JOURNAL OF BACTERIOLOGY, Mar. 1997, p. 2060–2064 Vol. 179, No. 6
0021-9193/97/$04.00+0

NOTES

Association of the Histone-Like Protein HBsu with the Nucleoid of *Bacillus subtilis*

PETRA KÖHLER AND MOHAMED A. MARAHIEL*

Biochemie/Fachbereich Chemie, Phillipps-Universität Marburg, 35032 Marburg, Germany

Received 12 November 1996/Accepted 16 January 1997

To investigate the physiological role of the essential histone-like protein of *Bacillus subtilis* (HBsu) in the nucleoid structure, a fusion to the green fluorescent protein (GFP) of *Aequorea victoria* was constructed. This purified fusion protein, HBsuGFP, showed a threefold-reduced affinity to DNA compared to unmodified HBsu; however, in gel mobility shift experiments HBsuGFP DNA-binding was greatly enhanced in the presence of low HBsu concentrations. Additional production of HBsu also had a positive effect on the retarded growth of a *B. subtilis* strain, PK9C8, which expresses only *hbs-gfp* (encoding HBsuGFP). HBsu seemed to influence not only growth but also nucleoid structure, as monitored by DNA staining and fluorescence microscopy. Without HBsu production, strain PK9C8 showed a relaxed nucleoid structure associated with HBsuGFP. However, a highly compact nucleoid structure that coincides with the fluorescence of the fusion protein was visualized when HBsu synthesis was induced. This provides the first evidence for in vivo association of HBsu in DNA packaging and its consequence on cell growth.

HBsu of *B. subtilis* belongs to a widespread family of histone-like proteins in microorganisms (8, 26, 33). This family represents a group of small, basic, and abundant proteins that bind DNA. Because of these properties, it was suggested that histone-like proteins play a role in DNA condensation in bacteria.

One of the best-studied nucleoid-associated proteins is HU of *Escherichia coli* (for a review, see reference 28). It is predominantly a heterodimer consisting of two different subunits, each with a molecular mass of about 10 kDa. The HU subunits HU-2 and HU-1 are encoded by *hupA* and *hupB*, respectively (18, 19). Nucleoids isolated at low ionic strength were found to be associated with HU (29). However, by techniques that allow in vivo localization of HU in *E. coli*, controversial results were obtained. Immunocytochemically, HU was found to be localized with the metabolically active DNA fraction but not in the nucleoid (9). In contrast, fluorescein-labeled HU that was introduced into *E. coli* cells was distributed throughout the nucleoid (34). Likewise, no results on the role of the integration host factor (IHF) of *E. coli* in DNA packaging were obtained. IHF shows sequence homology with HU and also consists of two subunits, IHF-α and IHF-β, encoded by *himA* and *hip*, respectively (12, 25).

Another important nucleoid-associated protein of *E. coli* is H-NS, encoded by the *hns* gene (11, 23). This homodimeric protein does not show sequence homology to HU or IHF; it is neutral and has a molecular mass of about 15 kDa. Its association with the nucleoid was shown by immunoelectron microscopy (10). Furthermore, overproduction of H-NS in *E. coli* resulted in loss of cell viability, indicating a strong condensation of the nucleoid associated with H-NS, and as a consequence, inhibition of RNA and protein synthesis was observed (35). In contrast, overexpression of HU has little influence on cell growth (24).

A model of nucleoid organization in *E. coli* suggested that H-NS and HU are located in two different domains (24). An internal domain is thought to contain rarely transcribed DNA associated with H-NS, while the coralline-appearing outer sphere seems to be composed of metabolically active DNA and HU (4). Although histone-like proteins appear to play special roles in cell physiology, *E. coli* strains lacking HU, IHF, or H-NS are viable (13, 16, 17, 37). Even though retarded in growth, cells can also tolerate the absence of two of these proteins. On the other hand, the absence of all three proteins is lethal (39).

In contrast, *B. subtilis* harbors only one histone-like protein, HBsu, encoded by the essential *hbs* gene (26, 27). To our knowledge genes encoding neither H-NS- nor IHF-like proteins have been identified in *B. subtilis* (36). The HBsu protein shows a high level of homology to HU from *E. coli*. It has 57 and 52% identical amino acid residues with HU-2 and HU-1, respectively. Like HU, HBsu was found to bind DNA unspecifically. In contrast to HU, which forms predominantly heterodimers, HBsu binds DNA as a homodimer. Furthermore HBsu enables β recombinase-mediated recombination by stabilizing a DNA secondary structure (1). In order to investigate the fate of HBsu encoded by *hbs* in *B. subtilis*, we used a technique that allowed us to look directly into the cell, avoiding the use of exogenous markers, as described for HU of *E. coli* (9, 34). The green fluorescent protein (GFP) of the jellyfish *Aequorea victoria* was previously used as a reporter protein not only to monitor gene expression (7) but also to study protein localization during development in *B. subtilis* (3, 22, 38). In this study, an HBsuGFP fusion protein was constructed and its intracellular localization was studied. Additionally, the influence of HBsu on nucleoid condensation in *B. subtilis* was analyzed.

Construction of an *hbs-gfp* fusion. In order to investigate a possible involvement of the histone-like protein HBsu of *B.*

* Corresponding author. Mailing address: Philipps-Universität Marburg, Biochemie/Fachbereich Chemie, Hans-Meerweinstr., D-35032 Marburg, Germany. Phone: 49-6421-285722. Fax: 49-6421-282191. E-mail: marahiel@ps1515.chemie.uni-marburg.de.

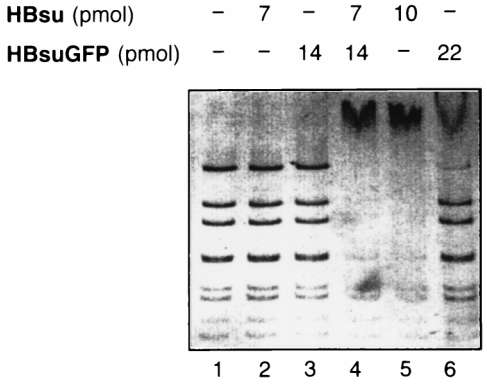

FIG. 1. Gel retardation experiment showing the diminished DNA-binding ability of (His_6)HBsuGFP compared to (His_6)HBsu and the complex formation of the two proteins. In each lane, 200 ng of *Hae*III-digested pBluescript, showing fragments of 767, 458, 434, 267, 243, 174, 142, 125, 102, 80, 79, 61, 18, and 11 bp, were mixed with different protein amounts: lane 1, no protein; lane 2, 7 pmol of (His_6)HBsu; lane 3, 14 pmol of (His_6)HBsuGFP; lane 4, 7 pmol of (His_6)HBsu and 14 pmol of (His_6)HBsuGFP; lane 5, 10 pmol of (His_6)HBsu; lane 6, 22 pmol of (His_6)HBsuGFP.

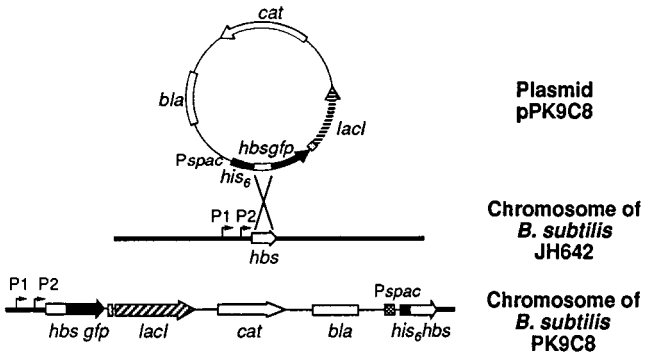

FIG. 2. Campbell-like integration of plasmid pPK9C8 containing the *hbs-gfp* fusion under the control of the IPTG-inducible *spac* promoter (P*spac*) into the chromosome of wild-type *B. subtilis*. In the resulting strain, PK9C8, the *hbs-gfp* fusion is under the control of the native *hbs* promoters P1 and P2 while *(his6)hbs* expression is controlled by the inducible *spac* promoter.

subtilis in DNA compaction, a fusion protein of HBsu and the easily detectable GFP was constructed (7). The *hbs* gene encoding HBsu was amplified without its stop codon by PCR using primers Bam5′hbs (5′CGC**GGATCC**ATGAACAAAAC AGAACTTATC) and Sma3′hbs (5′TCC**CCCGGG**GTTTTCC GGCAACTGCGT). After digestion with *Bam*HI and *Sma*I, the fragment was ligated into *Bam*HI- and *Sma*I-digested pCW8 (38), which contains the *gfp* coding sequence. The resulting plasmid, pPKC2, comprises an in-frame *hbs-gfp* fusion with a spacer of 10 codons between the last codon of *hbs* and the first codon of *gfp*. To ensure that the fusion with GFP does not influence the DNA-binding properties of HBsu, the fusion protein as well as HBsu was purified and used for DNA binding studies. A simple purification of HBsu and HBsuGFP, employing affinity chromatography, was established by fusing six histidine codons to the 5′ ends of *hbs* and *hbs-gfp*, respectively. For that purpose, PCR was used to amplify the *hbs* gene by using primers Bam5′hbs and Ava3′hbs (5′CTGC**ATGCAT** TTATTTTCCGGCAACTG). After digestion with *Bam*HI and *Ava*III, the fragment was ligated into *Bam*HI- and *Pst*I-digested pQE9 (Qiagen), resulting in pPK952, a plasmid carrying *(his6)hbs*. The 1,045-bp *Bam*HI/*Hin*dIII fragment from pPKC2, containing the *hbs-gfp* fusion, was also ligated into *Bam*HI/*Hin*dIII-digested pQE9 to create the *(his6)hbs-gfp* fusion in pPK9C2. Both gene fusions were placed under control of the T5 promoter, and their expression was induced by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside). For overproduction of (His_6)HBsu and (His_6)HBsuGFP, *E. coli* M15 [pREP4] containing pPK952 or pPK9C2 was grown in 2× YT (31) containing ampicillin (100 μg/ml) and kanamycin (25 μg/ml) at 30°C. Expression was induced by IPTG (1 mM) at an optical density at 600 nm (OD_{600}) of 0.7, and cells were grown for another 2 h and centrifuged at 6,000 × *g* and 4°C for 15 min. The pellet was resuspended in 0.025 volume of buffer A (300 mM NaCl, 50 mM Na_2HPO_4-NaH_2PO_4, [pH 8.0]), sonicated, and centrifuged at 18,000 × *g* and 4°C for 30 min. The supernatant was mixed with 30 mM imidazole and applied to a fast-performance liquid chromatography Ni^{2+} nitrilotriacetic acid agarose column (Pharmacia/Qiagen). The proteins were eluted with buffer A containing 50 mM imidazole at a flow rate of 0.75 ml/min. Their purity was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (32) (see Fig. 4), showing migration according to the predicted molecular masses of 11.3 and 39.2 kDa for the 104 amino acids (aa) constituting (His_6)HBsu and the 352 aa containing (His_6)HBsuGFP, respectively. The protein concentration was determined as described previously (5). (His_6)HBsu was used for immunization of rabbits to obtain polyclonal antibodies (Eurogentec, Seraing Belgium) against the (His_6)HBsu protein. Both proteins, (His_6)HBsu and (His_6)HBsuGFP, were used for gel retardation experiments after dialysis against TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]).

DNA-binding properties of (His_6)HBsu and (His_6)HBsuGFP. HBsu was reported to bind nonspecifically to DNA (14). We used a *Hae*III-digested pBluescript (Stratagene) plasmid (14 fragments; 767, 458, 434, 267, 243, 174, 142, 125, 102, 80, 79, 61, 18, and 11 bp) to investigate the DNA binding of recombinant (His_6)HBsu and (His_6)HBsuGFP. The fragments were mixed with different amounts of proteins (His_6)HBsu and (His_6)HBsuGFP in binding buffer B (50 mM NaCl, 20 mM Tris-HCl [pH 8.6], 5 mM $MgCl_2$, 6% glycerol). Samples with a mixture of both proteins were preincubated for 2 h at 0°C before mixing with DNA. The probes (total volume, 10 μl) were mixed and incubated for 30 min at 25°C. Probe buffer (10% glycerol, 0.1% bromophenol blue) (0.5 volume) was added, and the samples were loaded on a 10% native polyacrylamide gel containing TBE (135 mM Tris, 48 mM boric acid, 2.5 mM EDTA) and 6% glycerol. Polyacrylamide gel electrophoresis was performed at 4°C and 20 mA in TBE buffer. After electrophoresis, the gels were incubated for 5 min in TBE containing 40 ppm of ethidium bromide to stain the DNA. (His_6)HBsu (7 pmol) and (His_6)HBsuGFP (14 pmol) were not sufficient for DNA binding (Fig. 1, lanes 2 and 3). However, a preincubated mixture of 7 pmol of (His_6)HBsu and 14 pmol of (His_6)HBsuGFP exhibited the same ability to bind to DNA as did 10 pmol of (His_6)HBsu (Fig. 1, lanes 4 and 5). This increased DNA-binding capacity was only developed after preincubation of the two proteins, indicating a possible complex formation between (His_6)HBsu and (His_6)HBsuGFP. Nevertheless, (His_6)HBsuGFP could also bind to DNA without additional (His_6)HBsu. For the first step of retardation, the binding of the largest fragment of *Hae*III-digested pBluescript, about 22 pmol of (His_6)HBsuGFP was required (Fig. 1, lane 6). That is about three times more than the amount of (His_6)HBsu (8.2 pmol) needed for a comparable retardation (data not shown). This reduced DNA-binding ability might be due to the fusion of a 238-aa protein to the 92 aa of HBsu, even

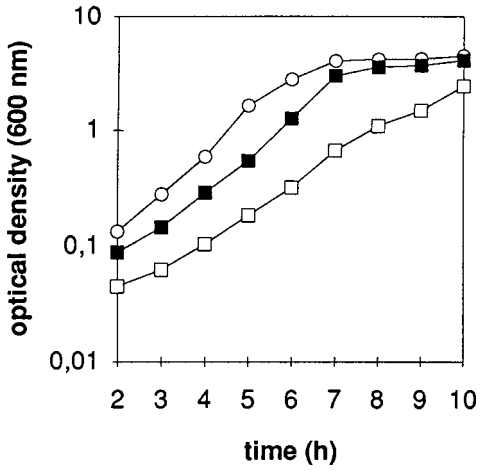

FIG. 3. Growth of *B. subtilis* dependent on HBsu production. Cells were grown in 2× YT medium at 30°C. The expression of *(his$_6$)hbs* in strain PK9C8 (P*spac(his$_6$)hbs*) was induced by the addition of IPTG. ○, wild-type *B. subtilis* JH642; □, strain PK9C8; ■, strain PK9C8 with IPTG.

though it was separated by a spacer of 10 aa. The observation that (His$_6$)HBsuGFP binds to DNA and that its DNA binding is enhanced in the presence of (His$_6$)HBsu, confirmed the usefulness of the fusion protein to detect its cellular localization in *B. subtilis.*

Integration of *hbs-gfp* fusion in the *B. subtilis* chromosome. In order to investigate the HBsuGFP localization within the cell as well as the influence of the histone-like protein HBsu on nucleoid structure, a strain allowing inducible expression of *hbs* was constructed. The *hbs-gfp* fusion was cloned downstream of the *spac* promoter in pDH88 (15) (Fig. 2). The plasmid pPK9C2 was digested with *Hin*dIII, the ends were filled in with DNA polymerase, and the resulting product was digested with *Xho*I, resulting in a 1,170-bp fragment carrying the fusion. This fragment was ligated with pDH88 digested with *Sph*I, the ends were filled in with DNA polymerase, and the resulting product was *Sal*I digested, creating pPK9C8. Competent *B. subtilis* JH642 cells were prepared and transformed as described previously (20) with pPK9C8. The plasmid was integrated Campbell-like into the *hbs* locus of the chromosome, resulting in *B. subtilis* PK9C8 (Fig. 2). This strain harbors the *hbs-gfp* fusion under control of the two original *hbs* promoters, P1 and P2 (27). Due to the recombination event, *hbs* was fused to the six histidine codons originating from the *hbs-gfp* gene fusion (Fig. 2). Expression of *hbs* was placed under control of the IPTG-inducible *spac* promoter. Integration was confirmed by PCR using primers Eco5′P2 (5′CCG**G AATTC**ACTTTGTTGGACAAA) and Sal3′gfp (5′C**GACGTC** GACTAAAATTTTGTATAGTTCATCCA). The amplified single 1.2-kb fragment containing the 5′ end of the *hbs* P2 promoter and the 3′ end of *gfp* was analyzed in sequencing reactions using AmpliTaq DNA Polymerase, FS (ABI). The reactions were analyzed on a model 310 genetic analyzer (ABI). In-frame fusion of *(his$_6$)* to *hbs* was additionally confirmed by (His$_6$)HBsu purification with affinity chromatography from *B. subtilis* PK9C8 cells grown in the presence of IPTG.

Growth of *B. subtilis* is dependent on *hbs* expression. The influence of HBsu on growth of *B. subtilis* cells was studied by using strain PK9C8 grown in the absence and in the presence of IPTG, which induced the expression of *(his$_6$)hbs* in this strain. As a control, wild-type *B. subtilis* was grown under the same conditions. In rich medium, growth of strain PK9C8 producing (His$_6$)HBsu was slightly retarded compared to that of the wild type (Fig. 3). Both strains reach the stationary phase of growth at the same time, at 7 h after the end of log phase (T_7). In contrast, strain PK9C8 not expressing *(his$_6$)hbs* grew much more slowly than under (His$_6$)HBsu-producing conditions (Fig. 3). Only about one-eighth of the cell density (OD_{600}, 0.5) was achieved at T_7 in the absence of (His$_6$)HBsu compared to that of the wild type (OD_{600}, 4.0). In contrast to wild-type cells, which reached late stationary phase at T_{10}, PK9C8 cells not expressing *(his$_6$)hbs* were still growing exponentially, showing only a twofold reduction in growth compared to wild-type cells (Fig. 3). Nevertheless, when cells were grown in M9 minimal medium (31) no large differences in growth rates were detected. Western blot analysis (6) of cell extracts from strains grown in different media showed that the amount of HBsuGFP was higher in cultures grown in minimal medium (not shown). The Western blot in Fig. 4B and the corresponding gel (Fig. 4A) show the levels of HBsu, HBsuGFP, and (His$_6$)HBsu production in *B. subtilis* wild-type, PK9C8 not expressing *(his$_6$)hbs*, and PK9C8 expressing *(his$_6$)hbs*, all grown in minimal medium. The antibodies raised against (His$_6$)HBsu reacted predominantly with HBsu and HBsu derivatives (Fig. 4B). Nonspecific reactions were ob-

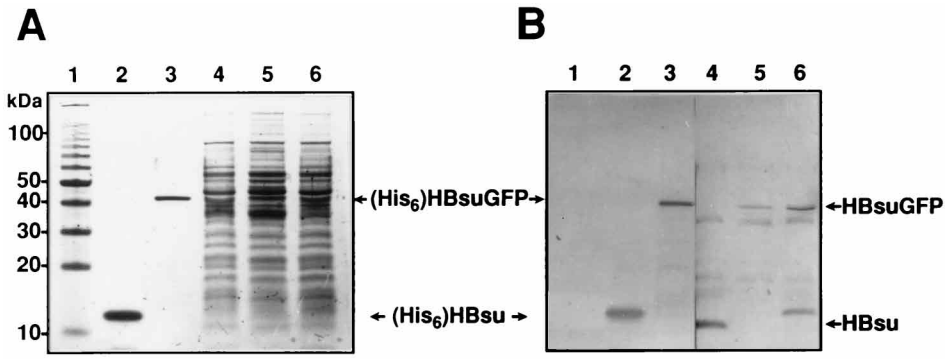

FIG. 4. Production of (His$_6$)HBsu and HBsuGFP in strain PK9C8. The expression of the *hbs-gfp* fusion is controlled by the native *hbs* promoters P1 and P2, whereas *(his$_6$)hbs* is under the control of the IPTG-inducible *spac* promoter. (A) Coomassie blue-stained 10% Tricine–polyacrylamide gel. (B) Western blot analysis of the gel shown in panel A, using polyclonal antibodies raised against HBsu. Lane 1, 10-kDa protein mass ladder; lane 2, purified (His$_6$)HBsu protein; lane 3, purified (His$_6$)HBsuGFP; lane 4, cell extracts of wild-type *B. subtilis* JH642; lane 5, cell extract of strain PK9C8 without induction of *(his$_6$)hbs* expression; lane 6, cell extract of strain PK9C8 with induced *(his$_6$)hbs* expression.

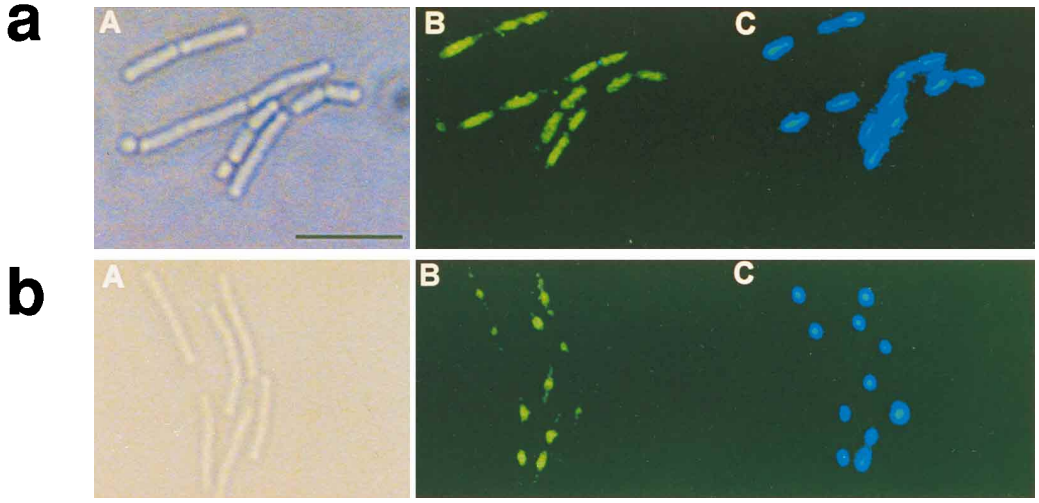

FIG. 5. Localization of HBsuGFP fusion protein in *B. subtilis* PK9C8. Cells shown in the upper panels (a) were grown without additional production of (His_6)HBsu. In the lower panels (b), cells grown in the presence of IPTG, which induces (His_6)HBsu production, are shown. (A) Phase-contrast image; (B) HBsuGFP green fluorescence; (C) DAPI fluorescence. The bar in aA represents 5 μm.

served with a 30-kDa *B. subtilis* protein (Fig. 4B, lanes 4 to 6), which appeared in all strains independently of *(his_6)hbs* expression and was therefore not further characterized.

Localization of HBsuGFP in *B. subtilis* cells. To visualize the localization of the HBsuGFP fusion protein and possible cellular changes depending on in *trans* expression of (His_6)HBsu, fluorescence microscopy and DNA staining were employed. PK9C8 cells carrying the *hbs-gfp* fusion grown in complete or sporulation medium did not produce large amounts of HBsuGFP, as determined by Western blot analysis and confirmed by fluorescence microscopy. In contrast, cells grown in minimal medium developed a bright green fluorescence. These cells were used to investigate the putative association of HBsuGFP to the nucleoid. The vegetative growing cells were centrifuged and resuspended in 0.05 volume of TE and a 3-μl aliquot was applied to a slide precoated with 0.005% polylysine (Sigma). The dried cells were treated with 10 μl of methanol, resuspended in 5 μl of distilled water, and mixed with 1 μl of DAPI (1 μg/ml; 4′,6-diamidino-2-phenylindol), and a coverslip was placed on the top. As observed for control experiments on viable cells (not shown), neither cell drying nor methanol treatment had an influence on the DNA structure but they were necessary to fix the cells on the slide. Cells were viewed with a Zeiss Axiovert epifluorescence microscope with a ×100 Plan-Neofluar oil immersion objective lens. GFP fluorescence was observed with a filter set for green light (excitation wavelength, 450 to 490 nm; long pass wavelength, 520 nm; emission wavelength, 510 nm), while DAPI fluorescence was visualized with a UV filter set (excitation wavelength, 365 nm; long pass wavelength, 397 nm; emission wavelength, 395 nm). Whole cells were viewed as phase-contrast images. In both types of PK9C8 cells grown with or without expression of *(his_6)hbs*, HBsuGFP-directed green fluorescence was always observed within the cell at exactly the same location as DAPI fluorescence (Fig. 5). This is, to our knowledge, the first in vivo evidence for the association of the histone-like protein HBsu as a fusion to GFP with the *B. subtilis* nucleoid. Furthermore, we observed that the additional production of (His_6)HBsu caused the formation of a more dense nucleoid (Fig. 5bC). This may be due to DNA compaction by (His_6)HBsu and HBsuGFP, as indicated by the compact green fluorescence associated with the highly condensed nucleoid. Wild-type cells showed the same DNA structure after DAPI staining as that observed in Fig. 5bC for strain PK9C8 grown with additional IPTG. Due to the absence of GFP, wild-type cells do not show any green fluorescence at all (not shown).

Conclusion. We used HBsuGFP as a marker for HBsu to investigate its interaction with the nucleoid of *B. subtilis*. HBsuGFP was shown to bind DNA in vitro (Fig. 1) and to be associated with the chromosome of *B. subtilis* (Fig. 5aC). Its in vitro as well as its in vivo DNA binding could be improved by addition of (His_6)HBsu (Fig. 1 and Fig. 5b). That nucleoid condensation depends on HBsu emphasizes its principal role in DNA packaging. Consequently, we infer that HBsu is essential for *B. subtilis*, as indicated by the failure to disrupt the *hbs* gene (27), due to its structural role for the organization of the chromosomal DNA.

Production of HBsuGFP alone in strain PK9C8 leads to the formation of the relaxed nucleoid (Fig. 5aC) and consequently to a growth slower than that of those cells producing additional (His_6)HBsu (Fig. 3). It is remarkable that *B. subtilis* cells are able to grow while expressing a histone-like protein (HBsuGFP) with reduced DNA-binding ability. However, one might assume that some other nonspecific DNA-binding proteins may complement this partial deficiency. Such proteins could be the small heat-stable, acid-soluble proteins which were isolated from the *B. subtilis* nucleoid and were shown to have DNA-binding capacity (30). The major heat-stable, acid-soluble protein, called HPB9, was found to be identical to HBsu (21). A second protein of this class, HPB12, with an estimated abundance of 20,000 monomers per cell, was found to be identical to the ribosomal protein L24 (2). It was proposed to participate in nucleoid formation by binding DNA as well as nucleoid-associated RNA. In the presence of an HBsu derivative with lowered DNA-binding ability, like HBsuGFP, HPB12 may support nucleoid formation but it seems not to be able to substitute for HBsu. In the absence of HBsu, DNA might not be packed adequately, causing inhibition of growth and development. These changes, however, might also be due to an altered gene expression, caused by low levels of HBsu. This system with inducible *(his_6)hbs* expression will allow us to study changes in promoter activity dependent on DNA packaging and to visualize DNA segregation during development.

We thank Erhard Bremer for comments on the manuscript, Richard Losick for providing the plasmid pCW8, and Reinhard Fischer and Matthias Ulrich for their expert help and the disposal of an epifluorescence microscope and a slide marker, respectively.

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

REFERENCES

1. **Alonso, J. C., C. Guitierrez, and F. Rojo.** 1995. The role of the chromatin-associated protein Hbsu in β-mediated DNA recombination is to facilitate the joining of distant recombination sites. Mol. Microbiol. **18:**471–478.
2. **Arnold-Schulz-Gahmen, B., V. Salti-Montesanto, J. Nguyen, L. Hirschbein, and F. Le Hégarat.** 1994. The *Bacillus subtilis* nucleoid-associated protein HPB12 strongly compacts DNA. J. Bacteriol. **176:**50–60.
3. **Barák, I., J. Behari, G. Olmedo, P. Guzmán, D. P. Brown, E. Castro, D. Walker, J. Westpheling, and P. Youngman.** 1996. Structure and function of the *Bacillus* SpoIIE protein and its localization to sites of sporulation septum assembly. Mol. Microbiol. **19:**1047–1060.
4. **Bohrmann, B., W. Villinger, R. Johansen, and E. Kellenberger.** 1991. Coralline shape of the bacterial nucleoid after cryofixation. J. Bacteriol. **173:** 3149–3158.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
6. **Burnette, N.** 1981. Western blotting: electrophoretic transfer of proteins from SDS-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112:** 195–203.
7. **Chalfie, M., Y. Tu, G. Euskirchen, W. W. Ward, and D. C. Prasher.** 1994. Green fluorescent protein as a marker for gene expression. Science **263:**802–805.
8. **Drlica, K., and J. Rouviere-Yaniv.** 1987. Histonelike proteins of bacteria. Microbiol. Rev. **51:**301–319.
9. **Dürrenberger, M., M.-A. Bjornsti, T. Uetz, J. A. Hobot, and E. Kellenberger.** 1988. Intracellular location of the histonelike protein HU in *Escherichia coli*. J. Bacteriol. **170:**4757–4768.
10. **Dürrenberger, M., A. La Taena, G. Citro, F. Venanzi, C. O. Gualerzi, and C. L. Pon.** 1991. *Escherichia coli* DNA-binding protein H-NS is localized in the nucleoid. Res. Microbiol. **142:**373–380.
11. **Falconi, M., M. T. Gualtieri, A. La Taena, M. A. Losso, and C. L. Pon.** 1988. Proteins from the procaryotic nucleoid: primary and quaternary structure of the 15 kD *Escherichia coli* DNA binding protein H-NS. Mol. Microbiol. **2:**323–329.
12. **Flamm, E. L., and R. A. Weisberg.** 1985. Primary structure of the *hip* gene of *Escherichia coli* and its product the beta subunit of integration host factor. J. Mol. Biol. **183:**117–128.
13. **Göransson, M., B. Sondén, P. Nilson, B. Dagdberg, K. Forsman, K. Emanuelsson, and B. E. Ulin.** 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature **344:**682–685.
14. **Groch, N., H. Schindelin, A. S. Scholtz, U. Hahn, and U. Heinemann.** 1992. Determination of DNA-binding parameters for the *Bacillus subtilis* histone-like HBsu protein through introduction of fluorophores by site-directed mutagenesis of a synthetic gene. Eur. J. Biochem. **207:**677–685.
15. **Henner, D. J.** 1990. Inducible expression of regulatory genes in *Bacillus subtilis*. Methods Enzymol. **185:**223–228.
16. **Huisman, O., M. Faelen, D. Girard, A. Jaffé, A. Toussaint, and J. Rouvière-Yaniv.** 1989. Multiple defects in *Escherichia coli* mutants lacking HU protein. J. Bacteriol. **171:**3704–3712.
17. **Kano, Y., and F. Imamoto.** 1990. Requirement of integration host factor (IHF) for growth of *Escherichia coli* deficient in HU protein. Gene **89:**133–137.
18. **Kano, Y., K. Osata, M. Wada, and F. Imamoto.** 1987. Cloning and sequencing of the HU-2 gene of *Escherichia coli*. Mol. Gen. Genet. **209:**408–410.
19. **Kano, Y., S. Yoshino, M. Wada, K. Yokoyama, M. Nobuhara, and F. Imamoto.** 1985. Molecular cloning and nucleotide sequence of the HU-1 gene of *Escherichia coli*. Mol. Gen. Genet. **201:**360–362.
20. **Klein, C., C. Kaletta, N. Schnell, and K.-D. Entian.** 1992. Analysis of genes involved in biosynthesis of the lantibiotic subtilin. Appl. Environ. Microbiol. **58:**132–142.
21. **Le Hégarat, F., V. Salti-Montesanto, Y. Hauck, and L. Hirschbein.** 1993. Purification and characterization of the HU-like protein HPB9 from the *Bacillus subtilis* nucleoid. Biochim. Biophys. Acta **1172:**101–107.
22. **Lewis, P. J., and J. Errington.** 1996. Use of green fluorescent protein for detection of cell-specific gene expression and subcellular protein localization during sporulation in *Bacillus subtilis*. Microbiology **142:**733–740.
23. **May, G., P. Dersch, M. Haardt, A. Middendorf, and E. Bremer.** 1990. The *osmZ (bglY)* gene encodes the DNA-binding protein H-NS (H1a), a component of the *Escherichia coli* K12 nucleoid. Mol. Gen. Genet. **224:**81–90.
24. **McGovern, V., N. P. Higgins, R. S. Chiz, and A. Jaworski.** 1994. H-NS over-expression induces an artificial stationary phase by silencing global transcription. Biochimie **76:**1019–1029.
25. **Mechulam, Y., G. Fayat, and S. Blanquet.** 1985. Sequence of the *Escherichia coli pheST* operon and identification of the *himA* gene. J. Bacteriol. **163:** 787–791.
26. **Micka, B., N. Groch, U. Heinemann, and M. A. Marahiel.** 1991. Molecular cloning, nucleotide sequence and characterization of the *Bacillus subtilis* gene encoding the DNA-binding protein HBsu. J. Bacteriol. **173:**3191–3198.
27. **Micka, B., and M. A. Marahiel.** 1992. The DNA-binding protein HBsu is essential for normal growth and development in *Bacillus subtilis*. Biochimie **74:**641–650.
28. **Nash, H. A.** 1996. The HU and IHF proteins: accessory factors for complex protein-DNA assemblies, p. 149–179. *In* E. C. C. Lin, and A. S. Lynch (ed.), Regulation of gene expression in *Escherichia coli*. R. G. Landes Company, Austin, Tex.
29. **Rouvière-Yaniv, J.** 1977. Localization of the HU protein on the *E. coli* nucleoid. Cold Spring Habor Symp. Quant. Biol. **42:**439–447.
30. **Salti, V., F. Le Hégarat, and L. Hirschbein.** 1985. Isolation and characterisation of small heat-stable acid-soluble DNA-binding proteins from *Bacillus subtilis*. J. Gen. Microbiol. **131:**581–590.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
32. **Schäggar, H. G., and G. von Jagow.** 1987. Tricine-sodium-dodecyl-sulfate polyacrylamide gel electrophoresis for the separation of proteins in a range from 1 to 100 kDa. Anal. Biochem. **166:**368–379.
33. **Schmid, M. B.** 1990. More than just 'histone-like' proteins. Cell **63:**451–453.
34. **Shellman, V. L., and D. E. Pettijohn.** 1991. Introduction of proteins into living bacterial cells: labeled HU protein in *Escherichia coli*. J. Bacteriol. **173:**3047–3059.
35. **Spurio, R., M. Dürrenberger, M. Falconi, A. La Taena, C. L. Pon, and C. O. Gualerzi.** 1992. Lethal overproduction of the *Escherichia coli* nucleoid protein H-NS: ultramicroscopic and molecular autopsy. Mol. Gen. Genet. **231:** 201–211.
36. **Ussery, D. W., J. C. D. Hinton, B. J. A. M. Jordi, P. E. Granum, A. Seirafi, R. J. Stephen, A. E. Tupper, G. Berridge, J. M. Sidebotham, and C. F. Higgins.** 1994. The chromatin-associated protein H-NS. Biochimie **76:**968–980.
37. **Wada, M., Y. Kano, T. Okazaki, and F. Imamoto.** 1988. Construction and characterization of the deletion mutant of *hupA* and *hupB* genes in *Escherichia coli*. J. Mol. Biol. **204:**581–591.
38. **Webb, C. D., A. Decatur, A. Teleman, and R. Losick.** 1995. Use of green fluorescent protein for visualization of cell-specific gene expression and subcellular protein localization during sporulation in *Bacillus subtilis*. J. Bacteriol. **177:**5906–5911.
39. **Yasuzawa, K., N. Hayashi, N. Goshima, K. Kohno, F. Imamoto, and Y. Kano.** 1992. Histone-like proteins are required for cell growth and constraint of supercoils in DNA. Gene **122:**9–15.