Molecular Cloning, Nucleotide Sequence, and Characterization of the Bacillus subtilis Gene Encoding the DNA-Binding Protein HBsu

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A homologous class of histonelike proteins which are believed to wrap the DNA and to condense the chromosome into highly folded nucleoid structures has been identified in different bacterial species. Bacillus subtilis encodes a homodimeric DNA-binding protein called HBsu. We have cloned the corresponding gene (hbs) on a 3.8-kb fragment. The gene was subcloned to a 1-kb fragment, sequenced, and characterized. It encodes a 92-amino-acid protein with a predicted molecular mass of 9,884 Da. Fortunately, analysis of the DNA sequence downstream of the 3' end of hbs revealed the location of the first 19 amino acid residues of MtrA. This finding located the hbs gene unequivocally to the 5' end of the mtr operon at about 204° on the standard genetic map of B. subtilis. Northern (RNA) blot analysis and primer extension studies indicated the presence of two distinct hbs transcripts, which were found to be initiated at two different sites located about 160 bases apart. Several attempts to replace the hbs gene in the B. subtilis chromosome with a cat-interrupted copy (hbs::cat) through marker replacement recombination were unsuccessful. In order to study whether hbs is an essential gene, we have constructed a strain containing a truncated copy of the gene behind its own promoter and another intact copy under control of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible spac-I promoter. In this strain (BM19), normal growth was found to depend on IPTG, whereas in the absence of IPTG, growth was severely affected. These results suggest an essential role for the hbs gene product for normal growth in B. subtilis.

In procaryotes, a number of abundant, small, basic, and heat-stable proteins have been identified. These proteins wrap DNA without obvious sequence specificity and are not homologous with the eucaryotic histones (for reviews, see references 6, 11, 37, 41, and 46). Among bacteria, the primary structures are highly conserved. They have been designated as histonelike DNA-binding proteins (41). The best-studied and most abundant protein is HU, present in Escherichia coli. It exists predominantly as a heterodimer (HU2 and HU1) encoded by the genes hupa and hupB (25, 26, 30, 32). The two subunits consist of 90 amino acid residues each and have 70% identical residues. Since HU protein alters the structure and topology of bound DNA (3, 6, 7, 37, 38), it affects several cellular processes by binding to DNA. Its role in DNA replication is especially well documented (5, 10, 35, 42). Also, a function as an accessory protein stimulating DNA recognition by other proteins has been postulated (9). In addition, recent studies of hupA and hupB null mutants revealed that growth, lysis, transposition, and cell division are affected in the absence of HU protein (19, 35).

Several proteins with properties and structures highly homologous to those of HU protein have been isolated from other bacteria and bacteriophages. Among some Bacillus species (B. caldolyticus, B. globigii, B. stearothermophilus, and B. subtilis), a protein highly homologous to HU, classified HB, was isolated and characterized (1, 4, 21, 27). In contrast to HU, HB exists as a homodimeric protein (44). This protein binds to both single-stranded and double-stranded DNA and RNA (4, 21, 22, 48). The HB protein of B. subtilis, HBsu, consists of 92 amino acid residues and differs only in one sequence position from the B. globigii HB protein (49). The identity of other nucleoid proteins of B. subtilis (34, 39) with HBsu remains unproven.

As an initial step towards understanding the physiological role of the B. subtilis protein, we have cloned, sequenced, and characterized the corresponding gene. The gene was identified from a genomic library constructed in Lambda Zap II by using a radiolabeled DNA probe synthesized by the polymerase chain reaction (PCR).

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. Bacterial strains are listed in Table 1. Plasmid pDH88 has been described by Henner (16). 2xYT and NZY media as well as SM buffer were described by Short et al. (43).

Construction of oligonucleotides. The DNA sequence of the

<table>
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<th>Strain</th>
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<tr>
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<td>trpC2 pheA1</td>
<td>J. Hoch</td>
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<tr>
<td>BM04</td>
<td>trpC2 pheA1 hbs+ hbs::cat</td>
<td>This paper</td>
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<tr>
<td>BM19</td>
<td>trpC2 pheA1 Psub::hbs'::cat::Pspac-hbs hbs+</td>
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<td>J. Messing</td>
</tr>
<tr>
<td>JM105</td>
<td>endA1 (lac pro) thi-1 strA sbcB15 hsdR4 (F' traD36 proA' B' lacZAM15)</td>
<td>J. Messing</td>
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* Corresponding author.
oligonucleotides used in the PCR is based on the amino acid sequence of HBSu protein (49). The oligomers represent two distinct parts of the chemically synthesized hbs gene (14). Both oligonucleotides were synthesized on an Applied Biosystems automatic DNA synthesizer and purified by hydrophobic interaction and anion-exchange fast protein liquid chromatography. Final purification was achieved by polyacrylamide gel electrophoresis. One oligomer, a 26-oligonucleotide sequence, 5'-CATAAAGCCGTGGCCGAAGCC AGCG-3', matches to the N-terminal HBSu sequence, and the other oligomer, a 50-mer, 5'-TTTTAAGCCTTGCAGGGTTTAAAGGCGGTACCTTGCTGCGCGAATT T-3', matches to the C-terminal amino acid sequence.

Amplification of bacterial DNA by PCR. PCR was used to amplify genomic DNA fragments of B. subtilis. Chromosomal DNA of JH642 (trpC2 pheA1) was prepared according to the method of Krause et al. (28). Genomic DNA as a template was mixed with the two oligonucleotides as primers. PCR was carried out in a 50-μl volume containing 1 μg of template DNA, 200 pmol of primer (100 pmol of each oligonucleotide), 50 mM Tris-HCl (pH 9.0 at 25°C), 15 mM ammonium sulfate, 7 mM magnesium chloride, 50 mM potassium chloride, 1 mM (each) deoxynucleoside triphosphates, 170 μg of bovine serum albumin per ml, and 1 U of *Thermus aquaticus* DNA polymerase (Anglian Biotech). The DNA was denatured at 93°C for 1 min, annealed at 50°C for 3 min, and extended at 72°C for 3 min. Following 30 cycles of amplification, 2 μl of the 50-μl reaction mixture was electrophoresed through a 1% agarose gel. The product of amplification was a DNA fragment 241 bp in length.

**Hybridization conditions of PCR product.** Nitrocellulose filters were prehybridized in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-10 mM EDTA (pH 8.0)-100 μg of denatured salmon sperm DNA per ml for 1 h at 65°C. The PCR probe was labeled with the Multiprime DNA-labeling system (Amersham). The probe was added to the prehybridization mix and hybridized for 5 h at 65°C. Southern blots and plaque- and colony-screening membranes were subjected to two washes: one in 2× SSC and 0.5% sodium dodecyl sulfate (SDS) for 15 min at room temperature and one in 2× SSC and 0.1% SDS for 15 min at 65°C. Filters were exposed to Kodak X-Omat film.

**Construction of a genomic library.** Genomic DNA of *B. subtilis* JH642 was partially digested with the restriction enzyme Sau3A. Bacteriophage Lambda Zap II was used as a vector to clone genomic DNA according to the instructions of the supplier (Stratagene). Ten micrograms of vector DNA was linearized by the restriction enzyme *XhoI*.

To ligate complementary ends, dCTP and dTTP were used to fill in the *XhoI*-digested vector, whereas dATP and dGTP were used to fill in Sau3A-digested chromosomal DNA. After polymerization, 1 μg of Lambda Zap II vector and 0.6 μg of insert DNA were packaged in Gigapack II Gold packaging extract (Stratagene). The ratio of nonrecombinant (blue) to recombinant (white) plaques was determined by plating 0.1 μl of the 500-μl DNA library with 200 μl of XL-1 Blue host cells on NZY plates containing 10 mM isopropyl-β-p-thiogalactopyranoside (IPTG) and 6.25 mg of X-Gal (5-bromo-4-chloro-3-indolyl-β-p-galactopyranoside) per ml (43). The JH642 library contained 4 - 10⁶ PFU/μg of DNA with 1 - 10⁴ blue background plaques.

The library was amplified and phages were transferred to nitrocellulose filters. Phage DNA was released from the phage head and denatured by standard procedures (2). Methods of hybridization and wash conditions are described.

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**FIG. 1.** Identification of the *hbs* gene of *B. subtilis*. (A) Restriction analysis of genomic DNA isolated from *B. subtilis* JH642. Chromosomal DNA has been digested with the restriction enzymes HindIII (lane 1), EcoRI (lane 2), and PstI (lane 3). The cleaved products were separated and visualized on a 0.8% agarose gel. (B) Southern blot analysis revealed one major band after digestion with HindIII (lane 1), EcoRI (lane 2), and PstI (lane 3) restriction enzymes and hybridization with the PCR probe. The probe recognized one fragment at about 4.3 kb in the HindIII digest and one fragment at about 5.4 kb in the EcoRI and PstI digests.

**FIG. 2.** Restriction map of DNA in the vicinity of the *hbs* gene. (A) Restriction map of the 3.8-kb DNA insert of plasmid pHS1. (B) Restriction map of the 1.0-kb DNA insert of subcloned plasmid pHS2. The location and orientation of the *hbs* coding sequence are indicated.
above. Promising plaques were isolated from the agar plate and transferred to 500 μl of SM buffer containing 20 μl of chloroform (43).

In vivo excision of pBluescript plasmid from Lambda Zap II vector. The Bacillus library has been constructed in Lambda Zap II to use the facility of in vivo excision of the cloned insert within this vector. The phagemid, pBluescript [SK(-)], can be excised by fl helper phage because Lambda Zap II incorporates the signals for both initiation and termination of DNA synthesis from the fl origin of replication (43). After in vivo excision of the lambda vector, colonies that appeared on plates were transferred to nylon filters and denatured by standard procedures (2). Methods of hybridization and wash conditions were as described previously (40).

DNA sequencing. DNA sequencing of cloned plasmid DNA denatured in alkali was carried out by the chain termination reaction (United States Biological). The chain-terminated products were separated on 6% sequencing gels and visualized by overnight autoradiography with Kodak X-Omat film.

RNA preparation and Northern (RNA) blot. Fifty-milliliter cell cultures of B. subtilis were collected at two different times during vegetative growth. Total cellular RNA was prepared by the procedure of Penn et al. (36) as modified by Igo and Losick (20). RNA pellets were suspended in 50 μl of diethylpyrocarbonate-treated water. The transfer of RNA from the gel to the blot was performed by the method of Igo and Losick (20).

Primer extension. Primer extension was used to map the 5' termini of mRNA as described in reference 40. The RNA was hybridized with an excess of single-stranded DNA primer. The primer, a 50-mer oligonucleotide, is complementary to the sequence located between nucleotides 111 and 158. Reverse transcriptase and nucleotides dATP, dGTP, dTTP, and radiolabeled [α-32P]dCTP were used to extend this primer to produce the cDNA, which was used in mapping the 5' termini of mRNA of hbs.

Strain constructions to interrupt the chromosomal copy of the hbs gene. The gene containing the 1991

FIG. 3. DNA sequence of the hbs gene and the deduced amino acid sequence. The hbs gene is 279 bp in length and encodes a protein of 92 amino acid residues. The −10 and −35 regions, the putative ribosome binding site (SD), and the sequence of a potential transcription terminator are underlined. Arrows indicate the 5' ends (P1 and P2) mapped by primer extension. The 5' sequence of the mtrA gene and the first 19 amino acid residues are shown.

FIG. 4. Northern blot analysis of total cellular RNA (5 μg) of B. subtilis prepared during two different periods of vegetative growth (optical densities at 600 nm of 0.5 and 1.0) (lanes 1 and 2, respectively). With a PCR probe, two transcripts of about 0.54 and 0.38 kb were identified. To the right are indicated the locations of the rRNAs.
the hbs gene and to place it under control of the spac-1 promoter. Plasmid pHBS2 was linearized within the hbs gene by using the restriction enzyme HincII. The cat gene localized on a 1.3-kb EcoRI fragment of pZΔ327 (50) was isolated. To ligate blunt-ended fragments, dATP and dTTP were filled into the EcoRI sites. After ligation, the hbs::cat plasmid (pHBS2cat) was transformed into competent cells of E. coli JM105 (40). About 2 \( \mu \)g of linearized pHBS2cat was used to transform competent cells of B. subtilis JH642 (15). Transformants were selected on 2xYT plates containing 5 \( \mu \)g of chloramphenicol per ml.

To place the hbs gene under the control of the spac-1 promoter within the integrative plasmid pDH88, a 191-bp fragment flanked by the restriction sites HindIII and SphI was synthesized by PCR. The HindIII-SphI PCR fragment contained the natural ribosomal-binding site (SD) and the first 59 of 92 codons of hbs. This truncated copy and pDH88 were digested with HindIII and SphI, ligated, and transformed into competent cells of E. coli JM105. The new construct, pDH88hbs', was transformed into competent cells of B. subtilis JH642. Transformants were selected on 2xYT plates containing 5 \( \mu \)g of chloramphenicol per ml and 1 mM IPTG.

Antisera. Antisera against the HB protein of B. globigii, HBgl, were raised in rabbits and used in a dilution of 1:500 without further purification. Polyclonal anti-HBgl antibodies cross-reacted with the B. subtilis HBsu protein. Total protein extracts of B. subtilis and of E. coli cells containing the

FIG. 5. (A) Construction and integration of pDH88hbs' by homologous recombination. The plasmid is indicated by solid lines, and the chromosomal DNA is indicated by dotted lines. The box indicates the hbs coding region, and the jagged ends of hbs' indicate a truncated copy of the gene. The positions of the promoters are shown by \( P_{spac} \) and \( P_{hbs} \). The locations of the restriction sites HindIII, EcoRI, and SphI are shown. The figure is not to scale. (B) Characterization of B. subtilis BM19 by Southern hybridization. Chromosomal DNAs of B. subtilis wild type (lane 1) and BM19 (lane 2) were digested with HindIII-EcoRI. The cleaved products were separated on a 0.8% agarose gel, transferred to a nitrocellulose filter, and hybridized to a PCR probe. Arrowheads indicate the locations of the hbs gene in the wild type (2.3 kb) and in the BM19 strain containing the truncated copy (6.5 kb) and the \( P_{spac-hbs} \) fusion (1.8 kb).
recombinant plasmid pHBS1 or pHBS2 were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (15% polyacrylamide) as described by Laemmli (29). Gels were stained with Coomassie brilliant blue R 250 or transferred to nitrocellulose for immunodetection analysis (Western blot) as described by Towbin et al. (45).

**Nucleotide sequence accession number.** Sequence data in this work have been assigned EMBL GenBank data base accession number X52418.

**RESULTS**

**Construction of the PCR probe and its hybridization to chromosomal DNA.** To verify the quality of the successfully amplified PCR probe (a 241-bp fragment of the chromosomal hbs gene), the labeled probe was hybridized to a chromosomal DNA blot and washed under stringent conditions as indicated in Materials and Methods. Chromosomal DNA that has been cleaved with the restriction enzymes HindIII, EcoRI, and PstI revealed in the corresponding Southern blot the presence of one major band in each digest (Fig. 1). The probe recognized one band of about 5.4 kb in the EcoRI and PstI chromosomal digests and a smaller fragment of about 4.3 kb in the HindIII digest. This suggested the presence of at least one hbs gene within these fragments.

**Cloning, sequencing, and transcriptional mapping of the hbs gene.** The efficiency of the constructed genomic library was very high, since less than 1% of the PFU were without inserted DNA. About 1 μl (0.002%) of the packaged material was amplified and gave rise to about 8 × 10^7 PFU/μl. The plaques were transferred to nylon membranes and screened with the PCR probe. Five plaques (L-1 to L-5) were identified. After purification by reinfecion, one of them (L-1) was subjected to the excision process. By using 10^2 phage particles of L-1, a few ampicillin-resistant colonies appeared. After colony filter hybridization and screening with the PCR probe, one promising clone was identified. A plasmid, designated pHBS1, containing a 3.8-kb DNA insert was identified. By Southern blot analysis, the hbs gene was assigned to a 900-bp EcoRI-NdeI fragment of the 3.8-kb insert of pHBS1 (Fig. 2A). Plasmid pHBS2, bearing the hbs gene within its 1-kb insert, was constructed by elimination of the 2.8-kb EcoRI insert of pHBS1 (Fig. 2B).

By using the T7 primer and synthetic oligonucleotides, the sequence of a major part of the inserted DNA in pHBS2 was determined. The hbs gene is 279 bp in length and encodes a protein of 92 amino acid residues with a molecular mass of 9,884 Da (Fig. 3). A strong potential ribosome-binding site (AGGAGGT) with a ΔG of −18.8 kcal (1 cal = 4.184 J) is located 7 bp upstream of the initiation codon AUG (33). The hbs open reading frame ends with three stop codons followed by a hairpin loop structure similar to rho-independent terminators. The deduced amino acid sequence of the hbs gene was found to be identical to that of the purified HBsu protein (49). Also, a part of the sequence of an open reading frame located downstream of the hbs gene was determined. This sequence was found to be identical to that of the 5' end of the mtrA gene which was determined by Gollnick et al. (12). As shown in Fig. 3, the determined sequence of mtrA, which is located to the 3' end of hbs, comprises 58 nucleotides which encode the first 19 amino acid residues of the MtrA protein (12). The sequence determined by Gollnick et al. at the 5' end of the mtrA gene overlaps with our sequence over a region of 453 bp. This region comprises the 3' end of hbs, the 5' end of mtrA, and a region of 175 bp located in between. These data revealed the exact location of the hbs gene to the 5' end of the mtr operon located at about 204° on the Bacillus chromosome.
In order to determine the level and size of the \( hbs \) transcript, Northern blot and primer extension analyses were carried out. Northern blot analysis of total cellular RNA prepared at different stages of vegetative growth (optical densities at 600 nm of 0.5 and 1.0) indicated the presence of two distinct \( hbs \) transcripts of almost constant level (Fig. 4). The approximate lengths of both transcripts were estimated to be about 0.54 and 0.38 kb. In addition, primer extension studies (not shown) confirmed that both \( S' \) ends of the transcripts are located about 160 bases apart.

Inspection of the DNA sequence (Fig. 3) upstream of the putative initiation site \( P1 \) at about \(-10 \) and \(-35 \) revealed consensus sequences which may be recognized by the vegetative form of RNA polymerase (Eo\(^{+}\)) in \( B. subtilis \). In contrast, the \(-10 \) and \(-35 \) regions upstream of \( P2 \) (not underlined in Fig. 3) show a good homology to the conserved consensus sequence recognized by a minor form of RNA polymerase containing sigma-32 (Eo\(^{-}\)) (24). However, additional studies are needed for a direct evaluation of those putative promoters.

**Interruption and placing the \( hbs \) gene under control of the \( spac-1 \) promoter.** Many attempts to interrupt the \( hbs \) gene directly by a double-crossover recombination event using the linearized pHBS2cat plasmid (see Materials and Methods) resulted only in the production of chloramphenicol-resistant \( hbs \)-partial diploids. Southern hybridization experiments (not shown) using digested chromosomal DNA of such transformants indicated that the \( hbs:cat \) construct was integrated by a Campbell-like single-crossover recombination event.

Our failure to interrupt \( hbs \) in the chromosome of \( B. subtilis \) suggests an essential role for the gene product. To test this possibility we have placed the \( hbs \) gene under the control of the \( spac-1 \) promoter (Fig. 5A) and tested the cell viability in relation to IPTG induction. As shown in Fig. 6, normal cell growth in strain BM19 depends highly on the presence of IPTG. In the absence of IPTG, cell growth is strongly affected. This limited growth switched to the normal level upon addition of 1 mM IPTG, which derepresses the expression of \( hbs \). It should also be noted that cells containing the \( P_{spac}\)-\( hbs \) fusion were able to grow in the absence of IPTG as a consequence of leakiness of the control system.

The integration of pHDH888\( hbs' \) by a homologous recombination event was confirmed by Southern hybridization experiments. As shown in Fig. 5B, in an HindIII-EcoRI digest of wild-type DNA, \( hbs \) is located on a single 2.3-kb fragment. In strain BM19, the fragment was split into a 6.5-kb fragment carrying the truncated gene of \( hbs \) and a 1.8-kb fragment containing the \( P_{spac}\)-\( hbs \) fusion.

**\( HBsu \) expression in \( E. coli \).** Anti-HBgl antibodies cross-reacted specifically with a single band of about 10 kDa from total \( B. subtilis \) protein extract (Fig. 7, lane 5). This band corresponds to the \( HBsu \) protein which has the same molecular mass as the purified HBgl protein (Fig. 7, lane 1). In contrast, anti-HBgl antibodies did not cross-react with protein extracts of the \( E. coli \) control strain (Fig. 7, lane 2) and \( E. coli \) containing pBluescript (Fig. 7, lane 3). However, one immunoreactive polypeptide was detected in protein extracts of \( E. coli \) JM105 cells bearing the recombinant plasmids pHBS1 (Fig. 7, lane 4) or pHBS2 (data not shown). The polypeptide synthesized by the \( E. coli \) strains carrying the recombinant plasmids has a molecular mass of 10 kDa, which is identical to that of the \( B. subtilis \) \( HBsu \) protein. This expression was independent of the external vector promoter; as in both plasmids, pHBS1 and pHBS2, the lacZ promoter is located at the 3' end of the \( hbs \) gene.

**DISCUSSION**

We have cloned, sequenced, and characterized the \( hbs \) gene encoding the \( B. subtilis \) histonelike protein \( HBsu \). According to our knowledge, \( hbs \) represents the first identified gene for a histonelike protein within this Bacillus species. The gene encodes a protein of 92 amino acid residues with a molecular mass of 9,884 Da. The deduced amino acid sequence of the \( hbs \) gene is identical to that determined from the purified \( B. subtilis \) protein and differs from \( B. globigii \) protein (HBgl) only at amino acid position 40. An aspartic acid residue in \( HBsu \) is replaced by lysine in HBgl (49). This relationship reflects the immunological cross-reactivity between anti-HBgl antibodies and the \( HBsu \) protein. In contrast, \( E. coli \) HU proteins (HU2 and HU1) which show a lower degree of homology (52 to 57% identity) to the \( HBsu \) protein do not cross-react with the anti-HBgl antibodies (21). Among the \( HBsu \) proteins of different Bacillus species so far sequenced, conservation is more than 80% (Fig. 8). However, the homology of \( B. subtilis \) bacteriophage SPO1-encoded TF-1 protein to HB proteins of different Bacillus species is less than 50% (13). TF-1 isolated from infected \( B. subtilis \) cells shares only 41% identical residues with \( HBsu \) (13). Most of those amino acid substitutions are within the conserved \( \alpha \)-helix region (Fig. 8). TF-1 binds preferentially to 5-hydroxymethyl-uracil-containing DNA as present in SPO1 DNA (23), and the divergence in its amino acid sequence from those of Bacillus HB proteins may have arisen from selective pressure to adapt to this novel function. However, when the nucleotide sequence of the TF-1 gene (13) was compared with that of \( hbs \), 57% identity was observed. This indicates a higher level of conservation at the DNA level than that observed on the amino acid sequence level. This may suggest a common ancestor for both genes. On the other hand, all histonelike proteins are lysine and arginine rich and are devoid of cysteine, tryptophan, and tyrosine, generally.
The data obtained from Southern hybridization and the sequence analysis suggest the presence of a single copy of the hbs gene in the B. subtilis chromosome. This copy is located to the 5' end of the mtrA gene which maps at 204° on the standard genetic map of B. subtilis. As determined from the DNA sequence (Fig. 3), the mtrA initiation codon is placed 175 bp downstream of the third hbs stop codon. The hbs gene is located on a 900-bp EcoRI-NdeI chromosomal fragment. In E. coli and Salmonella typhimurium, two genes were found to encode closely related proteins (HU2 and HU1) that form the heterodimeric protein HU (17, 25, 26, 30–32). The availability of the genes encoding HU in E. coli and S. typhimurium facilitated studies of its biological role in vivo. Recently, HU null mutants were constructed and shown to be defective in growth, cell division, lysis, and transposition of bacteriophage Mu (18, 19, 35, 47). This pleiotropy suggests an important role for the HU protein in the physiology of E. coli and S. typhimurium.

In order to understand the in vivo physiological role of the HBsu protein during growth and development of B. subtilis, we have constructed in vitro the hbs::cat mutation. However, we were unable to replace the hbs chromosomal copy by the hbs::cat construct by a double-crossover recombination event. Although the transformation efficiency was very low, several chloramphenicol-resistant transformants were analyzed by Southern hybridization to screen for the gene interruption. All transformants tested were found to be partial diploids; they contain one intact and one interrupted copy of hbs, which may have arisen by a Campbell-like integration mechanism.

Our failure to interrupt hbs within the B. subtilis chromosome by homologous recombination using the hbs::cat construct prompted us to use another strategy to control hbs expression in vivo. We have constructed a strain bearing a truncated hbs copy under the control of its own promoter and a second complete copy under the spac promoter control (Fig. 5A). In this strain, BM19, normal growth was found to depend upon IPTG induction, indicating an essential role for the intact HBsu protein in cell growth. Cells containing P spac-hbs fusion grew at least threefold more slowly than the same cells in the presence of 1 mM IPTG. The reduced growth in the absence of IPTG may be due to the leakiness of the inducible spac promoter. Similar results were also obtained when the spoOA gene was fused to the spac promoter with the same vector (8).

Is hbs an essential gene for viability in B. subtilis? The results mentioned above, including (i) the failure to interrupt hbs by homologous recombination, (ii) the IPTG-dependent growth in strain BM19 containing the hbs gene under spac promoter control, and (iii) the fact that the gene is expressed from two distinct promoters throughout growth, may suggest an important or an essential physiological role for the HBsu protein in B. subtilis. However, the final proof of whether HBsu is essential for growth and development awaits additional detailed studies, which are under way.

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REFERENCES


