-NS and StpA, notably the effects on RNA described above.

As a first step toward identifying a more independent role for StpA, we have therefore investigated the expression of the *stpA* gene under a variety of conditions in *hns* wild-type cells to determine if there are specific conditions under which StpA may be induced. We show here that during growth in rich medium, there is a transient burst of *stpA* expression during log phase and that in minimal medium, expression is significantly enhanced in a manner which is dependent on the leucine-responsive regulatory protein (Lrp). We have also identified a number of environmental conditions which can modulate *stpA* transcription. Both increases in medium osmolarity and, to a lesser extent, in growth temperature activate *stpA* expression, while carbon starvation causes a rapid repression of the gene. These responses may correlate with a sensitivity of the *stpA* promoter to DNA topology. We demonstrate that qualitatively similar responses to the H-NS and Lrp proteins are shown by an *stpA-lacZ* promoter gene fusion and have identified regions of the *stpA* promoter which may be involved in interactions with these proteins.

MATERIALS AND METHODS

Strains, plasmids, and media. The *E. coli* K-12 strains CSH50 [*ara* Δ(*lac pro*) *strA thi*] and MC4100 [*araD139* Δ(*arg lac*)U196 *rpsL150 relA1 ptsF25 rbsR flbB5301*] and their derivatives were used throughout this work. The *hns-205*::Tn*10* allele from GM230 (17) was moved by P1*cml* transduction into CSH50 (to generate strain CJD1091) and MC4100 (to generate strain CJD847). The *lrp-201*::Tn*10* allele from MEW26 (22) was moved by P1 *cml* transduction into

* Corresponding author. Mailing address: Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Republic of Ireland. Phone: 353-1-6082521. Fax: 353-1-6799294. E-mail: afree@acer.gen.tcd.ie.

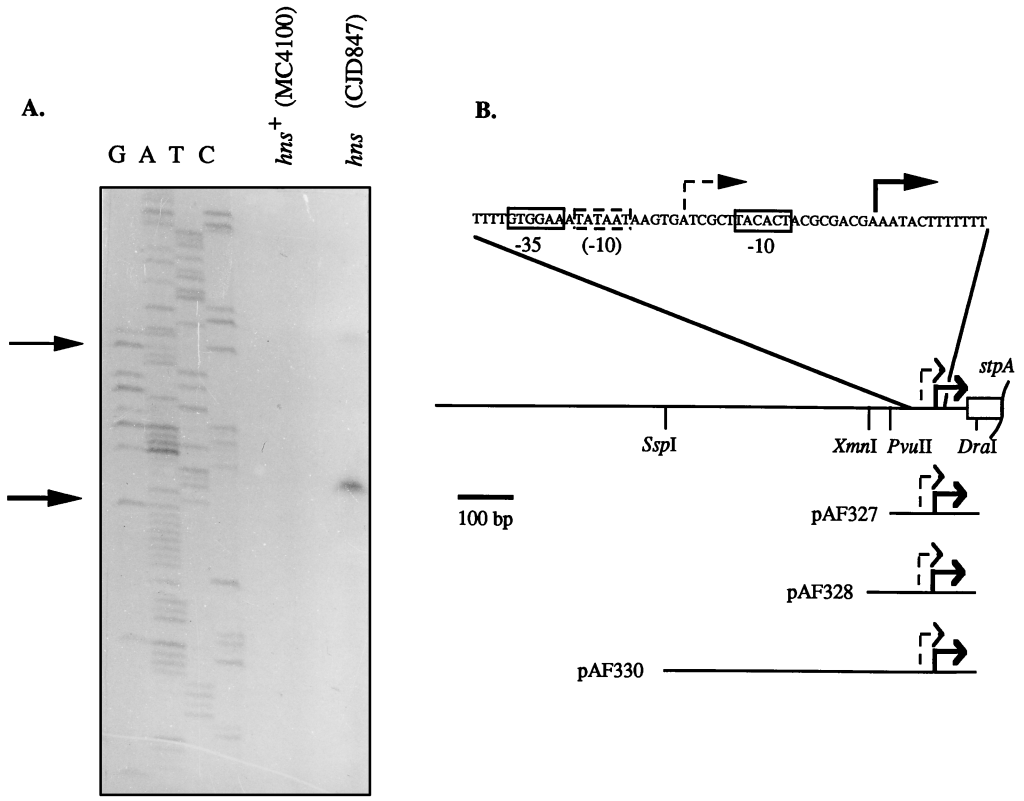

FIG. 1. (A) *stpA* primer extension analysis on RNA from strains MC4100 (*hns*$^+$) and CJD847 (*hns*). Sequencing lanes with the same primer and plasmid pAF301 as a template are shown alongside. Major and minor transcription start sites are indicated by large and small arrows, respectively. (B) Map of the *stpA* promoter region showing the start sites mapped in panel A and the potential −10 and −35 regions. The fragments of the promoter cloned into the *lacZ* reporter plasmid pQF50 (see text) are indicated below.

CSH50 to generate strain CJD1092. A second *hns* mutation, *hns-203*, which is caused by an insertion element within the 5′ coding region of *hns* (15a), was also used. A Tn*10* insertion which is 83% linked to *hns* (*zch-97*::Tn*10* [17]) was moved into the *hns-203* strain BRE2076 (17), and transductants which retained the *hns* mutation were selected on the basis of continued derepression of *proU* expression in this strain. The *zch-97*::Tn*10* insertion from one such transductant was then moved into CSH50 by using P1*cml*, and a transductant in which the *hns* allele had been cotransduced with the Tn*10* was selected on the basis of PCR amplification of the *hns* gene, using specific primers (5′-CAAAATAAAGAAC AATTTTGAATTC-3′ and 5′-AATAAATTAGGTTACATGCAGGCCT-3′), and designated CJD1101. For construction of an *hns lrp* double mutant strain, *hns-203* was transduced from BRE2076 into the *lrp-201*::Tn*10* mutant CJD1092, and transductants with derepressed expression of the *bgl* operon due to the introduction of this *hns* allele (17) were selected on MacConkey-salicin plates (containing 4% MacConkey agar base [Difco] supplemented with 0.4% salicin). The presence of the *hns* mutation was confirmed by PCR amplification as described above. A *fis* mutant derivative of CSH50 (CSH50 *fis*::*kan* [20]) was also used. The broad-host-range, medium-copy-number (≈36 copies/cell) *lacZ*-containing vector pQF50 (12) was digested with *Sma*I, which cuts twice within its polylinker region, and fragments of the *stpA* promoter region obtained by digestion of the *stpA*-containing plasmid pAF301 (13a) were cloned into these sites as indicated in Fig. 1 to generate pAF327, pAF328, and pAF330. The self-ligated vector backbone lacking part of the polylinker was designated pAF332. Strains were grown routinely in Luria broth (L broth) or L0 (L broth lacking NaCl) at 30 or 37°C as indicated or in M9 minimal medium supplemented with 0.4% (wt/vol) glucose at 37°C. To induce carbon starvation, cells were grown in MOPS minimal medium (26) supplemented with 0.05% (wt/vol) glucose. Minimal media were supplemented with 1 μg of thiamine per ml and, for growth of CSH50 and its derivatives, with 0.023% (wt/vol) proline. When cells were grown in the presence of 100 μg of leucine per ml, isoleucine and valine were also added to the medium at a concentration of 50 μg/ml as previously described (9, 22). Plasmids were selected for with carbenicillin at a concentration of 50 μg/ml.

RNA extraction and analysis. RNA was extracted from samples (10 to 120 ml) of cultures grown as described in the text by the method of Free and Dorman (14). The concentration and purity of the RNA samples were assessed by A_{260} and A_{280} measurements, and the samples were then used in primer extension or Northern blot analyses. For determination of the 5′ end of the *stpA* mRNA by primer extension, a synthetic oligonucleotide homologous to nucleotides 70 to 89 of the *stpA* open reading frame (5′-AGCATTTCTTCAAGAACGTC-3′) was end labeled with 25 μCi of [γ-^{32}P]ATP (10 mCi/ml; Amersham) and purified on a NAP 5 column (Pharmacia Biotech) according to the manufacturer's instructions. Three-tenths picomole of the labeled primer was annealed to 50 μg of total cellular RNA, and primer extension was performed as described previously (15). cDNA transcripts were electrophoresed on 7 M urea–5% polyacrylamide gels next to sequencing markers generated by dideoxy sequencing reactions using the same primer and the *stpA*-containing plasmid pAF301 as a template. Radioactive fragments on fixed, dried gels were visualized by autoradiography using Hyperfilm-MP film (Amersham). To generate an *stpA*-specific antisense RNA probe for use in Northern blotting, a 246-bp internal *Eco*RI-*Hin*cII fragment from the *stpA* gene was cloned into the SP6/T7 expression vector pSPT18 digested with *Eco*RI and *Sma*I; the resulting plasmid (pAF326) was linearized with *Pvu*II and used as a template for in vitro transcription by SP6 RNA polymerase, using digoxygenin-labeled UTP and the reagents and protocol supplied by Boehringer Mannheim. Five-microgram samples of total cellular RNA were denatured at 65°C, electrophoresed on 1.5% formaldehyde-agarose gels in 3(*N*-morpholino) propanesulfonic acid (MOPS) buffer, transferred to Hybond-N nylon filters (Amersham), and hybridized with a 1/100 aliquot of the denatured products of the in vitro transcription reaction. Following stringency washes, the digoxygenin-labeled probe was detected with the CSPD chemiluminescence detection system (Boehringer Mannheim) and Hyperfilm-MP film (Amersham). For analysis of *hns* transcription, 20-μg samples of total cellular RNA were electrophoresed and blotted as described above, and the *hns* mRNA was detected with a digoxygenin-labeled synthetic oligonucleotide probe as described previously (15).

Enzyme assays. β-Galactosidase assays were carried out on sodium dodecyl sulfate-$CHCl_3$-permeabilized cells as described previously (25). Duplicate assays were performed, and the data were expressed as the mean of the two measurements. Standard deviations were less than 10%.

RESULTS

H-NS represses the main chromosomal *stpA* promoter. A single transcription start site upstream of the *E. coli stpA* gene has previously been mapped by using a cloned copy of the gene

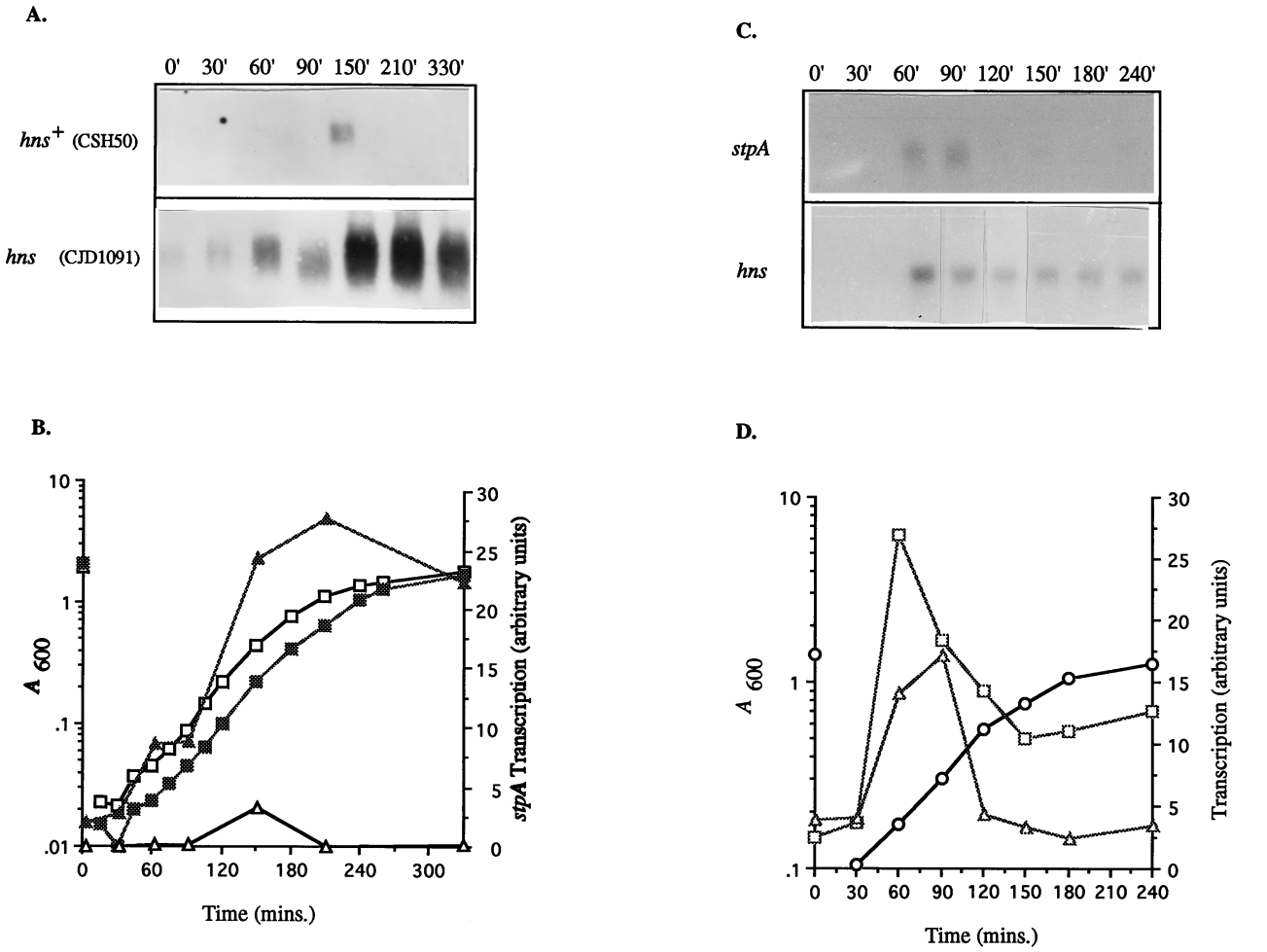

FIG. 2. (A) Northern blots of the *stpA* transcript from CSH50 (*hns*$^+$) and CJD1091 (*hns*) at various time points (minutes) throughout the growth curve. (B) Growth curves (squares) and densitometric analysis of the blots in panel A (triangles) for CSH50 (open symbols) and CJD1091 (closed symbols). (C) Northern blots of the *stpA* and *hns* transcripts from MC4100 at various time points throughout the growth curve. (D) Growth curve (circles) and densitometric analysis of the *stpA* (triangles) and *hns* (squares) blots in panel C. The relative levels of the two transcripts are not comparable.

situated on a multicopy plasmid (28). To confirm that the same promoter is the primary start site in the native chromosomal location and to investigate its regulation by the H-NS protein, total RNA was extracted from *E. coli* MC4100 and its *hns-205*::Tn*10* derivative CJD847 grown to late log phase (A_{600} of ≈0.8) and subjected to primer extension analysis using a primer specific for the *stpA* transcript (see Materials and Methods for details). Polyacrylamide gel electrophoresis of the resulting extension products (Fig. 1A) showed that there were no detectable start sites in the vicinity of the *stpA* gene in the wild-type strain. However, in the *hns* mutant CJD847, we detected significant transcription initiating at the A residue 41 bp upstream of the *stpA* open reading frame. This corresponds to the promoter previously mapped in the context of a multicopy plasmid and correlates with the presence of potential −10 and −35 sequences at appropriate spacings from this site (28) (Fig. 1B). There also appeared to be a much lower level of transcriptional initiation at the A residue located 20 bp upstream of the main start site. A potential −10 sequence (TATAAT) is centered 8 bp upstream of this minor start, although no obvious −35-like sequence is found at an appropriate spacing from this sequence. Both transcriptional starts are detectable only in RNA isolated from the *hns* mutant strain, consistent with the failure to detect *stpA* transcripts in a wild-type strain by Zhang et al. (34). Although it is possible that there are other promoters much further upstream which read through into the *stpA* region but which were not detected in this experiment due to inefficient extension over long distances, it seems likely that the transcriptional start at −41 is the primary *stpA* promoter in vivo and is strongly repressed by H-NS.

***stpA* is transiently expressed during exponential growth in a wild-type background.** It is known that the *hns* gene is strongly growth phase dependent in its regulation, its mRNA being highly expressed during exponential growth but repressed during stationary phase (11, 15). The H-NS protein negatively regulates its own gene, and this may help account for the pattern of growth phase-dependent regulation (10, 15), as may the contribution of the positive regulator FIS (11). As H-NS is a strong negative regulator of *stpA* expression, it was possible that *stpA* expression also varied with growth phase. Given that the level of *stpA* transcript detected by primer extension is fairly low even in an *hns* mutant, we used a digoxygenin-labeled antisense RNA probe to detect *stpA* transcription in a Northern blot (see Materials and Methods). Wild-type strain CSH50 and its *hns-205*::Tn*10* mutant derivative CJD1091 were grown overnight in L broth and then diluted 1:100 into fresh medium.

RNA samples were taken at various time points throughout the resulting growth curves and subjected to Northern blotting (Fig. 2A and B). In the *hns* mutant CJD1091, a significant level of *stpA* transcription was seen throughout the growth curve, with the highest level occurring during the transition to stationary phase. The transcript size (≈500 bp) is consistent with a monocistronic transcript initiating immediately upstream of the *stpA* open reading frame. In the wild-type strain, transcription was virtually undetectable in most of the samples except for a transient activation 150 min after subinoculation. This time point corresponds to an A_{600} for the culture of ≈0.4. The level of transcription at this point was approximately 10-fold lower than that in the *hns* mutant strain at the corresponding point in the growth curve but was nevertheless at least 10-fold higher than that in the wild-type strain at other points in the growth curve. The expression pattern found in this experiment is consistent with the absence of extension products in Fig. 1 from RNA sampled from an *hns*$^+$ culture at an A_{600} of ≈0.8.

To investigate this unusual transient expression phenomenon further, *stpA* expression was examined during exponential growth of another wild-type strain, MC4100. This is the same genetic background as was used for the primer extension analysis in Fig. 1. In this experiment, MC4100 was grown overnight in L broth and then diluted 1:40 into fresh medium. *stpA* was found to be expressed transiently between 60 and 90 min after subinoculation (Fig. 2C and D). Because the dilution of the overnight culture was less than in the previous experiment, this again corresponded to an A_{600} in the range 0.2 to 0.4. Thus, two different *E. coli* K-12 strains both express *stpA* in a transient manner during mid-exponential growth. As a control, the RNA samples from this experiment were probed for the *hns* transcript. As we have previously shown (15), *hns* transcription was found to peak in mid-log phase and then decline as the cells progressed toward stationary phase, but was more sustained than that of *stpA* (Fig. 2C and D).

FIS is not required for the temporal regulation of *stpA* expression. FIS has been shown to play a role in positive regulation of *hns* expression (10, 11), and the levels of FIS protein and *fis* mRNA are themselves strongly growth phase dependent, being maximal in early exponential phase (2). We therefore decided to investigate whether FIS contributed to the transient activation of *stpA* expression during growth in batch culture. Strain CSH50 and its *fis* derivative CSH50 *fis*::*kan* were grown overnight in L broth and then diluted 1:20 into fresh medium. In both strains, a transient peak of *stpA* transcription was seen 30 min after subinoculation, corresponding to an A_{600} of ≈0.3 (Fig. 3). The level of the peak seemed to be somewhat higher in the *fis*::*kan* strain; this may be due to the positive effect of FIS on *hns* expression causing an apparent negative effect on *stpA*. However, as the patterns of *stpA* transcription were virtually identical in the two strains, it seems that FIS is not required for the growth phase-dependent activation of the *stpA* promoter.

Transcription of *stpA* in minimal medium: role of the LRP protein. The transient nature of the activation of *stpA* expression described above suggested that environmental conditions might be involved in triggering transcription of this gene. As a first step toward examining this hypothesis, we studied the effect of growth medium on the production of the *stpA* transcript. *E. coli* CSH50 cells were grown overnight in M9 minimal medium containing 0.4% glucose and then diluted 1:100 into the same medium. RNA was sampled from the overnight culture and at A_{600} values of ≈0.1, 0.2, 0.4, and 0.8 and subjected to Northern analysis (Fig. 4A). Peak expression was at an A_{600} of ≈0.2 and reached a level similar to that attained in an *hns* mutant strain grown to a comparable A_{600} in L broth (15a).

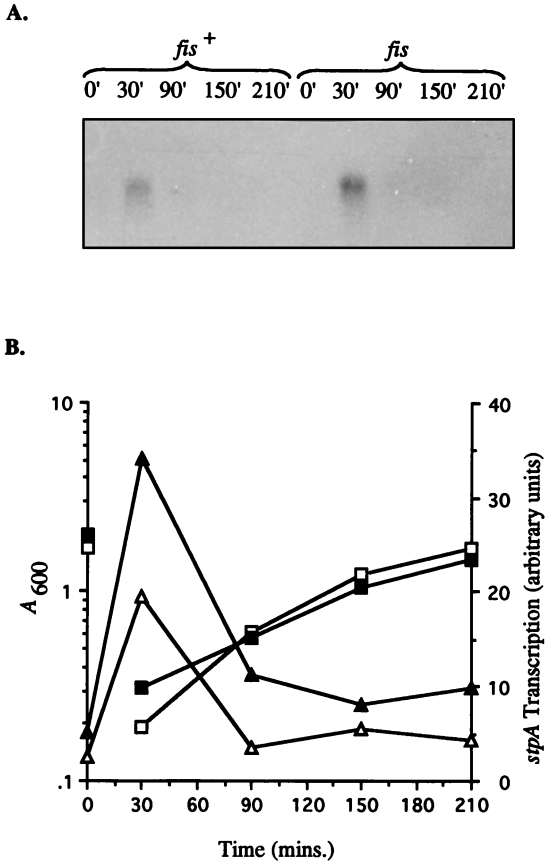

FIG. 3. (A) Northern blot of the *stpA* transcript from CSH50 and CSH50 *fis*::*kan* at various time points (minutes) throughout the growth curve. (B) Growth curves (squares) and densitometric analysis of the blots in panel A (triangles) for CSH50 (open symbols) and CSH50 *fis*::*kan* (closed symbols).

There was also a significant level of *stpA* transcription at other stages of exponential growth, although not in the stationary-phase culture; this marks a major difference from the situation in rich medium. One difference between growth in minimal medium and growth in rich medium is the presence of elevated levels of Lrp in the former case (23). We therefore asked whether Lrp contributes to the activation of *stpA* in minimal medium. The *lrp-201*::Tn*10* allele from MEW26 was introduced into CSH50 to generate strain CJD1092, and the experiment in Fig. 4A was repeated with this strain (Fig. 4B). In the presence of the *lrp* mutation, transcription of *stpA* was severely reduced at all points in the growth curve. The expression of some (but not all) Lrp-dependent genes can be affected by the presence of leucine in the growth medium (3). When CSH50 was grown in M9-glucose medium supplemented with 100 μg of leucine per ml, significant repression of *stpA* relative to the medium without leucine was observed (Fig. 4C), but the effect was not as dramatic as that of mutating Lrp. Therefore, it seems that in minimal medium, *stpA* transcription is elevated in an Lrp-dependent manner and is partially repressed by the presence of leucine. However, in rich medium, when levels of Lrp are reduced, we have found that the lower level and more transient expression of *stpA* is independent of Lrp (15a).

Effect of carbon starvation, osmolarity, and temperature on *stpA* expression. We then investigated the effect of a number of environmental stress conditions on the transcription of *stpA*. First, the effect of starving a growing culture for carbon in

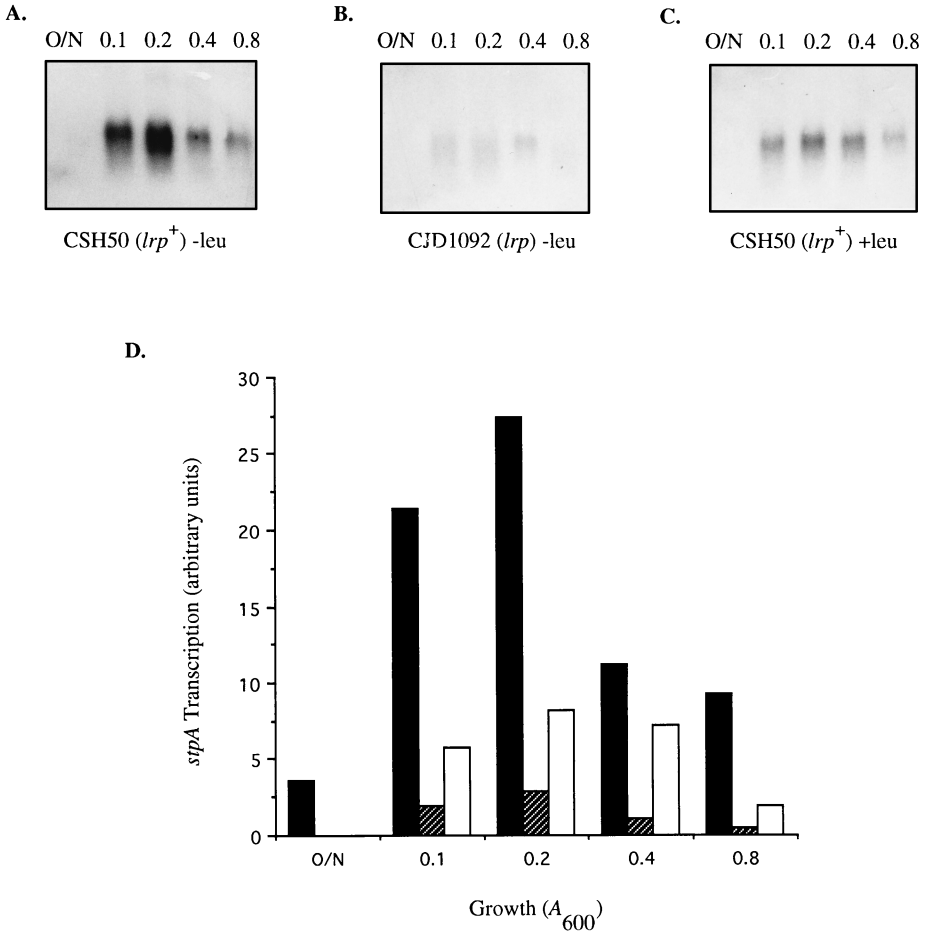

FIG. 4. (A) Northern blot of the *stpA* transcript from CSH50 (*lrp*$^+$) grown in M9-glucose without leucine at various A_{600} values. O/N, overnight culture. (B) Northern blot of the *stpA* transcript from CJD1092 (*lrp*) grown in M9-glucose without leucine at various A_{600} values. (C) Northern blot of the *stpA* transcript from CSH50 grown in M9-glucose with leucine (100 μg/ml) at various A_{600} values. (D) Densitometric analysis of the blots in panels A to C. Solid bars, CSH50 without leucine; hatched bars, CJD1092 without leucine; open bars, CSH50 with leucine.

mid-exponential phase was studied. Growing cells in MOPS minimal medium supplemented with limiting (0.05%) glucose leads to carbon starvation and a cessation of growth in mid-log phase (1). CSH50 and its *hns* derivative CJD1091 were grown overnight in MOPS medium supplemented with 0.2% glucose and were then diluted 1:100 into MOPS medium plus 0.05% glucose and grown at 37°C. This resulted in the cells reaching an A_{600} of ≈0.6 before growth rapidly ceased (Fig. 5B). RNA was isolated over a 2-h period before and during this nutritional downshift and probed for *stpA* transcription on a Northern blot (Fig. 5A). Significant *stpA* transcription was seen in both cultures prior to starvation; the level in the *hns* mutant CJD1091 was only ≈4-fold higher than that in the wild type, unlike the minimum 10-fold activation seen in L broth (Fig. 2), consistent with the Lrp-dependent activation of *stpA* in the wild type in minimal medium. In both strains, there was a rapid repression of *stpA* transcription following carbon starvation (Fig. 5B), which is obviously therefore H-NS independent.

The effects of the environmental parameters of extracellular osmolarity and growth temperature on *stpA* expression in rich medium were then studied. CSH50 was grown in L0 (L broth lacking salt) to an A_{600} of ≈0.3, the culture was split into two, and 0.4 M NaCl was added to one half. RNA samples were taken from both cultures for 90 min. In the starting culture grown in L0, little expression was observed. It can be seen (Fig. 6A and B) that the osmotic shock led to a very strong activation of *stpA* expression, which reached a maximum of ≈50-fold 60 min after the initial shock before declining again slightly. No activation of expression was seen in the control culture maintained in L0. We have determined that this is a genuine osmotic induction rather than a salt effect, as it can also be mediated by 0.6 M sucrose, which causes an osmotic shock identical to that caused by 0.4 M NaCl, and that as expected in rich medium, it is independent of the Lrp protein (15a). While *stpA* transcription is strongly activated in the absence of salt in the *hns* mutant CJD1091, some residual osmotic induction is still observable in this strain (15a). In a similar experiment, a culture of CSH50 grown in L broth at 30°C was split into two and one half was shifted to 37°C. In this case (Fig. 6C and D), the moderate level of transcription in the starting culture at 30°C was enhanced fourfold after 30 min at 37°C, while in the culture left at 30°C, expression had declined after this time. This activation of *stpA* expression was less dramatic and less sustained than that due to osmotic shock, perhaps because the temperature shift has a less severe effect on cell growth than the osmotic upshift, meaning that the culture at 37°C rapidly progressed toward stationary phase, when *stpA* is repressed. Nevertheless, these two environmental changes both led to an activation of the *stpA* gene.

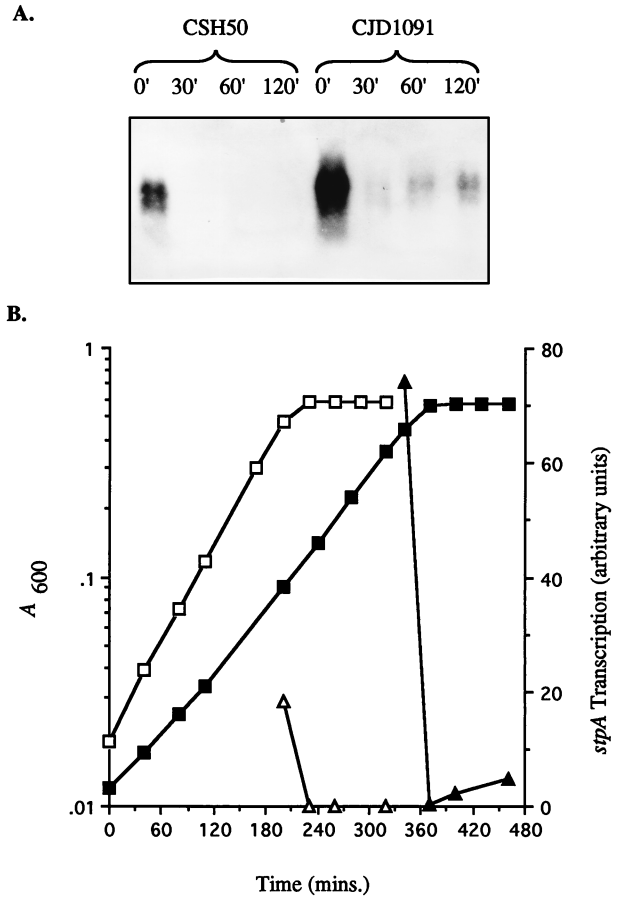

FIG. 5. (A) Northern blot of the *stpA* transcript from CSH50 (*hns*$^+$) and CJD1091 (*hns*) during carbon starvation in MOPS minimal medium with limiting (0.05%) glucose. The 0-min (0′) time point corresponds to 200 min for CSH50 and 340 min for CJD1091 on the *x* axis of the graph in panel B. (B) Growth curves (squares) and densitometric analysis of the graph in panel A (triangles) for CSH50 (open symbols) and CJD1091 (closed symbols).

Artificially modulating DNA topology alters *stpA* expression. A possible explanation for the foregoing results is that the *stpA* promoter responds to changes in DNA topology. It has been shown previously that a growth transition identical to that in the carbon starvation experiment described above leads to a severe increase in plasmid linking number (1). These authors propose that the reduction in negative DNA supercoiling upon carbon starvation may be an important factor in the resultant changes in global gene expression. Similarly, osmolarity and temperature both affect DNA topology. In *E. coli*, increases in growth temperature or in medium osmolarity lead to enhanced negative supercoiling of reporter plasmids (16, 17) which correlate with changes in the expression of supercoiling-sensitive promoters (8, 17). Thus, an environmental change which reduces negative supercoiling inhibits *stpA* transcription, while two conditions which increase negative supercoiling activate the gene.

We therefore attempted to define the response of the *stpA* promoter to artificially induced changes in DNA topology. To avoid the complications of the very brief period of *stpA* transcription in rich medium, cells were grown in minimal medium in which transcription of the gene is active for a longer period of time. *E. coli* MC4100 cells were grown to an A_{600} of ≈0.6 in minimal glucose medium in the presence of the DNA gyrase inhibitor novobiocin at concentrations of 0 to 100 μg/ml. Under identical conditions, we have previously shown that the supercoiling-sensitive tRNA promoters *tyrT* and *hisR* are induced in the presence of 12.5 μg of novobiocin per ml and inhibited at higher concentrations of the drug, while the *hns* promoter is unaffected (14). The increase in tRNA expression at the lowest concentration of novobiocin is probably due to the activation of the genes encoding DNA gyrase under these conditions (13). When the *stpA* transcript in such cultures was quantified (Fig. 7), very little effect on expression was seen at 12.5 μg of novobiocin per ml, while at 25 μg/ml and higher, inhibition occurred, reaching 50% at the higher drug concentrations. Novobiocin had no significant effect on growth at concentrations lower than 75 μg/ml. Therefore, while some supercoiling sensitivity of *stpA* transcription is suggested by these results, the effects under these conditions are not as dramatic as those seen for the strongly supercoiling-sensitive *tyrT* and *hisR* promoters (14). The possibility remains that other factors as well as DNA topology contribute to the environmental regulation of *stpA*.

H-NS- and Lrp-dependent regulation of *stpA* promoter fusion constructs. In an attempt to define *cis*-acting sequences which are responsible for the regulatory patterns of *stpA* transcription described above, fragments of DNA containing the *stpA* promoter and various portions of upstream DNA were cloned into the medium-copy-number *lacZ* reporter plasmid pQF50 (12) to generate transcriptional fusions. The 3′ end of each promoter fragment was the *Dra*I site within the *stpA* coding sequence, while different 5′ ends extending to the *Pvu*II, *Xmn*I, or *Ssp*I site upstream of *stpA* and containing 82, 111, or 477 bp, respectively, of DNA upstream of the transcription start site (Fig. 1) were cloned into *Sma*I-digested pQF50 to generate pAF327, pAF328, or pAF330, respectively. These plasmids and the self-ligated vector (pAF332) were then transformed into CSH50 and its *hns-205*::Tn*10* and *lrp* derivatives CJD1091 and CJD1092, the strains were grown overnight in L broth, and β-galactosidase was assayed. The larger promoter fragments (pAF328 and especially pAF330) gave rise to lower levels of β-galactosidase than the smallest fragment in pAF327 in the wild-type strain (Table 1). The same trend was observed in CJD1091, indicating that this repression was partially independent of H-NS. However, an additional *hns*-dependent repression of the two longest promoter fragments was observed; this was much greater (>3-fold) for pAF330 than for pAF328. *hns*-dependent repression was not observed for the shortest promoter fragment (pAF327). Thus, several hundred base pairs of upstream DNA are required for a significant repressive effect of H-NS on the *stpA* promoter to be seen. As might be predicted, the *lrp* mutation is seen not to affect the expression of these promoter constructs in rich medium. A second *hns* mutation, the more severe *hns-203* allele (17), which, unlike *hns-205*::Tn*10*, does not produce a truncated, partially functional H-NS protein (6a, 15a), caused a larger increase in the expression from the longest promoter fragment (pAF330 in CJD1101), consistent with the stronger derepression of *proU* and *bgl* expression in strains carrying this mutation than that caused by *hns-205*::Tn*10* (17). Combination of the *lrp* mutation with *hns-203* in CJD1102 had little effect compared to the *hns* mutation alone, an unsurprising finding given that the *lrp* mutation alone was found not to affect expression in rich medium. The vector control (pAF332) gives essentially no β-galactosidase activity in any of the strains.

The effects of minimal medium and leucine on the shortest and longest promoter constructs (pAF327 and pAF330) were then determined. CSH50 and its *hns-205*::Tn*10* and *lrp* derivatives transformed with pAF327 or pAF330 were grown over-

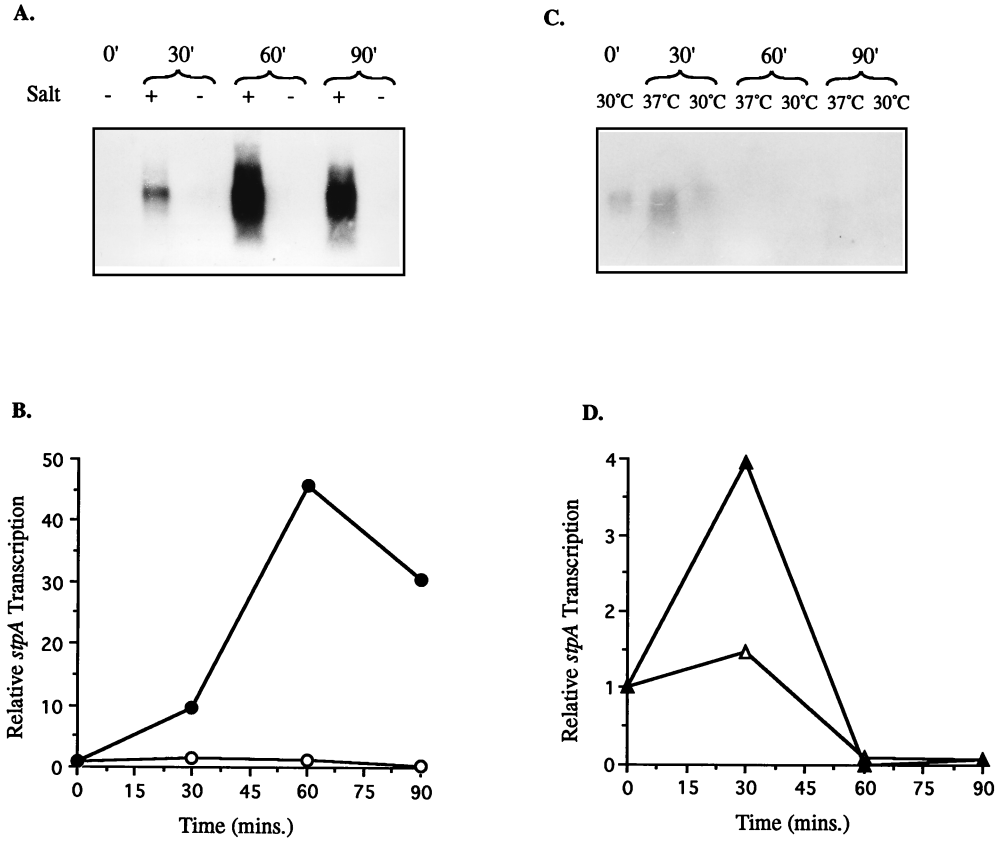

FIG. 6. (A) Northern blot of the *stpA* transcript in cultures of CSH50 in the presence (+) or absence (−) of 0.4 M NaCl added at 0 min (0′). (B) Densitometric analysis of the blot in panel A. Open symbols, no added NaCl; closed symbols, 0.4 M NaCl added. (C) Northern blot of the *stpA* transcript in cultures of CSH50 incubated at the indicated temperatures following growth to log phase at 30°C (0′). (D) Densitometric analysis of the blot in panel C. Open symbols, 30°C; closed symbols, 37°C.

night in M9 minimal glucose medium with or without 100 μg of leucine per ml. Expression from pAF330 in minimal medium was seen to be activated twofold over that in L broth; this increase was *lrp* dependent and could be largely overcome by the inclusion of leucine in the medium. In contrast, the shortest promoter construct (pAF327) was found if anything to be repressed in minimal medium relative to the situation in L broth and was unaffected by Lrp or leucine (Table 1). Expression from pAF330 in the presence of the second *hns* allele *hns-203* with or without functional Lrp was also examined. Once again the *hns-203* allele caused a larger increase in expression than *hns-205*::Tn*10*. The combination of the *lrp* mutation with *hns-203* reduced the level of expression somewhat, particularly in the absence of leucine, but the effect was not as severe as that of the *lrp* mutation in the presence of H-NS. Taken together, these results suggest that both H-NS and LRP may require DNA sequences located several hundred base pairs upstream of the primary *stpA* promoter in order to regulate its expression correctly and that Lrp may in part act to overcome the negative effect of H-NS at the *stpA* promoter.

DISCUSSION

Expression of the *E. coli stpA* gene has previously been shown to be strongly down-regulated by the product of the highly related *hns* gene, with the result that *stpA* transcription is virtually undetectable in a wild-type strain producing H-NS protein (34). We have shown here that this is due to a silencing effect of H-NS on a promoter immediately upstream of *stpA*. These results led to the proposal that *stpA* might function primarily as a molecular backup for H-NS. However, StpA has properties different from those of H-NS, particularly in its effect on RNA transactions (33, 34), and might thus have a more independent role. Here we demonstrate that rather than being completely silent in wild-type cells, the *stpA* gene is induced in a highly transient manner in mid-exponential phase (A_{600} of ≈0.2 to 0.4) during growth of a batch culture in rich medium. Moreover, *stpA* can be significantly induced in minimal medium (in an Lrp-dependent manner), and in rich medium by stresses such as osmotic shock and by shifts in temperature, while being repressed by carbon starvation in minimal medium. A response of the *stpA* promoter to DNA topology may contribute to some of these effects.

The transient expression of *stpA* observed during growth in rich medium is a highly unusual phenomenon. Its mechanism is not entirely clear, although our data show that it is not dependent on the FIS protein, levels of which are also strongly growth phase regulated. Given that H-NS seems to be a prime regulator of *stpA*, it is possible that changes in the repressive activity of H-NS play a role in the induction. It is certainly true that H-NS is important for growth phase regulation of *stpA*, as the pattern of regulation is radically different in an *hns* mutant. However, it is not readily apparent how H-NS acting alone might influence *stpA* in this manner. We have previously argued that derepression of *hns* from autoregulation by its protein product occurs during logarithmic growth due to the synthesis of the target of H-NS, DNA (15); however, it is

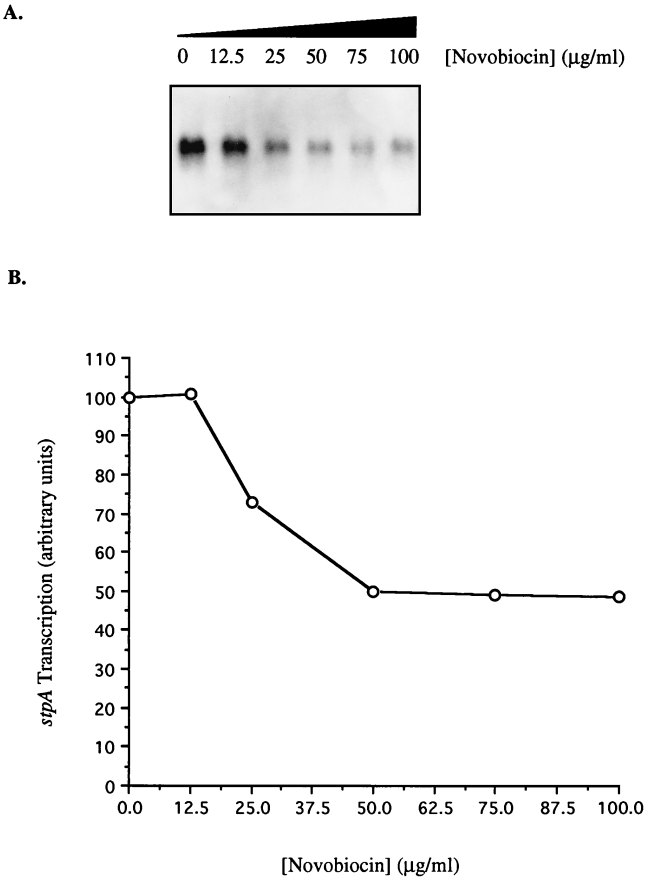

FIG. 7. (A) Northern blot of the *stpA* transcript in cultures of MC4100 grown to mid-log phase in minimal glucose medium with novobiocin added at the indicated concentrations. (B) Densitometric analysis of the blot in panel A.

predicted that only the *hns* promoter is sensitive to this sort of derepression—other H-NS-regulated promoters (e.g., the *proU* promoter [24]) are not derepressed during log phase. Furthermore, the pattern of *stpA* induction does not match that of *hns*, which is sustained throughout log phase (Fig. 2). It is perhaps more likely that another factor or factors serve to derepress *stpA* in this transient manner. One possibility is that the environmental sensitivity of the *stpA* promoter defined here serves to mediate the induction, given the continuous changes in parameters such as aerobiosis, pH, and nutrient availability during growth in batch culture. We show here that premature carbon starvation causes a rapid shutoff of *stpA* transcription which is independent of H-NS; this is consistent with the complete absence of *stpA* expression in stationary-phase cultures. This silencing of the gene upon carbon starvation and its 50-fold induction upon osmotic shock are particularly impressive, and some induction can also be achieved by a temperature shift. Changes in growth and environmental conditions are known to correlate with alterations in DNA topology (1, 7, 27), and it is thus possible that under certain conditions the DNA of the *stpA* promoter is in a favorable conformation for expression even in the presence of H-NS. It is noteworthy that many H-NS-regulated promoters can be induced by changes in DNA topology (8, 17), although that of *hns* itself is not affected by topological changes (14). However, a relatively small reduction in *stpA* transcription is achieved when supercoiling is decreased artificially by treatment with the DNA gyrase inhibitor novobiocin. Nevertheless, it is important to note that because of the extremely transient activation of *stpA* during growth in rich medium, we had to perform the latter experiment in minimal medium, when *stpA* is subject to *trans* activation by the Lrp protein. It is well known that *trans*-acting factors can overcome the requirement of several promoters for the correct DNA topology, both in vivo (3a) and in vitro (27a). Therefore, the absence of *trans* activation by Lrp in rich medium may make the *stpA* promoter much more sensitive to the changes in DNA topology induced by growth phase, osmotic shock, and temperature shift.

The enhanced expression of *stpA* in minimal medium is more readily explicable, being dependent on *trans* activation by the Lrp protein. Activation of *stpA* by Lrp can be largely eliminated by growth of the cells in the presence of leucine, thus placing *stpA* in the class of genes identified by Calvo and Matthews (3) which exhibit leucine-dependent regulatory pattern 1, along with the *ilvIH* operon. However, in the presence of leucine or in the absence of Lrp, a residual growth phase-dependent regulation of *stpA* can still be observed, and the Lrp-dependent activation of *stpA* in minimal medium is still affected by growth phase. This is probably due to a superposition of the effects of growth phase (via DNA topology?) and those of the transactivator. The data obtained by fusion of portions of the *stpA* promoter region to *lacZ* indicate that Lrp and H-NS require similar regions of upstream DNA to affect expression from the promoter and suggest that Lrp may partly

TABLE 1. Effects of growth medium, leucine, and *hns* and *lrp* mutations on expression of *stpA* promoter fusion constructs

Strain	Relevant genotype	Construct[a]	Broth	β-Galactosidase activity[b]
CSH50	Wild type	pAF327	L	532 (32.5)
CJD1091	*hns-205*::Tn*10*	pAF327	L	425 (4.24)
CJD1092	*lrp*	pAF327	L	522 (37.5)
CSH50	Wild type	pAF328	L	229 (7.78)
CJD1091	*hns-205*::Tn*10*	pAF328	L	389 (2.83)
CJD1092	*lrp*	pAF328	L	291 (11.3)
CSH50	Wild type	pAF330	L	79.2 (0)
CJD1091	*hns-205*::Tn*10*	pAF330	L	306 (4.95)
CJD1092	*lrp*	pAF330	L	89.0 (0.21)
CJD1101	*hns-203*	pAF330	L	475 (17.7)
CJD1102	*hns-203 lrp*	pAF330	L	523 (43.1)
CSH50	Wild type	pAF332	L	1.97 (0.09)
CJD1091	*hns-205*::Tn*10*	pAF332	L	2.29 (0)
CJD1092	*lrp*	pAF332	L	1.87 (0.06)
CJD1101	*hns-203*	pAF332	L	3.65 (0)
CJD1102	*hns-203 lrp*	pAF332	L	3.95 (0.06)
CSH50	Wild type	pAF330	M9	193 (3.54)
CJD1091	*hns-205*::Tn*10*	pAF330	M9	358 (4.24)
CJD1092	*lrp*	pAF330	M9	95.7 (2.05)
CJD1101	*hns-203*	pAF330	M9	473 (13.4)
CJD1102	*hns-203 lrp*	pAF330	M9	352 (28.3)
CSH50	Wild type	pAF330	M9 + leucine	121 (1.41)
CJD1091	*hns-205*::Tn*10*	pAF330	M9 + leucine	254 (10.6)
CJD1092	*lrp*	pAF330	M9 + leucine	99.8 (3.18)
CJD1101	*hns-203*	pAF330	M9 + leucine	488 (14.8)
CJD1102	*hns-203 lrp*	pAF330	M9 + leucine	412 (19.8)
CSH50	Wild type	pAF327	M9	371 (7.07)
CJD1091	*hns-205*::Tn*10*	pAF327	M9	341 (14.8)
CJD1092	*lrp*	pAF327	M9	344 (8.49)
CSH50	Wild type	pAF327	M9 + leucine	302 (0.71)
CJD1091	*hns-205*::Tn*10*	pAF327	M9 + leucine	367 (10.6)
CJD1092	*lrp*	pAF327	M9 + leucine	383 (36.8)

[a] See the text and Fig. 1B for details of the individual constructs.
[b] Values are given in Miller units. Standard deviations are shown in parentheses.

act to overcome the negative effect of H-NS. Thus, a shorter promoter construct which is not repressed by H-NS is not significantly repressed by the absence of Lrp or the presence of leucine. It is possible that H-NS and Lrp modulate each other's binding to the *stpA* promoter region, as has been suggested for other systems such as *ilvIH* (21). Lrp must still make a positive contribution to *stpA* transcription in the absence of H-NS, however, as leucine in the medium reduces transcription in an *hns* mutant, and an *hns lrp* double mutant gives lower expression in M9 medium than an *hns* single mutant (Table 1).

Although the data presented here give some indication of the means by which regulation of *stpA* is effected, the mechanistic details have yet to be elucidated. What is clear is that regions extending several hundred base pairs upstream of the transcription start site are probably involved in *stpA* regulation. Indeed, more than 500 bp of upstream DNA may be required for full regulation, as the effects of mutating Lrp and (particularly) H-NS on even our longest promoter fusion construct are severalfold smaller than the effects on the chromosomal promoter. It is interesting to note that no large open reading frames are located in the 1,180 bp between the 3′ end of an upstream unknown gene designated *ygaC* and the 5′ end of *stpA* (28). The 600 bp upstream of *stpA* are highly AT rich (an A+T content of >65%) and may therefore adopt an unusual DNA conformation in vivo. This could account for a sensitivity of the promoter to DNA topology, which may affect such a structure, and for its recognition by H-NS, which has a preferential affinity for the intrinsic DNA curvature often associated with long stretches of A and T residues. The Lrp protein has a degenerate AT-rich consensus binding sequence which has recently been refined (5′-YAGHAWATTWTDCTR-3′ [4]). The two best matches to this consensus sequence within the *stpA* upstream region (5′-TAGCGTATATCGCTG-3′ and 5′-TAGTCAGTTTTTGTA-3′) actually lie outside the region cloned in our longest promoter fusion construct, being centered at −539 and −490, respectively, but this far-upstream DNA may contribute to regulation as noted above. A weaker match (5′-CTTGAAATTATTCTG-3′) is centered at −107 with respect to the transcriptional start, and given the degeneracy of the Lrp consensus and the known contribution of flanking DNA to Lrp binding (5), it is likely that other binding sites are also present within the intervening sequences. To define such sites would require biochemical analysis of the interaction of Lrp with the *stpA* promoter; we are currently undertaking such studies.

The physiological relevance of the complex regulatory patterns defined here for *stpA* is of great interest. It is intriguing that unlike its homolog H-NS, StpA is strongly regulated by environmental conditions. *hns* transcription is unaffected by environmental conditions such as osmolarity and temperature which are known to modulate the expression of many H-NS-regulated genes (15a, 18, 19) and is likewise insensitive to DNA topology (14). Because of its high similarity to H-NS, it is likely that StpA has the ability to form hetero-oligomeric complexes with its more abundant relative; indeed, such interactions have recently been detected (31). Such complexes may differ in DNA-binding and/or gene-regulatory properties from those containing H-NS alone and may therefore mediate changes in the expression of other loci. For instance, the osmotic induction of StpA may contribute to the induction of some osmotically regulated, H-NS-repressed genes by altering the ability of the H-NS complexes at the promoters of those genes to repress transcription. How such a scenario relates to the situation in minimal medium, when StpA levels are probably more consistently elevated, is not clear. The transient expression of *stpA* during growth in rich medium probably leads to only a low level of StpA protein. Nevertheless, these few molecules of StpA may interact with H-NS complexes to change the properties of those complexes, thus subtly altering patterns of gene expression to the advantage of cells in batch culture, which are under constantly changing conditions. It will be of great interest to determine the cellular content of StpA protein under the various conditions defined here.

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ADDENDUM

After this report was submitted for publication, a second study of the regulation of *stpA* expression was published (28a). These authors also examined the expression of *stpA* in response to H-NS, Lrp, and temperature and the requirement for promoter-distal DNA for regulation, and they obtained results in good agreement with our own.

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