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The StpA Protein Functions as a Molecular Adapter To Mediate Repression of the *bgl* Operon by Truncated H-NS in *Escherichia coli*

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The mechanism of repression of the β-glucoside utilization (*bgl*) operon of *Escherichia coli* by a carboxy-terminally truncated derivative of the nucleoid-associated protein H-NS which is defective in DNA binding was investigated. The DNA-binding function of the H-NS-like protein StpA was found to be necessary for repression, which is consistent with a role for StpA as a DNA-binding adapter for mutant derivatives of H-NS.

The H-NS protein of *Escherichia coli* is a major component of the bacterial nucleoid and has been identified as a pleiotropic regulator of gene expression, recombination, and genome stability (reviewed in reference 22). Among other functions, H-NS was identified as a negative regulator of expression of the cryptic *bgl* operon (*bglY* [1]) and of the osmoregulated *proU* operon (*osmZ* [8]). The *bgl* operon encodes the functions required by the cell for the uptake and utilization of β-glucoside sugars, such as salicin, and can be activated by insertion sequence element insertions upstream of its promoter, point mutations in its upstream cyclic AMP receptor protein binding site, or mutations in DNA gyrase as well as by H-NS inactivation (12, 15). H-NS is necessary but not sufficient for in vitro repression of the *bgl* operon, which involves DNA elements located both upstream and downstream of the promoter (14, 15). Heterologous promoters placed into the *bgl* context are also repressed (14), and the repression of this operon is probably the eubacterial example closest to the classic eukaryotic process of gene silencing.

It was observed earlier that of *hns* alleles selected on the basis of *proU* derepression at low osmolarity, only a subset led to high-level expression of the *bgl* operon (2, 8). More recently, the nature of this effect was clarified as part of a systematic structure-function analysis of the H-NS protein (21). In that study it was found that while *proU*-derepressing point mutations near the N terminus of H-NS also caused *bgl* derepression, mutations near the C terminus of the protein, and also a C-terminal truncation of H-NS at amino acid 92, caused no *bgl* derepression despite derepressing *proU* significantly. Intriguingly, an in vitro analysis of the latter class of proteins showed that they appeared to have lost their ability to bind DNA, which is consistent with an earlier study suggesting that the DNA-binding function of H-NS may be localized in the C terminus (17). In contrast, the N-terminal mutants which did derepress *bgl* retained the ability to bind DNA, implying that they were specifically impaired in repression function. These observations led Ueguchi et al. (21) to conclude that *bgl* silencing employed a (possibly unique) mechanism which merely required the targeting of an N-terminal repression domain of H-NS to the *bgl* promoter via an interaction with a heterologous DNA-binding protein.

The *E. coli stpA* gene encodes a 15.3-kDa protein which is 58% identical to H-NS at the amino acid level. StpA and H-NS have similar in vitro DNA-binding properties, including a preference for intrinsically curved DNA, and can repress similar spectra of genes in vivo (16, 24). Although normally expressed at a low level in rich medium, *stpA* is strongly induced in *hns* mutants (5, 19, 24) and StpA may take over some of the functions of H-NS in these strains. Moreover, StpA has been shown both genetically in vivo and by protein-protein cross-linking in vitro to interact with H-NS, and these interactions seem to require the respective N termini of the proteins (23). Thus, StpA is a likely candidate for a molecular adapter which could target the N-terminal repression domain of a mutant or truncated H-NS derivative to the *bgl* promoter region. We have investigated this possibility by studying the derepression of *bgl* in the presence of various combinations of *hns* and *stpA* mutations.

***hns* mutations with differing effects on *bgl* expression.** In order to assess a possible adapter role for StpA in mediating *bgl* repression by truncated derivatives of H-NS, we used two different mutant alleles of *hns*. The first, *hns-205*::Tn*10*, is a well-characterized allele which harbors a Tn*10* insertion in codon 93 of *hns* (9) and produces both a truncated *hns* mRNA species (6) and a truncated N-terminal protein fragment (2, 3) consistent with the termination of *hns* transcription and translation close to this insertion point. In contrast, the *hns2* allele was isolated as a spontaneous *proU*-derepressing mutant in our laboratory and carries an ≈750-bp insertion (probably IS*1*) within the first 22 codons of the *hns* open reading frame. This allele produces no detectable RNA transcript or protein product (3, 6). Duplicate cultures of the *E. coli* strain GM37 (8) and its *hns-205*::Tn*10* and *hns2* derivatives GM230 and CJD829 were grown overnight at 37°C in M9 minimal medium supplemented with 0.4% (wt/vol) succinate, 0.2% (wt/vol) Casamino Acids, and 5 mM β-methyl-D-glucoside, and phospho-β-glucosidase activity was assayed in growing cells (13). The results (Table 1) show that the *hns-205*::Tn*10* allele causes no derepression of *bgl* expression, while the *hns2* allele allows a sigificant level of *bgl* expression. This is consistent with the model proposed by Ueguchi et al. (21) in which N-terminal mutations in H-NS lead to *bgl* derepression whereas the H-NS C terminus is dispensable for its repression function at this promoter.

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TABLE 1. Effects of *hns* and *stpA* mutations and plasmids on expression of the *bgl* operon as assayed by phospho-β-glucosidase activity

Strain	Relevant genotype	Phospho-β-glucosidase activity[a]
GM37	Wild type	0.78 (0.05)
CJD1124	Δ*stpA*::Tc^r	0.51 (0.03)
CJD898	*hnrG*::Ap^r	0.59 (0.05)
GM230	*hns-205*::Tn*10*	0.59 (0.04)
CJD899	*hns-205*::Tn*10 hnrG*::Ap^r	1.03 (0.02)
CJD1126	*hns-205*::Tn*10* Δ*stpA*::Tc^r *hnrG*::Ap^r	57.3 (0.42)
CJD1126(pYCStpA)	*hns-205*::Tn*10* Δ*stpA*::Tc^r *hnrG*::Ap^r (StpA$^+$)	0.54 (0.03)
CJD1126(pYCStpAΔ65C)	*hns-205*::Tn*10* Δ*stpA*::Tc^r *hnrG*::Ap^r (StpA$^-$)	64.5 (1.37)
CJD829	*hns2*	83.0 (3.17)
CJD1125	*hns2* Δ*stpA*::Tc^r	105 (5.66)

[a] Duplicate cultures were assayed; mean values, in relative units calculated as for β-galactosidase, are shown. Standard deviations are in parentheses.

We also examined the effect of these two *hns* mutations on expression of the *stpA* gene. This was to rule out the possibility that the above-mentioned effects on *bgl* expression could be explained by a differential induction of StpA by the two alleles, which might substitute for H-NS in repression of *bgl*. Strains GM37, GM230, and CJD829 were grown in L broth at 37°C to an optical density at 600 nm of ≈0.6, and total cellular RNA was isolated as described previously (4). Five-microgram aliquots of these RNA samples were electrophoresed on a 1.5% 3′-(*N*-morpholino)propanesulfonic acid (MOPS)-formaldehyde-agarose gel, transferred to a Hybond-N membrane, and probed for the *stpA* transcript, using a specific RNA probe by previously published methods (4, 5). The blot (Fig. 1) shows significant induction of the *stpA* transcript in both *hns* mutants; the level of induction is slightly higher in the *hns2* mutant CJD829 than in the *hns-205*::Tn*10* strain GM230. Western blotting with a polyclonal antiserum that recognizes both H-NS and StpA suggested that levels of StpA protein are also similar in the two *hns* mutant strains (data not shown). This is inconsistent with the continued repression of *bgl* in GM230 being due to an enhanced induction of StpA in this strain compared to that in CJD829.

StpA is necessary for repression of *bgl* by truncated H-NS. We then used P1*cml* transduction (18) to introduce the Δ*stpA*::Tc^r allele (24) into GM37 and also into the *hns* mutant CJD829, creating strains CJD1124 and CJD1125. Because both the Δ*stpA*::Tc^r allele and the *hns-205*::Tn*10* allele encode tetracycline resistance, we used a derivative of GM230 containing the linked *hnrG*::Ap^r allele (CJD899 [3]) as a P1*cml* donor to transfer the *hns-205*::Tn*10* allele into the Δ*stpA*::Tc^r strain CJD1124, generating CJD1126. The *hns-205*::Tn*10* and *hnrG*::Ap^r alleles are ≈95% linked, and cotransduction of *hns-205*::Tn*10* into CJD1126 was verified by Southern blotting (data not shown). Duplicate cultures of these strains were then grown overnight in M9 medium supplemented with 0.4% (wt/vol) succinate, 0.2% (wt/vol) Casamino Acids, and 5 mM β-methyl-D-glucoside, and phospho-β-glucosidase was assayed as before (Table 1). It can be seen that the *stpA* mutation on its own causes no derepression of *bgl* expression, which is consistent with the dominant effect of H-NS over StpA in many systems in wild-type strains (24) (see below). Likewise, the combination of the Δ*stpA*::Tc^r and *hns2* mutations leads to little extra derepression of *bgl* over that seen in the *hns2* single mutant strain despite the fact that *stpA* expression is strongly induced in this strain (Fig. 1), suggesting that StpA by itself is a poor repressor of *bgl*. However, when the *stpA* mutation is combined with the *hns-205*::Tn*10* allele, strong derepression of *bgl* expression to a level similar to that in the *hns2* mutant is observed. Thus, the continued repression of *bgl* by the truncated H-NS protein produced by *hns-205*::Tn*10* strains is absolutely dependent upon the presence of StpA. The most likely explanation for this observation is that StpA provides the DNA-binding function allowing this fragment of H-NS to be targeted to and repress the *bgl* promoter. However, it remains possible that StpA is merely required for the synthesis of a second protein which functions as a corepressor at the *bgl* promoter. As a control, we confirmed that the *hnrG*::Ap^r allele used as a transductional marker has no effect on *bgl* expression (Table 1).

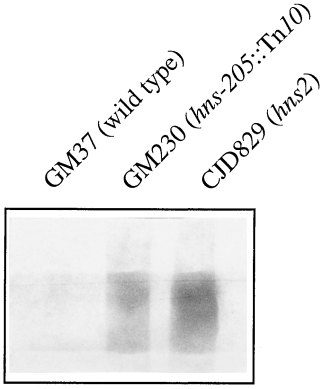

FIG. 1. Northern blot of the *stpA* transcript from wild-type strain GM37 and its *hns* mutant derivatives GM230 and CJD829.

To investigate further the role of StpA in the presence of the *hns-205*::Tn*10* mutation, we transformed CJD1126 with plasmid pYCStpA, a pACYC184 derivative carrying the wild-type *stpA* gene (23). pYCStpA was able to restore full repression of phospho-β-glucosidase production, as predicted if the plasmid-encoded StpA protein can act as a DNA-binding adapter for the truncated H-NS derivative produced in this strain. Moreover, when plasmid pYCStpAΔ65C, which encodes a truncated StpA derivative lacking the presumptive DNA-binding domain (23), was transformed into CJD1126, repression was not restored (Table 1), suggesting that DNA binding by StpA is essential for this repression mechanism to operate.

The combined effect of H-NS and StpA on *bgl* expression contrasts with that on *proU* expression. We sought to compare the effects of these combinations of *hns* and *stpA* alleles on *bgl* repression with their abilities to repress the *proU* operon at low osmolarity. The wild-type and mutant strains previously assayed for *bgl* expression were grown overnight in L broth; under these low-osmolarity conditions the *proU-lacZ* fusion in GM37 remains repressed (8) (Table 2). When β-galactosidase activity in the mutant strains was assayed (as described by Miller [11]), both the *hns-205*::Tn*10* and *hns2* alleles were seen to allow significant derepression of *proU*, although this was

TABLE 2. Effects of *hns* and *stpA* mutations on expression of the *proU-lacZ* fusion in GM37 and its derivatives as assayed by β-glucosidase activity

Strain	Relevant genotype	β-Galactosidase activity[a]
GM37	Wild type	8.57 (0.07)
CJD1124	Δ*stpA*::Tc^r	10.6 (0.29)
CJD898	*hnrG*::Ap^r	6.87 (0.07)
GM230	*hns-205*::Tn*10*	117 (2.83)
CJD899	*hns-205*::Tn*10 hnrG*::Ap^r	101 (0)
CJD1126	*hns-205*::Tn*10* Δ*stpA*::Tc^r *hnrG*::Ap^r	457 (8.49)
CJD829	*hns2*	355 (0.71)
CJD1125	*hns2* Δ*stpA*::Tc^r	409 (12.0)

[a] Assays were carried out in duplicate; mean values, in Miller units, are shown. Standard deviations are in parentheses.

greater in the latter case (Table 2). The Δ*stpA*::Tc^r mutation had no effect on *proU* expression when H-NS was present but led to further enhanced transcription of the fusion when combined with either of the *hns* alleles. This demonstrates that H-NS is the dominant repressor of *proU* in a wild-type cell, as is the case for *bgl*, but that StpA exerts a measurable negative effect on *proU* expression when it is induced in an *hns* mutant strain. The significant derepression of *proU* in the *hns-205*::Tn*10* strain GM230, in contrast to the continued repression of *bgl* in this strain, implies that StpA cannot provide a DNA-binding function to allow the truncated H-NS molecule to function as a repressor at this promoter, presumably because the H-NS′-StpA-DNA complex thus formed is not conformationally suited to repression (see, however, the discussion below). Interestingly, it has recently been shown that merely targeting H-NS to the vicinity of the *proU* promoter is insufficient to repress transcription (10), suggesting that the architecture of the repressive nucleoprotein complex formed by H-NS at this promoter is more subtle than that at *bgl*.

Conclusions. By combining two different *hns* mutations with a knockout allele of *stpA*, we have shown that the presence of the StpA protein is necessary for the ability of a truncated N-terminal fragment of H-NS to repress the *bgl* promoter. Derepression of *bgl* in this *hns-205*::Tn*10* Δ*stpA*::Tc^r double mutant can be complemented by wild-type StpA but not truncated StpA lacking the presumptive DNA-binding domain, expressed from a multicopy plasmid. This suggests that StpA acts as an adapter molecule which provides a DNA-binding function to target this truncated H-NS′ protein to the *bgl* regulatory region, although it remains possible that the effect of StpA is indirect. In contrast, in a strain containing a mutation in *hns* which leads to the production of no detectable H-NS protein fragment, the *stpA* mutation has only a limited derepressive effect on β-glucosidase production, suggesting that StpA by itself is a rather poor repressor of the *bgl* operon. This is despite the significant induction of the chromosomal *stpA* gene in this strain and implies that differences in the N-terminal regions of H-NS and StpA affect their respective repression abilities. These results contrast with those obtained when the same strains are assayed for derepression of the H-NS-regulated *proU* operon at low osmolarity. In this case the operon is derepressed no matter which *hns* mutation is present, although again StpA may partially substitute as a low-efficiency repressor in an H-NS-independent fashion at this promoter.

Although the situation in which StpA is coexpressed in the cell with a truncated derivative of H-NS is an artificial one, the implications of these results for wild-type cells are twofold. Firstly, they provide further evidence for the different mechanisms of H-NS-mediated repression in operation at the *bgl* and *proU* promoters. The conclusion of Ueguchi et al. (21) that an H-NS DNA-binding function is not necessary for repression of *bgl* is taken further by the demonstration that what is necessary is the DNA-binding function of a related protein (StpA) to substitute. Therefore, it seems that the *bgl* promoter is quite tolerant of substantial changes in the nature of the repressor-DNA complex in its regulatory region in a way that the *proU* promoter is not. In this context, it is interesting that a heterologous DNA curve-binding protein, Btcd from mouse cells, is able to substitute for H-NS in *bgl* regulation but not in *proU* regulation (20), confirming that the *bgl* regulatory system is much more amenable to manipulation. This may in itself aid mechanistic studies on the role of H-NS in repressing this promoter. Intriguingly, Btcd can also restore flagellar expression to an *hns* mutant strain (20), as can an H-NS-related protein from *Bordetella pertussis* (7), suggesting that the *bgl* system is not unique in possessing this flexibility.

The second implication of these results may be for that class of H-NS-regulated promoters which, like *proU*, are stringent in their requirements for H-NS-mediated repression. In these systems, any subtle changes in the nature of the H-NS-DNA complex could be sufficient to affect the regulation of the promoter and may be important for transcription under inducing conditions. It is noteworthy, though, that while *proU* is significantly (>10-fold) activated by the *hns-205*::Tn*10* allele, this activation is still 3- to 4-fold less than that caused by *hns2* or by combining *hns* and *stpA* mutations. Therefore, there may be a component of *proU* repression which can be mediated by StpA in conjunction with truncated H-NS, on top of which the more stringent H-NS-dependent repression works. Elucidation of the molecular mechanisms of *proU* repression and induction will be required in order to evaluate these hypotheses.

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REFERENCES

1. **Defez, R., and M. De Felice.** 1981. Cryptic operon for β-glucoside metabolism in *Escherichia coli* K-12: genetic evidence for a regulatory protein. Genetics **97:**11–25.
2. **Dersch, P., S. Kneip, and E. Bremer.** 1994. The nucleoid-associated DNA-binding protein H-NS is required for the efficient adaptation of *Escherichia coli* K-12 to a cold environment. Mol. Gen. Genet. **245:**875–889.
3. **Free, A.** 1995. H-NS and the regulation of transcription in *Escherichia coli*. Ph.D. thesis. Trinity College, University of Dublin, Dublin, Ireland.
4. **Free, A., and C. J. Dorman.** 1994. *Escherichia coli tyrT* gene transcription is sensitive to DNA supercoiling in its native chromosomal context: effect of DNA topoisomerase IV overexpression on *tyrT* promoter function. Mol. Microbiol. **14:**151–161.
5. **Free, A., and C. J. Dorman.** 1997. The *Escherichia coli stpA* gene is transiently expressed during growth in rich medium and is induced in minimal medium and by stress conditions. J. Bacteriol. **179:**909–918.
6. **Free, A., and C. J. Dorman.** Unpublished data.
7. **Goyard, S., and P. Bertin.** 1997. Characterization of BpH3, an H-NS-like protein in *Bordetella pertussis*. Mol. Microbiol. **24:**815–823.
8. **Higgins, C. F., C. J. Dorman, D. A. Stirling, L. Waddell, I. R. Booth, G. May, and E. Bremer.** 1988. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *S. typhimurium* and *E. coli*. Cell **52:**569–584.
9. **Hulton, C. S. J., A. Seirafi, J. C. D. Hinton, J. M. Sidebotham, L. Waddell, G. D. Pavitt, T. Owen-Hughes, A. Spassky, H. Buc, and C. F. Higgins.** 1990. Histone-like protein H1 (H-NS), DNA supercoiling and gene expression in bacteria. Cell **63:**631–642.
10. **Jordi, B. J. A. M., A. E. Fielder, C. M. Burns, J. C. D. Hinton, N. Dover, D. W. Ussery, and C. F. Higgins.** 1997. DNA binding is not sufficient for

H-NS-mediated repression of *proU* expression. J. Biol. Chem. **272:**12083–12090.
11. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Mukerji, M., and S. Mahadevan.** 1997. Characterization of the negative elements involved in silencing the *bgl* operon of *Escherichia coli*: possible roles for DNA gyrase, H-NS, and CRP-cAMP in regulation. Mol. Microbiol. **24:**617–627.
13. **Prasad, I., and S. Schaefler.** 1974. Regulation of the β-glucoside system in *Escherichia coli* K-12. J. Bacteriol. **120:**638–650.
14. **Schnetz, K.** 1995. Silencing of *Escherichia coli bgl* promoter by flanking sequence elements. EMBO J. **14:**2545–2550.
15. **Schnetz, K., and J. C. Wang.** 1996. Silencing of the *Escherichia coli bgl* promoter: effects of template supercoiling and cell extracts on promoter activity *in vitro*. Nucleic Acids Res. **24:**2422–2428.
16. **Shi, X., and G. N. Bennett.** 1994. Plasmids bearing *hfq* and the *hns*-like gene *stpA* complement *hns* mutants in modulating arginine decarboxylase gene expression in *Escherichia coli*. J. Bacteriol. **176:**6769–6775.
17. **Shindo, H., T. Iwaki, R. Ieda, H. Kurumizaka, C. Ueguchi, T. Mizuno, S. Morikawa, H. Nakamura, and H. Kuboniwa.** 1995. Solution structure of the DNA binding domain of a nucleoid-associated protein, H-NS, from *Escherichia coli*. FEBS Lett. **360:**125–131.
18. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Sondén, B., and B. E. Uhlin.** 1996. Coordinated and differential expression of histone-like proteins in *Escherichia coli*: regulation and function of the H-NS analog StpA. EMBO J. **15:**4970–4980.
20. **Timchenko, T., A. Bailone, and R. Devoret.** 1996. Btcd, a mouse protein that binds to curved DNA, can substitute in *Escherichia coli* for H-NS, a bacterial nucleoid protein. EMBO J. **15:**3986–3992.
21. **Ueguchi, C., T. Suzuki, T. Yoshida, K. Tanaka, and T. Mizuno.** 1996. Systematic mutational analysis revealing the functional domain organization of *Escherichia coli* nucleoid protein H-NS. J. Mol. Biol. **263:**149–162.
22. **Ussery, D. W., J. C. D. Hinton, B. J. A. M. Jordi, P. E. Granum, A. Seirafi, R. J. Stephen, A. E. Tupper, G. Berridge, J. M. Sidebotham, and C. F. Higgins.** 1994. The chromatin-associated protein H-NS. Biochimie **76:**968–980.
23. **Williams, R. M., S. Rimsky, and H. Buc.** 1996. Probing the structure, function, and interactions of the *Escherichia coli* H-NS and StpA proteins by using dominant negative derivatives. J. Bacteriol. **178:**4335–4343.
24. **Zhang, A., S. Rimsky, M. E. Reaban, H. Buc, and M. Belfort.** 1996. *Escherichia coli* protein analogs StpA and H-NS: regulatory loops, similar and disparate effects on nucleic acid dynamics. EMBO J. **15:**1340–1349.