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Lethal overproduction of the *Escherichia coli* nucleoid protein H-NS: ultramicroscopic and molecular autopsy

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Summary. The *Escherichia coli hns* gene, which encodes the nucleoid protein H-NS, was deprived of its natural promoter and placed under the control of the inducible lambda P_L promoter. An *hns* mutant yielding a protein (H-NSΔ12) with a deletion of four amino acids (Gly_{112}-Arg-Thr-Pro_{115}) was also obtained. Overproduction of wild-type (wt) H-NS, but not of H-NSΔ12, resulted in a drastic loss of cell viability. The molecular events and the morphological alterations eventually leading to cell death were investigated. A strong and nearly immediate inhibition of both RNA and protein synthesis were among the main effects of overproduction of wt H-NS, while synthesis of DNA and cell wall material was inhibited to a lesser extent and at a later time. Upon cryofixation of the cells, part of the overproduced protein was found in inclusion bodies, while the rest was localized by immunoelectron microscopy to the nucleoids. The nucleoids appeared condensed in cells expressing both forms of H-NS, but the morphological alterations were particularly dramatic in those overproducing wt H-NS; their nucleoids appeared very dense, compact and almost perfectly spherical. These results provide direct evidence for involvement of H-NS in control of the organization and compaction of the bacterial nucleoid in vivo and suggest that it may function, either directly or indirectly, as transcriptional repressor and translational inhibitor.

Key words: Nucleoid structure – DNA compaction – Cryofixation – Transcriptional repression – Translational inhibition

Introduction

Several DNA-binding proteins have been implicated in the condensation of the chromosome and in the organization of the bacterial nucleoid. Among these, the most abundant and best characterized are HU (NS) and H-NS (H1a) (for reviews see Gualerzi et al. 1986; Drlica and Rouviere-Yaniv 1987; Pettijohn 1988; Higgins et al. 1990). H-NS (15.4 kDa) is a neutral, heat-stable, dimeric protein (Falconi et al. 1988) localized by immunoelectron microscopy as being primarily within the nucleoid (Dürrenberger et al. 1991). Unlike HU, H-NS displays a higher affinity for double-stranded than for single-stranded DNA (Friedrich et al. 1988) and recent data show that it binds selectively to sharply curved DNA (Yamada et al. 1990; Tanaka et al. 1991). H-NS (H1a) has been reported to induce a strong condensation of DNA in vitro, without altering its topological properties (Spassky et al. 1984) and to repress selectively the expression of some genes (Bertin et al. 1990; Rimsky and Spassky 1990).

H-NS is encoded by the gene *hns* and was mapped at 27 min on the *Escherichia coli* chromosome (Göransson et al. 1990; Hulton et al. 1990; May et al. 1990). *hns* has been cloned and characterized in *E. coli* (Pon et al. 1988) and in other Enterobacteriaceae (La Teana et al. 1989). Mutations in the *hns* locus have highly pleiotropic effects (Diderichsen 1980; Defez and De Felice 1981; Spears et al. 1986; Higgins et al. 1988; Hromocky and Maurelli 1989; Dorman et al. 1990; Göransson et al. 1990; Lejeune and Danchin 1990; May et al. 1990; Falconi et al. 1991), of which the common underlying molecular basis may be altered rigidity and compaction of the genome. Finally, recent data have shown that *hns* is one of the genes (approximately 13 in number) whose expression is increased ca. fourfold) during the cold shock response in *E. coli* (La Teana et al. 1990).

In the present paper, we describe the construction of an expresssion vector for the massive overproduction of H-NS and demonstrate that this overproduction is lethal for the cells. Furthermore, by investigating both morphological and biochemical alterations induced by H-NS overproduction we searched for clues concerning the physiological role of this protein. The accumulation of H-NS was found to cause a severe and nearly immediate inhibition of RNA and protein syntheses, coupled to a dramatic compaction of the DNA into very dense

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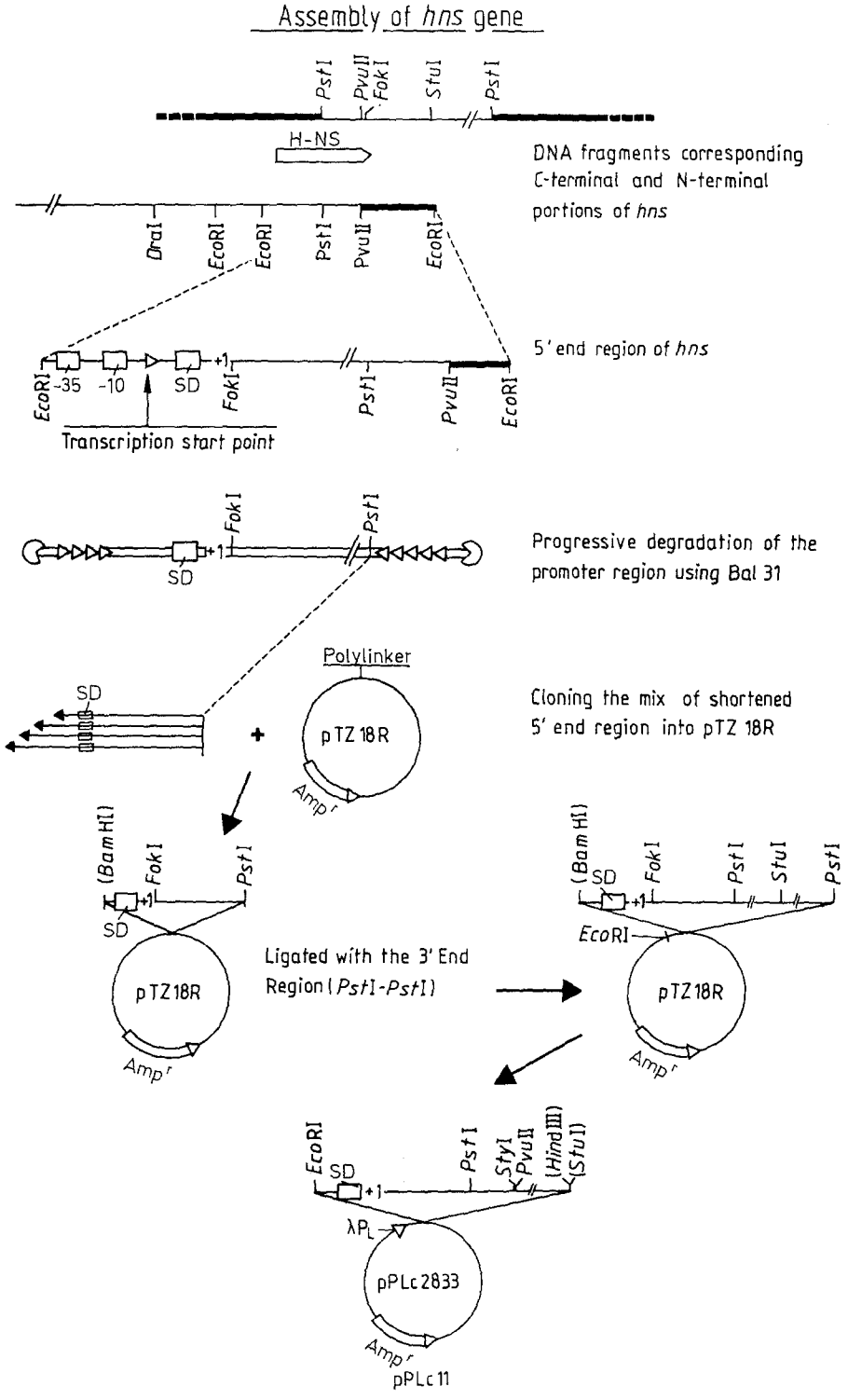


Fig. 1. Strategy for assembly of the *Escherichia coli hns* gene and for construction of the *hns* expression vector (pPLc11). The *thick lines* represent DNA sequences derived from the vector and the *thin lines* represent sequences derived from the *E. coli* chromosome. The *open arrow* indicates the direction of *hns* transcription. Experimental details are given in the Materials and methods

and spherical nucleoids. The possible relationship is discussed between these findings and H-NS function in vivo.

Materials and methods

Assembly of hns. The complete *hns* gene was assembled from the two original fragments which had been cloned into M13mp19 (Pon et al. 1988). First, a 440 bp fragment containing the promoter region and encoding the N-terminal portion of the protein was excised with *Eco*RI, subjected to Bal31 digestion to remove the promoter and then to cleavage with *Pst*I. Shortened fragments of ca. 270 bp in length were purified by agarose gel electrophoresis and ligated to pTZ18R digested with *Pst*I and *Bam*HI (filled-in). The desired clone (screened by size on agarose gel) was linearized with *Pst*I and the 1.6 kb *Pst*I fragment containing the 3' end of *hns* was inserted. The clones were screened by restriction

mapping to determine the orientation of the insert and DNA sequencing to determine the extent of the deletion (Fig. 1). A shortened clone with an insert beginning 27 bases in front of the ATG initiation codon (i.e. missing the *hns* promoter; La Teana et al. 1989) was finally selected for subcloning into an inducible expression vector.

For this purpose the desired insert was excised with *Eco*RI (from the pTZ18R polylinker) and *Stu*I and cloned downstream of the lambda P_L promoter in pPLc2833 (Remaut et al. 1983), after *Eco*RI and *Hin*dIII digestion and fill-in, to yield pPLc11, which was tranformed into *E. coli* K12ΔH1Δtrp (i.e. *E. coli* strain M72, Sm^r*lacZ*am *Δbio-uvrB ΔtrpEA2*(*λNam7-Nam-53*c*I857ΔH1*) as described by Bernard et al. 1979). A flowsheet describing the gene manipulations and the cloning strategy used to obtain pPLc11 is shown in Fig. 1.

Construction of pPLc12. The *Sty*I-*Pvu*II fragment was excised from *hns* in pPLc11 and the resulting deletion product was blunt-end ligated after filling-in the *Sty*I end, to yield pPLc12; upon transformation into *E. coli* K12ΔH1Δtrp and induction, pPLc12 was found to determine the overproduction of a shortened H-NS molecule (H-NSΔ12) lacking the Gly_{112}-Arg-Thr-Pro_{115} tetrapeptide.

Overproduction of wild-type H-NS or H-NSΔ12. E. coli K12ΔH1Δtrp harboring pPLc11 or pPLc12 was grown at 30° C in LB supplemented with ampicillin (50 μg/ml) to A_{620} ~0.7 and then divided into two fractons. One fraction was further incubated at 30° C (uninduced cells) and one at 42° C for 20 min to inactivate the temperature sensitive lambda repressor and for an additional 45 min at 37° C (induced cells). The cells were pelleted and resuspended in sample buffer for electrophoresis and different aliquots were loaded on SDS-polyacrylamide gels.

Incorporation of thymidine, uridine, histidine or acetylglucosamine. E. coli pop3184 (a kind gift of Dr. R. Calogero) harbouring pPLc11 or pPLc12 was grown in M9 minimal medium supplemented with 0.1% casamino acids, tryptophan (5 μg/ml) vitamin B1, $CaCl_2$, $MgSO_4$, glucose and ampicillin (50 μg/ml) at 30° C. When the A_{620} reached ~0.2, one of the following precursors was added to 2 ml aliquots of the culture: (i) 29.3 μCi methyl [^{3}H]thymidine (47 Ci/mmol) and 1.64 μg cold thymidine; (ii) 5.6 μCi [^{3}H]uridine (40 Ci/mmol) and 7.4 μg cold uridine; (iii) 20 μCi [^{3}H]histidine (51 Ci/mmol); (iv) 5.2 μCi N-acetyl[^{3}H]glucosamine (5.1 Ci/mmol) and 1.74 μg cold N-acetylglucosamine. the culture to which thymidine was added also contained adenosine (50 μg/ml). Induction of H-NS production was performed either 8 min before or 10 min after addition of the precursors. At the indicated times 100 μl aliquots were removed for determination of the incorporated acid-insoluble radioactivity.

Transcription and translation in vitro. Protein synthesis directed by poly(U) or MS2 RNA was assayed in vitro essentially as previously described (Gualerzi et al. 1989).

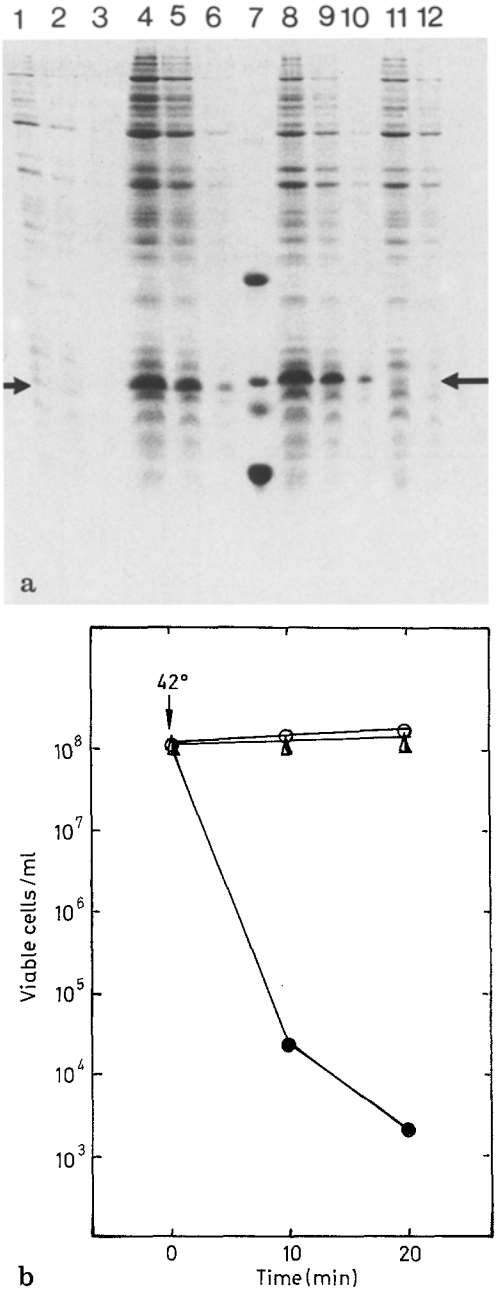


Fig. 2a and b. Overproduction of wild-type (wt) H-NS and H-NSΔ12 and its effect on cell viability. **a** SDS-PAGE (15%) electrophoretic analysis of different amounts of total cell extract derived from cell cultures of: uninduced (lanes 1–3) and induced (lanes 4–6) *E. coli* K12ΔH1Δtrp cells carrying pPLc12; induced (lanes 8–10) and uninduced (lanes 11, 12) *E. coli* K12ΔH1Δtrp cells carrying pPLc11. In lane 7 the following protein standards were loaded: IF3 (M_r=20.5 kDa), H-NS (M_r=15.4 kDa), lysozyme (M_r=14.3 kDa), HU (NS) (M_r=9.5 kDa). The *arrows* indicate bands corresponding to H-NS and H-NSΔ12. **b** Lethal effect of wt H-NS overproduction. *E. coli* K12ΔH1Δtrp cells harboring pPLc11 or pPLc12 were grown to A_{620}=0.8 and then divided into two fractions; one was further incubated at 30° C (uninduced cells) and one at 42° C (induced cells). At the indicated times, aliquots from each culture were removed and the number of viable cells determined by plating on LB agar (containing ampicillin) and incubating at 30° C. (○) uninduced and (●) induced cells carrying pPLc11 (△) uninduced and (▲) induced cells carrying pPLc12

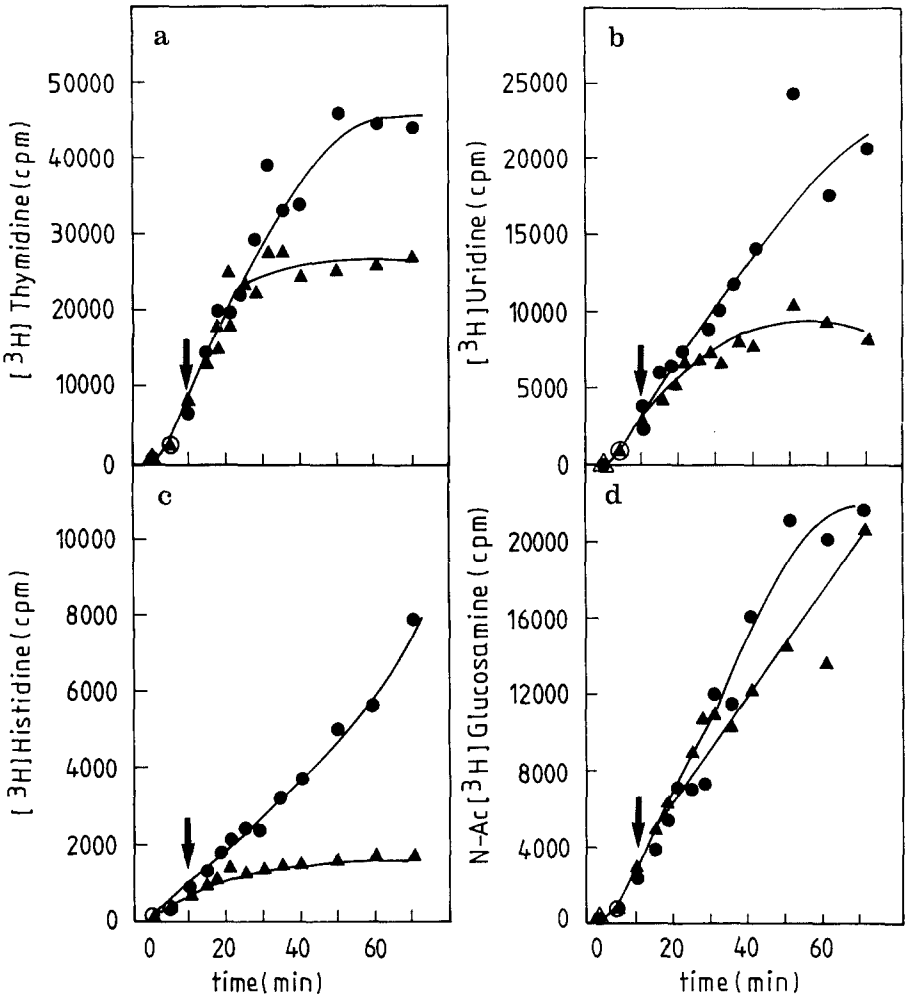


Fig. 3a–d. Effect of H-NS overproduction on the on-going macromolecular synthesis in vivo: **a** DNA, **b** RNA, **c** protein and **d** peptidoglycan. *E. coli* cells harbouring pPLc12 (●) or pPLc11 (▲). The *arrow* indicates the time of induction. Further experimental details are given in the Materials and methods

Each reaction mixture (100 µl) contained: 16 mM Tris-HCl, pH 7.7, 56 mM NH_4Cl, 10 mM magnesium acetate, 2 mM dithiothreitol, 2 mM ATP, 0.4 mM GTP, 10 mM phosphoenol pyruvate, 2 µg pyruvate kinase, 0.2 mM each of all amino acids but for the radioactive one, 50 pmol of 70S ribosomes and 7 µl of a crude post-ribosomal supernatant (S100). When appropriate, the reaction mixture also contained 16 µg of polyuridylic acid and 10 µmol [^{14}C]phenylalanine (500 mCi/mmol) or 0.8 A_{260} units of MS2 RNA, 0.12 mM citrovorum, 50 pmol each of the three purified initiation factors and 10 µmol [^{14}C]valine (285 mCi/mmol). The reaction mixture was incubated at 37° C for 30 min and 70 µl was then withdrawn for determination of the incorporated acid-insoluble radioactivity. In vitro transcription was carried out using a pBR322 derivative and purified *E. coli* RNA polymerase following an experimental protocol to be described elsewhere (La Teana et al. 1991).

Bacterial cryofixation and embedding. Overnight cultures of *E. coli* K12ΔH1Δtrp/pPLc11 were diluted 100-fold in 10 ml of LB supplemented with 50 µg/ml ampicillin and were aerated at 28° C until a cell density of 2×10^8/ml was reached. H-NS overproduction was induced as described above for either 15 or 30 min and the cells were then harvested by filtration on Nucleopore (0.22 µm) membrane filters. The deposit was transferred to JOB No. 807S cigarette paper and immediately frozen by slamming on a polished copper block at −268° C (Escaig 1982; Heuser et al. 1979). Note that the samples were not chemically fixed prior to cryofixation. The frozen bacteria were placed in a substitution medium containing either 3% glutaraldehyde (for Lowicryl embeddings) or 2% osmium tetroxide (for Epon embeddings) in acetone in the presence of a molecular sieve (0.4 nm Perlform, Merck) for 64 h at −90° C. The temperature was then raised to −40° C and the samples were kept at −40° C for 6 h. The Lowicryl HM20-embedded samples were infiltrated at −40° C by stepwise increases in the HM20 concentrations in acetone (Kellenberger et al. 1987). The following day, the samples were transferred into fresh HM20 and left for 4 h before being placed into gelatin capsules and polymerized with UV-light (254 nm) at −40° C for 24 h. Further hardening was achieved by UV-irradiation for 3 days at room temperature (Carlemalm et al. 1982; Acetarin et al. 1986).

For Epon embedding, the substitution temperature was raised within 4 h to room temperature. The samples were washed in pure acetone, then infiltrated by stepwise increases in the Epon concentration in acetone and poly-

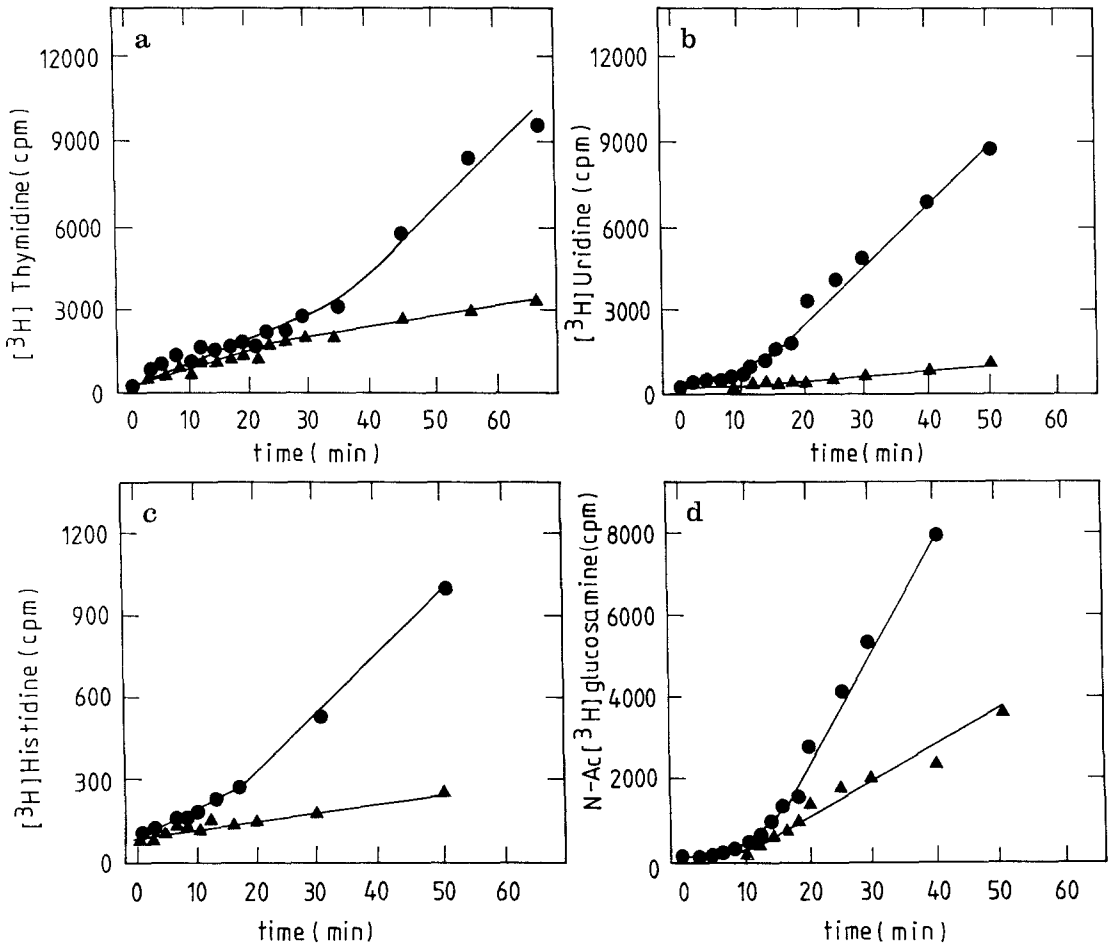


Fig. 4a–d. Effect of H-NS overproduction on the de novo macromolecular synthesis in vivo: **a** DNA, **b** RNA, **c** protein and **d** peptidoglycan syntheses. *E. coli* cells harbouring pPLc12 (●) or pPLc11 (▲). Further experimental details are given in the Materials and methods

merized for 3 days at 70° C. Thin sections (50–70 nm) were cut with a diamond knife on a Reichert Ultracut E microtome, mounted on 150 mesh Formvar-coated copper grids, immunolabelled and observed in a Philips EM 400 at 60 kV.

Immunocytochemistry. For labelling, the precoupling procedure (Dürrenberger 1989) was used; background label can be substantially reduced by this procedure. Two hundred microlitres of phosphate-buffered saline (PBS), pH 7.4, containing 1% bovine serum albumin (BSA; fraction V) were mixed with 2 µl anti-H-NS polyclonal rabbit serum. Twenty-five microlitres of concentrated ($OD_{520\ nm}=4$) protein A-gold (14 nm), prepared as described by Roth (1982), was then added and the mixture was incubated for 2 h at 25° C. The typical red colour of colloidal gold disappears during this time. The aggregates formed were centrifuged 2 min at $12000\times g$ and after discarding the supernatant, 100 µl PBS/BSA were added to resuspend the pellet. This washing was repeated twice. The last pellet was resuspended in 400 µl PBS/BSA and sonicated at 42 kHz for a few seconds until the red colour indicative of a colloidal sol reappeared. The sonication was continued for 1 min to ensure optimal dispersion. The solution to be applied to the grids had an OD_{520} of ~0.1. Grids with sections were incubated for 10 min on a drop of ovalbumin (2.5% in PBS), placed on a drop of freshly sonicated precoupled IgG-protein A-gold for 1 h and then jet-washed with PBS (on the section side only) and put for 5 min on a drop of PBS containing 1% glutaraldehyde to fix the complexes to the antigens. An intensive jet-wash (on the section side) with bidistilled water followed. The grids were briefly blotted on filter paper and stained on 2% aqueous uranyl acetate (45 min) and lead citrate (1 min).

Results

Upon induction of the lambda P_L promoter, cells harbouring pPLc11 or pPLc12 were found to produce large and quantitatively comparable amounts of wild type (wt) H-NS or H-NSΔ12, respectively. This can be clearly seen by comparing the SDS-PAGE patterns obtained with different amounts of cell extracts derived from induced and uninduced *E. coli* cells carrying either vector (Fig. 2a). However, while cells overproducing H-NSΔ12 remained fully viable, those overproducing wt H-NS were rapidly killed, viable counts being reduced by ap-

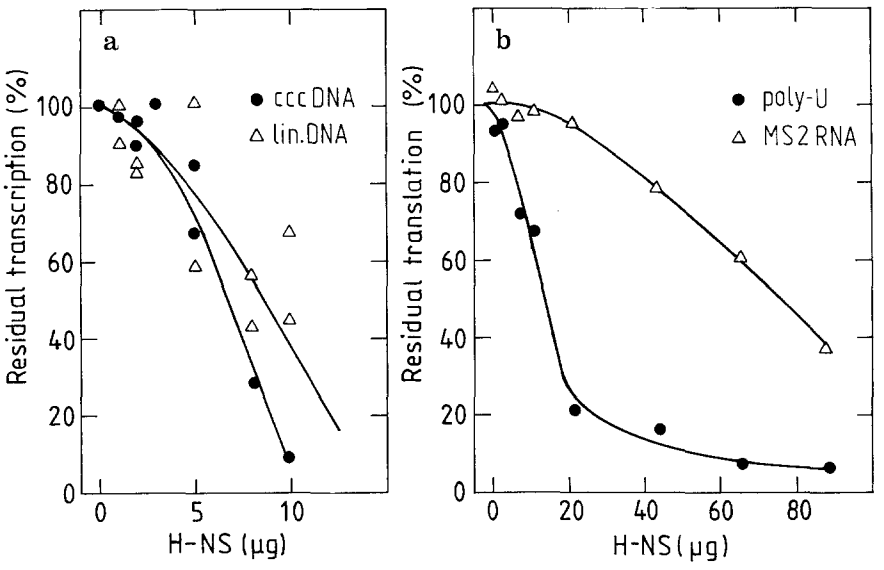


Fig. 5a and b. Effect of H-NS on transcription and translation in vitro. Dose response curve of the inhibition by H-NS of **a** incorporation of [α^{32}P]ATP into RNA using either cccDNA (●) or linearized DNA (△) as template. **b** Poly(U)-dependent synthesis of polyphenylalanine (●) and MS2 RNA-dependent incorporation of radioactive valine (△). Further experimental details are given in the Materials and methods

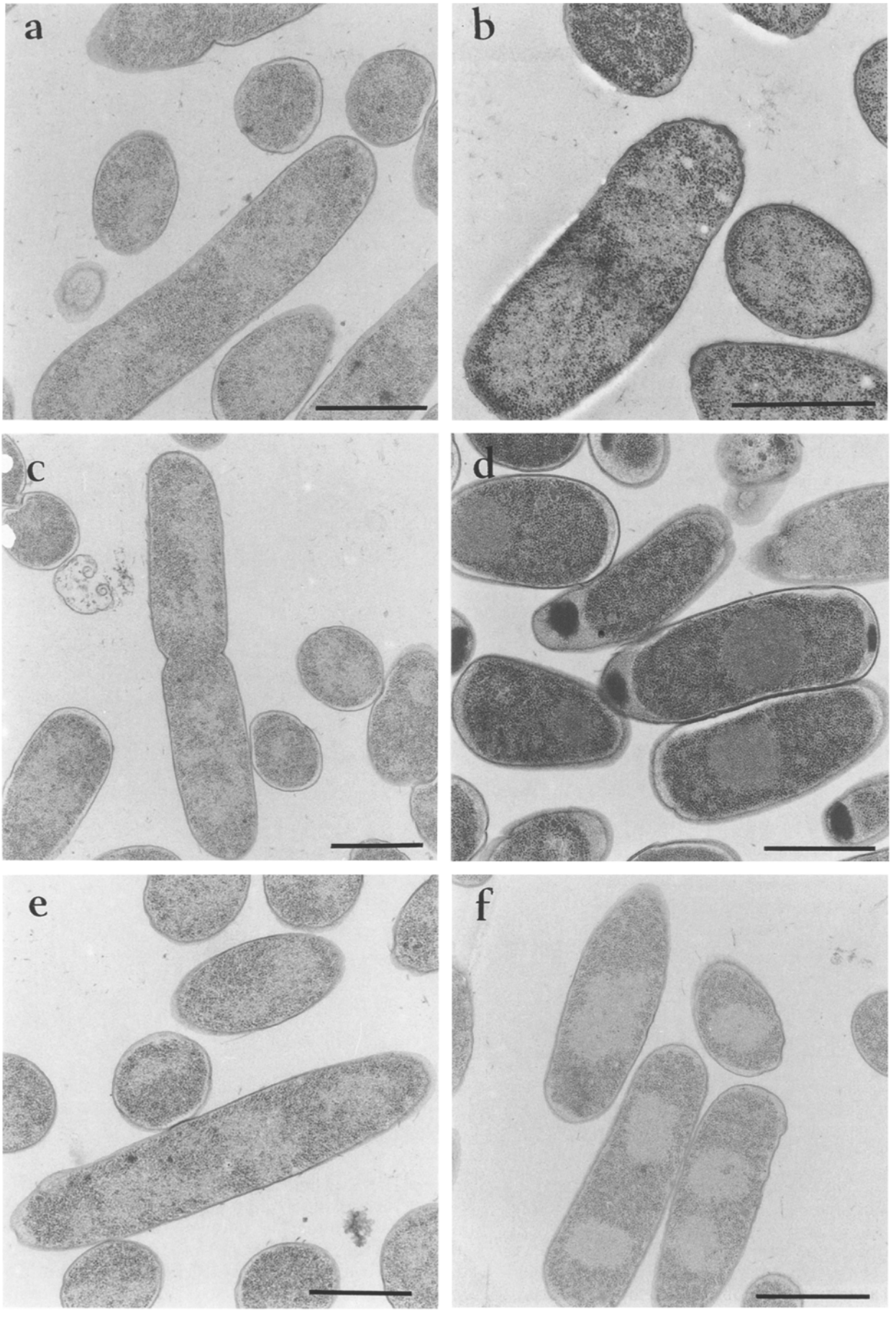
proximately four orders of magnitude within 10 min after induction (Fig. 2b); since the few surviving cells were found to be unable to overproduce H-NS (not shown), it can be safely concluded that the overproduction of wt H-NS is incompatible with cell survival, while that of H-NSΔ12 is not accompanied by cell death.

Consequently, we reasoned that important clues concerning the in vivo function of H-NS could be obtained by analysing both biochemical and morphological alterations brought about by the massive production of wt H-NS eventually leading to cell death. Thus, the time course of macromolecular syntheses (i.e. DNA, RNA, protein and peptidoglycan) was followed by measuring the incorporation of radioactive thymidine, uridine, histidine (an amino acid not present in H-NS) and N-acetyl-glucosamine into cells overproducing either the toxic wt H-NS or the non-toxic H-NSΔ12 to approximately the same extent. Two types of experiment were performed: in the first experiment (Fig. 3), the radioactive precursors were added before induction and syntheses followed before and after induction so that the effect of H-NS overproduction on total (i.e. on-going+ de novo) syntheses could be analysed. In the second experiment (Fig. 4), the radioactive precursors were given a few minutes after the induction of H-NS synthesis to determine the effect of an excess of this protein primarily on de novo syntheses. The first experiment clearly shows that the overproduction of the mutant H-NS, unlike that of wt H-NS has no major effect on macromolecular synthesis since the linearity of the incorporation of the precursors was not perturbed by the induction of pPLc12 expression. As to the effect of wt H-NS overproduction, both types of experiments led to the same basic conclusion. Thus, if one looks at the time at which the two curves (wt H-NS vs. H-NSΔ12) diverge and at the extent of the difference between them, it is clear that although synthesis of all four macromolecular classes is affected, RNA (Fig. 3b, and 4b) and protein (Fig. 3c and 4c) synthesis is inhibited earlier and to a greater extent than DNA (Fig. 3A and 4a) and cell wall (Fig. 3d and 4d) synthesis.

Transcription and translation are tightly coupled processes in bacteria and are inhibited at about the same time and to approximately the same extent by the excess wt H-NS. Therefore one cannot distinguish between an effect on transcription, on translation or on both by simply analysing the results of Figs. 3 and 4. Indeed, since it has been reported that H-NS inhibits transcription in vitro (Lammi et al. 1984; Spassky et al. 1984), the in vivo inhibition of protein synthesis could be a secondary effect of transcriptional inhibition in vivo. Nothing is known, on the other hand, of possible effects of H-NS on translation. The experiments shown in Fig. 5 demonstrates using in vitro systems in which transcription and translation are uncoupled, that H-NS can inhibit not only RNA (Fig. 5a) but also protein synthesis (Fig. 5b). Surprisingly, in these experiments poly(U)-dependent polyphenylalanine synthesis was found to be more strongly affected than MS2 RNA-dependent translation (see the discussion).

The morphology of cells overproducing comparable amounts of wt H-NS, H-NSΔ12 and initiation factor IF3 (a nucleic acid-binding protein) was examined by electron microscopy following cryosubstitution and embedding (Figs. 6 and 7). Cryosubstitution has the advantage that cells are fixed within milliseconds by a physical rather than by a chemical procedure, thus preserving the physiological conditions. Also, the samples are not warmed up until the end of the preparation, and all artefacts originating from chemical fixation and washing

Fig. 6a–f. Effect of H-NS and IF3 overproduction on the morphology of *E. coli* nucleoids. Specimens of *E. coli* K12ΔH1Δtrp carrying: **a** pIM320 (an expression vector for *E. coli* translation initiation factor IF3), uninduced cells; **b** pIM320, after 30 min induction; **c** pPLc11, uninduced; **d** pPLc11, after 20 min induction; **e** pPLc12, uninduced; **f** pPLc12, after 20 min induction. Conditions for cell growth, heat-induced overproduction of the corresponding proteins, cryosubstitution and electron microscopy techniques were as described in the Materials and methods. The *black bars* in each panel correspond to 1 µm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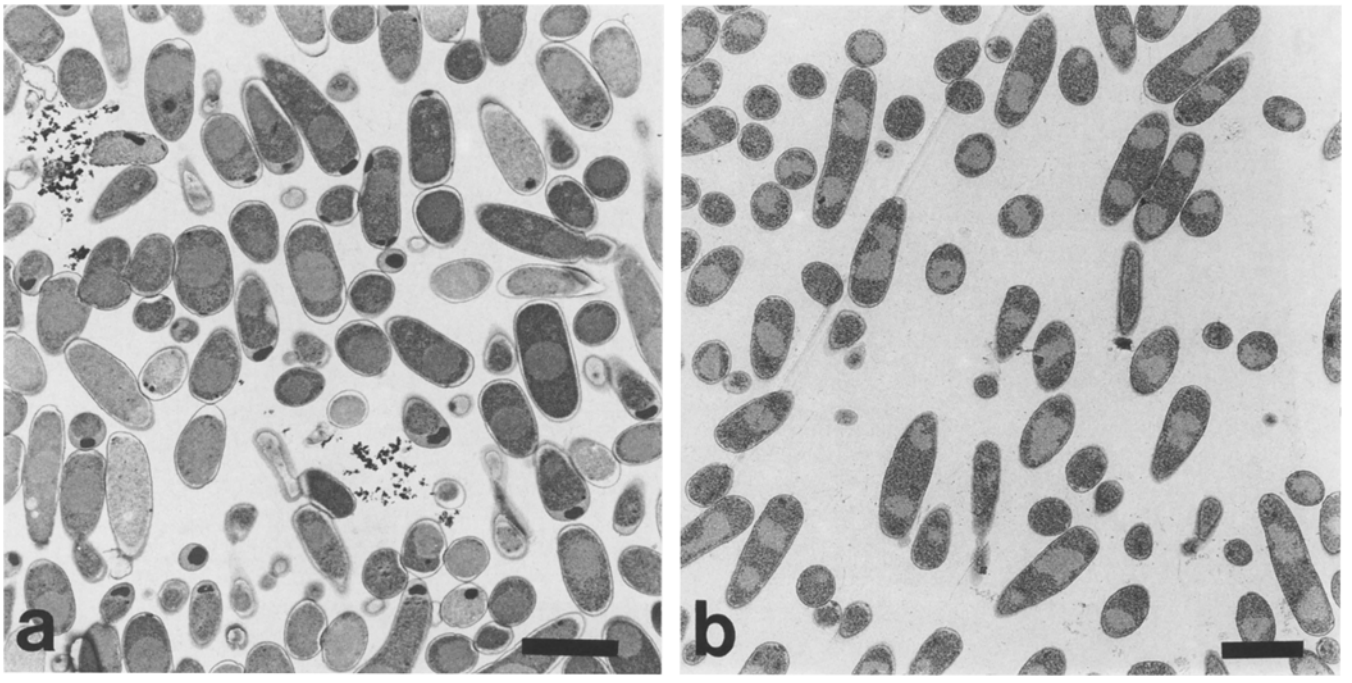

Fig. 7a and b. Comparison of the morphology of *E. coli* cells hyperproducing **a** wt H-NS and **b** H-NSΔ12. The samples were prepared as described in the legend to Fig. 6 and correspond to the specimens presented in Fig. 6e and f seen at lower magnification

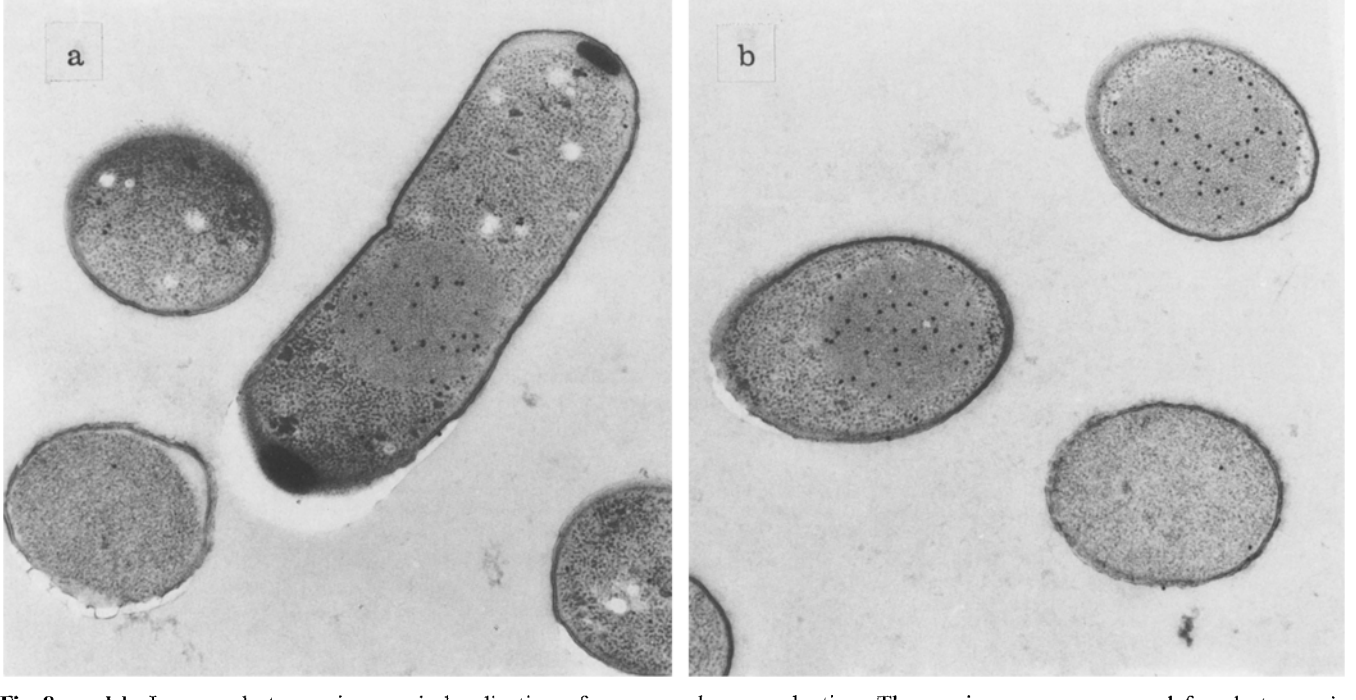

Fig. 8a and b. Immunoelectron microscopic localization of overproduced wt H-NS. Both panels represent specimens of *E. coli* K12ΔH1Δtrp carrying pPLc11 after 20 min induction of wt H-NS hyperproduction. The specimens were prepared for electron microscopy and subjected to immunogold labelling as described in the Materials and methods

are kept to a minimum. Therefore, the morphology revealed by this method is reliable down to $\sim$1 nm resolution.

Compared to the uninduced controls (Fig. 6a, c, e) and to the cells overproducing IF3 (Fig. 6b), clear morphological alterations were observed in the cells overproducing H-NS. Thus, in the cells overproducing H-NSΔ12, one can observe substantial condensation of the nucleoids, which remain, however, somewhat loose and irregular in shape; furthermore, most of the cells appear to contain two nucleoids or, at least, a nucleoid in the process of dividing (Figs. 6f and 7b). In the cells over-

producing wt H-NS, on the other hand, the morphological alteration of the nucleoids is much more dramatic. The chromosomes appear as extremely compact, dense bodies having nearly perfectly spherical shapes and superficially resemble the yolks in a section of hard-boiled eggs (Figs. 6d and 7a). In these nucleoids it is sometimes possible to recognize a mass of DNA fibres reminiscent of condensed eukaryotic chromatin. Furthermore, unlike the cells overproducing H-NSΔ12, nearly all cells including those in the process of dividing, were found to contain a single nucleoid. In some rare cases, however, the compaction process seemed to have "frozen" nucleoid segregation during cell fission (not shown).

Other morphological observations typical of cells overproducing wt H-NS are the presence of large inclusion bodies (lumps) mainly at the polar ends of the cells and an unusual condensation of the ribosomal area. Although the inclusion bodies were not recognized by anti-H-NS serum, presumably due to denaturation of the protein, it is very likely that these lumps contained a large portion of the overproduced H-NS. All the protein that reacted with anti-H-NS antibodies, on the other hand, was found to be localized in the nucleoids (Fig 8); a similar localization was also found for the overproduced H-NSΔ12 (not shown).

Discussion

It has been shown here that massive overproduction of the DNA-binding protein H-NS results in cell death accompanied by several biochemical and morphological alterations. The main effects involve strong inhibition of transcription and translation and extraordinary compaction of the genetic material into very dense and almost perfectly spherical "nuclei" and the formation of inclusion bodies. Other cell functions such as DNA duplication and cell wall synthesis were also inhibited but to a lesser extent and at a later time. Cell lethality could result from any one or a combination of the above-mentioned effects. However, the purpose of this work was not to determine the primary cause of death following an overproduction of H-NS, but rather to obtain clues to the in vivo function of this protein from the observation of the effects produced by abnormally high intracellular concentrations of this protein.

Unlike the case of wt H-NS, overproduction of a mutant H-NS molecule (i.e. H-NSΔ12) did not result in the inhibition of macromolecular synthesis or in cell death. Protein H-NSΔ12 lacks four amino acids (G-R-T-P) in a region of the molecule (residues 112–115) located very close to the tryptophan residue (position 108) whose intrinsic fluorescence is affected by the H-NS-DNA interaction (Friedrich et al. 1988). In spite of the deletion, H-NSΔ12 was found to retain substantial DNA-binding activity (i.e. it is bound as tightly by DNA cellulose as is wt H-NS). Thus neither the lethal effect of wt H-NS nor its ability to inhibit macromolecular syntheses can be correlated with DNA-binding capacity in any simple way. Hence, it seems clear that the DNA-binding capacity is probably a necessary but certainly not a sufficient condition to ensure full H-NS function. This is also true for the activity responsible for the compaction of DNA. The nucleoids of cells overproducing H-NSΔ12 are somewhat condensed compared to normal cells but are much less dramatically compacted than those seen after overproduction of wt H-NS.

While these results seem to indicate that DNA compaction (or at least a certain degree of compaction) is still compatible with cell viability, they also indicate, beyond any doubt, that H-NS can affect the level of DNA condensation in vivo. A special kind of nucleoid condensation has also been observed in cells treated with antibiotics that stop protein synthesis [e.g. chloramphenicol (Kellenberger et al. 1958, 1986), aureomycin and puromycin (M. Dürrenberger, unpublished results)]. There are, however, important differences between the nucleoids of cells in which protein synthesis has been arrested and nucleoids condensed by excess wt H-NS. In the latter case, the nucleoids are extremely dense and absolutely homogeneous with the appearance of filled spheres, whereas in the former case the nucleoids appear as hollow spheres giving rise to annular sections with the denser material in the outer region. This type of morphology more closely resembles that seen upon overproduction of H-NSΔ12. Thus, although the inhibition of protein synthesis may contribute to some extent to the unprecedentedly dramatic condensation of the nucleoids, this observation specifically underlies the DNA-binding capacity of wt H-NS, coupled to its known ability to affect the physical packaging of the genetic material (Spassky et al. 1984).

As mentioned above, inhibition of transcription by H-NS has already been observed in vitro and several *hns* alleles have altered phenotypes characterized by a substantial derepression of particular genes (see the Introduction). Thus, the transcriptional inhibition observed upon H-NS overproduction is not surprising, although its precise mechanism remains to be established. The inhibition could result from a direct interaction of H-NS with specific DNA sequences (Rimsky and Spassky 1990), from a generalized "tightening" of the chromosome (Lejeune and Danchin 1990), from a topological alteration of DNA (Higgins et al. 1988), or through some other mechanism such as, for instance, the potentiation of the activity of transcriptional repressors (Falconi et al. 1991). Regardless of the mechanism, the present data lend further support to the notion that one of the physiological functions of H-NS is to control, either directly or through its effect on the nucleoid structure, the level of transcription in the cell.

Inhibition of translation by H-NS, on the other hand, represents a new and somewhat unexpected finding; even more surprising is the finding that poly(U)-dependent polyphenylalanine synthesis was more affected than MS2 RNA-dependent translation. Since the addition of an excess of poly(U), $tRNA^{Phe}$, MS2 RNA or total tRNA mixture did not relieve the inhibition, binding of H-NS to either template or tRNA cannot explain the observed inhibition; moreover, a selective interaction with either poly(U) or $tRNA^{Phe}$ cannot explain the stronger effect on the poly(U)-programmed cell-free sys-

tem. The results seem to indicate instead that the target of the H-NS inhibition is the ribosome and, therefore, it is not trivial to recall that H-NS was originally purified in our laboratory from a high-salt ribosomal wash (Lammi et al. 1984). Furthermore, we recently found that H-NS can bind to ribosomes, primarily to the small subunit (unpublished observations). If access of H-NS to its ribosomal binding site is less hindered by a non-structured template like poly(U) than by the structurally more complex MS2 RNA, then the two translational systems might be expected to display different sensitivities to H-NS.

The present data do not allow us to decide whether the inhibition of translation is restricted to the case in which a massive excess of H-NS is present in the cell or may have a physiologically relevant effect may arise under special conditions in which the level of the protein is moderately increased, such as, for instance, during the cold shock response (La Teana et al. 1991; Dürrenberger et al. 1991). It is conceivable, however, that under these "stress" conditions, the increased number of H-NS molecules might serve a multipurpose function, such as that of attaining an increased compaction of the nucleoid and of slowing down both transcription and translation. Considering the abundance of H-NS, only a small fraction of the molecules accumulated under conditions of cold shock could cause a temporary and reversible inactivation of a substantial portion of the approximately 15000 ribosomes present in an actively growing cell.

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