The EMBO Journal vol.15 no.18 pp.4970–4980, 1996

Coordinated and differential expression of histone-like proteins in *Escherichia coli*: regulation and function of the H-NS analog StpA

Berit Sondén and Bernt Eric Uhlin[1]

Department of Microbiology, Umeå University, S-901 87 Umeå, Sweden

[1]Corresponding author

The histone-like protein H-NS has been shown to influence the regulation of gene expression at the transcriptional level in several *Escherichia coli* operons. We have examined the regulation of the *stpA* gene, which encodes a protein sharing 58% identity with H-NS, by mRNA analysis and by using *stpA–lacZ* operon fusions. The expression of *stpA* is temperature dependent, with 2-fold higher expression at 37°C than at 26°C. In addition, *stpA* expression is stimulated by the global regulator Lrp. In an *hns* mutant *E.coli* derivative *stpA* expression is derepressed, suggesting that regulation of the two genes is coupled. Overproduction of the StpA protein affects expression from at least four *hns* regulated operons (the *papB*, *proU*, *bgl* and *hns* operons), in both the presence and absence of H-NS. The construction of *E.coli* strains carrying mutations in both *stpA* and *hns* demonstrated that the absence of both proteins affects growth rate and viability of the cells. Our work establishes that *E.coli* can express two H-NS-like proteins with coordinated yet differential regulation. Evidently, these proteins have both overlapping and distinct functions in the cell, and they are both important for normal cell growth and gene control.

Keywords: *Escherichia coli*/gene regulation/nucleoid-associated proteins

Introduction

The bacterial chromosome can be isolated as a nucleoid in which the DNA is associated with a number of proteins presumed to be important for DNA organization in the cell (reviewed by Drlica and Rouviére-Yaniv, 1987; Pettijohn, 1988). One of the most abundant nucleoid-associated proteins in *Escherichia coli* is H-NS, with ~20 000 copies per cell (Spassky *et al.*, 1984; reviewed in Higgins *et al.*, 1990; Ussery *et al.*, 1994). H-NS binds double-stranded DNA nonspecifically, but with a preference for curved DNA (Yamada *et al.*, 1991; Owen-Hughes *et al.*, 1992). Overproduction of H-NS has severe effects on cell viability and results in a striking condensation of the bacterial chromosome (Spurio *et al.*, 1992). The amount of H-NS within the cell is therefore likely to be under tight control, which is presumably achieved by the negative autoregulation of the *hns* gene (Dersch *et al.*, 1993; Ueguchi and Mizuno, 1993). Expression of *hns* is influenced further by binding of the FIS protein to the *hns* promoter region (La Teana *et al.*, 1991; Falconi *et al.*, 1996). The *hns* gene is cold-shock regulated such that transcription is enhanced 3- to 4-fold following a shift from 37 to 10°C (La Teana *et al.*, 1991). This induction involves the binding of CspA, the major cold-shock protein of *E.coli*, to the *hns* promoter region (La Teana *et al.*, 1991). The intracellular level of H-NS has also been reported to increase 3-fold at the stationary phase (Spassky *et al.*, 1984; Dersch *et al.*, 1993; Ueguchi and Mizuno, 1993), although this finding has not been observed in other reports (Hinton *et al.*, 1992; Yasuzawa *et al.*, 1992; Free and Dorman, 1995). Recent data further suggest that expression of the *hns* gene is coupled to DNA synthesis (Free and Dorman, 1995).

In recent years it has become apparent that the H-NS protein is involved, directly or indirectly, in a number of cellular functions. Mutations in *hns* have been shown to result in altered frequencies of transposition, chromosomal deletions and site-specific recombination events (Lejeune and Danchin, 1990; Falconi *et al.*, 1991). Mutations in *hns* are also highly pleiotropic, affecting the expression of a number of genes (Göransson *et al.*, 1990; Hulton *et al.*, 1990; May *et al.*, 1990; Olsén *et al.*, 1993; Yamashino *et al.*, 1995). The genes affected by H-NS are dispersed throughout the chromosome and are often regulated in response to changes in environmental conditions. Among the genes affected by *hns* mutations are the *pap* genes, encoding di-galactoside binding pili in uropathogenic *E.coli.* We have shown previously that a mutation in *hns* leads to the loss of *pap* thermoregulation and an increase in transcription at both 37 and 26°C (Göransson *et al.*, 1990).

stpA, a putative homolog to the gene encoding the H-NS protein in *E.coli*, was first identified as a multicopy suppressor of a Td^- phenotype in phage T4 (Zhang and Belfort, 1992). More recently, Shi and Bennett (1994) identified the *stpA* gene as a multicopy suppressor that could transcomplement *hns* mutants in the modulation of arginine decarboxylase gene expression in *E.coli.* The high sequence identity (58%) between the protein predicted to be encoded by the *stpA* gene and the H-NS protein suggested that the two proteins could have similar functions. In this study we performed experiments to characterize the regulation and function of the *stpA* gene and its product. In particular, we studied its influence on the transcriptional regulation of the *pap* operon and other operons known to be H-NS regulated. The results show that StpA can substitute for H-NS and presumably acts as a backup in hitherto studied *hns* mutants of *E.coli.*

Results

Expression of stpA is affected by H-NS and temperature

To determine whether the expression of the *stpA* gene was affected by H-NS and temperature at the transcriptional

© Oxford University Press

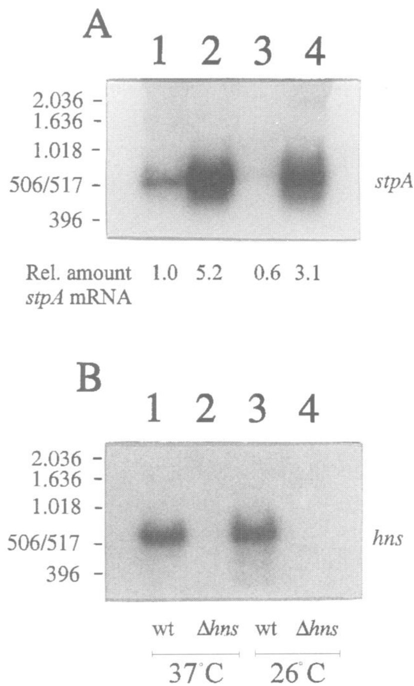


Fig. 1. Northern blot hybridization of total RNA isolated from strains grown in LB medium to logarithmic phase at 37 and 26°C. Lane 1, HMG11 (wt), 37°C; lane 2, HMG9 (Δ*hns*), 37°C; lane 3, HMG11 (wt), 26°C; lane 4, HMG9 (Δ*hns*), 26°C. (**A**) Hybridization with a *stpA*-specific probe. (**B**) Hybridization with an *hns*-specific probe.

level, Northern blot hybridization experiments were performed on RNA from wild type and an *hns*-deleted strain of *E.coli* in logarithmic phase at 37 and 26°C. As seen in Figure 1A, the 700 nucleotide *stpA* transcript is expressed at low levels at 37°C, but was hardly detectable at 26°C. In the *hns* mutant the expression of *stpA* was derepressed and the level of transcription was increased at both 26 and 37°C. Quantification of the *stpA* signal showed an ~2-fold effect of temperature on the regulation of transcription in both strains. Figure 1B shows the result obtained after the same membrane was hybridized with the *hns*-specific probe. The *hns* gene is expressed at the same level both at 37 and 26°C (Göransson *et al.*, 1990). A quantitative analysis of the relative amount of StpA protein in the cell, by immunoblotting with a polyclonal antiserum recognizing both StpA and H-NS, confirmed that the temperature regulation of StpA was also manifested at the protein level (data not shown).

Characterization of the stpA gene regulatory region

The *stpA* gene maps to the region carried by Kohara phage λ444 (Kohara *et al.*, 1987). A 9 kb *Kpn*I–*Hin*dIII subclone (pBSN43) was used as a source of *stpA* DNA in our studies (Figure 2A). To study the regulation of *stpA* we characterized the upstream region of the gene (Figure 2B). A short transcriptional *stpA–lacZ* fusion (pBSN32) was constructed by PCR. This fusion contains the *stpA* upstream region required for the expression of *stpA* according to the report by Zhang and Belfort (1992). Because the fusion (pBSN32) showed only a low level of β-galactosidase activity when grown in LB medium at 37°C, we mapped the *stpA* promoter(s) by primer extension. A primer extension analysis was performed on total RNA from both a wild-type and an *hns*-deleted strain grown at 37°C to logarithmic growth phase in LB medium. Four extension products were produced in both strains (Figure 3). The 5′ ends of the *stpA* mRNA seemed to be identical in an *hns* mutant compared with a wild type. The two most prominent transcription start points appeared to be at the A and the G residues 41 and 42 bp upstream respectively of the *stpA* translational start codon (Figure 2B). The two other start sites were located at the A residue at position 61 and the T residue 83 bp upstream of the *stpA* start codon. The positions of the extension products suggested that *stpA* has at least three promoters. Postulated promoter sequences for the prominent start points are indicated in Figure 2B. The start point 41 bp upstream of the *stpA* translational start codon is identical to the one identified by Shi and Bennett (1994).

Two *stpA–lacZ* fusions containing longer upstream regions were constructed in the vector pRZ5202. The endpoints of the different *lacZ* fusion constructs are shown in Figure 2B. The level of β-galactosidase activity was measured during growth in LB medium at 37 and 26°C. The results, summarized in Figure 4A, suggest that sequence information sufficient for transcriptional activation and temperature regulation of the *stpA* gene is present within the 332 bp *stpA* upstream region in plasmid pBSN58. The longer ~0.7 kb fusion construct pBSN57 gave essentially identical results. In contrast, the shortest fusion, pBSN32, seemed to lack the temperature regulation and showed only a low basal β-galactosidase activity at both 37 and 26°C.

A sequence analysis of the *stpA* upstream region revealed that it is very AT-rich (Figure 2B). A computer prediction of the DNA structure (using the BEND programme of the DNASTAR software package) suggested that it may contain intrinsic curvature (data not shown). The regulatory region upstream of *hns* has also been shown to contain intrinsic curvature, where H-NS has been shown to bind and, by autoregulation, affect its own expression (Dersch *et al.*, 1993; Falconi *et al.*, 1993). The regulatory features of the *stpA* operon, being affected by H-NS and by temperature, resemble those found in other cases of transcriptional control subject to temperature-dependent regulation. For example, the *pap*, *cfa* and *sfa* fimbrial adhesin operons are regulated in a similar fashion (Göransson *et al.*, 1990; Jordi *et al.*, 1992; Morschhäuser *et al.*, 1993). Furthermore, such operons have been shown to be regulated by the global regulator Lrp, which is involved in the regulation of at least 30 different proteins in *E.coli* (Ernsting *et al.*, 1992; reviewed in Newman *et al.*, 1992; D'Ari *et al.*, 1993; Calvo and Matthews, 1994). To determine whether s*tpA* is regulated by Lrp, we introduced the *stpA–lacZ* fusion constructs into a *lrp* mutant strain (BEU683) and its wild-type parent strain (BEU602). The strains were grown in LB medium at 37°C and the β-galactosidase activity measured. As shown in Figure 4B, there was only a relatively small difference in expression from the *stpA* promoters, but both pBSN57 and pBSN58 showed an ~20% reduction of β-galactosidase units in the *lrp* mutant strain in comparison with the wild type. Furthermore, the fusions were introduced into an *hns* mutant strain (BEU601) and an *hns*, *lrp* double mutant strain (BEU681). The H-NS deficiency caused derepressed *stpA–lacZ* expression in the case of the short fusion

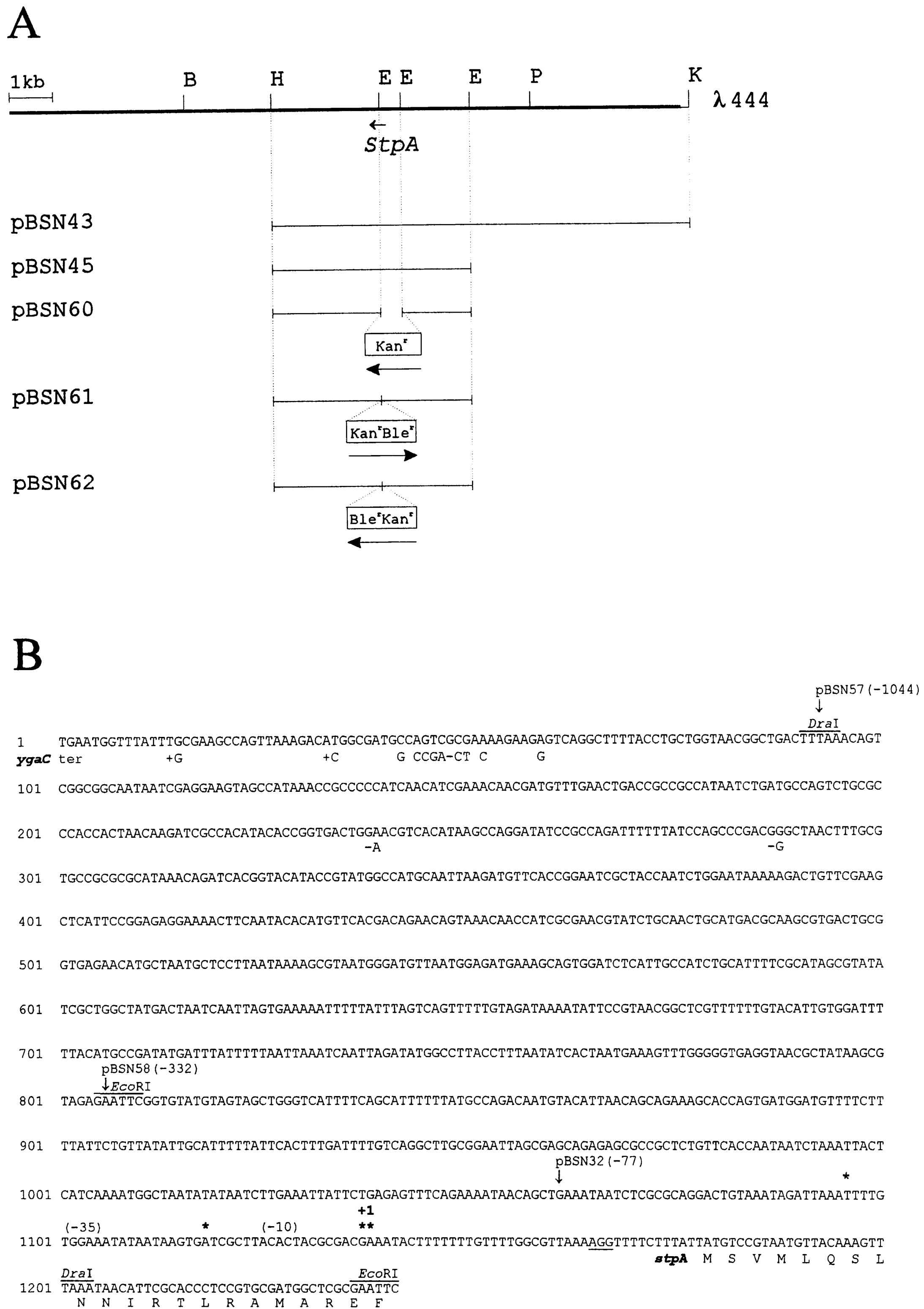


Fig. 2. (**A**) Map of the constructs used in cloning, nucleotide sequencing and mutagenesis of the *stpA* gene. The locations of the restriction sites are indicated as follows: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I. (**B**) Nucleotide sequence of the *stpA* upstream region. The transcriptional start points determined by primer extension are denoted by asterisks, and predicted promoter sequences (–35 and –10 regions) are indicated for the strongest extension. The 5′ ends of the *stpA* operon fusions pBSN57, pBSN58 and pBSN32 are indicated by arrows. Our nucleotide sequence analysis largely confirmed the sequence reported by Shi and Bennett (1994), but a few differences (indicated by letters below the sequence) were noted in the upstream region. The restriction sites *Eco*RI and *Dra*I used in the cloning are included.

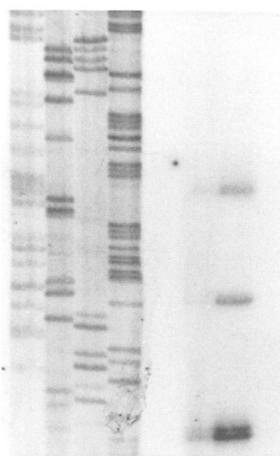


Fig. 3. Primer extension analysis of the start point of the *stpA* transcripts. Total RNA isolated from HMG11 (wt) (lane 1) and HMG9 (Δ*hns*) (lane 2) was used for primer extension. Lanes A, C, G and T show the sequence analysis of plasmid pBSN43 using the same primer as a reference.

construct (pBSN32). Despite repeated attempts, we were unable to establish the pBSN57 and pBSN58 fusion constructs in the *hns* mutant strain. We were, however, able to establish the longer fusions in the *hns*, *lrp* double mutant strain, and the results are consistent with the observation that LRP stimulates *stpA* expression at the transcriptional level in a manner involving the upstream region carried by the *stpA–lacZ* fusion pBSN58 and pBSN57. Presumably the high copy number of these plasmids caused detrimentally high levels of expression from the longer fusions in the absence of H-NS because we could establish low copy number *stpA* fusions (pBSN157 and pBSN158 based on the mini F vector pFZY1) in an *hns* mutant strain (Figure 4C). The low copy number fusions showed a 10-fold derepression of expression in an *hns* mutant compared with the wild-type strain, consistent with the above-described results from a Northern blot analysis. The finding that the shortest fusion construct, pBSN32, showed a strong derepression in the *hns* mutant strains suggests that H-NS exerts a significant negative effect by interacting close to or within the promoter region of *stpA*.

The response to leucine differs among LRP-regulated operons in *E.coli*, and positive, negative and neutral effects have been found. Furthermore, the level of LRP has been shown to be lower in rich media than in minimal media (Calvo and Matthews, 1994, and references therein). Therefore we investigated how leucine might effect *stpA* expression. A Northern blot analysis was performed with RNA extracted from a wild type (W3110), a *lrp* mutant (BSN21) and an *hns* mutant (BSN22), grown at 37°C in glucose minimal medium supplemented with isoleucine and valine in the presence or absence of 10 mM leucine. Table I summarizes the results of this experiment. The level of *stpA* transcription showed a 2-fold reduction in an *lrp* mutant strain. Furthermore, the transcription of *stpA* was reduced to the same degree by the presence of leucine in the growth medium, indicating that leucine repression is mediated via Lrp.

Table I. Relative amounts of the *stpA* transcript in strains grown in glucose minimal media, 0.4 mM isoleucine and 0.4 mM valine with and without 10 mM leucine at 37°C

Strain	Relative level of *stpA* mRNA[a]	
	–Leucine	+Leucine
W3110 (wt)	1.0	0.31
BSN21 (*lrp*::Tn*10*)	0.44	0.36
BSN22 (*hns*::*cat*)	3.33	2.01

[a]Quantification of the *stpA* mRNA levels was performed as described in Materials and methods.

Binding of Lrp and H-NS protein to the stpA regulatory region

To test whether Lrp and H-NS interacts directly with the *stpA* regulatory region, gel mobility shift assays were performed. [^{32}P]ATP-end-labeled fragments representing different parts of the presumed *stpA* regulatory region were mixed with purified Lrp and H-NS protein. The mixtures were then subjected to gel mobility shift assays. Gel mobility shifts with purified Lrp protein were detected with fragments I (covering the *stpA* promoter region) and II (covering the *stpA* upstream region farther up) (Figure 5A). Similar shifts were seen when using crude extract from an Lrp-over-producing strain, but not with protein extract from an *lrp*-deleted strain (data not shown). Gel mobility shift assays with purified H-NS protein also detected shifted mobility with fragments I and II (Figure 5B). The findings from the gel mobility shift assays, that both Lrp and H-NS can interact directly with sequences in the *stpA* regulatory region, are consistent with the effects described above on *stpA* expression in *hns* and *lrp* mutant strains.

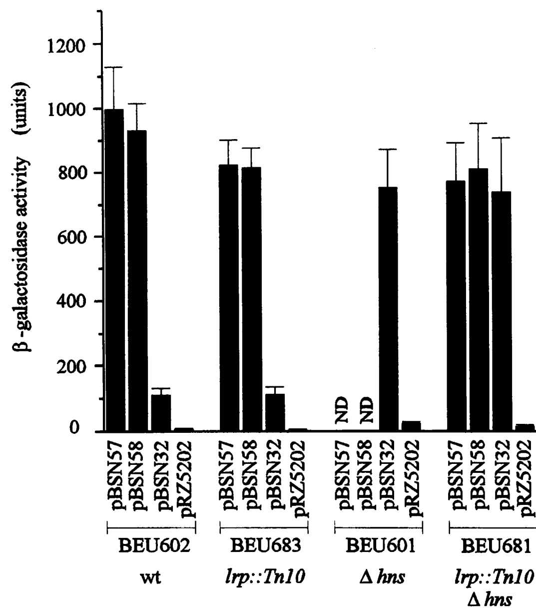
Effect of induced expression of the stpA gene product

Experiments with the *stpA* gene cloned in a plasmid vector suggest that it may at least partially complement mutations in *hns*. Our clone carrying the intact *stpA* gene with its native promoter resulted in normal growth at low temperature (26°C) of a Δ*hns* mutant strain, which otherwise shows a cold-sensitive phenotype (data not shown). To examine how StpA might affect the transcription of different operons, we constructed an expression plasmid from which the StpA protein could be expressed and overproduced in a regulated manner. For this purpose the *stpA* gene was cloned into the low copy number vectors pBSN47 and pMMB66HE, such that their expression was dependent on the IPTG-inducible *tac* promoter. Because high levels of H-NS are known to be lethal to the cell (Spurio *et al.*, 1992; our unpublished data) and because we made similar observations with StpA (unpublished data), the level of StpA was induced only partially in the following experiments to a level still allowing normal cell growth. To determine whether StpA could affect *hns* expression, we measured β-galactosidase activity in strains harboring a translational *hns–lacZ* fusion (pBSN2), together with the StpA expression plasmid (pBSN63). As shown in Figure 6A, the induction of StpA overproduction caused a significant decrease in the expression of H-NS. This was also confirmed directly by an immunoblot analysis of cell extracts from the strains overproducing StpA using a polyclonal antiserum recognizing H-NS (data

A

Fusion plasmid		β-galactosidase activity MC1029 37°C	β-galactosidase activity MC1029 26°C
stpA-lacZ		(Miller units)	
(-1044)	pBSN57	549	219
(-332)	pBSN58	504	209
(-77)	pBSN32	27	34
	pRZ5202	5	3

B



C

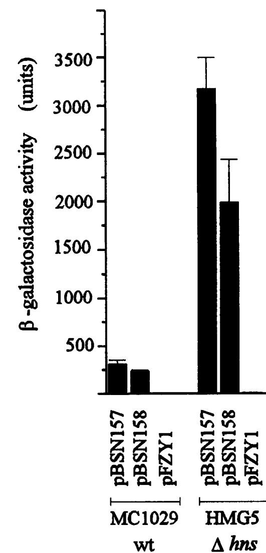


Fig. 4. (**A**) Expression of β-galactosidase activity by *stpA–lacZ* operon fusions pBSN57, pBSN58 and pBSN32 containing different lengths of *stpA* upstream sequence in strain MC1029. The strains were grown to logarithmic phase in LB medium at 37 and 26°C. (**B**) Expression of β-galactosidase activity by *stpA–lacZ* operon fusions in BEU601 (wt), BEU683 (*lrp*), BEU602 (*hns*) and BEU681 (*lrp, hns*) grown in LB medium to logarithmic phase at 37°C. ND, not determined. (**C**) Expression of β-galactosidase activity by low copy number *stpA–lacZ* operon fusions pBSN157 and pBSN158 in MC1029 (wt) and HMG5 (*hns*) grown in LB medium to logarithmic phase at 37°C.

not shown). The StpA-overproducing plasmid (pBSN63) was also introduced into strain MC1029, together with *lacZ* operon fusion plasmids containing the *pap* operon promoter (pHMG118) and the *alaS* promoter (pHMG117) as a reference. As shown in Figure 6B, the β-galactosidase activity from the *pap–lacZ* fusion showed a reduced expression upon induction of StpA, but no effect was seen on the expression from the *alaS* promoter. The H-NS protein is also known to negatively affect transcription from the osmoregulated *proU* promoter (Higgins *et al.*, 1988; Hulton *et al.*, 1990; May *et al.*, 1990). The StpA-overproducing plasmid pBSN42 was therefore introduced into strain BEU693, which contains a chromosomal *proU: lacZ* fusion. In this test we also included a Δ*hns* derivative based on strain BEU693. As shown in Figure 6C, the overproduction of StpA by plasmid pBSN42 caused a clear reduction in *proU–lacZ* expression in both wild-type and Δ*hns* strains. The induced StpA evidently exerts its negative effect on transcription from the *proU* promoter independently of H-NS.

A similar effect of StpA overproduction was obtained with the β-glucoside operon. The (*bgl*) operon is cryptic in wild-type strains and derepressed in H-NS-deficient mutants of *E.coli* (Defez and Defelice, 1981). By monitoring the phenotype of the StpA-overproducing strains growing on plates containing a chromogenic β-glucoside, we found that StpA could repress the *bgl* operon in the absence of a functional H-NS-encoding locus (data not shown). Taken together, the results demonstrate that StpA can silence four known H-NS-regulated operons, and that it can function as a silencer in both the presence and absence of H-NS. Furthermore, it is evident from the above experiments (and the Northern blot analysis) that StpA can control the expression of *hns* and vice versa.

Construction of stpA gene disruption mutants

As StpA seemed to have a function similar to H-NS, and the expression of the *stpA* gene was found to be somewhat derepressed in an *hns* mutant strain, it is possible that StpA might compensate for the loss of H-NS in *hns*-

negative mutants. To examine the possible effects of mutations abolishing the *stpA* gene, we constructed three different *stpA* gene disruption mutants. This was carried out by inserting a kanamycin and bleomycin resistance gene cassette from Tn*5* into the *Eco*RI site situated within the cloned *stpA* gene (plasmid pBSN45) to generate plasmids pBSN61 and pBSN62 (Figure 2A). We also constructed a plasmid (pBSN60), in which we inserted a kanamycin resistance cassette from Tn*903* and deleted the small *Eco*RI fragment containing the *stpA* promoters (Figure 2A). The constructs were transferred to the MC1061 chromosome by the λ replacement method (Kulakauskas *et al.*, 1991), and putative mutants were checked and confirmed by a Southern blot hybridization analysis (data not shown). The mutations were subsequently moved by P1-mediated transduction to strain HMG11. The different alleles were denoted *stpA60::km* (BSN6), *stpA61::km* (BSN9) and *stpA62::km* (BSN12). The transductants were checked for typical *hns* mutant phenotypes such as growth deficiency at lower temperatures and the expression of β-glucosidase activity, but they did not show any of these phenotypes on rich medium. Double mutant *hns*, *stpA* derivatives grew slower than the parents and showed typical H-NS-deficient phenotypes. Figure 7 shows growth curves and viable counts of a wild-type (HMG11) strain, a Δ*hns* mutant (HMG9) strain, the *stpA60::km* mutant (BSN6) strain and a Δ*hns*, *stpA60::km* mutant (BSN7) strain all grown in broth medium at 37°C. The different strains were allowed to grow to a stationary phase, and then diluted into fresh medium and their growth monitored with optical density and viable count over a 12 h batch culture cycle. The double mutant strain (BSN7) showed a clear reduction in growth rate (monitored as optical density during the

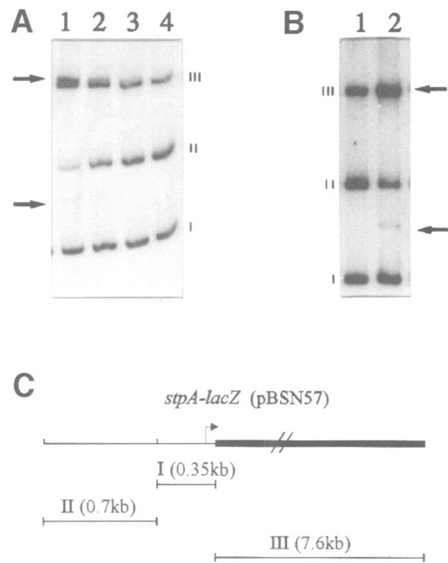


Fig. 5. Gel mobility shift analysis of the *stpA* regulatory region. DNA-binding reactions were performed with *Eco*RI/*Bam*HI-cut pBSN57 labeled with [^{32}P]ATP. The positions of unbound fragments are indicated by I, II and III, and the positions of the different gel-retarded bands are indicated by arrows. (**A**) End-labeled fragments were mixed with purified Lrp. Lane 1, 80 ng; lane 2, 16 ng; lane 3, 8 ng; lane 4, no protein. (**B**) End-labeled fragments were mixed with purified H-NS. Lane 1, no protein; lane 2, 1 µg. (**C**) Schematic representation of DNA fragments.

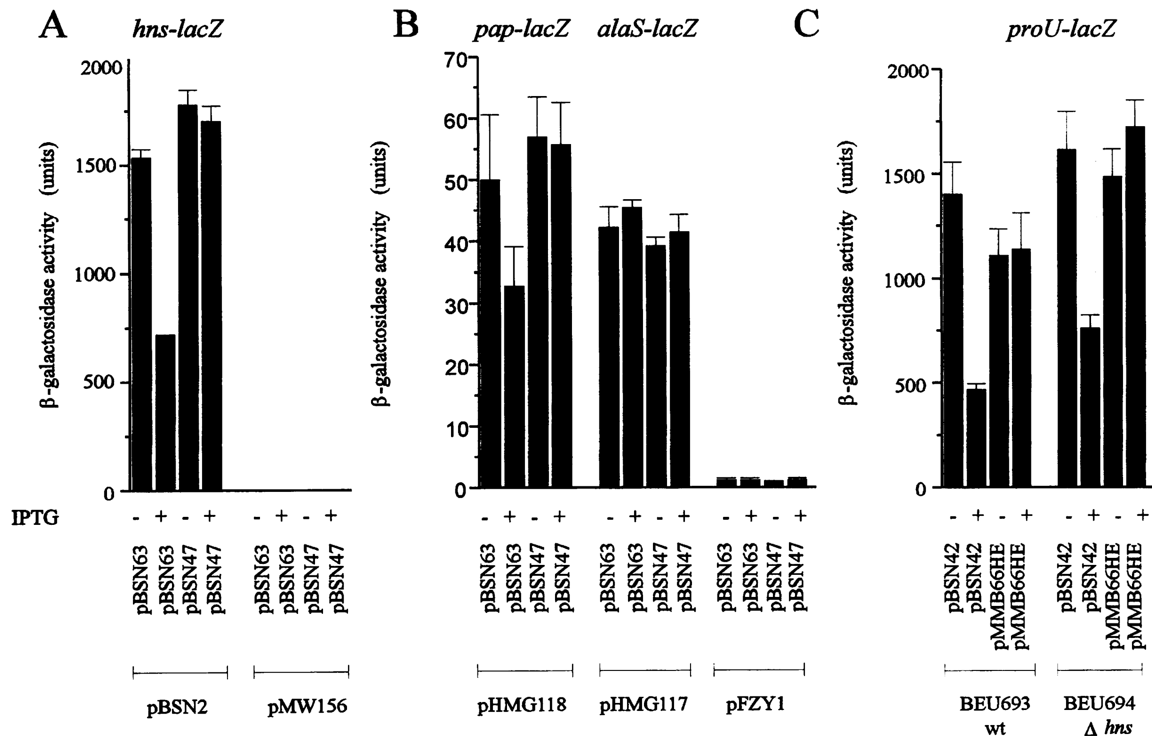


Fig. 6. Effect of overexpression of StpA on different *E.coli* operons. (**A**) Expression of β-galactosidase activity by *hns::lacZ* (pBSN2) and vector control (pMW156) upon overexpression of StpA from plasmid pBSN63. (**B**) Expression of β-galactosidase activity by *pap–lacZ* (pHMG118), *alaS–lacZ* (pHMG117) and vector control (pFZY1) upon overexpression of StpA from plasmid pBSN63. (**C**) Expression of β-galactosidase activity by a *proU–lacZ* chromosomal fusion in a wild-type (BEU693) and a Δ*hns* mutant (BEU694) strain upon overexpression of StpA from plasmid pBSN42.

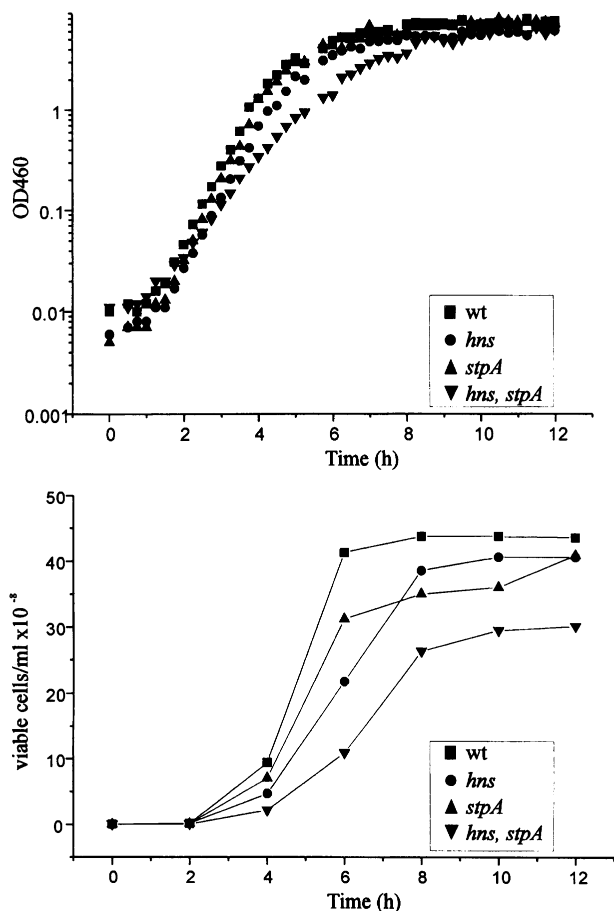


Fig. 7. Growth curve and viable count of HMG11 (wt), HMG9 (Δ*hns*), BSN6 (*stpA60::km*) and BSN7 (Δ*hns*, *stpA60::km*) grown in LB medium at 37°C.

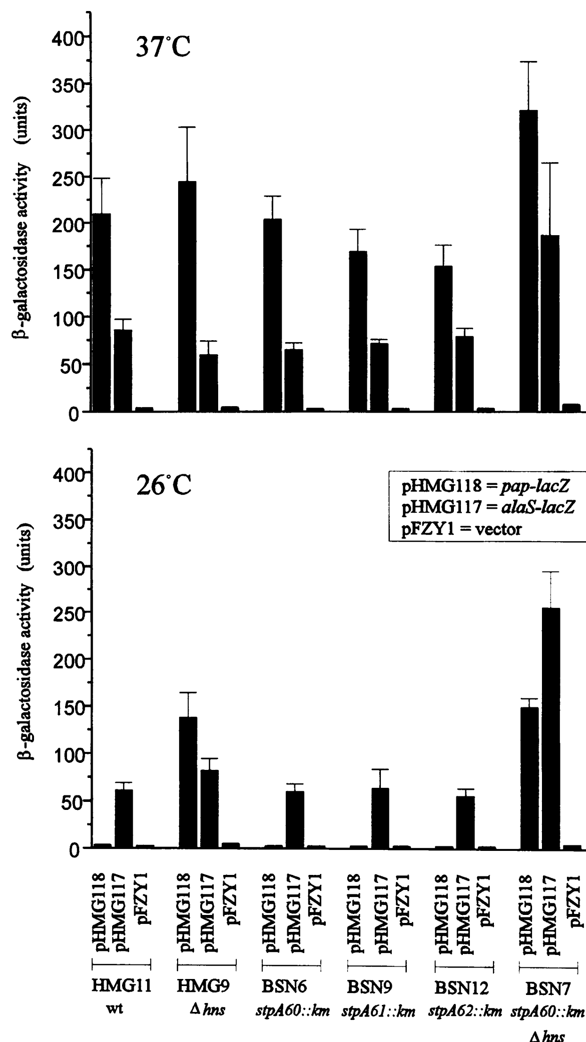


Fig. 8. Expression of β-galactosidase activity by *pap–lacZ* (HMG118) and *alaS–lacZ* (HMG117) fusions in strains HMG11 (wt), HMG9 (Δ*hns*), BSN6 (*stpA60::km*) and BSN7 (Δ*hns*, *stpA60::km*) grown in LB medium at 26 and 37°C.

logarithmic growth phase), with a doubling time of 47 min, compared with an *hns* mutant with a doubling time of 30 min and the wild type and the *stpA* mutant with doubling times of 26 and 25 min, respectively. Similar results were obtained with a set of derivatives based on strain MC4100, although the generation times were longer (data not shown). The double mutant strain stopped growing at a markedly lower cell density than the other strains. As seen in Figure 7, there was also a clear reduction of viable cells when the bacteria had been grown to the stationary phase.

Effect of stpA and hns mutations on pap transcription and thermoregulation

Our earlier studies established that *pap* transcription is derepressed in strains which are H-NS negative. In particular, this can be observed at low (<30°C) growth temperatures. To assay the effect on *pap* transcription in the different *stpA* mutants and in a double *stpA*, *hns* mutant derivative of strain HMG9, we transformed the strains with a low copy number *pap–lacZ* operon fusion plasmid. In addition to the *pap* promoter regulatory region (pHMG118), we included the *alaS* promoter (pHMG117) which does not show the same temperature dependence as *pap* (Göransson *et al.*, 1989). The activity from the different fusions was assayed after growth in LB medium at 37 and 26°C. The results are summarized in Figure 8A and B. The transcription of the *pap* operon was not affected by either of the different *stpA* mutant alleles alone. The thermoregulatory effect was similar to that of the wild-type strain and only in the case of the Δ*hns* mutant derivatives was *pap–lacZ* expression observed at 26°C. In addition, in the combined *stpA*, Δ*hns* mutant derivatives (strain BSN7) the *pap* transcription was derepressed at 26°C to a level similar to that of the wild-type strain HMG11 at 37°C. The *stpA* mutant allele appeared not to affect *pap* expression under these conditions, whereas the *alaS–lacZ* expression was clearly higher in the double mutant at 26°C. It should be noted that growth of the double mutant was severely impaired at low temperature and that the unexpected effect on *alaS* expression may be caused by the more general perturbation of the cells in that case. With respect to *pap* regulation, we concluded that StpA plays a minor role during logarithmic growth under the conditions tested here.

Discussion

The H-NS protein has been determined to play an important role in enterobacteria as a component of the bacterial

nucleoid affecting recombination, transposition and transcription. Mutant bacteria with a defective *hns* gene show a loss of regulatory features in some operons, and the ability of the cell to respond and adapt to different environmental growth conditions is severely impaired. The identification of StpA as an analog to the H-NS protein necessitated the reinterpretation of studies with *hns* mutant strains. In this work we have established that there is coupled regulation of the expression of H-NS and StpA in *E.coli*. Furthermore, our data suggest that the StpA protein can to some extent substitute for H-NS.

Studies of the expression of *stpA* by a Northern blot analysis of mRNA and *lacZ* operon fusion constructs showed that the transcription of the *stpA* gene is somewhat temperature regulated. A low level of *stpA* expression occurs in LB medium at 37°C during logarithmic growth, which is lower at 26°C. In an *hns* mutant cell, *stpA* transcription was derepressed, leading to increased levels of StpA protein. This higher level probably partially compensates for the loss of H-NS. The fact that *hns* mutants often show a temperature-dependent, cold-sensitive phenotype (Dersch *et al.*, 1994; our unpublished data) might be due to the lower level of *stpA* expression at 26°C in comparison with that at 37°C.

Our experiments with Lrp mutant strains and our analysis of protein–DNA interactions by gel mobility shift assays suggest that *stpA* expression is directly stimulated by Lrp. Lrp has been shown to be involved in the adaptation of *E.coli* to shifts in environmental conditions. The stimulation or repression exerted by Lrp on different operons in *E.coli* varies considerably, and effects between 2- and 50-fold have been found (Calvo and Matthews, 1994). The Lrp stimulation of *stpA* expression was ~2-fold, and exogenous leucine caused a 2-fold repression (Table I). By sequence comparison, using the Lrp consensus sequence of Rex *et al.* (1991), putative Lrp-binding sites could be identified in the *stpA* control region (data not shown). The target DNA sites in different operons shown to be bound by Lrp or H-NS have features in common and are often naturally bent DNA regions; several protein molecules may bind to such curved DNA (Yamada *et al.*, 1990, 1991; Newman *et al.*, 1992; Owen-Hughes *et al.*, 1992; Calvo and Matthews, 1994; Zuber *et al.*, 1994). In the case of the *ilvIH* operon, it has also been shown that the binding of Lrp induces further bending to the DNA (Wang and Calvo, 1993). Many genes where expression is affected by Lrp have actually been shown to be subjected to the effects, either directly or indirectly, of H-NS, e.g. *papB*, *fimB*, *ilvIH* and *lysU* (Kawula and Orndorff, 1991; Van der Woude *et al.*, 1992; Ito *et al.*, 1994; Levinthal *et al.*, 1994).

By overexpressing the StpA protein in a regulated manner we have shown that it can silence transcription in that it caused reduced expression of at least four operons: the *pap*, *hns*, *proU* and *bgl* operons. The silencing effect caused by StpA was seen independent of H-NS, suggesting that in an H-NS mutant strain StpA can still partially silence the transcription. The present construction of *stpA* insertion/deletion mutants showed no obvious phenotype when grown under standard laboratory conditions. This might explain why the gene has not been found in mutant searches, such as those that identified mutations in the H-NS-encoding locus at minute 27 in *E.coli*. The lack of

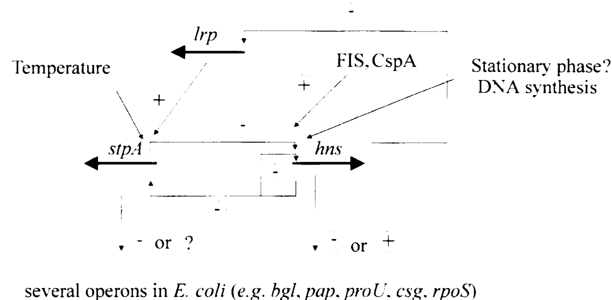


Fig. 9. Schematic summary of the regulatory network controlling the expression of *hns* and *stpA* (see text for references).

a recognizable phenotype of *stpA* mutants resembles the case of HU. Mutations in either of the genes encoding the two HU subunit (*hupA* and *hupB*) genes in *E.coli* give little phenotype (Wada *et al.*, 1988), presumably because each of the HU subunits can substitute for each other, requiring the double mutant to give a clear HU^- phenotype. It is most likely that an *stpA* mutant lacks a phenotype because the *E.coli* cell normally contains large amounts of H-NS. This could be taken as evidence that StpA is a redundant but less important component than H-NS. However, it is possible that the real function of StpA is not quite manifested under standard laboratory conditions. Recently Zhang *et al.* (1995) reported on the function of StpA in promoting group I RNA splicing. The StpA protein was found to stimulate self-splicing by promoting RNA assembly *in vitro*, suggesting that StpA also has some RNA chaperone activity. So, despite the similar features shown by the H-NS and StpA proteins presented here, evidently there are also differences in function between the two proteins. Double mutants with defects in both *stpA* and *hns* are viable under laboratory conditions, but growth and cell division are perturbed. The cells have a tendency to accumulate secondary site mutations leading to faster growing subpopulations of cells. Microscopy of the double *stpA*, *hns* mutant strain shows that it exhibits a filamentous phenotype, especially in early log phase, if compared with the wild type, the *stpA* mutant and the *hns* mutant strain (unpublished results). This might suggest that the StpA protein could play a particular role in the cell during transition from the stationary phase to logarithmic growth. Further experiments are in progress to elucidate if the levels of StpA vary with growth phase.

Our results, together with previous studies, indicate that there is an intriguing regulatory network controlling expression of the *hns* and *stpA* genes. As depicted in Figure 9, the StpA, FIS and CspA proteins seem to influence the autoregulatory system of *hns* (La Teana *et al.*, 1991; Dersch *et al.*, 1993; Ueguchi and Mizuno, 1993; Falconi *et al.*, 1996; this paper). The expression of H-NS also seems to be coupled to DNA synthesis (Free and Dorman, 1995). The different regulatory circuits provide the cell with a mechanism for fine tuning of the intracellular level of StpA and H-NS, and thus allow adjustment of the nucleoid structure and precise adaptation to physiological changes.

Materials and methods

Bacterial strains, media and phages

For routine strain manipulations, cells were grown in LB medium (Bertani, 1951) with appropriate antibiotics when needed. *E.coli* strains

used in this study are listed in Table II. Generalized transduction mediated by P1 was performed according to Willets *et al.* (1969). Transductants were routinely checked for characteristic *hns* phenotypes such as poor growth at 26°C and positive Bgl phenotype. BEU601 (Δ*hns*) and BEU602 (wt) were derived from MC1063 (*trp*::Tn*10*). MC1063 was transduced with P1 grown on HMG9 (Δ*hns*), and Trp$^+$ transductants were isolated on casa amino acid plates. BEU683 (*lrp*::Tn*10*) and BEU681 (*lrp*::Tn*10*, Δ*hns*) were derived from BEU602 and BEU601. The strains were transduced with P1 grown on SS5357 (*lrp*::Tn*10*), and transductants were isolated by selection for tetracycline resistance. BEU603 (Δ*hns*, *trp*::Tn*10*) was derived from BEU601. BEU601 was transduced with P1 grown on MC1063 (*trp*::Tn*10*), and transductants were selected for growth on tetracycline plates. Strains BEU693 (*trp*::Tn*10*) and BEU694 (*trp*::Tn*10*, Δ*hns*) were derived from strain GM37. The strain was transduced with P1 grown on BEU603 (*trp*::Tn*10*, Δ*hns*). The transductants were isolated by selection for tetracycline resistance. Strain BEU630 (*hns*::*cat*, *trp*::Tn*10*) was derived from BSN1 (*hns*::*cat*). BSN1 was transduced with P1 grown on MC1063 (*trp*::Tn*10*), and transductants were selected on tetracycline plates. Strain BSN21 (*lrp*::Tn*10*) was constructed by transduction of W3110 with P1 grown on SS5357 (*lrp*::Tn*10*), and transductants were isolated on TYS plates by selection for tetracycline resistance. Strain BSN22 (*hns*::*cat*) was constructed by transduction of W3110 with P1 grown on BEU630 (*hns*::*cat*, *trp*::Tn*10*). Transductants were selected on chloramphenicol plates and checked for tetracycline sensitivity.

Table II. *E.coli* strains used in this study

Strain	Relevant characteristics	Reference/source
MC1029	*recA*	Casadaban and Cohen (1980)
MC1061	Δ*lac*	Casadaban and Cohen (1980)
MC1063	MC1061, *trp*::Tn*10*	M.Casadaban
HMG5	MC1029, (*drdX*) Δ*hns*	Göransson *et al.* (1990)
HMG11	MC1029, *recA*$^+$	Göransson *et al.* (1990)
HMG9	HMG5, *recA*$^+$	Göransson *et al.* (1990)
GM37	*proU–lacZ*	May *et al.* (1986)
SS5357	*lrp*::Tn*10*	S.A.Short
BSN1	M182, *hns*::*cat*	Forsman *et al.* (1992)
W3110	F$^-$ prototroph	F.C.Neidhart
BEU601	MC1063, *trp*$^+$, (*drdX*) Δ*hns*	this study
BEU602	MC1063, *trp*$^+$	this study
BEU603	BEU601, *trp*::Tn*10*	this study
BEU630	BSN1, *trp*::Tn*10*	this study
BEU681	BEU601, *lrp*::Tn*10*	this study
BEU683	BEU602, *lrp*::Tn*10*	this study
BEU694	GM37, (*drdX*) Δ*hns*, *trp*::Tn*10*, *proU–lacZ*	this study
BEU693	GM37, *trp*::Tn*10*, *proU–lacZ*	this study
BSN6	HMG11, *stpA60*::*km*	this study
BSN7	HMG9, *stpA60*::*km*, (*drdX*) Δ*hns*	this study
BSN9	HMG11, *stpA61*::*km*	this study
BSN12	HMG11, *stpA62*::*km*	this study
BSN21	W3110, *lrp*::Tn*10*	this study
BSN22	W3110, *hns*::*cat*	this study

DNA manipulations and construction of plasmids

Standard recombinant procedures were performed essentially as described by Sambrook *et al.* (1989). The plasmids used in this study are listed in Table III. The *stpA* gene was mapped to λ445, λ444 by Southern hybridization to a Kohara membrane (gene mapping membrane containing a recombinant λ library from *E.coli* K-12 strain W3110; Takara Shuzo Co. Ltd, Kyoto, Japan) using a PCR fragment (primers 40 and 41) as a probe. DNA from Kohara phage λ444 was subsequently purified and cut with *Hin*dIII–*Kpn*I. A 9 kb fragment was ligated into the *Hin*dIII–*Kpn*I cut plasmid pCL1921 to generate plasmid pBSN43. To construct pBSN45, plasmid pBSN43 was partially digested with *Eco*RI. The StpA-overproducing plasmid pBSN42 was constructed by PCR amplifying a fragment containing the *stpA* gene without its native promoters from strain MC1029. The primers 52 (5′-GGGAAGCTT-ATGTCCGTAATGTTACAAAG-3′) and 41 (5′-GGGGTCGACCTTTG-TTGGTGCCGGGTTACTG-3′) were designed with a *Hin*dIII and a *Sal*I restriction site at the 5′ ends. The PCR fragment was cloned into pT/blue(R) T-vector (Novagen) to produce plasmid pBSN40. To ensure the absence of PCR mistakes, the insert was sequenced. A *Hin*dIII fragment containing the *stpA* gene was ligated into *Hin*dIII-cut pMMB66HE to generate the plasmid pBSN42 and into *Hin*dIII-cut pBSN47 to generate the plasmid pBSN63. To monitor the expression of *stpA*, different regions from the *stpA* upstream sequence were transcriptionally fused to the *lacZ* gene. Plasmid pBSN32 was constructed in two steps. The *stpA* gene was PCR amplified with its promoters using primer 40 (5′-GGGGGTACCGAAATAATCTCGCGCAGGACTG-3′ with a designed *Kpn*I restriction site at its 5′ end) and primer 41 (see above). The PCR-generated DNA fragment was cloned into pT/blue(R) to make pBSN30. To ensure that no PCR mutations had occurred, the insert was sequenced. pBSN30 was then cut with *Eco*RI and ligated to *Eco*RI-cut pRZ5202 to generate pBSN32. To construct pBSN57, pBSN45 was cut with *Dra*I and a 1.1 kb fragment was cloned into *Sma*I-cut pRZ5202. pBSN58 was constructed by an *Eco*RI cutback of pBSN57. To construct pBSN157 and pBSN158, DNA from pBSN57 and pBSN58 were cut with *Eco*RI–*Bam*HI and the *stpA* promoter fragments cloned into *Eco*RI–*Bam*HI-cut pFZY1. The *hns* translational fusion plasmid pBSN2 was constructed by cutting pHMG409 with *Eco*RI–*Rsa*I and ligating with *Eco*RI–*Sma*I-cut pMW156.

Allelic replacement of the chromosomal stpA gene and construction of stpA, Δhns strains

The method used for allelic replacement was essentially as described by Kulakauskas *et al.* (1991). The three plasmids used to construct *stpA* insertion mutations were constructed as follows. Plasmid pUC4K (Pharmacia) was cut with *Eco*RI and ligated with *Eco*RI-cut pBSN45 to generate the plasmid pBSN60. Plasmid pUC4-KIXX (Pharmacia) was cut with *Eco*RI and ligated with *Eco*RI-cut pBSN45 to generate plasmids pBSN61 and pBSN62, with the Tn*5* kan^r ble^r resistance cassette in opposite directions (see also Figure 2A). The plasmids were introduced into MC1061 and propagated with the wild-type λ444 to create recombinant λ phage carrying the kanamycin cassette. These phages were then used to infect MC1061 to induce the allelic replacement of the *stpA* allele on the chromosome. The three different *stp*A::*Km* mutants were then moved by P1 transduction to HMG11. The different chromosomal *stpA* mutations were confirmed by Southern blotting using a PCR-amplified DNA fragment (primers 40 and 41) covering the *stpA* gene as a probe. To construct *stpA*, *hns* double mutants, we also moved the different *stpA* insertion mutations to the Δ*hns* mutant strain HMG9 by phage P1-mediated transduction. Transductants were obtained with the *stpA60*::*km* and *stpA62*::*km* alleles. Such transductants grew slower than the parent Δ*hns*. No transductants were obtained with the phage lysate grown on the *stpA61*::*km* mutant. The problem in constructing the *stpA61*::*km*, Δ*hns* double mutant strain was probably not a result of the loss of the *stpA* gene product because we were unable to transduce the *stpA61*::*km* mutant allele into HMG9 even in the presence of plasmid pBSN42, which contained and expressed the wild-type *stpA* gene under an inducible promoter. We also moved the different *stpA* mutant alleles into the *E.coli* strain MC4100. We obtained single *stpA* mutant strains in all cases but were unable to introduce any of the *stpA* mutations into a Δ*hns* derivative of MC4100. Therefore we tested the reciprocal experiment and transduced the Δ*hns* mutation (by cotransduction with a *trp*::Tn*10* allele) into derivatives already carrying the different *stpA* mutant alleles. In these transductions we obtained progeny with similar frequencies, as when transducing Δ*hns* alleles into a StpA$^+$ strain.

PCR amplification

PCR amplification was carried out in an SDS thermocycler for 35 cycles with denaturation at 94°C for 30 s, annealing at 55°C for 1 min and extension for 1 min at 72°C. PCR-amplified products were analyzed on a 0.7% agarose gel.

DNA sequencing

A sequence analysis was performed on CsCl-purified double-stranded plasmid pBSN43 according to the protocol for T7 sequencing (TM kit; Pharmacia). Both strands of the DNA were completely sequenced. Primers used in sequencing were: 45, 5′-GAACGTCAATGGAGAA-TTCGCGA-3′; 49, 5′-GTCCTGCGCGAGATTATTTC-3′; 54, 5′-CACTGGTGCTTTCTGCTG-3′; 56, 5′-TAATGAAAGTTTGGGGG-TGAGG-3′; 57, 5′-ATCATATCGGCATGTAAAAA-3′; 58, 5′-CTA-AACAACCATCGCGAACG-3′; 59, 5′-TTCCGTAACGGCTCGTT-TTT-3′; 60, 5′-GCATTGCATGTTCTCACCGC-3′; 61, 5′-GGCGG-ATATCCTGGCTTATGTG-3′; 62, 5′-GCCAGTCTGCGCCCACCA-CTA-3′; 65, 5′-CGAAGCTCATTCCGGAGAGG-3′; 66, 5′-CAC-

Table III. Plasmids used in this study

Plasmids	Relevant characteristics	References
pCL1921	low copy number, cloning vector, Spec^r/Str^r	Lerner and Inouye (1990)
pT/blue(R)	T-vector for cloning of PCR fragments, Cb^r	Novagen
pMMB66HE	broad-host range expression vector, Cb^r	Furste *et al.* (1986)
pRZ5202	transcriptional *lacZ* fusion vector, Cb^r	Reznikoff and McClure (1986)
pFZY1	transcriptional *lacZ* fusion vector, Cb^r	Koop *et al.* (1987)
pMW156	translational *lacZ* fusion vector, Km^r	Wikström *et al.* (1992)
pHMG409	*hns* clone	Göransson *et al.* (1990)
pHMG452	*hns* clone	Göransson *et al.* (1990)
pBSN2	translational fusion *hns* in pMW156	this study
pBSN30	PCR-amplified *stpA* clone in pT/blue. Primers 40–41	this study
pBSN32	transcriptional fusion *stpA* in pRZ5202	this study
pBSN40	PCR-amplified *stpA* clone in pT/blue. Primer 52–41	this study
pBSN42	*tac* promoter-linked *stpA* clone in pMMB66HE	this study
pBSN43	*Hin*dIII–*Kpn*I λ444 cloned in pCL1921	this study
pBSN45	*Hin*dIII–*Eco*RI subclone of pBSN43	this study
pBSN47	pMMB66HE with Cm^r inserted in the *Dra*I site, Cb^s	this study
pBSN57	transcriptional fusion *stpA* in pRZ5202	this study
pBSN58	transcriptional fusion *stpA* in pRZ5202	this study
pBSN60	Kan^r cassette inserted in the *Eco*RI–*Eco*RI site in the *stpA* promotor region	this study
pBSN61	Kan^r/Ble^r cassette inserted in the *Eco*RI site within *stpA*	this study
pBSN62	as plasmid pBSN61 but with the Kan^r/Ble^r cassette in the opposite orientation	this study
pBSN63	*tac* promoter-linked *stpA* clone in pBSN47	this study
pHMG117	transcriptional fusion with the *alaS* promoter in pFZY1	M.Göransson *et al.* (unpublished data)
pHMG118	transcriptional fusion with *pap* promoter in pFZY1	M.Göransson *et al.* (unpublished data)
pBSN157	transcriptional fusion with *stpA* promoter in pFZY1	this study
pBSN158	transcriptional fusion with *stpA* promoter in pFZY1	this study

CAATAATCTAAATTAC-3′; and 67, 5′-GCGTGTTGCGCTTGAACG-TTA-3′.

RNA extraction and Northern blot analysis

RNA was extracted from bacterial cultures grown to logarithmic growth phase as described in Båga *et al.* (1988). For the temperature Northern blot the bacteria were grown in LB medium at 37 or 26°C. For the *lrp* Northern blot the bacteria were grown at 37°C in glucose minimal medium supplemented with 0.4 mM isoleucine and 0.4 mM valine in the presence or absence of 10 mM leucine, according to Ernsting *et al.* (1992). 20 μg of each RNA sample were loaded onto the gel. RNA blotting and hybridization were performed as described in Båga *et al.* (1985, 1988). The *stpA* probe used was a PCR-amplified DNA fragment covering the *stpA* gene (primers 40 and 41). The *hns* probe was a *Kpn*I–*Pst*I DNA fragment purified from plasmid pHMG452 covering 175 bp of the *hns* gene. The probes were labeled using a Megaprime DNA kit (Amersham). To correct for possible differences in the amount of RNA loaded in each lane, we included an analysis of a reference transcript, the 2.1 kb *trmD* transcript, which was characterized by Byström *et al.* (1989). The *trmD* probe was a [γ-^{32}P]dATP kinase-labeled oligonucleotide (5′-GCGCCTTCACCCGCCGCGGCTTTTGCTGCAT-GAATGGCGTCCCGCAAGGGT-3′). Washing of the membranes was performed in a Hybaid oven according to the protocol supplied by the manufacturer. Bands were visualized and quantified using a phosphorimager (Molecular Dynamics). The amount of *stpA* mRNA was correlated to the *trmD* level, and the relative values were calculated. The level of *stpA* signal in minimal media without leucine was set to 1.0.

Primer extension analysis

Primer extension was performed according to Göransson *et al.* (1989). The oligonucleotide used for the primer extension analysis was primer 45 described above. Dideoxy sequencing reactions (T7 sequencing TM kit; Pharmacia) using plasmid pBSN43 and primer 45 were loaded in parallel to determine the endpoint of the extension product.

Protein–DNA binding assay

The gel mobility shift assays were performed as described previously (Forsman *et al.*, 1989; Göransson *et al.*, 1989) The [α-^{32}P]dATP-labeled fragments used were obtained by cutting plasmid pBSN57 or pBSN58 with *Eco*RI and *Bam*HI and subsequently labeling the fragments by fill-in. Lrp was purified from a polyacrylamide gel essentially according to the method described by Hager and Burgess (1980) for the purification of the sigma subunit of *E.coli* RNA polymerase. The gel-purified Lrp protein was checked for binding to the *pap* intercistronic region (known previously to bind the Lrp protein; Braaten *et al.*, 1992) in gel mobility shift assays. Protein concentrations were measured using the Bio-Rad protein assay reagent. Purified H-NS was a kind gift from Dr C.Gualerzi.

β-Galactosidase assay

The specific activity of β-galactosidase was determined as described by Miller (1972). Values represent the mean of at least three separate measurements. The standard error was calculated and is indicated in each case by bars in the figures. In the experiments with *stpA* low copy number fusions, the number of plasmid-containing cells was measured. Samples were taken and spread on plates without carbenicillin. The colonies formed after incubation overnight were printed onto new plates with and without 25 μg/ml carbenicillin. The frequency of plasmid-containing cells was thus obtained, and the level of β-galactosidase activity compensated for the number of cells containing plasmid.

To assess β-galactosidase activity upon overexpression of StpA, the strains were inoculated to 1 klett unit and grown to 12 klett units. The cultures were divided into two. One was induced with IPTG. The cultures were then allowed to grow to 50 klett units and the β-galactosidase activities measured. Strain MC1029 containing the *hns::lacZ* (pBSN2) fusion was grown in LB medium supplemented with 50 μg/ml kanamycin and 10 μg/ml chloramphenicol. The cultures were induced with 1×10^{-6} M IPTG. For the assay of β-galactosidase activity by *pap–lacZ* (pHMG118) and *alaS–lacZ* (pHMG117) upon overexpression of StpA, the strains were grown in LB medium supplemented with 10 μg/ml chloramphenicol and 25 μg/ml carbenicillin. The cultures were induced with 1×10^{-5} M IPTG. For the assay of β-galactosidase activity by the *proU–lacZ* chromosomal fusion upon overexpression of StpA, cultures were grown in minimal medium A supplemented with 0.3 M NaCl (May *et al.*, 1990) and 50 μg/ml carbenicillin. The cultures were induced with 1×10^{-6} M IPTG.

Acknowledgements

We are grateful to Monica Persson for assistance with the experiments. We thank Dr E.Bremer for kindly supplying strain GM37, Dr S.Short for the kind gift of strain SS5357 and Dr C.Gualerzi for the kind gift of purified H-NS protein. This work was supported by grants from the Swedish Natural Science Research Council, the Swedish Medical Research Council and the Göran Gustafsson Foundation for Natural Science and Medical Research.

References

Båga,M., Göransson,M., Normark,S. and Uhlin,B.E. (1985) *EMBO J.*, **4**, 3887–3893.
Båga,M., Göransson,M., Normark,S. and Uhlin,B.E. (1988) *Cell*, **52**, 197–206.
Bertani,G. (1951) *J. Bacteriol.*, **62**, 293–300.
Braaten,B.A., Platko,J.V., van der Woude,M.W., Simons,B.H., de Graaf,F.K., Calvo,J.M. and Low,D.A. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 4250–4254.
Byström,A.S., von Gabain,A. and Björk,G.R. (1989) *J. Mol. Biol.*, **208**, 575–586.
Calvo,J.M. and Matthews,R.G. (1994) *Microbiol. Rev.*, **58**, 466–490.
Casadaban,M.J. and Cohen,S.N. (1980) *J. Mol. Biol.*, **138**, 179–207.
D'Ari,R., Lin,R.T. and Newman,E.B. (1993) *Trends Biochem. Sci.*, **18**, 260–263.
Defez,R. and Defelice,M. (1981) *Genetics*, **97**, 11–25.
Dersch,P., Kneip,S. and Bremer,E. (1994) *Mol. Gen. Genet.*, **245**, 255–259.
Dersch,P., Schmidt,K. and Bremer,E. (1993) *Mol. Microbiol.*, **8**, 875–889.
Drlica,K. and Rouviére-Yaniv,J. (1987) *Microbiol. Rev.*, **51**, 301–319.
Ernsting,B.R., Atkinson,M.R., Ninfa,A.J. and Matthews,R.G. (1992) *J. Bacteriol.*, **174**, 1109–1118.
Falconi,M., McGovern,V., Gualerzi,C., Hillyard,D. and Higgins,N.P. (1991) *New Biol.*, **3**, 615–625.
Falconi,M., Higgins,N.P., Spurio,R., Pon,C.L. and Gualerzi,C.O. (1993) *Mol. Microbiol.*, **10**, 273–282.
Falconi,M., Brandi,A., La Teana,A., Gualerzi,C.O. and Pon,C.L. (1996) *Mol. Microbiol.*, **19**, 965–975.
Forsman,K., Göransson,M. and Uhlin,B.E. (1989) *EMBO J.*, **8**, 1271–1277.
Forsman,K., Sondén,B., Göransson,M. and Uhlin,B.E. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 9880–9884.
Free,A. and Dorman,C.J. (1995) *Mol. Microbiol.*, **18**, 101–113.
Furste,J.P., Pansegrau,W., Frank,R., Blocker,H., Scholz,P., Bagdasarian,M. and Lanka,E. (1986) *Gene*, **48**, 119–131.
Göransson,M., Forsman,K., Nilsson,P. and Uhlin,B.E. (1989) *Mol. Microbiol.*, **3**, 1557–1565.
Göransson,M., Sondén,B., Nilsson,P., Dagberg,B., Forsman,K., Emanuelsson,K. and Uhlin,B.E. (1990) *Nature*, **344**, 682–685.
Hager,D.A. and Burgess,R.R. (1980) *Anal. Biochem.*, **109**, 76–86.
Higgins,C.F., Dorman,C.J., Stirling,D.A., Waddell,L., Booth,I.R., May,G. and Bremer,E. (1988) *Cell*, **52**, 569–584.
Higgins,C.F., Hinton,J.C., Hulton,C.S., Owen-Hughes,T., Pavitt,G.D. and Seirafi,A. (1990) *Mol. Microbiol.*, **4**, 2007–2012.
Hinton,J.C., Santos,D.S., Seirafi,A., Hulton,C.S., Pavitt,G.D. and Higgins,C.F. (1992) *Mol. Microbiol.*, **6**, 2327–2337.
Hulton,C.S. *et al.* (1990) *Cell*, **63**, 631–642.
Ito,K., Oshima,T., Mizuno,T. and Nakamura,Y. (1994) *J. Bacteriol.*, **176**, 7383–7386.
Jordi,B.J., Dagberg,B., de Haan,L.A., Hamers,A.M., van der Zeijst,B.A., Gaastra,W. and Uhlin,B.E. (1992) *EMBO J.*, **11**, 2627–2632.
Kawula,T.H. and Orndorff,P.E. (1991) *J. Bacteriol.*, **173**, 4116–4123.
Kohara,Y., Akiyama,K. and Isono,K. (1987) *Cell*, **50**, 495–508.
Koop,A.H., Hartley,M.E. and Bourgeois,S. (1987) *Gene*, **52**, 245–256.
Kulakauskas,S., Wikstrom,P.M. and Berg,D.E. (1991) *J. Bacteriol.*, **173**, 2633–2638.
La Teana,A., Brandi,A., Falconi,M., Spurio,R., Pon,C.L. and Gualerzi, C.O. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 10907–10911.
Lejeune,P. and Danchin,A. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 360–363.
Lerner,C.G. and Inouye,M. (1990) *Nucleic Acids Res.*, **18**, 4631.
Levinthal,M., Lejeune,P. and Danchin,A. (1994) *Mol. Gen. Genet.*, **242**, 736–743.
May,G., Faatz,E., Villarejo,M. and Bremer,E. (1986) *Mol. Gen. Genet.*, **205**, 225–233.
May,G., Dersch,P., Haardt,M., Middendorf,A. and Bremer,E. (1990) *Mol. Gen. Genet.*, **224**, 81–90.
Miller,J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Morschhäuser,J., Uhlin,B.E. and Hacker,J. (1993) *Mol. Gen. Genet.*, **238**, 97–105.
Newman,E.B., D'Ari,R. and Lin,R.T. (1992) *Cell*, **68**, 617–619.
Olsén,A., Arnqvist,A., Hammar,M., Sukupolvi,S. and Normark,S. (1993) *Mol. Microbiol.*, **7**, 523–536.
Owen-Hughes,T.A., Pavitt,G.D., Santos,D.S., Sidebotham,J.M., Hulton, C.S., Hinton,J.C. and Higgins,C.F. (1992) *Cell*, **71**, 255–265.
Pettijohn,D.E. (1988) *J. Biol. Chem.*, **263**, 12793–12796.
Rex,J.H., Aronson,B.D. and Sommerville,R.L.J. (1991) *J. Bacteriol.*, **173**, 5944–5953.
Reznikoff,W.S. and McClure,W.R. (1986) *E. coli* promoters. In Reznikoff,W. and Gold,L. (eds), *Maximizing Gene Expression*. Butterworths, Boston, MA, pp. 1–33.
Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Shi,X. and Bennett,G.N. (1994) *J. Bacteriol.*, **176**, 6769–6775.
Spassky,A., Rimsky,S., Garreau,H. and Buc,H. (1984) *Nucleic Acids Res.*, **12**, 5321–5340.
Spurio,R., Durrenberger,M., Falconi,M., La Teana,A., Pon,C.L. and Gualerzi,C.O. (1992) *Mol. Gen. Genet.*, **231**, 201–211.
Ueguchi,C. and Mizuno,T. (1993) *EMBO J.*, **12**, 1039–1046.
Ussery,D.W. *et al.* (1994) *Biochimie*, **76**, 968–980.
Van der Woude,M.W., Braaten,B.A. and Low,D.A. (1992) *Mol. Microbiol.*, **6**, 2429–2435.
Wada,M., Kano,Y., Ogawa,T., Okazaki,T. and Imamoto,F. (1988) *J. Mol. Biol.*, **204**, 581–591.
Wang,Q. and Calvo,J.M. (1993) *EMBO J.*, **12**, 2495–2501.
Wikström,P.M., Lind,L.K., Berg,D.E. and Björk,G.R. (1992) *J. Mol. Biol.*, **224**, 949–966.
Willets,N.S., Clark,A.J. and Low,B. (1969) *J. Bacteriol.*, **97**, 244–249.
Yamada,H., Muramatsu,S. and Mizuno,T. (1990) *J. Biochem.*, **108**, 420–425.
Yamada,H., Yoshida,T., Tanaka,K., Sasakawa,C. and Mizuno,T. (1991) *Mol. Gen. Genet.*, **230**, 332–336.
Yamashino,T., Ueguchi,C. and Mizuno,T. (1995) *EMBO J.*, **14**, 594–602.
Yasuzawa,K., Hayashi,N., Goshima,N., Kohno,K., Imamoto,F. and Kano,Y. (1992) *Gene*, **122**, 9–15.
Zhang,A. and Belfort,M. (1992) *Nucleic Acids Res.*, **20**, 6735.
Zhang,A., Derbyshire,V., Galloway Salvo,J.L. and Belfort,M. (1995) *RNA*, **1**, 783–793.
Zuber,F., Kotlarz,D., Rimsky,S. and Buc,H. (1994) *Mol. Microbiol.*, **12**, 231–240.

Received on February 9, 1995; revised on June 10, 1995

Note added in proof

Recently, data on the expression and function of StpA and H-NS have also been reported by Zhang *et al.* [Zhang,A., Rimsky,S., Reaban,M.E., Buc,H. and Belfort,M. (1996) *EMBO J.*, **15**, 1340–1349].