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# Construction and Characterization of Mutations in *hupB*, the Gene Encoding HU-β (HU-1) in *Escherichia coli* K-12

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**Plasmid pJMC21 contains *Escherichia coli* chromosomal DNA encoding Lon protease, HU-β (HU-1), and an unidentified 67,000-dalton protein. A kanamycin resistance cassette was used in the construction of insertion and deletion mutations in *hupB*, the gene encoding HU-β on plasmid pJMC21. The reconstructed plasmids were linearized and used to introduce *hupB* chromosomal mutations into JC7623 (*recBC sbcBC*). These mutations, as expected, mapped in the 9.8-min region of the *E. coli* chromosome by P1 transduction (16% linkage to *proC*$^+$). Southern blot hybridization of chromosomal fragments verified that *hupB*$^+$ was replaced by the mutant allele, with no indication of gene duplication. All the mutant strains had growth rates identical to that of wild-type *E. coli*, were resistant to UV irradiation and nitrofurantoin, and supported the in vivo transposition-replication of bacteriophage Mu, Mu lysogenization, Tn*10* transposition from lambda 1098, and lambda replication-lysogenization. The only observable phenotypic variation was a reduced Mu plaque size on the *hupB* mutant strains; however, the yield of bacteriophage Mu in liquid lysates prepared from the mutant strains was indistinguishable from the yield for the wild type.**

The protein HU is a small, basic, histonelike protein isolated from the *Escherichia coli* nucleoid (for a recent review, see reference 5). Hu is composed of a heteromultimer of two nonidentical subunits, HU-α (HU-2) and HU-β (HU-1), of 9,000 daltons with a large degree (69%) of amino acid homology (5). The protein HU is required for in vitro transposition of bacteriophage Mu, stimulates in vitro replication on M13*oriC26* RF1 DNA, and enhances flagellar-phase variation in *Salmonella typhimurium* (5, 23, 30). HU has also been implicated in transcription from bacteriophage λ DNA, Tn*10* transposition, and phage λ DNA replication (5, 23).

Kano et al. (13) have sequenced a 460-base-pair (bp) fragment of the *E. coli* chromosome containing *hupB*, the gene encoding HU-β. Sequencing data were used to demonstrate that the gene lies immediately adjacent to *lon* at 9.8 min on the *E. coli* chromosome, with the initiation codon residing approximately 200 bp from the *lon* terminator (12). Both *lon* and *hupB* are transcribed in the same orientation, clockwise on the *E. coli* chromosome (6, 12, 20). Kano et al. suggest that the regulatory regions for *hupB* may overlap the *lon* terminator, and they postulate that the genes may be coregulated (12).

Although the in vitro function of HU has been addressed by several studies, minimal information pertaining to its role in vivo is available. In this paper, we describe the in vitro construction of *hupB* mutations and the introduction of these mutations into the bacterial chromosome. In addition, the phenotype of the *hupB* mutant strains is investigated.

## MATERIALS AND METHODS

**Media, bacterial strains, and plasmid constructions.** The bacterial strains and viruses used in this study are listed in Table 1. P1 transductions were performed as described previously (22). Bacteria were routinely grown in a complex medium (LB; 22) or in M9 minimal medium (22) without $CaCl_2$ supplemented with amino acids (50 μg/ml) as necessary. Cells were grown in minimal medium supplemented with proline, tryptophan, and 0.5% methionine assay medium (Difco Laboratories) for [$^{35}$S]methionine labeling of total cellular proteins. Nitrofurantoin (NF) sensitivity was measured on LB agar containing 2 μg of NF per ml (LB-NF; 14). Kanamycin and carbenicillin were each used at a concentration of 50 μg/ml for agar media and 25 μg/ml for liquid media; tetracycline hydrochloride was used at a concentration of 10 μg/ml.

The plasmids described in this report are illustrated in Fig. 1. The starting plasmid for the constructions was pJMC21 (29). Plasmid pDSC104 was prepared by digestion of pJMC21 with *Eco*RV and subsequent ligation to create a 2.8-kilobase (kb) deletion. A 1.4-kb kanamycin resistance (Km$^r$) cassette from pUC-4K (33) was isolated by *Hin*cII digestion and blunt end ligated into the *Eco*RV site of *hupB* on pJMC21 and into the *Eco*RV site of pDSC104. The orientation of the Km$^r$ cassette was determined by restriction enzyme analysis.

**In vivo protein labeling and polyacrylamide gel electrophoresis.** Maxicell strain CSR603 was transformed with plasmid DNA and prepared by the method of Sancar et al. (27). The cells were labeled with [$^{35}$S]methionine (5 μCi/ml) for 1 h at 37°C. Labeled cell extracts were electrophoresed on a 10 to 30% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gradient gel (15). For two-dimensional gel electrophoresis, cells were grown to an optical density at 600 nm of ~0.3 and total cellular proteins were labeled for 5 min at 37°C with 30 μCi of [$^{35}$S]methionine per ml. Cell extracts were prepared as described by Miller and Nash (21) and subjected to nonequilibrium two-dimensional gel electrophoresis (25). The first dimension was electrophoresed for 1,600 V · h, with 3.5 to 10 Ampholines (LKB Instruments, Inc.). Radioactive proteins were visualized by fluorography.

**Isolation of DNA.** Strain DH5α (*recA1*) was transformed by the $CaCl_2$ method (16). Plasmid DNA was routinely isolated from minipreparations (8). Large-scale isolation of plasmid DNA was performed as described by Holmes and Quigley (9), followed by CsCl density gradient centrifugation. Chromosomal DNA was obtained by the method of Harris-Warrick et al. (7) and quantitated by fluorometry.

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TABLE 1. Bacterial strains and viruses

| Strains and viruses | Relevant genotype[a] | Source |
|---|---|---|
| *E. coli* K-12 | | |
| JC7623 | *recB21 recC22 sbcB15 sbcC201* | A. J. Clark (36) |
| CSR603 | *recA1 uvrA6* | 27 |
| DH5α | *recA1* φ80d*lacZ*ΔM15 | Bethesda Research Laboratories |
| X7102 | *proC trp* | J. Beckwith via D. Court |
| DSC90 | JC7623 *hupB1*::Km$^r$> | Transformed with linearized pDSC102 |
| DSC91 | JC7623 *hupB1*::Km$^r$< | Transformed with linearized pDSC103 |
| DSC92 | JC7623 Δ*hupB2*::Km$^r$> | Transformed with linearized pDSC105 |
| DSC93 | JC7623 Δ*hupB2*::Km$^r$< | Transformed with linearized pDSC106 |
| DSC99 | X7102 *proC*$^+$ *hupB1*::Km$^r$> UV$^s$ NF$^s$ | X7102 × P1(DSC90) |
| DSC100 | X7102 *proC*$^+$ *hupB1*::Km$^r$> UV$^r$ NF$^r$ | X7102 × P1(DSC99) |
| DSC101 | X7102 *proC*$^+$ *hupB1*::Km$^r$< UV$^r$ NF$^r$ | X7102 × P1(DSC91) |
| DSC102 | X7102 *proC*$^+$ Δ*hupB2*::Km$^r$> UV$^r$ NF$^r$ | X7102 × P1(DSC92) |
| DSC103 | X7102 *proC*$^+$ Δ*hupB2*::Km$^r$< UV$^r$ NF$^r$ | X7102 × P1(DSC93) |
| Phages | | |
| P1 *vir* | | Laboratory stock |
| λ1098 | p*tac* mini-*tet* cI857 | N. Kleckner (34) |
| λQLP24 | p*lac3* (*lacZ*$^+$ *lacY*$^+$) *int*$^+$ cI857 Δ*cF1* (*nin5*) | J. A. Shapiro |
| Mucts62amp1 | Mucts62amp1 (Ap$^r$) | N. Symonds via M. Casadaban |

[a] The symbol > or < indicates that the Km$^r$ cassette is transcribed in the same or opposite orientation as *hupB*, respectively. Km and Ap refer to kanamycin and ampicillin, respectively.

**DNA sequence analysis.** The 870-bp *Eco*RV-*Kpn*I fragment of chromosomal DNA (Fig. 1A, probe 2) was sequenced by the dideoxy chain termination method of Sanger et al. (28) by using a T7 DNA polymerase Sequenase kit from United States Biochemical Corp.

**Southern blot analysis.** Chromosomal DNA (1 μg) and plasmid DNA (100 ng) were digested with *Eco*RI and *Kpn*I and electrophoresed on a 0.7% agarose gel. The DNA was capillary blotted onto GeneScreen (New England Nuclear Corp.) as recommended by the manufacturer. Fragments used as hybridization probes were purified by agarose gel electrophoresis and recovered by electroelution with an Elutrap device (Schleicher & Schuell, Inc.). The DNA was hybridized with a 192-bp *Rsa*I fragment (Fig. 1A, probe 1) labeled with [α-$^{32}$P]dCTP by using an oligolabeling kit (Pharmacia, Inc.) as specified by the supplier. This 192-bp fragment contains sequences immediately 5′ to *hupB* and extends into the *hupB* coding region (13). Hybridization and posthybridization washes were performed at high stringency as recommended by New England Nuclear. Fragments hybridizing to the radiolabeled probes were detected by autoradiography by using an intensifying screen. The radiolabeled probe was stripped from the membrane as recommended by New England Nuclear and reprobed with an [α-$^{32}$P]dCTP labeled 870-bp *Eco*RV-*Kpn*I chromosomal DNA fragment (Fig. 1A, probe 2) from pJMC21. The fragment encodes most of the *hupB* open reading frame and sequences 3′ to *hupB* (13).

**UV and NF sensitivity.** Stationary-phase cells were tested for UV sensitivity as described previously (19). NF sensitivity was evaluated by washing overnight bacterial cultures with phosphate buffer (19) and plating diluted suspensions on LB and LB-NF. The plates were incubated overnight at 36°C, and the relative number of survivors on LB-NF was compared with the number of colonies obtained on LB.

**Bacteriophage Mu transposition-replication, lambda replication-lysogenization, and Tn*10* transposition.** The titers of λQLP24 were determined on X7102 and the X7102 *hupB* mutant strains as previously described (1). The number of PFU was determined after overnight incubation at 37°C. The efficiency of lysogenization was determined by infecting the strains with λQLP24 at a multiplicity of infection of 5 as previously described (1). The number of lactose-positive lysogens was determined after incubation at 32°C for 24 h on minimal medium containing 1% lactose as a carbon source. Induction of Mucts62amp1 lysogens, preparations of lysate, and determinations of Mu titers were performed as previously described (3). Yields of bacteriophage Mucts62amp1 were determined as described by Ross et al. (26), except the titers of lysates were determined on X7102 with LB agar plates containing 1 mM $CaCl_2$ and 2.5 mM $MgSO_4$ (3). Lysogenization was quantitated by infecting the strains with a Mucts62amp1 lysate (multiplicity of infection of 0.5) for 15 min at 32°C; the cultures were then diluted 1:10 in LB and shaken at 32°C for 30 min prior to plating dilutions onto LB containing carbenecillin. The number of carbenecillin-resistant lysogens were counted after a 24-h incubation at 32°C. Tn*10* transposition from λ1098 was performed as previously described (34). The transposition efficiency was determined by selection on LB-tetracycline medium at 42°C for 18 h.

## RESULTS

**Construction of *hupB* mutants.** The location of *hupB* on plasmid pJMC21 was confirmed by DNA sequence analysis of the *Eco*RV-*Kpn*1 restriction fragment encoding most of the *hupB* open reading frame (Fig. 1) (13; data not shown). Plasmids pDSC102 and pDSC103 contained a Km$^r$ cassette inserted into the *Eco*RV site of *hupB* (Fig. 1). Insertion into this site disrupted the reading frame at codon 15. Plasmids pDSC105 and pDSC106 were deleted from the *Eco*RV site in *hupB* to an *Eco*RV site downstream from *hupB*, resulting in deletion of 83% of the 3′ terminus of the *hupB* gene and sequences 3′ to *hupB*. A Km$^r$ cassette was inserted into the *Eco*RV site on the plasmids to serve as a marker for the integration experiments described below.

Insertion and deletion mutations generated in the plasmid-encoded *hupB* were used to generate chromosomal mutations in *E. coli*. The plasmids (pDSC102, pDSC103, pDSC105, and pDSC106), linearized with *Eco*RI, were used to transform JC7623 (*recBC sbcBC*) to Km$^r$ by the procedure outlined by Winans et al. (36). The Km$^r$ transformants were

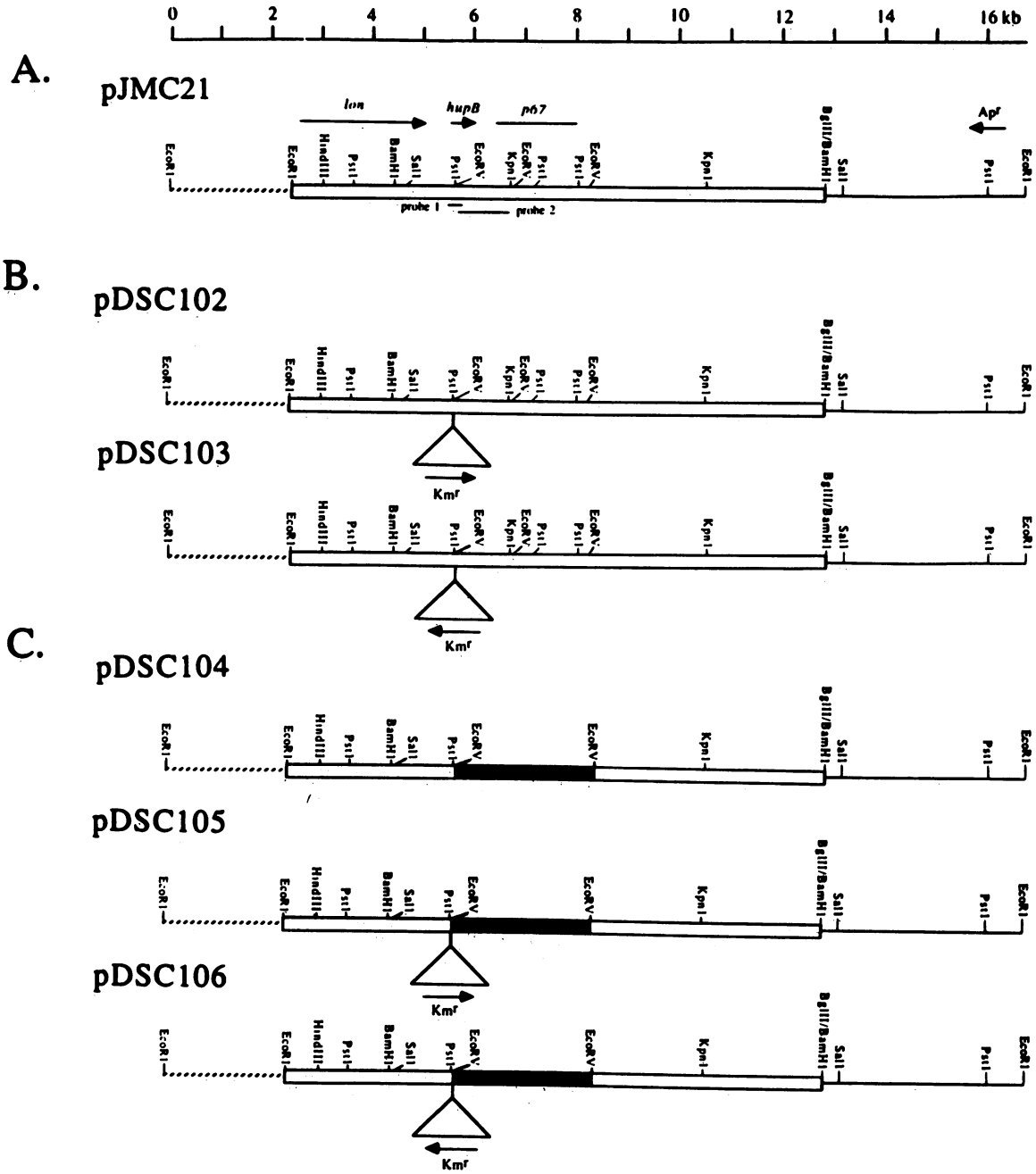


FIG. 1. Schematic diagram of the plasmids used in this study. (A) Starting plasmid pJMC21 (29). Symbols: ▭, chromosomal DNA; . . . . , mini-ColE1 sequences; ——, pBR322 sequences; →, orientation of transcription. The orientation of p67 is unknown. The thin lines under the diagram represent the regions of chromosomal DNA used as probes for Southern hybridization. Probe 1 is a 192-bp *Rsa*I fragment, and probe 2 is an 870-bp *Eco*RI-*Kpn*I fragment. The locations of the open reading frames are approximate. (B) Construction of the *hupB* insertion mutations by insertion of a 1.4-kb Km$^r$ cassette into the *Eco*RV site of *hupB*. (C) Construction of the *hupB* deletion and insertion of the Km$^r$ cassette. The solid band represents deleted regions of chromosomal DNA. kb, Kilobases.

then tested for carbenecillin sensitivity to verify plasmid loss. Homologous recombination with the chromosome presumably occurred in the 9.8-min region of the *E. coli* chromosome, near the *proC*$^+$ locus based on the map locations of *lon* and *hupB* (10, 12, 17). The location of the aminoglycoside-3′-phosphotransferase (Km$^r$) gene and presumably the disrupted *hupB* locus was verified by transducing *E. coli* X7102 (*proC*) to *proC*$^+$ with P1 grown on the carbenecillin-sensitive JC7623 (*proC*$^+$ *hupB* Km$^r$) strains. The *proC*$^+$ transductants were scored for Km$^r$. Linkage was approximately 16%, which is consistent with *lon* linkage to *proC*$^+$ (17).

**Characterization of plasmid-encoded proteins.** Proteins encoded by pJMC21 and the reconstructed plasmids encoding for Km$^r$ were characterized by using maxicells (Fig. 2). Plasmid pJMC21 expressed Lon (94 kilodaltons [kDa]), HU-β (9 kDa), β-lactamase (30 kDa), and an unidentified 67-kDa protein (p67; 29). Plasmids pDSC102 and pDSC103

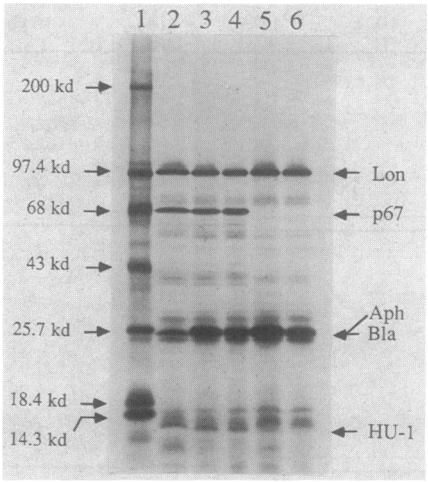


FIG. 2. Characterization of the plasmid-encoded proteins in maxicells. Lane 1 contains $^{14}$C-labeled molecular weight standards (Bethesda Research Laboratories, Inc.). Lanes 2 through 6 are $^{35}$S-labeled proteins encoded by plasmids pJMC21, pDSC102, pDSC103, pDSC105, and pDSC106, respectively. The film was intentionally overexposed to facilitate visualization of the small polypeptides. kd, Kilodaltons; APH, aminoglycoside-3′-phosphotransferase; Bla, β-lactamase.

encoded Lon, β-lactamase, p67, aminoglycoside-3′-phosphotransferase (30 kDa) and a small polypeptide approximately the same size as HU. This small peptide may be a truncated protein resulting from insertion of the Km$^r$ cassette or a fusion protein attributed to readthrough from the Km$^r$ cassette, or it may result from a restart within *hupB* initiating downstream of the Km$^r$ cassette. It is unlikely that the small peptide can be attributed to readthrough from the Km$^r$ cassette because the same polypeptide was encoded by plasmids containing the Km$^r$ cassette in both orientations. Because insertion of the cassette disrupted *hupB* at codon 15, a truncated protein would be 1,800 Da. This is significantly smaller than the protein expressed from the plasmids. Plasmids pDSC105 and pDSC106 are deleted for *hupB* and sequences 3′ to the gene (Fig. 1). The small peptide expressed from pDSC102 and pDSC103 was not expressed by the deleted plasmids; however, a 10- to 11-kDa protein was expressed from both pDSC105 and pDSC106. We were unable to ascertain the source of this protein. The deletion of the *Eco*RV fragment resulted in failure to express p67. Km$^r$ cassette inserts into the *Pst*I site 3′ to the p67 open reading frame (Fig. 1A) did not alter p67 expression, but Km$^r$ cassette insertions in the *Pst*I site located within the p67 open reading frame (Fig. 1A) resulted in the expression of a truncated p67 protein of 43,000 Da (data not shown). Therefore, we conclude that the gene encoding p67 resides entirely on the deleted *Eco*RV fragment.

**Southern blot analysis of the chromosomal DNA preparations.** Chromosomal DNA isolated from X7102 and the X7102 *hupB* mutants and digested with *Eco*RI and *Kpn*I was hybridized with probe 1 (Fig. 1A), the 192-bp *Rsa*I fragment (Fig. 3A). As predicted, the probe hybridized strongly with a 4.2-kb fragment from X7102. This fragment migrated with an identical $R_f$ to the 4.2-kb band of pJMC21 that encodes Lon, HU-β, and a portion of p67. The significance of the additional plasmid DNA fragments that hybridized to the probe is discussed below. The 4.2-kb fragment is not evident in the mutant strains or in plasmids pDSC102 and pDSC105. The absence of a 4.2-kb fragment demonstrates that the region of the chromosome encoding *hupB* was disrupted. As predicted, the *Eco*RI-*Kpn*I fragment is 1.4 kb larger in *hupB* mutant strains DSC99, DSC100, and DSC101 (and in plasmid pDSC102) because of insertion of the 1.4-kb Km$^r$ cassette. Strains DSC102 and DSC103, as well as plasmid pDSC105, have a 6.5-kb fragment that hybridizes with the *hupB* probe. The size of the fragment is in agreement with the calculated size resulting from deletion of the 2.8-kb *Eco*RV fragment and subsequent insertion of the Km$^r$ cassette (Fig. 1). To demonstrate that *hupB* was deleted in strains DSC102 and DSC103, the nylon membrane was stripped and rehybridized with probe 2 (Fig. 1A), the 870-bp *Eco*RV-*Kpn*I fragment from pJMC21 (Fig. 3B). Strain X7102 and the insertion mutant strains (DSC99, DSC100, and DSC101) all have fragments that hybridize with the probe, as predicted. The size of the fragments is in agreement with the data obtained

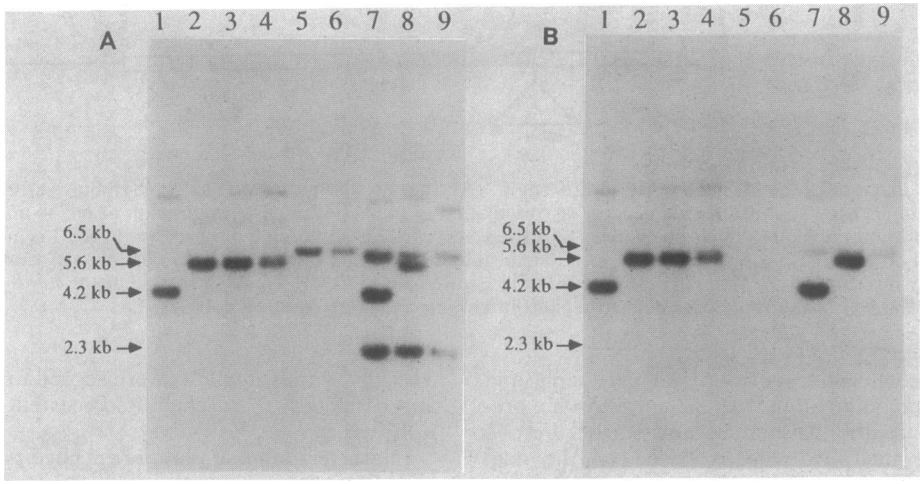


FIG. 3. Southern blot hybridization of chromosomal and plasmid DNAs. Approximately 1 μg of chromosomal DNA or 100 ng of plasmid DNA was digested with *Eco*RI and *Kpn*I, electrophoresed, and blotted as described in the text. The blot in panel A was hybridized with a 192-bp *Rsa*I fragment (Fig. 1A, probe 1) isolated from pJMC21. (B) The same blot stripped and reprobed with an 870-bp chromosomal DNA fragment (Fig. 1A, probe 2) from pJMC21. Lanes 1 through 6 contain chromosomal DNA isolated from X7102, DSC99, DSC100, DSC101, DSC102, and DSC103, respectively. Lanes 7 through 9 contain plasmid DNA from pJMC21, pDSC102, and pDSC105, respectively.

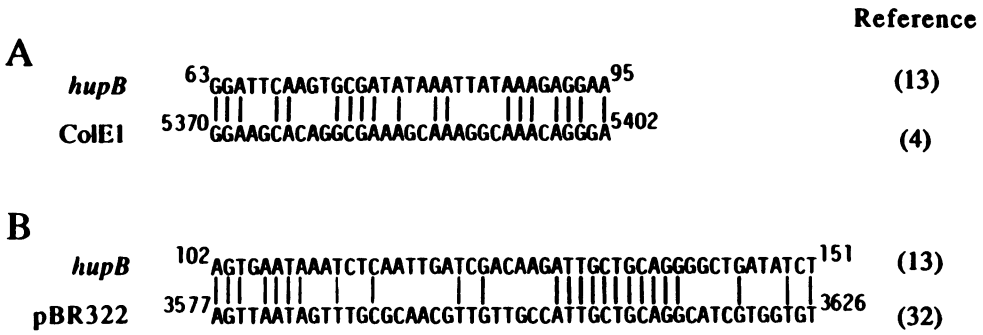


FIG. 4. DNA sequences aligned to show homology between *hupB* and mini ColE1 (A) and between *hupB* and pBR322 (B). DNA sequence analysis was performed on a Digital VAX computer by using the UWGCG WORDSEARCH program (35). The comparison is limited to the portion of *hupB* contained on the 192-bp *Rsa*I fragment. The *hupB* DNA sequence from bp 63 to 95 is located immediately 5′ to the *hupB* open reading frame. The *hupB* sequence from bp 102 to 151 is located within the *hupB* open reading frame.

with the 192-bp probe. DNA isolated from the mutants deleted for *hupB* (DSC102 and DSC103) failed to hybridize with the probe (Fig. 3B, lanes 5 and 6), indicating that the 2.8-kb fragment has been deleted from the chromosome, as expected. The Southern assay data confirm that *hupB* is disrupted and that duplication arising from the recombination event did not occur.

Surprisingly, the *hupB* probe 1 (Fig. 1A) also hybridizes to the 2.3-kb *Eco*RI and 6-kb *Eco*RI-*Kpn*I fragments of pJMC21, pDSC102, and pDSC105, but not with the 3.8-kb *Kpn*I fragment containing chromosomal DNA downstream of the gene encoding p67. The 2.3-kb *Eco*RI fragment contains mini ColE1, and the 6-kb *Eco*RI-*Kpn*I fragment contains chromosomal and pBR322 sequences. Comparative sequence analysis confirmed that mini ColE1 and pBR322 have sequence homology with the portion of *hupB* used as a hybridization probe (Fig. 4).

**Two-dimensional gel electrophoresis.** Labeled protein extracts from X7102 and DSC102 (Δ*hupB2*::Km$^r$) were subjected to nonequilibrium, two-dimensional polyacrylamide gel electrophoresis (Fig. 5). The spot(s) corresponding to HU-α and HU-β was identified in the X7102 extract by comparison with the profiles obtained by Neidhardt et al. (24). The spot is also present in the DSC102 extract. Because HU-α and HU-β have similar amino acid compositions and the same net charge, it was not possible to conclusively identify two separate proteins; subunits of purified HU are difficult to resolve by two-dimensional gel electrophoresis (31). As predicted, the spot corresponding to p67 was absent from the DSC102 extract, verifying that the gene encoding p67 was deleted from the chromosome.

**Characterization of X7102 *hupB* mutant strains.** Insertion and deletion mutations of *hupB* in X7102 did not adversely affect cell growth. All the *hupB* mutants had growth rates comparable to that of X7102 and were neither cold (15°C) nor heat (42°C) sensitive.

The *hupB* mutant strains were evaluated for sensitivity to the mutagenic agents UV radiation and NF. The *hupB* mutant strains were as resistant to UV irradiation as X7102 (*hupB*$^+$) and were not inhibited by the presence of 2 μg of

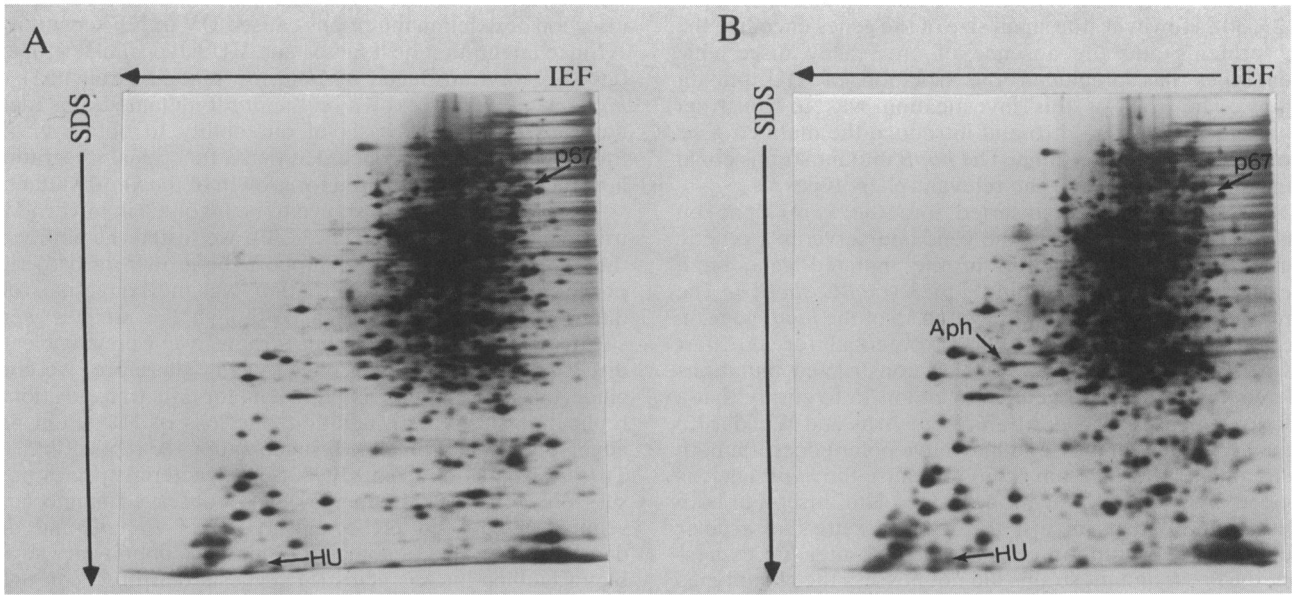


FIG. 5. Comparison of the two-dimensional gel electrophoresis profiles of X7102 and DSC102 (Δ*hupB2*::Km$^r$). (A) X7102: The locations of p67 and HU are shown. (B) DSC102: The locations of HU-α, aminoglycoside-3′-phosphotransferase, and the deleted spot corresponding to p67 are identified. The film was intentionally overexposed to permit visualization of HU. Aph, Aminoglycoside-3′-phosphotransferase; SDS, sodium dodecyl sulfate; IEF, isoelectric focusing.

NF per ml, as judged by their ability to form colonies. The colonies obtained on LB-NF were the same size as those observed for X7102. One of the insertion mutants (DSC99) was moderately UV and NF sensitive as previously reported (D. R. Storts, P.-L. Ho, and A. Markovitz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, H99, p. 156); however, X7102 transduced with P1 grown on strain DSC99 yielded only transductants as UV and NF resistant as X7102, indicating that the strain was UV and NF sensitive because of a secondary mutation that was not linked to $Km^r$.

The effect of the insertion and deletion mutations in *hupB* on viral replication and lysogenization and on Tn*10* transposition was examined. The mutations in *hupB* did not adversely affect λ lysogenization or replication. Strain X7102 and the *hupB* mutants yielded virtually identical titers ($3.6 \times 10^9 \pm 0.2 \times 10^9$ PFU/ml) when infected with a λQLP24 lysate. There was no qualitative difference in the plaques; all the strains yielded large, clear plaques after overnight incubation at 37°C. Furthermore, lactose-positive lysogens were generated at identical frequencies in all the strains ($2.1 \times 10^9 \pm 0.3 \times 10^9$ lysogens per ml of lysate). Bacteriophage Mucts62amp1 lysogenization and replicative transposition were unaffected by the mutation, as evidenced by the phage titer and the number of carbenecillin-resistant lysogens obtained: $1.5 \times 10^8 \pm 0.2 \times 10^8$ and $1.9 \times 10^7 \pm 0.3 \times 10^7$ per ml of lysate, respectively. Although the Mu phage titers were identical on the *hupB* mutant strains and X7102, the *hupB* mutant strains yielded plaques that were consistently smaller than those observed on X7102. However, no differences in yield of Mucts62amp1 phage were detected. Bacteriophage Mucts62amp1 lysogens of strains X7102, DSC100, and DSC102 yielded $1.2 \times 10^9 \pm 0.2 \times 10^9$ PFU/ml of induced culture after 1 h of induction at 42°C. Tn*10* transposition from λ1098 occurred at identical frequencies in X7102 and the *hupB* mutant strains, yielding $1.1 \times 10^4 \pm 0.1 \times 10^4$ tetracycline-resistant colonies per ml of lysate.

## DISCUSSION

Based on in vitro studies, HU is an important component in several reactions involving DNA conformational changes (5, 23, 30). However, the small size of the genes encoding the HU proteins and the absence of any easily discernible phenotypes has precluded the isolation of HU mutant strains. The goal of this investigation was to construct mutations in *hupB* in vitro and introduce the mutated gene into the *E. coli* chromosome. The *hupB* mutant strains could then be used to evaluate the relevant phenotypes.

A $Km^r$ cassette was inserted into the *hupB* gene on plasmid pJMC21 to disrupt the gene and serve as a genetic marker for the introduction of the mutated gene on a linearized plasmid into strain JC7623 (*recBC sbcBC*). The $Km^r$ cassette was inserted into codon 15 of the *hupB* gene on plasmid pJMC21. Because of the potential for a restart downstream of the insert, we also constructed mutations deleted for all the *hupB* gene 3′ to codon 15 to ensure that a functional peptide was not translated. Arps and Winkler (2) argued that the $Km^r$ cassette may have polar effects on both upstream and downstream genes. Because the *hupB* allele is in close proximity to *lon*, we generated $Km^r$ inserts in both orientations to determine if the $Km^r$ cassette had a polar effect on *lon*. Disruption of *hupB* did not alter *lon* expression; the *hupB* mutant strains did not possess the phenotypes associated with *lon* mutations (UV and NF sensitivity and mucoidy; reviewed in reference 18).

The Southern assay data obtained from X7102 transduced to $Km^r$ with P1 grown on the JC7623 $Km^r$ strains conclusively demonstrate that the linearized plasmids containing *hupB* mutations integrated into the JC7623 chromosome, replacing the wild-type allele. Hybridization with the 192-bp *Rsa*I fragment that contains a portion of *hupB* that was not deleted confirms that the chromosomal *hupB* was disrupted, as evidenced by an alteration in the size of the *Eco*RI-*Kpn*I chromosomal fragments. Furthermore, a probe specific for the 3′ deleted region of *hupB* failed to hybridize with chromosomal DNA isolated from strains deleted for that 3′ portion of *hupB*. We also expected to observe cross-hybridization with HU-α because of the large degree of amino acid homology with HU-β (5). Surprisingly, the hybridization probes failed to detect the gene encoding HU-α. Even assuming the gene encoding HU-α were located on a similar-size *Eco*RI-*Kpn*I fragment, we expected to see a fragment hybridize to the *Eco*RV-*Kpn*I probe (encoding the majority of the *hupB* open reading frame) in the strains deleted for *hupB*, but we did not see this (Fig. 3B). In addition, DNA sequence analysis of the *Eco*RI-*Kpn*I fragment (Fig. 1A) did not detect any sequences capable of encoding HU-α (data not shown), and the presence of a spot corresponding to HU-α on the two-dimensional gels suggests that the gene encoding HU-α was not deleted.

Although we were unable to demonstrate the absence of HU-β by two-dimensional gel electrophoresis because HU-α and HU-β have the same size and charge (31), we were able to demonstrate that p67 was deleted. The absence of p67 verifies that the integration caused a replacement of the chromosome in the homologous region, in agreement with the Southern assay data.

The disrupted region of the chromosome was transduced from the JC7623 *hupB* mutant strains (DSC90 through DSC93) into X7102 to permit phenotypic evaluation of the strains outside of the *recBC sbcBC* genetic background. The *hupB* mutant strains had the same generation time as X7102, suggesting that the loss of HU-β was not detrimental to the cells. Because HU is a DNA-binding protein and it is conceivable that the loss of one of the HU subunits increases sensitivity to mutagenic agents, we evaluated the UV and NF sensitivity of the *hupB* mutant strains. None of the insertion or deletion mutations caused UV or NF sensitivity.

Since integration host factor and HU have similar properties (5), we transduced a Δ*himA81* mutant strain (11) to *hupB*::$Km^r$ with P1 grown on the *hupB* mutant strains (data not shown). On the basis of our ability to obtain viable transductants, it was concluded that a functional integration host factor was not required for growth of the *hupB* mutants.

Because HU has been shown to be involved in in vitro Mu transposition-replication (5, 23, 30), we tested the ability of the *hupB* mutant strains to support the growth of bacteriophage Mu. Mu plaque formation was not quantitatively different in the *hupB* strains. Although the plaques were slightly smaller in the mutant strains, we were unable to discern any differences in phage yield. Therefore, we conclude that one subunit is sufficient for Mu transpositional replication in vivo. In addition, the loss of HU-β did not adversely affect Mu or λ lysogenization, λ replication, or Tn*10* transposition from λ1098. Some bacterial species possess only one HU subunit (5). The conserved amino acid sequences between HU-α and HU-β of *E. coli* and the demonstration that oligomers of identical subunits are capable of binding nucleic acids (5) suggests that in *E. coli* only one subunit may be required for functional activity; our results prove that only one subunit is required for growth and the activities tested.

We failed to detect any DNA sequences adjacent to *hupB*

capable of encoding HU-α; therefore, it is likely that the second gene arose by tandem duplication and subsequent translocation. We can conclude that the genes encoding HU-α and HU-β do not constitute an operon. Investigation of the mode of regulation for synthesis of the subunits and the potential involvement of *lon* in the regulation of *hupB* expression (12) may help to elucidate why *E. coli* continues to maintain two functional genes for HU.

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