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Primary Structure and Mapping of the *hupA* Gene of *Salmonella typhimurium*

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In bacteria, the complex nucleoid structure is folded and maintained by negative superhelical tension and a set of type II DNA-binding proteins, also called histonelike proteins. The most abundant type II DNA-binding protein is HU. Southern blot analysis showed that *Salmonella typhimurium* contained two HU genes that corresponded to *Escherichia coli* genes *hupA* (encoding HU-2 protein) and *hupB* (encoding HU-1). *Salmonella hupA* was cloned, and the nucleotide sequence of the gene was determined. Comparison of *hupA* of *E. coli* and *S. typhimurium* revealed that the HU-2 proteins were identical and that there was high conservation of nucleotide sequences outside the coding frames of the genes. A 300-member genomic library of *S. typhimurium* was constructed by using random transposition of MudP, a specialized chimeric P22-Mu phage that packages chromosomal DNA unidirectionally from its insertion point. Oligonucleotide hybridization against the library identified one MudP insertion that lies within 28 kilobases of *hupA*; the MudP was 12% linked to *purH* at 90.5 min on the standard map. Plasmids expressing HU-2 had a surprising phenotype; they caused growth arrest when they were introduced into *E. coli* strains bearing a *himA* or *hip* mutation. These results suggest that IHF and HU have interactive roles in bacteria.

Biochemical and genetic studies of DNA gyrase and topoisomerase I (ω protein) have provided important information about chromosome structure, revealing that DNA topology affects DNA replication, transcription, and recombination. In addition to the catalytic topoisomerases, bacteria also contain proteins that organize supercoiled DNA into the folded and condensed nucleoid structure (5, 9, 31). The roles of these small, basic, heat-stable, histonelike proteins in replication, transcription, and recombination are vague.

The most abundant histonelike protein in bacteria, HU, is thought to organize a portion of the bacterial chromosome into solenoids, or superhelical domains reminiscent of eucaryotic nucleosomes (3, 5, 29). In *Escherichia coli*, HU (also called NS and DBPII) is composed of nonidentical 10-kilodalton monomer subunits that form stable heterodimers in dilute solution (19, 28, 34). The two subunits are encoded by the *hupA* and *hupB* genes. In other bacterial species, HU is composed of dimers of identical polypeptides (36).

The physiological roles of HU have not been investigated in vivo, but HU stimulates several notable reactions in vitro, including initiation of *oriC* replication in *E. coli* (1), inversion of the flagellar gene regulatory sequences (13), and transposition of bacteriophage Mu (4). Genes for two HU subunits from *E. coli* were recently cloned and sequenced (14, 15). We report here the cloning, sequencing, and mapping of *Salmonella typhimurium hupA* (encoding HU-2) by a novel strategy that employs synthetic oligonucleotides and transposable locked-in P22 phage. *hupA* maps near *purH*, which is located at 90.5 min on the standard *Salmonella* genetic map. *hupA* from *S. typhimurium* and *E. coli* encoded identical proteins, and 5′-flanking sequences, which included two unusual homopurine-homopyrimidine tracts, showed extreme conservation. Evidence that HU and IHF proteins interact in vivo came from the observation that *E. coli* strains carrying mutations in *himA* and *hip* were hypersensitive to HU-2 overexpression.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and enzymes. Bacterial strains are listed in Table 1. Plasmids used for cloning were pUC19 (38) and pTTQ18 (32). Restriction enzymes and calf intestinal phosphatase were obtained from Boehringer Mannheim Biochemicals. Bacteriophage T4 DNA ligase and radioactive isotopes were obtained from Dupont-NEN Research Products. Bacteriophage T4 polynucleotide kinase was purified from T4-infected cells by the method of Panet et al. (27).

Genetic methods and library constructions. The construction and properties of the locked-in P22 prophage, MudP, have been described previously (39). MudP contains a chloramphenicol drug resistance gene, a functional P22 origin of replication, and P22 DNA replication and packaging genes cloned between 1-kilobase (kb) fragments containing *attR* and *attL* ends of phage Mu. Mitomycin C induction of MudP prophages results in P22-mediated replication and efficient directional packaging of three consecutive headsful of chromosomal DNA. The first headful contains 16 kb of the MudP element plus 28 kb of host chromosome adjacent to the inserted prophage; the second and third headsful each contain 42 kb of contiguous host DNA.

A library of random MudP insertions in the *Salmonella* chromosome was generated by transposition using the donor DH1602. MudP contains no transposition proteins, and it transposes only when Mu *A* and *B* gene products are provided in *trans*. Transduction of MudP from DH1602 (which contains a source of Mu *A* and *B* closely linked to the MudP) to strains containing the *his-3050* deletion results in efficient transpositional inheritance of a MudP in a genetic background free of transposase activity (12). Three hundred independent chloramphenicol resistant colonies were iso-

TABLE 1. Bacterial strains

Strain	Genotype	Source
S. typhimurium		
LT2	Wild type	J. Roth
TR5654	*thrA9*	J. Roth
TR5655	*leu-485*	J. Roth
TR5656	*proA36*	J. Roth
TR5658	*pyrC7*	J. Roth
TR5660	*pyrF146*	J. Roth
TR5662	*hisO1242 his-2236*	J. Roth
TR5663	*purF145*	J. Roth
TR5644	*cysA533*	J. Roth
TR5665	*cysC519*	J. Roth
TR5666	*serA13*	J. Roth
TR5667	*cysG439*	J. Roth
TT292	*purH*::Tn*10*	J. Roth
TR5688	*purA*	J. Roth
TR5671	*pyrA*	J. Roth
DH2233	*zja-5401*::MudP	This study
DH2700	*zja-5401*::MudP *purH*::Tn*10*	This study
DH1602	*hisD9953*::MudP *hisA9944*::Mudl	K. Nielson
E. coli		
N99	*galK* Str^r	H. Nash
HN414	N99, *himA42*	H. Nash
DH5α	*endA1, hsdR17* (r_K^- m_K^+)*supE44 thi-1 recA1 gyrA96 relA1 Δ(argF-lacZYA)* U169 φ80*dlacZ ΔM15* λ$^-$	Bethesda Research Laboratories
LE392	*hsdR514* ($r_K^-m_K^-$ *supE44 supF58Δ(lacIZY)6 galK2 galT22 metB1 trpR55*λ$^-$	J. Roth
MH4386	*gal rpsL himA42 zdh-201*::Tn*10*	M. Howe
NH272	*himAΔ82*	D. Friedman
NH273	*himA79*(Am)	D. Friedman
NH274	*supE supF hip*::Cam^r	R. Weisberg

lated, grown in 30-ml cultures to a density of 3 × 10^8 cells per ml, and induced with 1 μg of mitomycin C per ml. After cell lysis and incubation with chloroform, phage heads and DNA were purified as described previously (39). DNA yields ranged from 15 to 100 μg per culture.

A size-selected library of LT2 was constructed by cleaving 50 μg of bacterial DNA with 100 U of *Pst*I. The products were separated on a 1% agarose gel next to bacteriophage λ DNA that was cut with *Hin*dIII. DNA fragments comigrating near the 2,032-base-pair (bp) fragment of λ were eluted from the gel, adsorbed to a NACS column (Bethesda Research Laboratories, Inc.), eluted from NACS in high-salt buffer (2 M NaCl, 10 mM Tris hydrochloride, 1 mM EDTA), and ethanol precipitated. Ligation into *Pst*I-cleaved pUC19 DNA and transformation into *E. coli* DH5 cells was carried out by standard procedures (18).

Oligonucleotides and hybridization conditions. Oligonucleotides were synthesized with an Applied Biosystems model 380A DNA synthesizer and used without further purification. HU1d is a 60-bp oligonucleotide, 5'-CGCAACCCGCA GACCGGTAAAGAGATCACCATCGCTGCTGCTAAAG TACCGAGCTTCCGT-3', that matches the *hupB* sequence of *E. coli* at amino acids 41 to 60 (15). An oligonucleotide made to recognize the *hupA* gene, DH32, contained the sequence 5'-GG(C/T)TT(C/T)GG(C/T)AC(A/G/C/T)TT(C/T)-3', where the bases within parentheses were mixed in equal proportions. This oligonucleotide matched DNA predicted by amino acids that are highly conserved among all type II DNA-binding proteins (6) at positions 46 to 50 for the *hupA* protein (see Fig. 2). Oligonucleotide DHOG20, 5'-CGGATCG AAGACGAAGTAGC-3', corresponded to a sequence that lies approximately 1,700 bp upstream of the initiation codon for *hupA* (N.P.H., unpublished data). Oligonucleotide primers used for sequencing were the universal sequencing primer (U.S. Biological Corporation no. 70705), the reverse-sequencing primer (New England BioLabs, Inc., no. 1201), and oligonucleotides matching *hupA* sequences at 55 to 75 and 96 to 117 (see Fig. 2).

Oligonucleotide labeling was carried out as described previously (18). Radioactive oligonucleotides were bound to NACS columns (Bethesda Research Laboratories) in buffer containing 10 mM Tris hydrochloride–100 mM NaCl–1 mM EDTA (pH 7.5) and eluted in buffer containing 10 mM Tris hydrochloride–1 M NaCl–1 mM EDTA (pH 7.5) before hybridization. Hybridization reactions with 25 μCi of ^{32}P-labeled oligonucleotides were performed in 25 ml of solution composed of 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 10× Denhardt solution, 100 μg of denatured salmon sperm DNA per ml, and 1% sodium dodecyl sulfate (SDS) (18). Hybridization reactions initiated at 65°C were slowly cooled over 2 h to 42°C and then incubated at 42°C overnight. Colony lifts, phage library membranes, and Southern blots were all subjected to four wash cycles of 1 h each. The washing conditions were 4× SSC and 0.1% SDS at 49°C for DH32, 4× SSC and 0.1% SDS at 53°C for DHOG20, and 0.2× SSC and 0.1% SDS at 65°C for HU1d. Filters were stripped by being washed in 0.2× SSC and 0.1% SDS at temperatures above the T_m of the respective oligonucleotide.

Blots. Bacterial DNA (2.5 μg) digested with 5 U of restriction enzyme was extracted twice with an equal volume of phenol-chloroform (50:50), precipitated with ethanol, suspended, and subjected to electrophoresis for 14 h at 10 V/cm in horizontal slab gels. DNA was applied to Zeta probe nylon membranes by capillary transfer in 0.4 N NaOH.

Colonies from the library of 2- to 3-kb LT2 *Pst*I fragments cloned into pUC19 were grown overnight at 37°C. After transfer to 28-mm nitrocellulose filter discs (BA85; Schleicher & Schuell, Inc.), membranes were placed colony side up on an L-broth plate containing 100 μg of chloramphenicol per ml and a second nitrocellulose membrane was sandwiched on top. The plates were incubated at 37°C for 6 h, and then both filters were prepared for hybridization.

Phage head DNAs (90-μl portions) were slot blotted onto positively charged Biotrace (Gelman Sciences, Inc.) nylon membranes. After treatment for 5 min with 0.4 M NaOH, the filters were neutralized, air dried, and baked at 85°C for 1 h. Membranes were washed twice in a solution of 0.5× SSC and 0.1% SDS at 65°C for 30 min and then hybridized.

DNA sequencing. DNA sequencing of covalently closed plasmid DNA denatured in alkali was carried out by the chain termination method (30) using phage T7 DNA polymerase (35) and [^{35}S]dATP as suggested by the U.S. Biological Corporation. The chain-terminated products that separated on 6% sequencing gels run at 65°C were visualized by overnight autoradiography with Eastman Kodak X-Omat film.

RESULTS

***S. typhimurium* has two genes corresponding to *hupA* and *hupB* of *E. coli*.** The HU proteins from *E. coli* and from

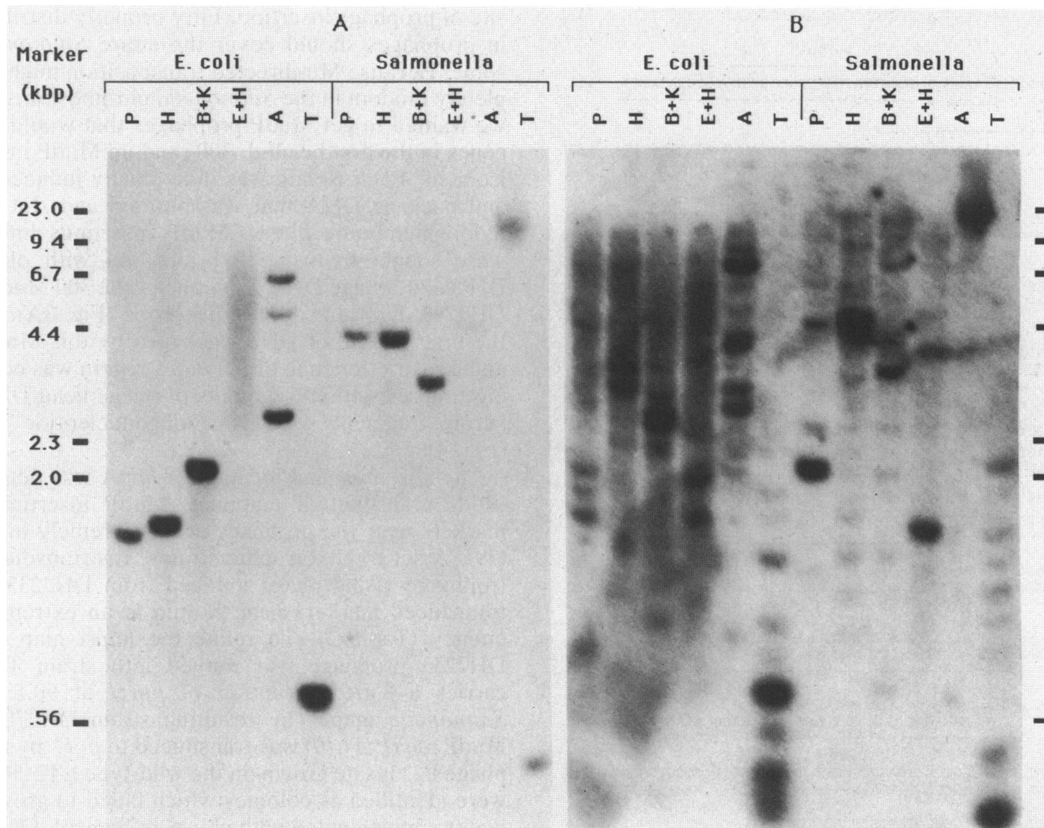

FIG. 1. Southern blot analysis of HU genes. A nylon membrane was blotted with restricted DNA from LE392 and LT2 and then probed with radioactive oligonucleotides containing sequences of the HU genes. Lanes contained DNA digested with *Pst*I (lanes P), *Hpa*I (lanes H), *Bam*HI plus *Kpn*I (lanes B + K), *Eco*RI plus *Hin*dIII (lanes E + H), *Acc*I (lanes A), and *Taq*I (lanes T). (A) The membrane was hybridized to oligonucleotide HU1d that corresponded to the coding region for amino acids 41 to 60 of *E. coli hupB*. (B) The same membrane was hybridized to the mixed oligonucleotide DH32 that recognizes DNA encoding consensus amino acids of the type II DNA-binding proteins (see Fig. 2). Markers indicate bands of λ *Hin*dIII fragments with lengths of 23, 9.4, 6.7, 4.4, 2.3, 2.0, and 0.56 kb.

several other organisms have been isolated and sequenced (5, 19, 34). A probe was made to the DNA sequence of the *E. coli hupB* (15). HU1d is an oligonucleotide that corresponds to 60 bp (from 181 to 240) of the *hupB* sequence. An alkaline transfer to a Zeta probe nylon membrane of LT2 and LE392 DNA that had been cleaved with several restriction enzymes was hybridized to the HU1d oligonucleotide and washed under stringent conditions (see Materials and Methods). Both LE392 and LT2 had a well-resolved major band in lanes containing DNA digested with *Pst*I, *Hpa*I, *Bam*HI plus *Kpn*I, and *Taq*I (Fig. 1A). Digestion with *Acc*I resulted in partial cleavage with three bands in the lane containing DNA from LE392 and an unresolved smear for LT2. No band was apparent in lanes digested with *Eco*RI plus *Hin*dIII, and the fragments produced by this combination of enzymes appeared too large to be resolved during electrophoresis or to be transferred efficiently to the membrane (Fig. 1). This Southern blot profile suggested that a single *hupB* gene was present in LT2. This conclusion was strengthened when, after the filters were stripped, the same size bands reappeared after hybridization to two different oligonucleotides that matched the *E. coli hupB* DNA sequence at positions 1 to 60 and 121 to 150 (data not shown).

HU is a member of a family of proteins (type II DNA-binding proteins) that contain two patches of highly conserved amino acids (6). To detect the *hupA* gene, an oligonucleotide was made that fit the first consensus region of the type II DNA-binding proteins. Oligonucleotide DH32 hybridized to several bands in lanes containing DNA from both LE392 and LT2 (Fig. 1). In each case, one relatively weak band corresponded to the *hupB*-specific band detected by the HU1d probe described above, but there were several additional bands. One prominent *Pst*I fragment from LT2 was 2 kb in length. A fragment of similar size was also detected by two different oligonucleotides that were made to correspond to amino acids 1 to 5 and 50 to 54 of *E. coli hupA* (data not shown). These data and the results of hybridization with the *hupB*-specific probes indicated that LT2 contained two genes that corresponded to *E. coli hupA* and *hupB*.

Cloning and sequencing *Salmonella hupA*. A library of 2-kb *Pst*I fragments from LT2 was cloned directly into the *Pst*I site of pUC19. Ampicillin-resistant colonies were transferred to filters and hybridized with the DH32 oligonucleotide. One plasmid, pHH6, was chosen for further analysis from eight independent clones that had identical inserts. *hupA* was located on a 720-bp *Sac*I-*Pst*I fragment (see Fig. 4) of pHH6, which was isolated, subcloned into pUC19, and sequenced.

LT2 *hupA* plus 170 bp of 5′-flanking sequence and 104 bp of 3′-flanking sequence was very similar to the homologous region from *E. coli* (Fig. 2). *E. coli* and *Salmonella* genes encoded identical amino acids, although base changes occurred at 13 neutral third codon positions. Only 2-bp differences between *E. coli* and *S. typhimurium* were found in a 130-bp region immediately upstream of *hupA*; this was the most highly conserved −1 to −130 region we found in the Genbank data base. The same interval for *cysB* (26), *hisR* (2,

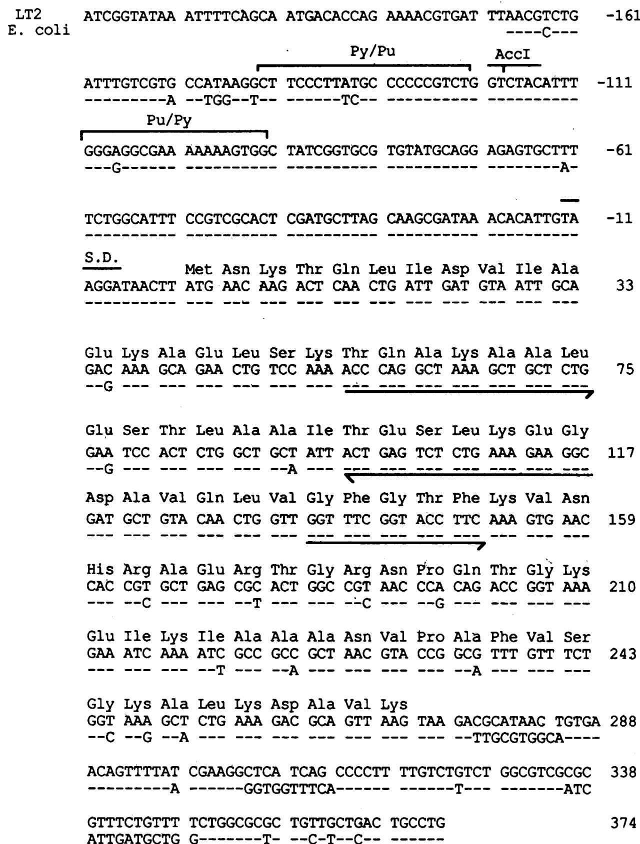

FIG. 2. DNA sequence comparison of *hupA* from LT2 and *E. coli*. *E. coli* sequences that are identical with LT2 are indicated (−). In 210 bp of 5′-flanking sequence, there were two unusual sequences of homopyrimidine from −142 to −122 and of homopurine from −110 to −92. Oligonucleotides used for sequencing (at bp 55 to 75 and 97 to 117) and for detecting *hupA* in the pUC19 library (DH32 at bp 136 to 150) are underlined, and orientations of the 3′ ends are indicated by the slanted lines (see Materials and Methods). The *Acc*I site used in construction of pPH100 (see Fig. 5) cleaves at −118. The ribosome-binding sequence (S.D.) was located 6 bp upstream of the initiating AUG codon.

11), *pyrBI* (20, 37), and *ompA* (8, 22) contained 34, 14, 28, and 5 mismatches, respectively. The highly conserved *ilv-GEDA* leader promoter contained 10 changes in 90 bp of 5′-flanking DNA (10). Two long, unusual homopurine and homopyrimidine tracts were embedded in the 5′-flanking region of *hupA*. A 19-bp segment from positions −92 to −110 contained 17 purines in one strand, and at −142 there was a 21-bp segment with 19 pyrimidines in the same strand (Fig. 2). Homopurine-homopyrimidine tracts can adopt unusual secondary conformations when placed under negative supercoil tension, and similar sequences are often found near eucaryotic promoter elements (R. D. Wells, D. A. Collier, H. C. Hanvey, M. Shimuzu, and F. Wohlrab, FASEB J., in press).

Mapping *hupA*. A selective chloramphenicol marker was introduced into the *Salmonella* chromosome near the HU genes using MudP (39). MudP is a Mu-P22 chimera that integrates at random by transposition when Mu *A* and *B* gene products are provided in *trans*. Once integrated, the element is locked in to the chromosome by virtue of defects in P22 integration and excision functions. Locked-in P22 prophages can be induced to replicate by using the P22 origin and initiation proteins and then to efficiently package approximately 100 kb of chromosomal DNA that lies adjacent to the site of prophage insertion. Fifty properly distributed locked-in prophages should cover the entire *Salmonella* chromosome. Because Mu-directed transposition might not be completely random in the *Salmonella* chromosome, and because we wanted to get MudP prophages that would package HU genes in the first headful, 300 random MudP insertions were isolated. Each isolate was individually induced to replicate and package DNA that was purified and slot blotted onto nylon membrane filters. MudP insertions linked to *hupA* were sought by using hybridization with oligonucleotide DHOG20. Phage DNA from one of the 300 MudP insertions, DH2233, hybridized with this probe (Fig. 3A). *hupA* was in the first headful of DNA packaged by this MudP insertion, and a restriction map of the *hupA* region was constructed by blotting restriction fragments of phage head DNA and measuring fragment sizes after oligonucleotide hybridization (Fig. 4).

The chromosomal location of *hupA* was determined with MudP transduction mapping. MudP insertions transduce markers near the prophage at an extremely high frequency (39). A set of auxotrophic strains was transduced to prototrophy by using phage induced from DH2233. This phage transduced markers near 90 min at an extremely high frequency (Table 2). To refine the *hupA* map position, the DH2233 prophage was moved into strain TT292, which carries a Tn*10* disruption of *purH* at 90.5 min on the *Salmonella* map. The resulting strain DH2700 (*zja-5401*::MudP *purH*::Tn*10*) was transduced to pur^+ by using a donor phage P22 lysate grown on the wild-type LT2. Recombinants were identified as colonies which failed to grow on minimal plates supplemented with chloramphenicol. Of 300 prototrophic transductants, 36 were chloramphenicol sensitive, so that the linkage of *zja-5401*::MudP to *purH* was 12%.

Expression of HU-2 protein retards growth of IHF mutants of *E. coli*. HU and IHF are type II DNA-binding proteins that play important roles in Mu transposition. In vitro studies have shown that HU stimulates the Mu transposition reaction (4) and that IHF enhances transcription of replication-transposition proteins (17). To test whether overexpression of HU might suppress the block of Mu replication in IHF mutants, we tried to introduce pHH6 into *E. coli* strain HN414, which carries the *himA42* allele. We could not build the strain. Whereas the transformation efficiencies of HN414 and of him^+ N99 were equal when pUC19 was used, N99 was 500-fold higher than HN414 in transformation efficiency when pHH6 DNA was used. To test whether the exclusion of pPH100 from HN414 could be due to a compensatory mutation that arose in HN414 during strain propagation, P1 was grown on *E. coli* MH4386, which carries a Tn*10* tightly linked to *himA42* (M. Howe, personal communication). One fresh tetracycline-resistant transductant of N99 that was also non-permissive for Mu replication was made competent and transformed with pUC19 and pHH6. A 500-fold difference in transformation frequency of pUC19 and pHH6 was again observed in the *himA42* derivative compared with the him^+ parent, implying that the pHH6 plasmid severely inhibited *himA42* mutants of *E. coli*.

hupA was not the only gene contained on the 2.1-kb LT2 insert in pHH6. To determine whether *hupA* expression could be directly responsible for inhibition of cell growth, a plasmid was constructed that placed *hupA* behind the inducible *tac* promoter of plasmid pTTQ18 (Fig. 5). The cloning utilized an *Acc*I site that eliminated the homopurine tract that begins 121 bp upstream from the start of HU-2 translation (Fig. 2). pTTQ18 has the vector sequences and polylinker of pUC18 but also contains a $lacI^q$ allele to diminish a

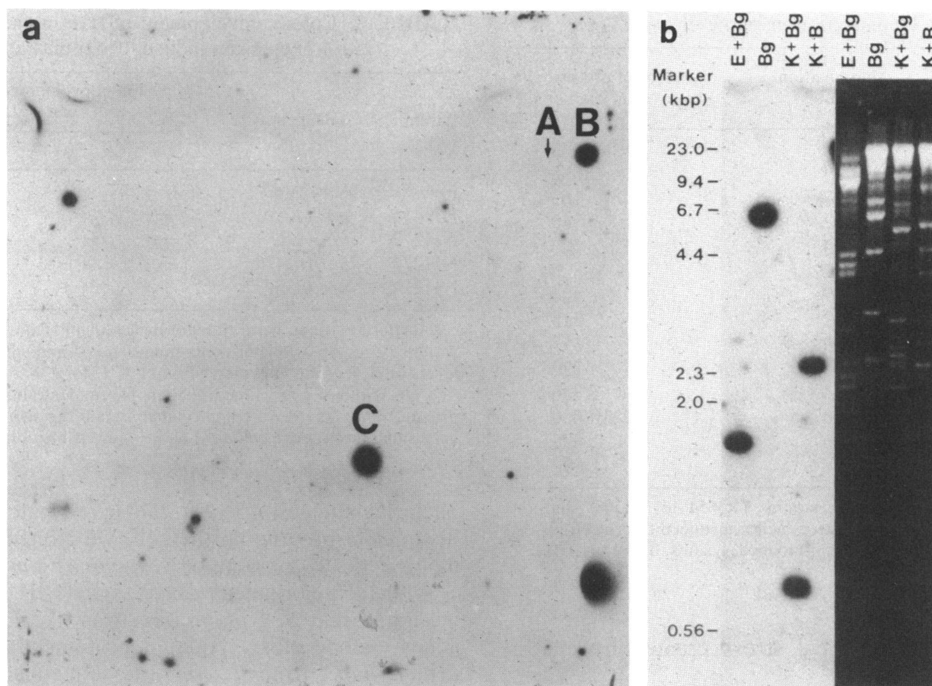

FIG. 3. Identification of a *hupA*-linked MudP insertion and analysis of the purified phage head DNA. (a) A nylon membrane with phage DNA from 300 random MudP insertions in the *Salmonella* chromosome was probed with oligonucleotide DHOG20 that contains a sequence from the 5′ end of the pHH6 plasmid insert. Positions of LE392 and LT2 DNA and purified DH2233 phage head DNA are shown by the letters A, B, and C, respectively. (b) Southern blot and restriction enzyme analysis of purified phage DNA isolated from strain DH2233 (see Materials and Methods section on oligonucleotides and hybridization conditions). The gel revealed major ethidium-staining bands from the first headful and minor bands from the second and third headfuls of DNA (shown on the right) after digestion with *Eco*RI (E), *Bam*HI (B), *Bgl*II (Bg), and *Kpn*I (K) restriction enzymes. After transfer to a nylon membrane, hybridization was carried out with oligonucleotide DHOG27 that encodes amino acids 56 to 63 of the *hupA* gene (shown on the left). kbp, Kilobase pair.

significant problem of unregulated expression from *tac* or *lac* promoters cloned on multicopy plasmids (32). The resulting plasmid, pPH100, was introduced into N99 (*him*⁺) and HN414 (*himA42*) by transformation. Unlike pHH6, pPH100 transformed HN414 at the same frequency as N99, and the problem that prevents stable maintenance of the *hupA* gene in pHH6 was largely eliminated. To test whether overexpression of *hupA* could explain the toxicity of pHH6 in HN414, we measured growth of fresh overnight cultures of pPH100 transformants in the presence of different amounts of isopropyl-β-D-thiogalactopyranoside (IPTG). All strains tested had similar growth on LB plates lacking inducer, but *hupA* induction created a dramatic differential growth response in IHF mutants. Strains with *himA42* or *himA*Δ*82* alleles had impaired growth at 90 μM IPTG, and at 125 μM IPTG, colony formation was totally blocked (Table 3). The *him*⁺ strain produced colonies at 200 μM IPTG (Table 3). IHF has two subunits. The gene for the β subunit, *hip*, is unlinked to *himA* (16). To find whether *hupA* expression was toxic only for strains carrying *himA* mutations, pPH100 was introduced into a strain (NH274) carrying a disrupted *hip* gene. NH274 transformants carrying pPH100 were also hypersensitive to IPTG, though they were not quite as sensitive as *himA* mutants (Table 3). Thus, IHF appears to

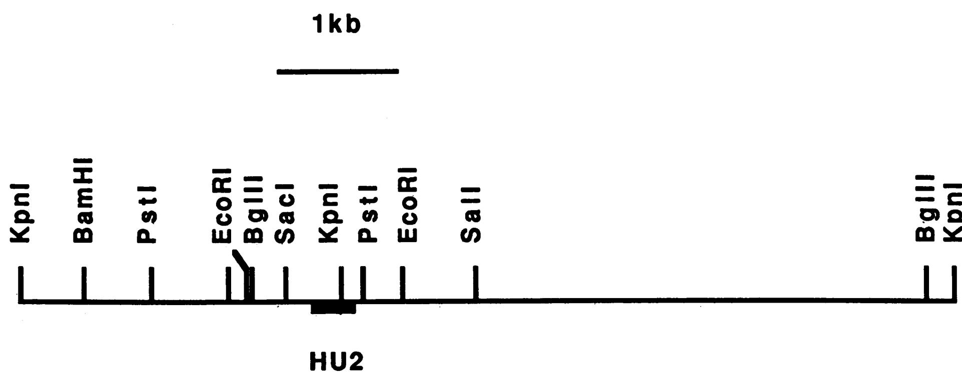

FIG. 4. Restriction map made from phage head DNA (39) isolated from DH2233 showing restriction sites for *Kpn*I, *Bam*HI, *Pst*I, *Bgl*II, *Sac*I, and *Sal*I in DNA flanking *hupA* in *LT2*. After being transferred to nylon membranes, bands containing *hupA* sequences were identified by hybridization to oligonucleotide probes (see oligonucleotides and hybridization section of Materials and Methods).

TABLE 2. Transduction mapping of *hupA*[a]

Auxotrophic recipient (transduction site)	Map position (minutes)	No. of prototrophic transductants
TR5654 (*thrA*)	0	1,185
TR5655 (*leu*)	3	3,065
TR5656 (*proA*)	7	163
TR5658 (*pyrC*)	22	60
TR5660 (*pyrF*)	34	196
TR5662 (*his*)	42	0
TR5654 (*purF*)	49	404
TR5664 (*cysA*)	52	318
TR5665 (*cysC*)	60	355
TR5666 (*serA*)	63	729
TR5667 (*cysG*)	73	1,826
TT292 (*purH*)	90	>10,000
TR5688 (*purA*)	96	1,823
TR5671 (*pyrB*)	98	1,092

[a] The set of auxotrophic-mapping strains TR5654 to TR5688 and the *purH*::Tn*10*-containing strain TT292 were each transduced to prototrophy by using phage prepared from mitomycin-induced lysates of strain DH2233 (*zja-5401*::MudP).

partially relieve a physiological stress created by excess *hupA* transcription.

DISCUSSION

The folded bacterial nucleoid is thought to contain DNA wrapped around solenoidal cores (or spindles) of histonelike proteins. HU is the most abundant histonelike protein of the

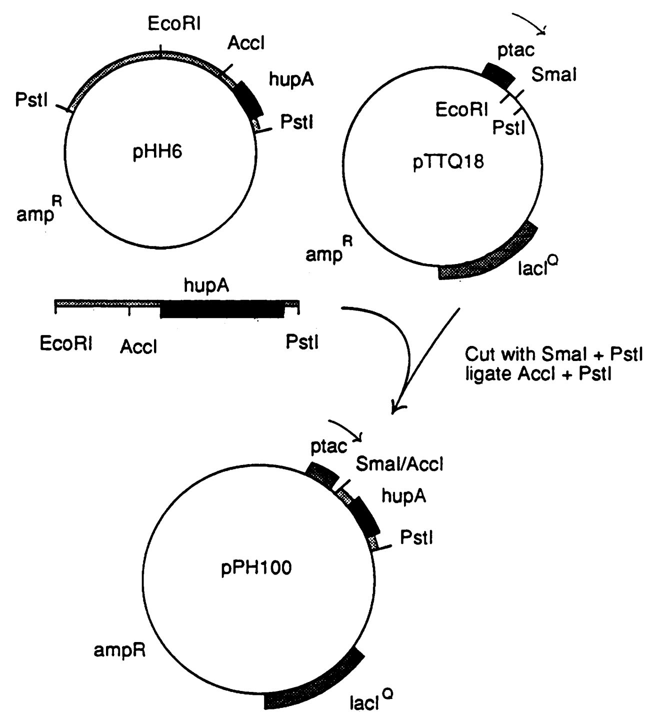

FIG. 5. Construction of a plasmid for *lacI*^{q}-regulated HU-2 protein synthesis. Plasmid pHH6 was digested with *Acc*I and the cohesive ends were filled with a Klenow fragment of DNA polymerase I. After cleavage with *Pst*I, a 500-bp *hupA* fragment was purified and ligated to pTTQ18 DNA that was digested with *Sma*I and *Pst*I. pPH100 carries a copy of *lacI*^{q} to regulate the *tac* promoter that drives HU-2 biosynthesis.

TABLE 3. Colony development of IHF mutants with differential *hupA* expression induced from plasmid pPH100[a]

Strain	IHF genotype	Colony development[b] with the following amt of IPTG (μM):				
		0	90	125	160	200
N99	Wild type	++	++	++	++	+
HN414	*himA42*	++	+	−	−	−
NH272	*himA*Δ*82*	++	+	−	−	−
NH273	*himAam79*	++	++	+	+/−	+/−
NH274	*hip*::Cam^r	++	++	+/−	−	−

[a] Bacterial strains were transformed with pPH100, and colony formation was measured on LB plates containing 50 μg of ampicillin per ml and indicated levels of IPTG following incubation at 37°C for 24h.

[b] ++, Colony size comparable to parental strains without the pPH100 plasmid; +, colonies were approximately half the size of ++ colonies; +/−, very small translucent colonies; and −, no visible colony development.

bacterial nucleoid (5, 31). To investigate the roles of the histonelike proteins in the cell, we cloned, sequenced, and mapped the *hupA* gene of *S. typhimurium*.

While some organisms have a single HU polypeptide (36), we found that *S. typhimurium* was like *E. coli* in that two genes encode closely related proteins that form HU. Recently, *hupA* from *E. coli* was sequenced (14). *hupA* was conserved between *E. coli* and *S. typhimurium*; the proteins encoded by the two species were identical (Fig. 2). More surprising was the high conservation of DNA sequence outside the coding frame of the gene. The DNA sequence 130 bp upstream of the initiating AUG was more highly conserved than was most of the coding frame, which contained 13 neutral mutations at third-codon positions; the −1 to −130 region contained fewer changes than several other tightly regulated genes, including *pyrBI*, *hisR*, *ilvGEDA*, *ompA*, and *cysB*. The conservation extended 170 bp upstream, where the reported sequence for the *E. coli hupA* ended. DNA from the 3′ end of the gene 100 base pairs downstream was related but had patches of nonhomology. The extreme conservation suggests that DNA flanking *hupA* on the 5′ side has important functions. Two stretches of homopurine and homopyrimidine sequences were unusual features of this DNA. In other contexts, such sequences adopt unusual supercoil-dependent secondary conformations (Wells et al., in press), and these tracts could have a role in HU regulation.

Our random MudP insertion mapping of HU genes was a direct extension of methods developed by Youdarian et al. (39). Because MudP prophages are stable chromosomal constructions that can efficiently package cosmid-sized patches of DNA, they are excellent for recloning sequences adjacent to a gene, for targeting transposon and chemical mutagenesis to a limited chromosomal segment, and for constructing physical and genetic maps of a region (Fig. 4). We located *Salmonella hupA* at 90.5 min, near *purH*. *hupA* was also found at this location in *E. coli* (Y. Kano, M. Wada, and F. Imamoto, Gene, in press).

A library of 300 random MudP insertions has a good probability of containing any other *Salmonella* gene in a highly accessible form that is 100-fold purified from other bacterial sequences. The membrane shown in Fig. 3 was stripped and reprobed with synthetic oligonucleotides that detect other genes. MudP phages near *Salmonella hupB* and *himA* have recently been identified.

Plasmids that express *hupA* exhibited a phenotype in *E. coli*. pHH6, which produced *hupA* from its own promoter, blocked growth of *E. coli* strains that carry a *himA* mutation.

We could not introduce pHH6 into LT2 or several other *Salmonella* strains, and so it seems that overexpression of *hupA* was toxic for even wild-type *S. typhimurium*. Evidence that toxicity was a consequence of HU-2 synthesis came from experiments with plasmid pPH100, which makes HU-2 under *lac* repressor control (Table 3). IHF mutants with defective *himA* or *hip* subunits were hypersensitive to *hupA* transcription from pPH100.

IHF is involved in numerous regulatory reactions in the cell, including λ integration and excision (21, 23, 24), Mu transcription (17), plasmid replication (33), utilization of xylose (7), and synthesis of the *ilvB* gene product (7). Both IHF and HU are type II DNA-binding proteins; our data provide evidence that they interact. Both proteins bend DNA (3, 33), so they might control alternative structures at key positions in the folded chromosome. The ability to modulate the expression of both IHF (25) and HU in vivo may be a useful tool for investigating chromosome structure and function.

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ADDENDUM IN PROOF

The *hupB* gene was recently subcloned from the *S. typhimurium* MudP library, and its sequence has been determined (M. Marsh and D. Hillyard, Nucleic Acids Res. **16**:7196, 1988).

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