Mol Gen Genet (1994) 245:255–259

SHORT COMMUNICATION

Petra Dersch · Susanne Kneip · Erhard Bremer

The nucleoid-associated DNA-binding protein H-NS is required for the efficient adaptation of *Escherichia coli* K-12 to a cold environment

Received: 23 March 1994 / Accepted: 6 May 1994

Abstract The *hns* gene is a member of the cold-shock regulon, indicating that the nucleoid-associated, DNA-binding protein H-NS plays an important role in the adaptation of *Escherichia coli* to low temperatures. We show here that the ability to cope efficiently with a cold environment (12°C and 25°C) is strongly impaired in *E. coli* strains carrying *hns* mutations. Growth inhibition is much more pronounced in strains carrying the *hns*-206 allele (an ampicillin resistance cassette inserted after codon 37) than in those carrying the *hns*-205 mutation (a Tn10 insertion located in codon 93). A protein fragment (H-NS*) is synthesized in strains carrying the *hns*-205::Tn10 mutation, suggesting that this truncated polypeptide is partially functional in the cold adaptation process. Analysis of the growth properties of strains harbouring four different low-copy-number plasmid-encoded *hns*' genes that result in the production of C-terminally truncated H-NS proteins supports this proposal. H-NS* proteins composed of 133, 117 or 94 amino-terminal amino acids partially complemented the severe cold-sensitive growth phenotype of the *hns*-206 mutant. In contrast, synthesis of a truncated H-NS protein with only 75 amino-terminal amino acids was insufficient to restore growth at low temperature.

Key words Adaptation to low temperature
DNA-binding protein H-NS · *hns* mutants
Truncated proteins

In *Escherichia coli*, an abrupt switch in the growth temperature from 37°C to 10°C arrests growth for 4 to 5 h. During this lag time most protein synthesis is inhibited, but the production of at least 13 proteins is transiently increased (for an overview see Jones and Inouye 1994). One of these cold shock proteins, CspA (CS 7.4), appears to play a central role in coordinating the cellular response to a low-temperature environment. CspA is a DNA-binding protein whose nucleic acid-binding domain shares high sequence identity with eukaryotic gene regulatory transcription factors that recognize a CCAAT (Y-box) DNA sequence motif (Wistow 1990; Schindelin et al. 1993). The CspA protein acts as a transcriptional activator for at least two members of the *E. coli* cold-shock response network: *gyrA* and *hns* (La Teana et al. 1991; Jones et al. 1992).

The *hns* gene encodes an abundant protein (H-NS) which is associated with the bacterial nucleoid (Spurio et al. 1992). H-NS is a high-affinity, DNA-binding protein that binds relatively nonspecifically to double-stranded DNA but displays a certain preference for curved and highly AT-rich DNA segments (Yamada et al. 1990; Lucht et al. 1994). The H-NS protein not only serves a role in structuring the chromosomal DNA, but is also actively involved in a variety of specific cellular functions (for an overview see Higgins et al. 1990). Several environmental factors and regulatory circuits provide the cell with a finely tuned control over the intracellular amount of H-NS. Expression of the *hns* gene is growth-phase controlled and and is subjected to negative autoregulation (Dersch et al. 1993; Ueguchi et al. 1993). An important environmental determinant regulating the expression of *hns* is the growth temperature. Transcription of *hns* is enhanced (3- to 4-fold) following a severe cold shock. In addition, the *hns* regulatory region contains a Y-box sequence motif and binding of the central cold shock regulatory protein CspA to the *hns* promoter region has been demonstrated *in vitro* (La Teana et al. 1991). The enhanced expression of *hns* after cold shock and its positive control by CspA suggest an important role for H-NS in the adaptation of *E. coli* to a cold environment. One would therefore expect that *hns* mutants would exhibit defects in their ability to cope efficiently with low temperature. We show here that mutations in *hns* confer a marked cold-sensitive growth phenotype. The degree of cold sensitivity is dependent on the particular *hns* allele employed and cor-

Communicated by J. F. Lengeler

P. Dersch · S. Kneip · E. Bremer (✉)
Max-Planck-Institut für Terrestrische Mikrobiologie,
Karl-von-Frisch Str., D-35043 Marburg, Germany

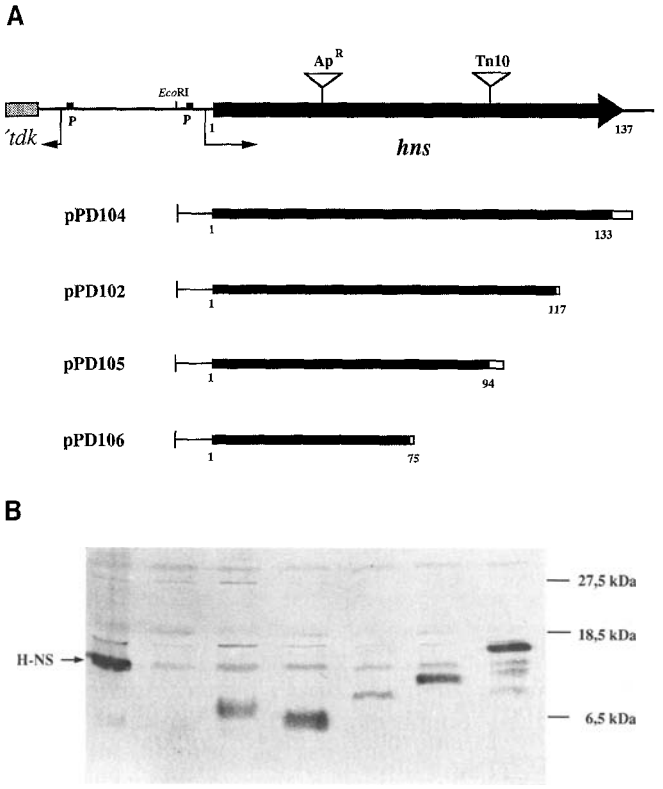

Fig. 1A, B Genetic and physical organization of the *hns*$^+$ gene, its mutant derivatives and immunological detection of H-NS related proteins. **A** The structure of *hns* and the beginning of the divergently described *tdk* gene, together with the position of the promoters and the transcription initiation sites of both *hns* and *tdk*, are shown. The location of the Ap^r cassette insertion and the position of the Tn10 element within the *hns* coding region in the *hns*-206::Ap^r and *hns*-205::Tn10 mutations is indicated. The extent of *hns* material present in the truncated *hns'* genes on plasmids pPD102, pPD104, pPD105 and pPD106 is indicated. The number shown at the end of each *hns'* gene refers to the number of *hns* codons present in the truncated *hns'* construct, and the *box* drawn at the 3' end of these genes indicates that a few codons contributed by material from the insertion element IS1 are present in the *hns'* reading frame. **B** Cultures were grown overnight at 37°C in LB medium. Whole cell protein extracts were prepared from the *hns*$^+$ strain MC4100 (lane 1), the *hns*-206::Ap^r strain PD32 (lane 2), the *hns*-205::Tn10 strain PD145 (lane 3), PD32 (pPD106) (lane 4), PD32 (pPD105) (lane 5), PD32 (pPD102) (lane 6) and PD32 (pPD104) (lane 7). The proteins were electrophoretically separated on a 15% SDS-polyacrylamide gel, and H-NS related proteins were immunologically detected as described by Dersch et al. (1993). The positions of molecular mass standards are indicated

relates with the synthesis of H-NS proteins truncated at their carboxy-terminal end.

Insertion mutations in *hns* cause a growth defect at low temperature

We followed the growth of two *hns* mutants, strains PD145 and PD32, and their *hns*$^+$ parent, strain MC4100 (Casadaban 1976), at 12°C to test whether alterations in the *hns* gene cause any growth defect at low temperature. The cultures were grown in LB medium (Sambrook et al. 1989) in a shaking waterbath (200 rpm) for several days and the density of the cultures was monitored spectrophotometrically. Strain PD145 carries the *hns*-205::Tn10 allele (originally designated as *osmZ*-205::Tn10; Higgins et al. 1988; Dersch et al. 1993), in which a copy of the transposable element Tn10 is inserted into codon 93 of the 137 codons comprising the *hns* structural gene (Hulton et al. 1990; May et al. 1990). Strain PD145 was constructed by transducing the *hns*-205::Tn10 allele from strain GM230 (Higgins et al. 1988) into strain MC4100 using phage P1*vir* and selecting for tetracycline-resistant colonies. The *hns*-206::Ap^r mutation present in strain PD32 carries an ampicillin resistance cassette engineered into the *hns* gene downstream of codon 37 (Dersch et al. 1993) (Fig. 1A). Both *hns* mutations caused a cold-sensitive growth phenotype, but the cold sensitivity of strain PD32 was much more pronounced than that of strain PD145 (Fig. 2A). Both mutants also exhibited sensitivity to the less extreme growth temperature of 25°C (Fig. 2B) but were not strongly retarded in growth at 37°C (Fig. 2B) and at 42°C (data not shown). Hence, both mutations impair the ability of *E. coli* to cope efficiently with a cold environment.

A severe cold shock results in a rapid killing of 70–90% of the cells of a *hupA hupB* strain lacking the major nucleoid-associated DNA-binding protein HU (Wada et al. 1988). To determine whether such a treatment affects the viability of *hns* mutants, we grew strains MC4100, PD145 and PD32 to mid-log phase in LB medium at 37°C, shifted the cultures to 4°C, withdrew samples at various times (1 h, 2 h, 3 h and 4 h) and plated appropriate dilutions on LB plates (incubated at 37°C overnight). There was no significant reduction in the number of colony-forming units obtained from the two *hns* mutants relative to the wild-type parental strain (data not shown). We also rapidly froze log-phase cells at −80°C, stored them for 48 h at this low temperature, and then determined the number of the surviving cells by plating on LB agar plates. This procedure drastically reduces cell viability of a *B. subtilis* mutant deficient in the production of the major cold shock protein CspB (Willimsky et al. 1992), but again, there was no difference in viability between the *hns* and the *hns*$^+$ *E. coli* strains in this test (data not shown). Hence, although *hns* mutations strongly affect growth of *E. coli* at low temperature, our data show that the H-NS protein is not absolutely essential for survival in a cold environment.

The *hns*-205::Tn10 insertion results in the synthesis of a truncated H-NS protein

The otherwise isogenic *hns* mutants PD145 (*hns*-205::Tn10) and PD32 (*hns*-206::Ap^r) differ significantly in cold sensitivity (Fig. 2). Since the Tn10 insertion is located in the 3' segment of the *hns* gene (Fig. 1A), we reasoned that a truncated H-NS protein might be syn-

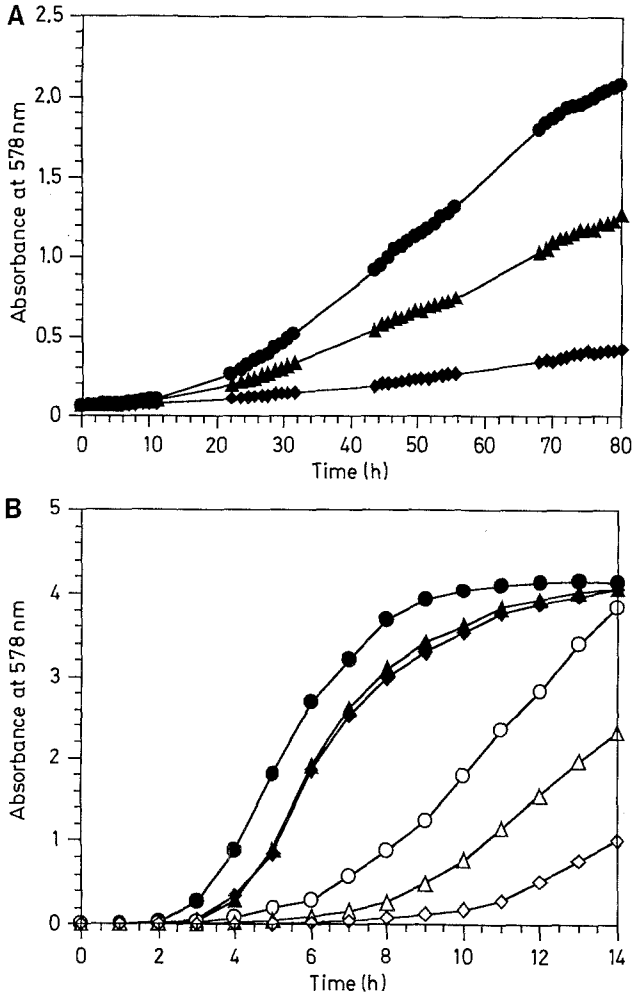

Fig. 2A, B Growth curves of the *hns*$^+$ strain and its mutant *hns*-205::Tn10 and *hns*-206::Ap^r derivatives. **A** Overnight cultures of strain MC4100 (*hns*$^+$, *circles*), PD145 (*hns*-205::Tn10, *triangles*) and PD32 (*hns*-206::Ap^r, *diamonds*), grown in LB medium at 37°C, were inoculated at a 1:1000 dilution into 100 ml of fresh LB medium in a 500-ml Erlenmeyer flask and incubated in a shaking waterbath (200 rpm) at 12°C. Samples of the cultures were withdrawn at the indicated times and their absorbance at 578 nm was determined in a spectrophotometer. One absorbance unit at 578 nm corresponds to 1×10^9 cells per ml culture (Sambrook et al. 1989). **B** Growth curves of strains MC4100 (*circles*), PD145 (*triangles*) and PD32 (*diamonds*) were determined under the growth conditions described in **A** at 25°C (*open* symbols) and 37°C (*closed* symbols), respectively

thesized in strain PD145 and that this protein fragment might retain function partially. To detect such a truncated H-NS polypeptide, we probed total cellular protein extracts prepared from the *hns*$^+$ strain MC4100 and the two *hns* mutant strains with a polyclonal rabbit antiserum raised against the *E. coli* H-NS protein using Western blotting (Dersch et al. 1993). The antiserum specifically recognized the H-NS protein produced in strain MC4100 (Fig. 1B). In strain PD32, the position of the ampicillin resistance cassette in *hns* permits maximally the synthesis of only the first 37 amino acids of H-NS (Fig. 1A); predictably, H-NS was absent in whole cell extracts of this strain (Fig. 1B). In strain PD145, we detected a truncated form of H-NS (H-NS*) (Fig. 1B). Its apparent molecular mass (9 kDa) is in good agreement with the molecular mass of the amino-terminal H-NS fragment (10.6 kDa) predicted from the position of the Tn10 insertion in codon 93 of the *hns* gene (Fig. 1A). Thus, it is very likely that the synthesis of the 93-amino acid, truncated H-NS* protein is responsible for the reduced cold sensitivity observed for strain PD145 in comparison with strain PD32 (Fig. 2). Hulton et al. (1990) did not detect a truncated protein in a strain carrying the *hns*-205::Tn10 allele, possibly owing to differences in their antiserum or detection method. Since a major antigenic determinant of H-NS is located close to its carboxy-terminus (Hulton et al. 1990), the visualization of the H-NS* fragment required an extended detection reaction time. Under these conditions several proteins cross-react unspecifically with the H-NS antiserum (Fig. 1B). One of these proteins migrates at the position of the H-NS protein (15.5 kDa) (Fig. 1B). We speculate that this cross-reacting band in the *hns*-206::Ap^r strain PD32 corresponds to the *E. coli* StpA protein (15.3 kDa), which shows extensive identity (58% identical residues) to H-NS (Zhang and Belford 1992).

Amino-terminal fragments of H-NS partially rescue the cold-sensitive growth phenotype of the *hns*-206::Ap^r mutant

To determine whether truncated derivatives of the H-NS protein might possess partial function, we investigated the influence of alterations at the carboxy-terminal end of H-NS on the adaptation of *E. coli* to low temperatures. For this purpose we used four mutant *hns* genes (*hns'*) that encode truncated H-NS proteins with 133, 117, 94 and 75 amino-terminal amino acids (Fig. 1A). These shortened *hns'* genes were initially isolated during a project unrelated to this study and their detailed characterization will be described elsewhere (P. Dersch and E. Bremer, manuscript in preparation). Each of these *hns'* genes resulted from the insertion of a copy of the transposable element IS1 into *hns*; DNA sequence analysis of their *hns*::IS1 junctions revealed that 8, 1, 6 and 1 codons, respectively, derived from the IS1 material are fused to the *hns* reading frame before a stop codon is encountered (Fig. 1A). The *hns'* genes were cloned into the low-copy-number T7 expression plasmid pPD100 (Dersch et al., submitted for publication) in such a way that most of the IS1 insertion element was removed but the *hns'* genes could still be expressed under the control of the *hns* promoter (Fig. 1A). We found that the presence of each of these *hns'* genes in strain PD32 resulted in the synthesis of a polypeptide that cross-reacted with the H-NS antiserum (Fig. 1B). Cells carrying three of the mutant *hns'* genes (plasmids pPD102, pPD105 and pPD106) synthesized truncated H-NS polypeptides with a lower apparent molecular mass than the wild-type protein. In contrast, the 133-codon mutant *hns'* gene (plasmid pPD104), which is

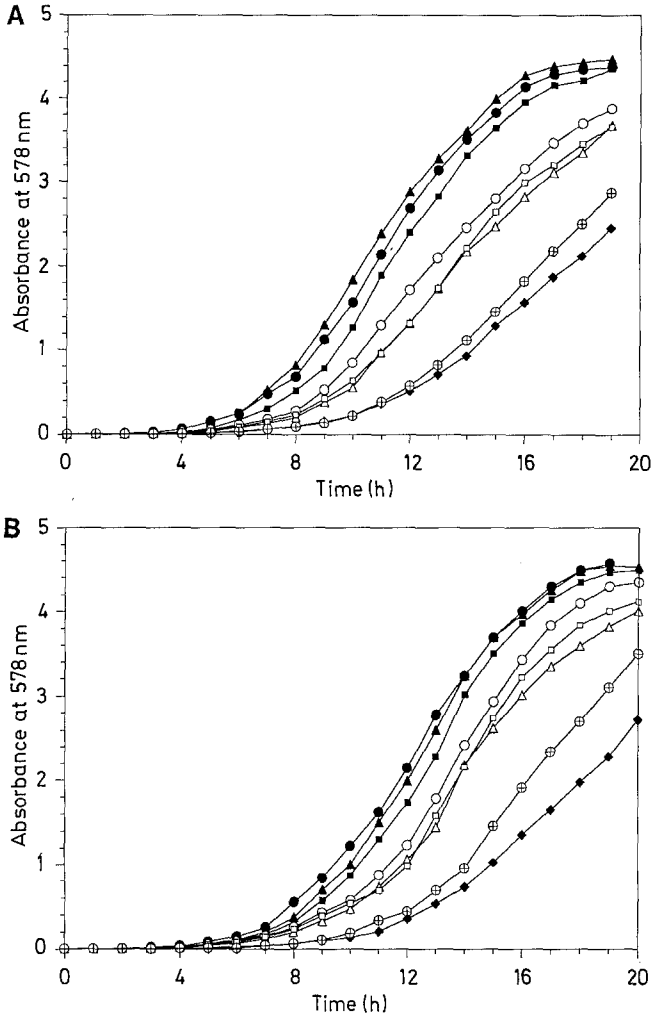

Fig. 3A, B Growth curves of *hns*-206::Ap^r mutant strains in the presence of plasmid-encoded *hns'* genes. **A** Growth curves of cultures of the *hns*$^+$ strain MC4100 and its *hns* mutant derivative PD32 and **B** the *recA* derivatives PD117 (MC4100 *recA*56 *srl*::Tn10 Φ[*proU-lacZ*] hyb2) and PD119 (PD32 *recA*56 *srl*::Tn10 Φ[*proU-lacZ*] hyb2) were determined at 25°C under the conditions described in the legend to Fig. 2A. (*filled diamonds*) PD32 or PD119 with pPD100, (*open triangles*) PD32 or PD119 with pPD102, (*open circles*) PD32 or PD119 with pPD104, (*open squares*) PD32 or PD119 with pPD105, (*crossed circles*) PD32 or PD119 with pPD106, (*filled squares*) PD32 or PD119 with pGM7, (*filled circles*) MC4100 or PD117 with pPD100, (*filled triangles*) MC4100 or PD117 with pGM7

fused to 8 IS1-encoded codons, directed the synthesis of an H-NS protein with a larger molecular mass than the wild-type protein (Fig. 1B; lane 7).

We monitored the growth of strain PD32 carrying the various *hns'* plasmids in LB medium at 25°C. Under these conditions, strain PD32 harbouring the vector pPD100 showed a strong growth defect that was corrected when the low-copy-number *hns*$^+$ plasmid pGM7 (May et al. 1990) was introduced into the mutant strain. Growth of strain PD32 (pGM7) was similar to that of the *hns*$^+$ parent strain MC4100 carrying either pGM7 or the vector pPD100 (Fig. 3A). Synthesis of truncated H-NS proteins with either 133 (pPD104), 117 (pPD102) or 94 (pPD105) *hns*-encoded amino-terminal amino acids partially rescued the cold-sensitive growth phenotype of strain PD32 (Fig. 3A). These *hns'* plasmid-carrying strains thus show growth characteristics similar to those of strain PD145 (*hns*-205::Tn10) in which the chromosome-encoded H-NS* protein with 93 amino-terminal amino acids is produced (Fig. 2B). In contrast, the truncated H-NS protein with only 75 amino-terminal amino acids synthesized by strain PD32 (pPD106) (Fig. 1A) was incapable of restoring growth at low temperature (Fig. 3A). We repeated the growth experiments with *recA* derivatives of strains MC4100 and PD32. The growth pattern of the *recA* strains at 25°C (Fig. 3B) in each case resembled that of the corresponding *recA*$^+$ parents strains (Fig. 3A), thus excluding the possiblity that the growth characteristics of strains carrying the various *hns'* plasmids resulted from homologous recombination between the plasmid-encoded, truncated *hns'* sequences and the *hns*-206::Ap^r mutation present in the bacterial chromosome.

Truncated H-NS proteins differentially influence the manifestation of *hns*-associated effects on *bgl* and *proU* expression

The data presented above show that a substantial carboxy-terminal part of the H-NS protein can be removed without abolishing the function of H-NS in the adaptation process of *E. coli* to low growth temperatures. We therefore tested whether any of the plasmid-encoded *hns'* genes (Fig. 1A) could partially correct two phenotypes characteristic of many *hns* mutants: derepression of the otherwise cryptic *bgl* operon and increased expression of the osmoregulated *proU* operon of *E. coli* (May et al. 1990; Göransson et al. 1990; Hulton et al. 1990). The Bgl phenotype of strain PD32 harbouring the various *hns'* plasmids was scored on agar indicator plates containing bromothymol blue and salicin (BS) (May et al. 1990). Strain PD32 shows a strong Bgl$^+$ phenotype (orange-yellow colonies) on these BS plates, which changes to a Bgl$^-$ character (blue-green colonies) when the *hns*$^+$ plasmid pGM7 is introduced into this strain, whereas the pPD100 vector has no influence on the derepressed expression of the *bgl* genes. The synthesis of the various truncated H-NS proteins modulated the Bgl phenotype of strain PD32 to different extents. The shortest H-NS fragment (75 aa; pPD106) had no influence on *bgl* expression, while the larger fragments (133 aa; pPD104 and 117 aa; pPD102) repressed *bgl* expression significantly. The 94-aa H-NS fragment (pPD105) resulted in an intermediate Bgl$^+$ phenotype, which is very similar to the Bgl phenotype exhibited by the chromosomal *hns*-205::Tn10 insertion strain. We also scored the influence of the truncated H-NS protein fragments on the expression of the osmoregulated chromosomal *proU-lacZ* gene fusion present in strain PD114 (MC4100 *hns*-206::Ap^r Φ[*proU-lacZ*] hyb2 [λp*lac*Mu15]) by assaying the Lac phenotype on lactose

MacConkey plates. None of the *hns*' genes caused significant repression of the deregulated transcription of the Φ(*proU-lacZ*) hyb2 fusion, whereas the presence of the *hns*$^+$ plasmid pGM7 resulted in a strong repression of the *proU-lacZ* fusion.

The data presented here show that certain H-NS proteins truncated at their carboxy-terminus not only partially restore growth at low temperatures (Fig. 3) but also exhibit partial function in the control of *bgl* expression. For both phenotypes, a 94-aa amino-terminal segment of H-NS is sufficient, whereas the 75-aa fragment appears to be nonfunctional (Fig. 1A). The difference between the properties of these truncated H-NS proteins might lie in their ability to dimerize and/or to bind to double-stranded DNA. The *hns*-205::Tn10 mutation, originally identified in our laboratory (Higgins et al. 1988), has been used in a number of studies to assess the function of the H-NS* protein on a variety of cellular processes. Our finding that a 93-aa truncated H-NS protein, synthesized in strains carrying this *hns* allele, is partially functional in cold adaptation and *bgl* control emphasizes that this *hns*-205::Tn10 insertion cannot be regarded as a true null mutant.

Acknowledgements We are grateful for the help of V. Koogle in preparing the manuscript and thank R. Thauer for his continued support. Financial support for this study was provided by the Max-Planck Gesellschaft and the Fonds der Chemischen Industrie. P. D. gratefully acknowledges the receipt of a fellowship from the Boehringer Ingelheim Foundation.

References

Casadaban MJ (1976) Transposition and fusion of the *lac* genes to selected promotors in *Escherichia coli* using the bacteriophage lambda and Mu. J Mol Biol 104:541–555

Dersch P, Schmidt K, Bremer E (1993) Synthesis of the *Escherichia coli* K-12 nucleoid-associated DNA-binding protein H-NS is subjected to growth-phase control and autoregulation. Mol Microbiol 8:875–889

Göransson M, Sonden B, Nilsson P, Dagberg B, Forsman K, Emanuelsson K, Uhlin BE (1990) Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature 344:682–685

Higgins CF, Dorman CJ, Stirling DA, Wadell L, Booth IR, May G, Bremer E (1988) A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *Salmonella typhimurium* and *Escherichia coli*. Cell 52:569–584

Higgins CF, Hinton JCD, Hulton CSJ, Owen-Hughes T, Pavitt GD, Seirafi A (1990) Protein H1: a role for chromatin structure in the regulation of bacterial gene expression and virulence? Mol Microbiol 4:2007–2012

Hulton CS, Seirafi A, Hinton JCD, Sidebotham JM; Wadell L, Pavitt GD, Owen-Hughes T, Spassky A, Buc H, Higgins CF (1990) Histone-like protein H1 (H-NS), supercoiling, and gene expression in bacteria. Cell 63:631–642

Jones PG, Inouye M (1994) The cold-shock response – a hot topic. Mol Microbiol 11:811–818

Jones PG, Krah R, Tafuri SR, Wolffe AP (1992) DNA gyrase, CS7.4, and the cold shock response in *Escherichia coli*. J Bacteriol 174:5798–5802

La Teana A, Brandi A, Falconi M, Spurio R, Pon CL, Gualerzi CO (1991) Identification of a cold shock transcriptional enhancer of the *Escherichia coli* gene encoding nucleoid protein H-NS. Proc Natl Acad Sci USA 88:10907–10911

Lucht JM, Dersch P, Kempf B, Bremer E (1994) Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled *proU* operon of *Escherichia coli*. J Biol Chem 269:6578–6586

May G, Dersch P, Haardt M, Middendorf A, Bremer E (1990) The *osmZ* (*bglY*) gene encodes the DNA-binding protein H-NS (H1a), a component of the *Escherichia coli* K-12 nucleoid. Mol Gen Genet 224:81–90

Sambrook J, Fritsch EF, Maniatis TE (1989) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Schindelin H, Marahiel MA, Heinemann U (1993) Universal nucleic acid-binding domain revealed by crystal structure of the *B. subtilis* major cold shock protein. Nature 364:161–168

Spurio R, Dürrenberger M, Falconi M, La Teana A, Pon CL, Gualerzi CO (1992) Lethal overproduction of the *Escherichia coli* nucleoid protein H-NS: ultramicroscopic and molecular autopsy. Mol Gen Genet 231:201–211

Ueguchi C, Kakeda M, Mizuno T (1993) Autoregulatory expression of the *Escherichia coli hns* gene encoding a nucleoid protein: H-NS functions as a repressor for its own transcription. Mol Gen Genet 236:171–178

Wada M, Kano Y, Ogawa T, Okazaki T, Imamoto F (1988) Construction and characterization of the deletion mutant of *hupA* and *hupB* genes in *Escherichia coli*. J Mol Biol 204:581–591

Willimsky G, Bang H, Fischer G, Marahiel MA (1992) Characterization of *cspB*, a *Bacillus subtilis* inducible cold shock gene affecting cell viability at low temperatures. J Bacteriol 174:6326–6335

Wistow G (1990) Cold shock and DNA binding. Nature 344:823–824

Yamada H, Muramatsu S, Mizuno T (1990) An *Escherichia coli* protein that preferentially binds to sharply curved DNA. J Biochem 108:420–425

Zhang A, Belfort M (1992) Nucleotide sequence of a newly identified *Escherichia coli* gene *stpA*, encoding an H-NS-like protein. Nucleic Acids Res 20:6735