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The *Escherichia coli* Histone-Like Protein HU Affects DNA Initiation, Chromosome Partitioning via MukB, and Cell Division via MinCDE

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***Escherichia coli hupA hupB* double mutants, lacking both subunits (HU1 and HU2) of the histone-like protein HU, accumulate secondary mutations. In some genetic backgrounds, these include mutations in the *minCDE* operon, inactivating this system of septation control and resulting in the formation of minicells. In the course of the characterization of *hupA hupB* mutants, we observed that the simultaneous absence of the HU2 subunit and the MukB protein, implicated in chromosome partitioning, is lethal for the bacteria; the integrity of either HU or MukB thus seems to be essential for bacterial growth. The HU protein has been shown to be involved in DNA replication in vitro; we show here that its inactivation in the *hupA hupB* double mutant disturbs the synchrony of replication initiation in vivo, as evaluated by flow cytometry. Our results suggest that global nucleoid structure, determined in part by the histone-like protein HU, plays a role in DNA replication initiation, in proper chromosome partitioning directed by the MukFEB proteins, and in correct septum placement directed by the MinCDE proteins.**

The HU protein, one of the histone-like proteins of *Escherichia coli*, is a small, abundant basic protein associated with the nucleoid (16, 42, 43), maintaining the global structure of the DNA by constraining DNA supercoils (8). By bending DNA, histone-like proteins can facilitate the formation of DNA-protein complexes and participate positively or negatively in gene regulation (17; for a review, see reference 15). The HU protein is a heterodimer of two homologous components, HU1 and HU2, products of the *hupB* and *hupA* genes, respectively (27–29). In vitro and in vivo studies suggest that HU is involved in several fundamental biological functions, including the initiation of DNA replication (3, 13, 22), site-specific DNA inversion (34, 49), and transposition (21, 34, 49). Mutants defective in both HU1 and HU2 are altered in cell division and chromosome partitioning, and they accumulate anucleate cells at a significant level (14, 21, 48).

Another set of proteins affecting cell division and chromosome partitioning is the MinB system, which in *E. coli* ensures that cell division occurs at midcell after replicated chromosomes have moved apart (1, 47). The *minB* operon consists of three genes: *minC*, *minD*, and *minE* (11). The MinC and MinD proteins together can block cell division at all potential division sites (11). The MinE protein was postulated to be a topological specificity factor which prevents the MinCD complex from acting at midcell, allowing cell division to occur (11). Mutants altered in the *minC* or *minD* gene or in which the *minB* operon is deleted give rise to a mixed cell population containing nucleate normal-size and elongated cells, anucleate spherical minicells formed by polar divisions, and a small proportion of anucleate rod-shaped cells (1, 9, 23, 24, 47). The block to cell division caused by overproduction of the MinC and MinD proteins (or by underproduction of MinE) can be relieved by increasing the amount of MinE protein (11) or by overexpressing the key cell division protein FtsZ. The FtsZ protein was suggested to be the target of the *minC* and *minD* gene products, interacting directly with them (5, 6, 10, 40). We and others have observed that *minB* mutant cells occasionally have an abnormal nucleoid distribution (2, 24, 35). Moreover, the DNA supercoiling of some reporter plasmids is reduced in a *min* mutant and in the presence of excess Min proteins to the same extent as in *gyrB* mutants, affected in the B subunit of DNA gyrase (35). This, together with the formation of anucleate rods in *min* mutants, indicates that the MinCDE proteins could interact with the chromosome partitioning system.

The chromosome partitioning system requires the integrity of the MukF, MukE, and MukB proteins (38, 53). By its structure, the MukB protein belongs to the family of motor proteins, like myosin (38), and it may participate in generating the force necessary to separate daughter chromosomes before the onset of cell division. The *mukB* gene codes for a 170-kDa protein (52) which possesses DNA binding activity and ATP-GTP binding activity (37). Cultures of *mukB* mutants contain a mixed population of nucleate and normal-size anucleate cells. Occasionally, two nucleoids move to the same half of the mother cell, generating one anucleate and one binucleate cell of the same size, and sometimes the septum appears to cut part of the nucleoid itself, the so-called guillotine effect (18, 38). Recently it was shown that the *mukB* gene belongs to an operon consisting of four open reading frames, *smtA*, *mukF*, *mukE*, and *mukB* (53). Mutants in which the *mukF*, *mukE*, or *mukB* gene is deleted produce anucleate cells at high frequency; they are viable at 25°C, but at high temperatures cell division is inhibited (38, 53). It is not known how the MukF, MukE, and MukB proteins interact to ensure correct partitioning of *E. coli* chromosomes.

We previously reported that some *hupAB* double mutants form colonies of heterogeneous size (21). After several steps of purification, these colonies generate cell populations differing with respect to their anucleate cell content, suggesting that *hupAB* double mutants are unstable and accumulate compen-

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TABLE 1. Bacterial strains used in this study

Strain	Genotype	Origin or reference
PB103	*dadR trpE61 trpA62 tna-5 leu-6*	12
OHP176	*pro trp-31 his-1 argG6 lacZ xyl-17 mtl tonA2 tsx rpsL104 supE44*	21
OHP 190	OHP176 *hupA*::Cm *hupB*::Km	21
GC7324	OHP176 *hupA*::Cm *hupB*::Km *minDE106*	This work
GC7528	PB103 *mukB*::*kan*	38
GC7588	OHP176 *minDE106 zcf-117*::Tn*10*	This work
GC7614	PB103 *zcf-117*::Tn*10*	Laboratory collection
GC7615	PB103 *hupA*::Cm	Laboratory collection
GC7646	PB103 *argE*::Tn*10 purH* Rif^r	Laboratory collection
GC7664	GC7646 *mukB*::*kan*	Laboratory collection
GC7796	PB103 *minC103 zcf-117*::Tn*10*	This work
GC7797	PB103 *hupA*::Cm *hupB*::Km *minC101*	This work
GC7798	OHP176 *hupA*::Cm *hupB*::Km *minC103*	This work
GC7802	PB103 *minCDE105 zcf-117*::Tn*10*	This work
GC7803	OHP176 *hupA*::Cm *hupB*::Km *minCDE105 zcf-117*::Tn*10*	This work
GC7804	PB103 *minC101 zcf-117*::Tn*10*	This work

satory mutations. More recently, it has been shown that certain *gyrB* mutations can suppress the colony size instability of a *hupAB* mutant (32). The possibility that gyrase and the MinCDE proteins exert similar effects on nucleoid structure will be discussed below.

In this work, we confirm that *hupAB* double mutants accumulate secondary mutations, and we show that these include mutations at the *minB* locus. We also show that the absence of HU subunits results in an abnormal distribution of chromosome origins per cell, as determined by flow cytometry. In addition, a mutant in which the MukB protein is defective cannot survive in the absence of the HU2 protein. These results suggest that the HU protein, possibly by controlling in part the global structure of the nucleoid, is involved in DNA replication initiation, that it participates in chromosome partitioning (as discussed in reference 51) via the MukB system, and that it affects septation via the MinCDE proteins.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used in this work, all *E. coli* K-12 derivatives, are listed in Table 1. The *hupA*::Cm and *hupB*::Km derivatives were obtained by P1*vir* transduction, selecting for resistance to 10 μg of chloramphenicol per ml and 40 μg of kanamycin per ml, respectively.

The p^{lac}::*minB* fusions are inserted in λNT5(imm21), carrying the *bla* gene (11). The *lac* operon inducer isopropyl-β-D-thiogalactopyranoside (IPTG) was used at a final concentration of 10^{-3} M, and ampicillin was used at 25 μg/ml.

Bacteria were grown in Luria-Bertani (LB) medium (33). For flow cytometry, M63 was used (33), supplemented with 0.5% glucose, 1 μg of thiamine per ml, 0.4% Casamino Acids, and 100 μg of tryptophan per ml. Phage Mu was grown on plates containing (per liter) 5 g of Bacto Tryptone, 8 g of Bacto Peptone, 5 g of NaCl, 10 mg of B1, 10 ml of 1 M $MgSO_4$, and 12 g of Bacto Agar.

Microscopy. Cell shape and nucleoids were observed by the fluophase combined method of Hiraga et al. (20).

Phage. Growth of phage Mu was detected by spotting serial dilutions of the phage Mu*gem2ts* (formerly Mu*lig2ts* [39]) on lawns of the bacteria to be tested.

DNA techniques and plasmids. Chromosomal DNA was extracted according to the method of Silhavy et al. (44). PCR amplification was carried out with *Taq* polymerase from Gibco. Synthetic primers (5′ to 3′) used to amplify the *minCDE* operon were primer A (GGGGAATTCTGAATAAATGGGAGGGTGACT) and primer B (GGGGAATTCTTTACCACAACGTGGATGAGA). Three independent reaction mixtures for each strain were pooled, and the resulting 2-kb band was extracted from a low-melting-point agarose gel with β-agarase (Biolab). The *minC* and *minD* genes were sequenced with suitable primers and the Sequenase kit (U.S. Biomedical).

Flow cytometry analysis. Flow cytometry analysis was performed on cultures grown to exponential phase at 37°C in minimal glucose medium supplemented with Casamino Acids and tryptophan and then treated with rifampin (200 μg/ml) for 5 h. Samples were fixed, washed, and resuspended in staining solution containing the DNA-specific fluorescent dye mithramycin (10 μg/ml) and ethidium bromide (5 μg/ml) (45). A culture of PB103 grown in M63 glycerol (0.5%) and tryptophan at 37°C was analyzed in parallel as a standard for cells containing one and two chromosome equivalents. Samples were analyzed with an Epics Elite flow cytometer (Coulter) using an excitation wavelength of 457 nm and an emission spectrum of 550 to 640 nm.

RESULTS

Minicell production in *hupAB* mutants. We previously found (21) that after successive colony purifications of a *hupAB* mutant, OHP190, derived from strain OHP176, the cell population contained normal-size and filamentous nucleate cells, normal-size anucleate cells, and, in addition, tiny spherical anucleate cells (minicells).

The same construction by P1 transduction of the *hupA*::Cm and *hupB*::Km alleles was repeated, using strain OHP190 as the donor and OHP176 as the recipient. The disrupted *hupA*::Cm gene was introduced first, followed by transduction of *hupB*::Km. After two nights' incubation at 37°C, the OHP176 *hupAB* transductants were heterogeneous in colony size (from tiny to large). Eleven transductants were purified. After the first purification, the descendants were still heterogeneous. We then systematically chose medium-size colonies for the next two purification steps, reaching homogeneous colony size. The 11 OHP176 *hupAB* transductants were inoculated in LB medium and observed by phase-contrast microscopy. Eight of them contained minicells (three are analyzed in this work), two contained a majority of filamentous cells, and one did not grow in liquid medium. The production of minicells in *hupAB* transductants was found only among *hupAB* double mutants; *hupA*::Cm and *hupB*::Km single mutants did not generate minicell producers. Two types of minicell-producing strains are known: mutants affected at the *minB* locus (1, 9) and strains overproducing the key division protein FtsZ (50). We show below that in *hupAB* strains, *min* mutations are responsible for minicell production.

Accumulation of minicell producers in *hupAB* mutants depends on the genetic background. The same transduction experiments were performed in parallel in two other genetic backgrounds, PB103 and C600. In the PB103 background, no minicells were detected in the primary *hupAB* transductants, but the cultures contained filamentous cells. One transductant was kept in stabs at room temperature. After several months' storage, this PB103 *hupAB* transductant was streaked on plates from the stab, and of 32 colonies observed in the microscope, all produced minicells, unlike the original culture. One minicell-producing clone was used for further analysis (GC7797). It is obvious that some time is necessary before *hupAB* mutants begin to generate minicells, in both the OHP176 and the PB103 backgrounds.

In the C600 background, *hupAB* double mutants formed microcolonies composed of a mixed population of nucleate and anucleate cells, as has been observed previously (21, 48). Accumulation of minicells was not observed in *hupAB* mutants derived from C600, even after several months' storage in stabs.

Genetic characterization of the secondary mutations causing minicell production in *hupAB* strains. Ward and Lutkenhaus (50) showed that three- to fivefold overproduction of the FtsZ protein leads to an increase in the frequency of septation events; populations of such strains contain minicells and nucleate cells whose mean cell volume is smaller than that of control cells harboring the vector plasmid. In contrast, cultures of *min* mutants show a heterogeneous cell population including nucleate normal-size, elongated, and filamentous cells and

TABLE 2. Effect of Min proteins on the phenotype of *min* mutants

Strain	Phenotype with induced Min protein(s)[a]:					
	MinC	MinD	MinE	MinCD	MinDE	MinCDE
PB103	WT	WT	WT	Sep⁻	WT	WT
GC7588	Min⁻	Min⁻	Min⁻	Sep⁻	WT	WT
GC7796	WT	Min⁻	Min⁻	Sep⁻	Min⁻	WT
GC7802	Min⁻	Min⁻	Min⁻	Sep⁻	Min⁻	WT
GC7804	WT	Min⁻	Min⁻	Sep⁻	Min⁻	WT

[a] The *min* mutants lysogenic for different λp^{lac}::*min* phages were grown at 37°C in LB medium supplemented with ampicillin and 10^{-3} M IPTG. Cultures were observed by phase-contrast microscopy after 6 to 8 h of growth in the presence of the inducer. A low level of polar septa and of free minicells was taken to indicate a wild-type phenotype (WT); Min^- and Sep^- refer to minicell and filamentous phenotypes, respectively.

tiny spherical minicells lacking chromosomal DNA. The minicells are the product of aberrant placement of the septum near the cell poles. Observation in the microscope of three OHP176 *hupAB* mutants and of the PB103 *hupAB* mutant (GC7797) revealed cell populations heterogeneous in size, similar to those of *min* mutants. To see whether these strains carried *min* mutations, a Tn*10* transposon which is 40% cotransducible with the *minB* locus was introduced into four *hupAB* mutants. One tetracycline-resistant, minicell-producing transductant of each *hupAB* mutant was used as the donor to transduce the *minB zcf-117*::Tn*10* region into a wild-type strain by selecting for tetracycline resistance. Among tetracycline-resistant transductants from all four donor strains, about 50% produced minicells, strongly suggesting that the secondary mutations appearing in *hupAB* mutants were located at the *minB* locus.

The *minB* operon consists of three genes, *minC*, *minD*, and *minE*. To confirm that the secondary mutations were indeed at the *minB* locus and to determine which genes were affected, the Min^- transductants were lysogenized with λ phages carrying a series of IPTG-inducible p^{lac}::*minB* fusions: λp^{lac}::*minC*, λp^{lac}::*minD*, λp^{lac}::*minE*, and combinations of *min* genes (11). The tests were performed at 37°C in the hup^+ tetracycline-resistant transductants showing minicell production and carrying the *minB* region of the four *hupAB* mutants (GC7588, GC7796, GC7802, and GC7804). As shown in Table 2, induction of the MinC and MinD proteins without MinE inhibited cell division in all cases, as expected (11). The phage λp^{lac}::*minCDE*, carrying the entire *minB* operon, restored a Min^+ phenotype to all four transductants in the presence of the inducer IPTG (Table 2). Two transductants became Min^+ by induction of the MinC protein (GC7796 and GC7804), one became Min^+ by induction of the MinDE proteins (GC7588), and one became Min^+ only by induction of the MinCDE proteins (GC7802). These results confirm that the secondary mutations appearing in *hupAB* mutants were at the *minB* locus.

Additional experiments were carried out to determine the nature of the *min* mutations. PCR amplification of the *minCDE* operon using primers A and B (see Materials and Methods) produced a single DNA fragment of 2 kb, the expected length, when the template was chromosomal DNA extracted from PB103, GC7796, or GC7804. In the case of GC7802, this band was not produced but two predominant smaller bands of approximately 1 kb were observed on agarose gels (data not shown). This indicates that strain GC7802 carries a rearrangement of the *minCDE* operon, probably with a duplication of the sequences corresponding to the primers used in the amplification. We sequenced the *minC* gene of strains PB103, GC7796, and GC7804. The sequence obtained from PB103 was 100% identical to the sequence extracted from the data bank (g146868). We found that the *minC* gene in strains GC7796 and GC7804 had a single nucleotide change (G710A and G661A, respectively) resulting in an arginine-to-histidine and a glutamate-to-lysine substitution, respectively, in the MinC protein.

Growth of bacteriophage Mu on the *hupAB min* mutants. An intact HU protein is essential for the growth of bacteriophage Mu (21, 26). We wished to see whether the *min* mutations which accumulated in the four *hupAB* mutants restored Mu growth. The parental strains OHP176 and PB103, the hup^+ *min zcf-117*::Tn*10* transductants, and the four *hupAB* strains which had spontaneously mutated at the *minB* locus were tested for their capacity to grow phage Mu at 37°C. Plaque formation was not affected in the parental strains or the hup^+ *min* single mutants. The *hupAB min* mutants, in contrast, did not allow Mu to develop. Thus, the *min* mutations that accumulate spontaneously in *hupAB* mutants do not suppress the inability of Mu phage to develop in the absence of HU protein.

Restoration of a Min^+ phenotype in a *hupAB min* mutant. As described above, the *min* mutations from the *hupAB min* mutants could be corrected by induction of the appropriate Min proteins. To define the phenotype of the original *hupAB* min^+ mutant, the strain PB103 *hupAB minC101* (GC7797) was lysogenized with phage λp^{lac}::*minCDE* and analyzed in the presence and absence of the inducer IPTG; in the former condition, the mutant is phenotypically $HupAB^-$ Min^+. There was no difference in the colony-forming ability of this lysogen on LB plates with or without IPTG. The lysogen was grown for 6 h in LB medium at 37°C in the presence or absence of IPTG and monitored for cell morphology by microscope observation and for anucleate cell production by 4′,6-diamidino-2-phenylindole (DAPI) staining. In the absence of inducer, the cell population was typical of a *min* mutant, with anucleate minicells and nucleate cells of a range of sizes. At 4.5 h, 12.5% of the cells showed polar septa. At 6 h, DAPI staining revealed 20% anucleate normal-size rod-shaped cells. The culture grown in parallel in the presence of IPTG for 6 h showed 0.5% of cells with polar septa; it contained 18% anucleate normal-size rod-shaped cells, with the rest of the population consisting of normal-size and elongated nucleate cells, in some of which a defect in nucleoid partitioning could be observed. The population, although not like a wild-type strain, was not as filamentous as the original *hupAB* transductant. Other mutations may have accumulated. Alternatively, the increased MinCDE concentration, which affects DNA structure in the same way as the absence of Min proteins (35), could partially compensate for the defect of the *hupAB* mutant, as discussed below.

Replacement of the *minCDE105* allele by min^+ in the *hupAB minCDE105* mutant. Another way to restore the original Hup^- phenotype was to introduce the min^+ allele into a *hupAB min* mutant with P1 phage grown on a min^+ *zcf-117*::Tn*10* donor, selecting for tetracycline-resistant transductants. Strain OHP176 *hupAB minCDE105* was used as recipient. Among 16 tetracycline-resistant transductants, six had normal colony morphology and contained minicells; these had probably just received the Tn*10* transposon. Ten were flat colonies which did not contain minicells when observed in the microscope. After two successive purifications of eight flat colonies, one did not grow in liquid medium, five formed minicells and showed the typical phenotype of *min* mutants, and two contained a mixture of filaments and lysed cells. These results indicate that replacing the *minCDE105* mutation by the wild-type $minCDE^+$ genes makes the *hupAB* mutant again accumulate mutations (probably including *min* mutations); these new mutants were not analyzed.

It should be noted that the two ways of making *min* mutants

Min$^+$, lysogenization with λp^{lac}::*minCDE* and replacement of the mutated *min* gene by the wild type, are not necessarily equivalent in terms of the amount of MinCDE protein synthesized and, as just pointed out, an excess of Min proteins has an effect on DNA structure similar to that of the absence of Min proteins (35).

The absence of the MukB protein is incompatible with the absence of HU2. The MukB protein has been shown to participate in the control of partitioning of *E. coli* chromosomes. The *mukB*::*kan* deletion mutant is viable at low temperature and spontaneously produces anucleate normal-size, rod-shaped cells (19). As we have shown, *hupAB* derivatives also accumulate anucleate rod-shaped cells. To test whether defects in these two systems, HU and MukB, were additive, we tried to introduce the *hupA*::Cm mutation into the *mukB*::*kan* mutant (GC7528) by P1 transduction at 25°C, but without success. To demonstrate that the two genes *hupA* and *mukB* could not be simultaneously inactivated under conditions in which both single mutants are viable, we performed crosses using cotransduction with the nearby *purH*$^+$ marker as selection. P1 phage grown on the *hupA*::Cm *purH*$^+$ donor strain (GC7615) was used to transduce *purH mukB*$^+$ (GC7646) and *purH mukB*::*kan* (GC7664) recipient strains. With the *purH mukB*$^+$ recipient, among 34 Pur$^+$ transductants tested, 27 had become chloramphenicol resistant (*hupA*::Cm). In contrast, with the *purH mukB*::*kan* recipient, among 34 Pur$^+$ transductants, no chloramphenicol-resistant clones were found. These results lead us to conclude that in the absence of the MukB protein, *E. coli* needs the HU2 protein. This may reflect the fact that in the absence of HU2, the amount of HU1 is severely reduced, leaving the cell with very few active HU (homo)dimers (41).

Flow cytometric analysis. In wild-type strains, DNA initiation is well regulated, occurring simultaneously at all origins within each cell, exactly once per cell cycle in steady-state growth (31). When rounds of replication are allowed to run to completion, the number of chromosomes per cell is 2^n ($n = 0$, 1, 2, 3, etc.). When initiations are asynchronous, as in *dnaA*(Ts) initiation mutants at the permissive temperature and in the *dam* mutant (7, 46), the presence of a different number of chromosome equivalents (three, five, six, etc.) was detected by flow cytometry. Since the HU protein has been shown in vitro to stimulate initiation of chromosome replication at *oriC* (13), flow cytometry analysis was performed on a culture of the *hupAB minC101*(λp^{lac}::*minCDE*) strain grown in the presence of IPTG to induce the MinCDE proteins and make the mutant phenotypically Min$^+$. Ampicillin was added to the medium to maintain the λ prophage. Residual division could not be readily inhibited because of the *bla* gene carried by the λp^{lac}::*minCDE* prophage.

The single mutant PB103 *minC101*(λp^{lac}::*minCDE*), growing in the same conditions, was used as control. The *hupAB* lysogen had a lower growth rate than the *hupAB*$^+$ strain. Samples were treated with rifampin for 5 h to let ongoing rounds of replication terminate and then prepared for the flow cytometer as described in Materials and Methods. The *hupAB minC101* (λp^{lac}::*minCDE*) mutant exhibited a different distribution of DNA per cell than the *hup*$^+$ *minC101*(λp^{lac}::*minCDE*) lysogen, with an additional peak corresponding to three chromosome equivalents (Fig. 1). The presence of cells containing a number of chromosomes different from 2^n suggests that the *hupAB* mutant has a defect in the synchrony of replication initiation. The *hupAB* mutant population also had a higher proportion of cells containing only one chromosome equivalent than in the *hup*$^+$ control strain, possibly reflecting its lower growth rate.

The *hupAB* mutant is defective in chromosome partitioning, as shown by irregular nucleoid localization and the production of anucleate cells. The above results suggest that it also has a slight defect in synchronizing replication initiation.

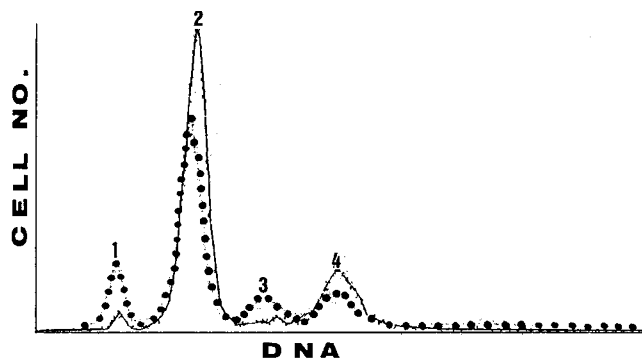

FIG. 1. Number of chromosome equivalents per cell measured by flow cytometry in cultures of PB103 *hupAB minC101*(λp^{lac}::*minCDE*) (●) and PB103 *minC101*(λp^{lac}::*minCDE*) (——) in the presence of IPTG. The number of chromosome equivalents is indicated above the peaks.

DISCUSSION

The nucleoid-binding proteins of *E. coli*, assimilated to histone-like proteins, include HU, H-NS, and IHF. They possess common features, among them the ability to bend DNA, inducing changes in nucleoid structure and affecting the expression of certain genes by promoting DNA structures that increase or decrease recognition by various regulatory proteins (17). These three histone-like proteins must have overlapping functions, since any two can be eliminated but simultaneous loss of all three is lethal (54).

In this work, we have focused our attention on the possible involvement of HU in chromosome partitioning in *E. coli*. Mutants deficient in HU or H-NS show a Muk$^-$ phenotype, with aberrant nucleoid positioning and the accumulation of anucleate rod-shaped cells (25). This phenotype is also observed in mutants with altered MukFEB proteins, which have been shown to participate in chromosome partitioning in *E. coli* (38, 53). In the case of *hupAB* mutants, anucleate cell production could have been caused either by an abnormal nucleoid structure per se or by altered expression of proteins involved in partitioning or septation.

Wild-type *E. coli* cells, in which replication is initiated synchronously at all *oriC* sequences present, normally contain 2^n origins ($n = 1$, 2, or 3), detected by flow cytometry as 2^n chromosomes after completion of replication rounds. The presence of cells with a number of origins different from a power of 2 (3, 5, or 6, etc.) can be indicative of lack of synchrony in replication initiation; this is the case, for example, with *dnaA*(Ts) and *dam* mutants (7, 46). The *hupAB* mutant also generated a significant proportion (10.8%) of cells with three origins (Fig. 1). The simplest interpretation would be that the HU protein, known to participate in replication initiation in vitro, is required for proper synchrony of initiation in vivo. Some caution is in order, however, because of the pleiotropic effects of loss of HU and the accumulation of undefined secondary mutations.

There is a relationship between HU and the MinB system, as shown by the fact that in the absence of both HU subunits, secondary mutations accumulate, among them *min* alleles. The spontaneous *min* mutations included *minC* and *minD* alleles and a rearrangement of the *minCDE* locus, showing that there is no specific Min protein that must be inactivated to compensate for the lack of HU. This is in contrast to suppressors of

overproduction of the *dicB* gene product (a division inhibitor [4]), which are all located in the *minC* gene (30, 36). The fact that *min* mutations appear spontaneously in some genetic backgrounds when HU is absent confirms our earlier observation that *hupAB* mutants accumulate secondary mutations (21). Recently, it was shown that specific alleles of the *gyrB* gene or overproduction of the GyrB protein can also compensate for the lack of HU protein (32). These *gyrB* mutations eliminate the relaxation caused by the absence of HU. The absence or overproduction of Min proteins also changes the superhelical density of reporter plasmids (35). These results suggest that the MinCDE proteins act together with gyrase in the maintenance of proper DNA superhelical density.

It is interesting that Malik et al. (32) did not find any *min* mutants among their *hupAB* transductants. This may reflect a strain difference (cf. our results with C600) or a bias in colony selection (medium versus large colony size). In our own study, we did not analyze non-minicell-producing *hupAB* transductants for possible *gyrB* mutations.

Several possibilities could explain the appearance of *min* mutations in the absence of HU. First, a change in chromosome superhelicity in primary *hupAB* transductants may increase the MinCD/MinE ratio, causing division inhibition observed in the original *hupAB* transductant that gave rise to strain GC7797, making the inactivation of MinC or MinD advantageous for *hupAB* mutants; this possibility is under investigation. Second, *min* mutations could affect the superhelicity of the nucleoid, changing the pattern of gene expression to one which is more advantageous to the *hupAB* mutant.

We show here that the inactivation of both MukB and HU2 proteins is lethal for the bacteria. In the absence of the MukB protein, cells are able to grow and divide at low temperature, although not at temperatures of 30°C or more (38); the additional inactivation of the HU2 subunit prevents the *mukB*::*kan* mutant from growing even at low temperature. It is conceivable that the MukB protein, like HU, is necessary to maintain correct chromosome topology.

Our results suggest that the HU protein, the Min proteins, and the MukB protein play interconnected roles in different steps of the *E. coli* life cycle, among them DNA replication initiation, nucleoid topology, nucleoid partitioning, and septation.

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