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Inhibition of Cell Division in *hupA hupB* Mutant Bacteria Lacking HU Protein†

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Escherichia coli hupA hupB **double mutants that lack HU protein have severe cellular defects in cell division, DNA folding, and DNA partitioning. Here we show that the *sfiA11* mutation, which alters the SfiA cell division inhibitor, reduces filamentation and production of anucleate cells in AB1157 *hupA hupB* strains. However, *lexA3*(Ind⁻) and *sfiB(ftsZ)114* mutations, which normally counteract the effect of the SfiA inhibitor, could not restore a normal morphology to *hupA hupB* mutant bacteria. The LexA repressor, which controls the expression of the *sfiA* gene, was present in *hupA hupB* mutant bacteria in concentrations half of those of the parent bacteria, but this decrease was independent of the specific cleavage of the LexA repressor by activated RecA protein. One possibility to account for the filamentous morphology of *hupA hupB* mutant bacteria is that the lack of HU protein alters the expression of specific genes, such as *lexA* and *fts* cell division genes.**

Protein HU is the most abundant DNA-binding protein in *Escherichia coli*, yet its biological function is not understood (5, 12, 40, 42, 44, 46, 51). Recent findings have revealed an involvement of HU protein in various reactions in vitro, including initiation of DNA replication, binding of repressors, and transposition of bacteriophage Mu (14, 31, 48, 55). In each of these processes, it has been proposed that the role of HU protein is to stabilize higher-order nucleoprotein structures, thereby conferring specificity in DNA transactions. Protein HU is composed of two closely related 10-kDa monomer subunits that form stable heterodimers in solution (4, 45). The *hupA* and *hupB* genes encoding the HU-α and HU-β subunits are located at 90.5 and 9.7 min, respectively, on the *E. coli* map (23, 52). Double mutants lacking HU protein were constructed, and cultures of *hupA hupB* mutant bacteria were found to contain filaments, anucleate cells, and a large fraction of cells that fail to form visible colonies (22, 23, 43, 52). Although these alterations imply that DNA partitioning and cell division are disturbed in the absence of HU protein, it is not clear how these alterations occur. In particular, a delay in cell division may result from at least four different processes affecting either temporal or topological regulation. (i) Filamentation occurs when some balanced expression of the essential cell division genes, which are clustered in the 2-min region of the map, is perturbed by conditional mutations or overexpression. The products of these genes are thought to act sequentially in the order FtsZ-FtsQ-FtsA-FtsI. The function of these proteins is unknown except for that of FtsI, the penicillin-binding protein 3, which is involved in septum formation (18, 27, 49). (ii) Filamentation may result from unbalanced expression of the MinC, MinD, and MinE division inhibitors, which normally prevent septation at cell poles but may also inhibit septation at all potential division sites when MinE protein is absent or *minC* or *minD* is overexpressed (10, 24). (iii) When DNA replication is transiently blocked, for example, by UV lesions, RecA protein is activated and promotes the cleavage of LexA protein and λcI repressor if the strain is lysogenic. Cleavage of λ repressor results in phage development and cell lysis, whereas cleavage of LexA repressor enhances the expression of several genes, including *lexA*, *recA*, and *sfiA* (30, 35, 47, 53). The SfiA protein interacts with the membrane protein FtsZ and thereby inhibits cell division (19, 21, 26). (iv) When the progression of the replication fork is not blocked but only slowed down, for example, during partial starvation for thymine or dNTP, the generation of putative signals required for cell division is delayed, resulting in an overall increase in cell length. Although the factors implicated in this process have not been identified, it is known that the activity of the *sfiA* gene is not required (7, 25, 50).

Although it has been shown that overproduction of one HU subunit (HU-α or HU-β) induces cell filamentation through activating RecA protein and expressing the SfiA inhibitor-dependent process (process iii) (43), it is not clear what mechanism is responsible for filamentation in bacteria lacking HU protein (22, 23, 52). Wada et al. (52) have recently suggested that filamentation in *hupA hupB* mutants is independent of the activation of RecA protein and may result from some alteration in DNA replication triggering the SfiA-independent process (process iv). On the basis of a variety of experimental approaches, we propose a different mechanism by which the lack of HU protein might perturb expression of cell division genes, thereby triggering cell filamentation and production of anucleate cells.

MATERIALS AND METHODS

Bacterial strains and phages. The bacterial strains used are listed in Table 1. Transduction with P1 *vir* followed procedures described by Miller (32). The presence of the *sfiA11* mutation in ENZ258 was confirmed by backcrossing it into a *recA441* strain and testing for suppression of filamentation at 42°C. Strain ENZ258 was lysogenized with phage λ p*sfiA*::*lac* as described by Huisman and D'Ari (20).

Media and growth conditions. Bacteria were grown in LB (32), LB-Glu (LB supplemented with 0.2% [wt/vol] glucose), or MOPS medium (36). Tetracycline hydrochloride (Boehringer Mannheim), chloramphenicol (Cm) (Sigma Chemical Co.), and kanamycin (Km) (Bristol Laboratories) were used at 12.5, 30, and 40 μg/ml, respectively. Growth was moni-

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† This paper is dedicated to the memory of Olivier Huisman.

TABLE 1. Bacterial strains

Strain	Relevant genotype	Reference or source
AB1157	F⁻ *thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2* λ⁻ *rac hisG4*(Oc) *rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	1
DM1623	*sfiA11* (*sulA211*)	15
DM7012	*malB45 zja-505*::Tn*10 lexA3*(Ind⁻)	38
ENZ134	AB1157 *sfiA100*::Tn*5 pyrD*	AB1157 × P1.GC2605
ENZ257	AB1157 *hupA*::Cm^r *hupB*::Km^r	AB1157 × P1.OHP109.OHP96
ENZ258	AB1157 *sfiA11*	ENZ134 × P1.DM1623
ENZ262	AB1157 *sfiA11 hupA*::Cm^r *hupB*::Km^r	ENZ258 × P1.OHP109.OHP96
ENZ271	AB1157 *sfiB*(*ftsZ*)*114 hupA*::Cm^r *hupB*::Km^r *leu*$^+$	GC2638 × P1.OHP109.OHP96
ENZ276	IC1637 *hupA*::Cm^r *hupB*::Km^r	IC1637 × P1.OHP109.OHP96
ENZ280	AB1157 *sfiA11* Δ*recA306*::Tn*10*	ENZ258 × P1.GY5902
ENZ281	AB1157 *sfiA11* Δ*recA306*::Tn*10 hupA*::Cm^r *hupB*::Km^r	ENZ262 × P1.GY5902
ENZ283	AB1157 *sfiA11 malB45 zja-505*::Tn*10 lexA3*(Ind⁻)	ENZ258 × P1.DM7012
ENZ296	AB1157 *malB45 zja-505*::Tn*10 lexA3*(Ind⁻)	AB1157 × P1.DM7012
ENZ298	AB1157 *sfiA11 malB45 zja-505*::Tn*10 lexA3*(Ind⁻) *hupA*::Cm^r *hupB*::Km^r	ENZ262 × P1.DM7012
ENZ343	AB1157 *lexA3*(Ind⁻) *hupA*::Cm^r *hupB*::Km^r	ENZ296 × P1.OHP109.OHP96
ENZ347	AB1157 *sfiA11*(λp*sfiA*::*lac*)	ENZ258 lysogenized with λp*sfiA*::*lac*
ENZ353	AB1157 *sfiA11*(λp*sfiA*::*lac*) *hupA*::Cm^r *hupB*::Km^r	ENZ347 × P1.OHP109.OHP96
ENZ356	AB1157 *sfiA11*(λp*sfiA*::*lac*) *malB45 zja-505*::Tn*10 lexA3*(Ind⁻)	ENZ347 × P1.DM7012
ENZ357	AB1157 *sfiA11*(λp*sfiA*::*lac*) *lexA3*(Ind⁻) *hupA*::Cm^r *hupB*::Km^r	ENZ356 × P1.OHP109.OHP96
GC2605	*sfiA100*::Tn*5 pyrD*	9
GC2638	AB1157 *sfiB*(*ftsZ*)*114 leu*$^+$	28
GY5902	Δ*recA306*::Tn*10*(pGY5353 *recA*$^+$)	13
IC1637	*thr leu thi proA argE3 galK sup-37 rspL sfiB*(*ftsZ*)*114*	3
OHP96	*hupB*::Km^r	22
OHP109	*hupA*::Cm^r	22

tored with a Perkin Elmer Lambda 1 spectrophotometer. All incubations were performed at 37°C.

Rate of DNA synthesis. Bacteria were grown in MOPS medium to approximately 10^8 bacteria per ml, and then 6 μCi of [*methyl*-^{3}H]thymidine per ml (86 Ci/mmol) (Amersham International plc) was added and incubation was continued for 10 min. Every 2 min, 400-μl samples were chilled in ice after the addition of 100 μl of cold 50% trichloroacetic acid. Precipitated counts were measured in a Packard scintillation counter.

Assay of *PsfiA*::*lacZ* fusion expression. Bacteria grown in LB-Glu into the late-logarithmic growth phase (optical density at 600 nm = 3.5 to 4.5; 6×10^8 to 8×10^8 bacteria per ml) were processed as described previously (34).

Immunoblotting procedures to measure LexA and RecA protein cellular levels. Bacteria grown in LB-Glu to an optical density at 600 nm of 3.5 to 4.5 were processed as described previously (33). For the quantitative determination of the amount of proteins, the blots were analyzed with a Digital Design image processor. RecA and LexA proteins were purified and antisera were raised in rabbits as described previously (33).

Fluorescence microscopy. Bacteria were grown in LB to an optical density at 600 nm of 1, fixed with 0.1% (wt/vol) OsO_4, and incubated for 20 min at room temperature in Veronal-acetate buffer (pH 6.0) containing 20 μM fluorochrome, Hoechst 33342 (Sigma Chemical Co.) (50). Bacteria were examined in a Reichert-Jung Polyvar microscope and photographed by using T-Max 400 films (Eastman Kodak Co.).

RESULTS

Suppression of filamentation by *sfiA11* mutation. The filamentous morphology of bacteria lacking HU protein is thought to be independent of the induction of the *lexA-recA-sfiA* regulon and hence of the activity of the SfiA division inhibitor (52). This conclusion is based on the observation that prophage λ is stably maintained in *hupA hupB* (λ) lysogens, suggesting that RecA protein-promoted cleavage of repressors such as λ*c*I protein and LexA protein is not enhanced in the absence of HU protein (52; data not shown). However, LexA protein being more susceptible to cleavage than is λ*c*I repressor (30), we wondered whether *hupA hupB* mutants could exist in a partly induced state, resulting in the induction of at least some of the LexA-controlled genes, such as *recA* and *sfiA*, while prophage λ remained in a dormant state (33, 39, 41, 53).

To test for a possible role for the *sfiA* gene product in filamentation in bacteria lacking HU protein, isogenic strains carrying mutations in *sfiA* and *hup* genes were constructed and examined by phase-contrast microscopy and by fluorescence microscopy to reveal DNA. The *sfiA11* mutation used has been shown to abolish specifically SfiA-dependent division inhibition (20, 28). Figure 1 shows that the introduction of *hupA* and *hupB* mutations altered, as expected, the morphology of AB1157 *sfiA*$^+$ bacteria. Most notably, the size of *hupA hupB* mutant bacteria was heterogeneous, ranging from approximately one to ten times the length of *hup*$^+$ cells, and DNA appeared unfolded. In contrast, in the AB1157 *sfiA11* strain, the introduction of *hupA* and *hupB* mutations did not lead to gross alterations in cell morphology but DNA was still generally unfolded (Fig. 2), suggesting that cell filamentation in *sfiA*$^+$ *hupA hupB* mutant bacteria may result from the induction of the *sfiA* gene following defects in DNA replication. One surprising aspect of the phenotype of *sfiA11 hupA hupB* mutant bacteria, however, was that the frequency of anucleate cells was lower in *sfiA11 hupA hupB* mutants (1.8%) than in *sfiA*$^+$ *hupA hupB* mutants (10%) (Table 2). Indeed, in contrast to our experimental results, the absence of SfiA-dependent division inhibition has been associated in various mutants with the production of anucleate rods (24, 25, 37).

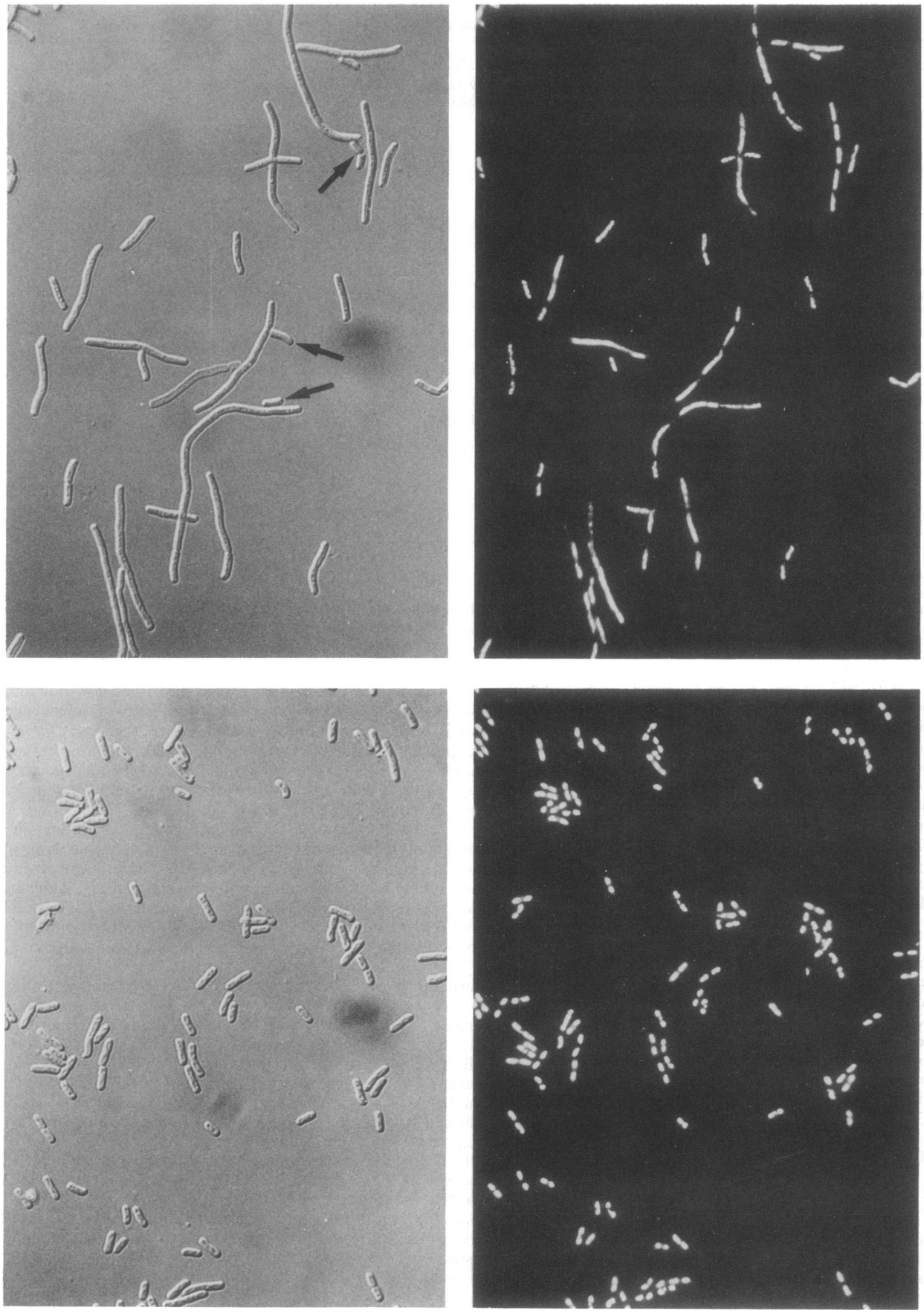

FIG. 1. Effect of *hupA hupB* mutations on cell morphology and nucleoid segregation. AB1157 (*hup*$^+$) (left panels) and ENZ257 (*hupA hupB*) (right panels) bacteria were subjected to light (upper panels) and fluorescence (lower panels) microscopic analysis. Arrows indicate anucleate cells. Bar, 10 μm.

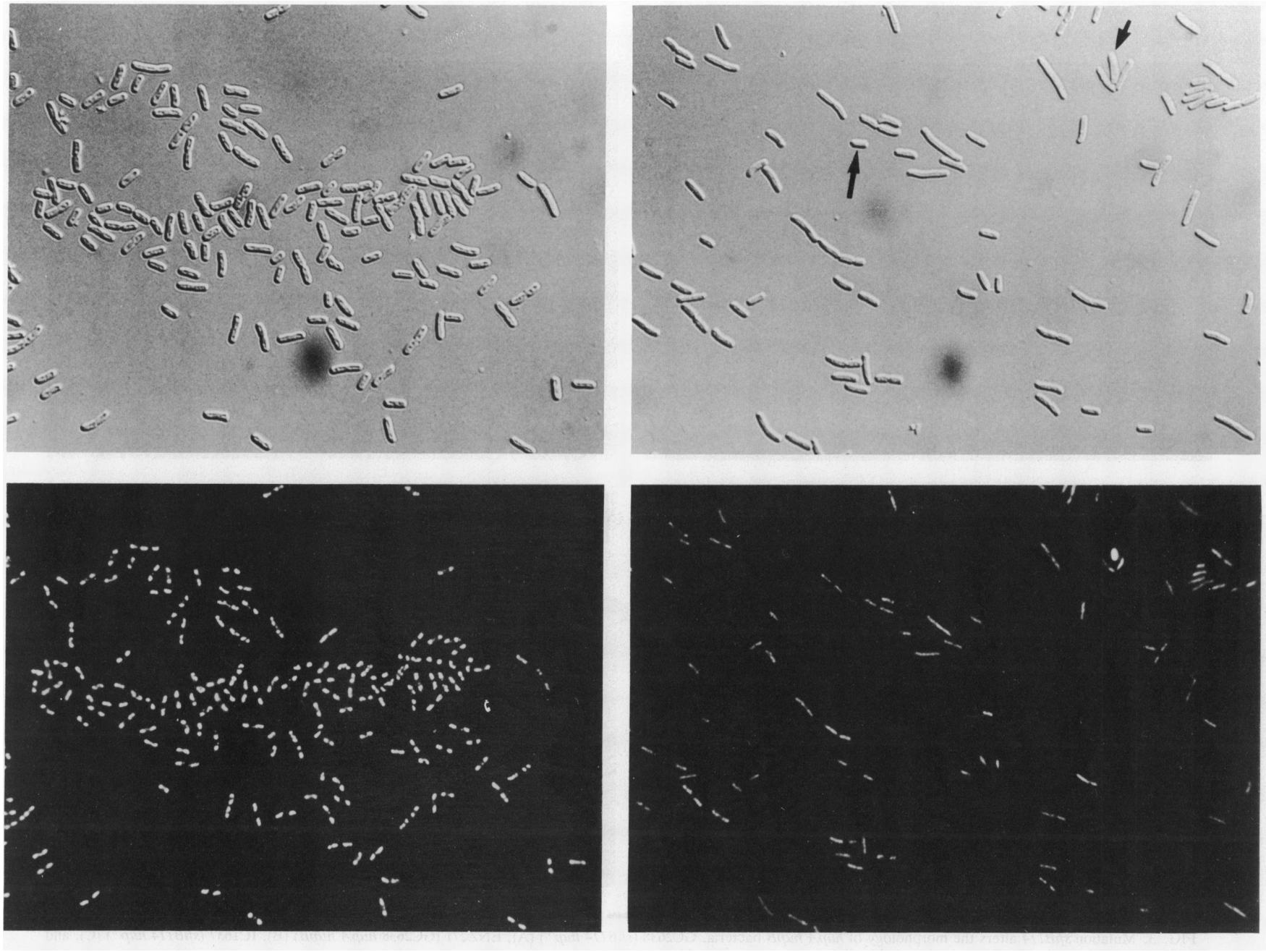

FIG. 2. Mutation *sfiA11* suppresses filamentation in a *hupA hupB* strain. ENZ258 (*sfiA11 hup*$^+$) (left panels) and ENZ262 (*sfiA11 hupA hupB*) (right panels) bacteria were observed by light and fluorescence microscopy. Arrows indicate abnormal cells. Bar, 10 μm.

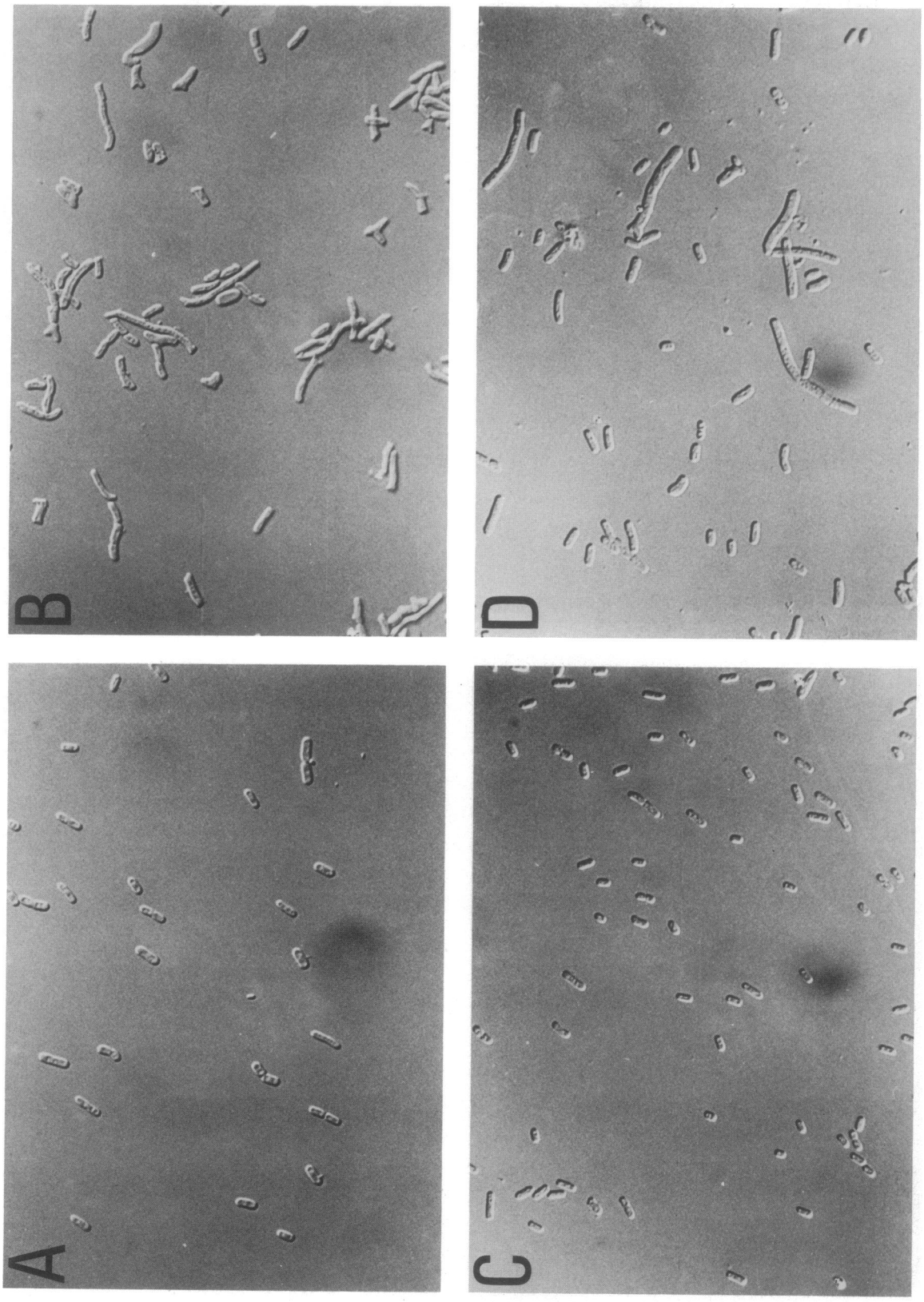

FIG. 3. Mutation *sfiB114* alters the morphology of *hupA hupB* bacteria. GC2638 (*sfiB114 hup*$^+$) (A), ENZ271 (GC2638 *hupA hupB*) (B), IC1637 (*sfiB114 hup*$^+$) (C), and ENZ276 (IC1637 *hupA hupB*) (D) were observed by light microscopy. Bar, 10 μm.

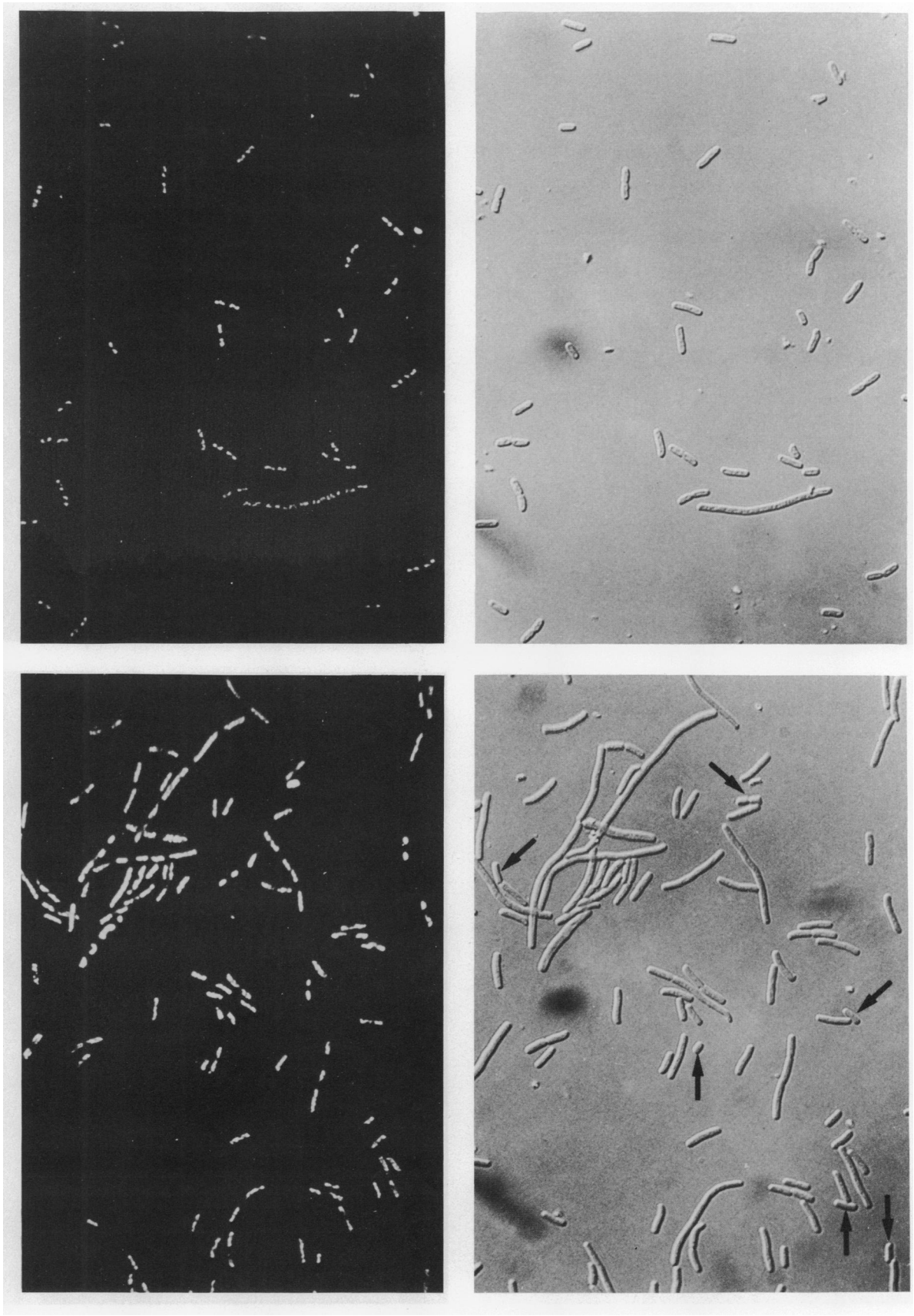

FIG. 4. Mutation *lexA3*(Ind$^-$) does not prevent filamentation of *hupA hupB* bacteria. ENZ296 (*lexA3 hup*$^+$) (left panels) and ENZ343 (*lexA3 hupA hupB*) (right panels) were observed by light (upper panels) and fluorescence (lower panels) microscopy. Arrows indicate anucleate cells. Bar, 10 μm.

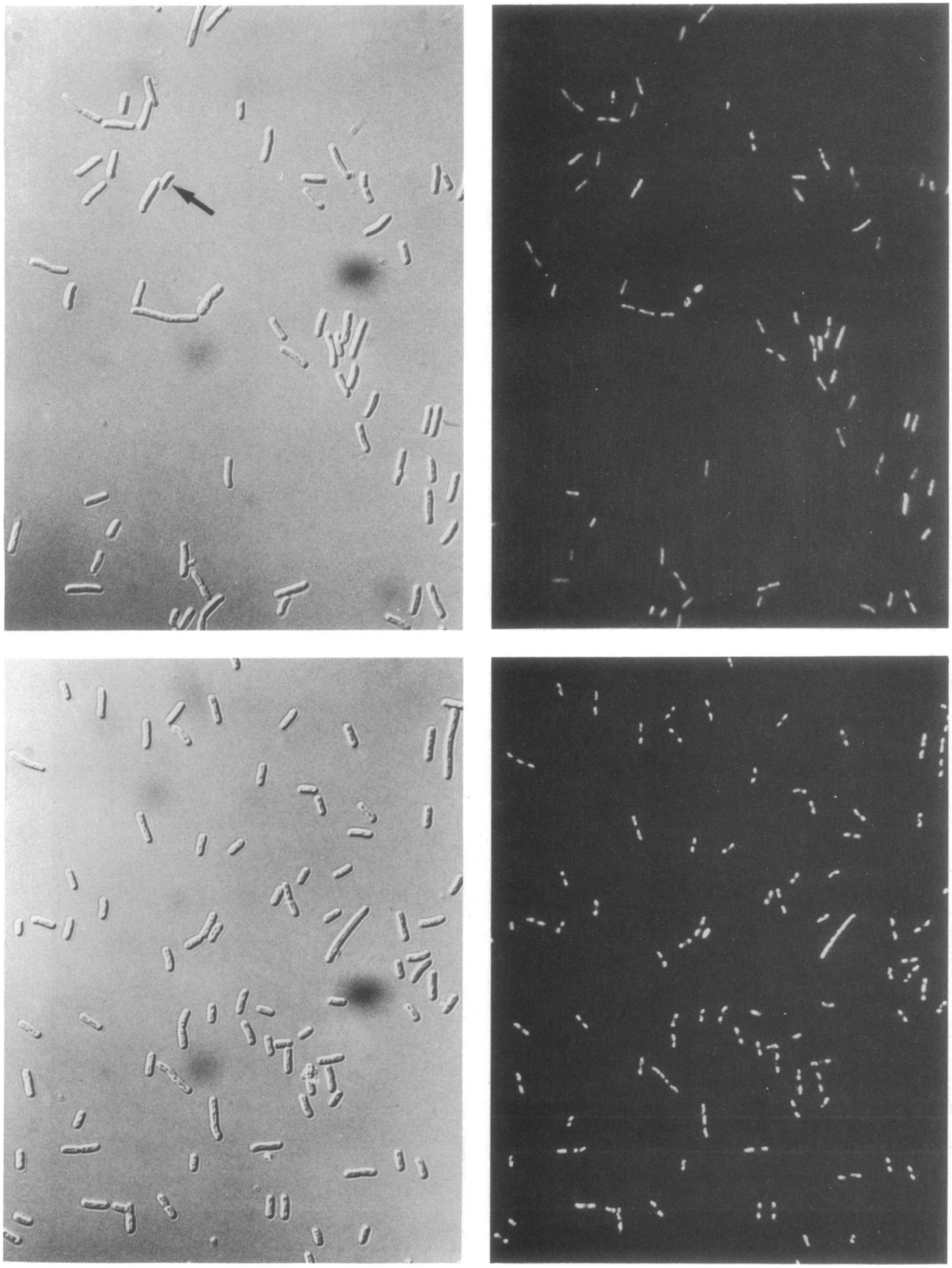

FIG. 5. Abnormal nucleoid segregation in *sfiA11 lexA3 hupA hupB* bacteria. ENZ283 (*sfiA11 lexA3 hup*$^+$) (left panels) and ENZ298 (*sfiA11 lexA3 hupA hupB*) (right panels) bacteria were observed by light and fluorescence microscopy. The arrow indicates an anucleate cell. Bar, 10 μm.

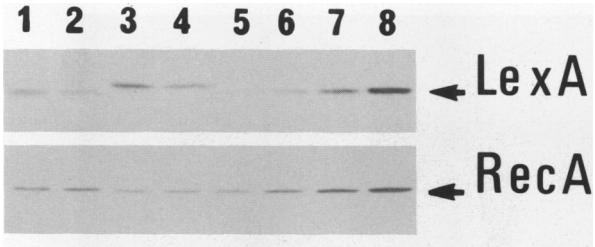

FIG. 6. Measurement of the cellular contents of RecA and LexA proteins by immunoelectrophoresis. Lanes: 1, ENZ258 (*sfiA11 hup*$^+$); 2, ENZ262 (*sfiA11 hupA hupB*); 3, ENZ283 (*sfiA11 lexA3 hup*$^+$); 4, ENZ298 (*sfiA11 lexA3 hupA hupB*); 5 to 8, purified LexA protein (2.5, 5, 10, and 20 ng) or purified RecA protein (1.25, 2.5, 5, and 10 ng). LexA$^+$ and LexA3 proteins have slightly different mobilities under the electrophoresis conditions used.

Effects of *lexA3*(Ind$^-$) and *sfiB114*(Rsa) mutations on cell morphology. If inhibition of cell filamentation by the *sfiA11* mutation truly reflects an overproduction of SfiA inhibitor in *sfiA*$^+$ *hupA hupB* bacteria, then cell filamentation should be inhibited in these strains by the introduction of two other mutations, *lexA3*(Ind$^-$) and *sfiB114*(Rsa). The *lexA3* mutation prevents cleavage of the LexA repressor and hence induction of LexA-controlled genes such as *sfiA* when DNA replication is perturbed and RecA protein is activated (Ind$^-$ phenotype) (29). The *sfiB114*(*ftsZ114*) mutation confers resistance to SfiA (Rsa phenotype) by altering the cell division membrane protein FtsZ, the target of the SfiA inhibitor (2). First, we found that the introduction of the *sfiB*(*ftsZ*)*114* mutation led to a dramatic alteration in the morphology of *sfiA*$^+$ *hupA hupB* bacteria. The same effect was found in strains ENZ271 and ENZ276, which have different genetic backgrounds (Fig. 3). Second, we found that the introduction of the *lexA3*(Ind$^-$) mutation failed to prevent filamentation and production of anucleate rods in *sfiA*$^+$ *hupA hupB* mutant bacteria (Fig. 4). However, introduction of the *lexA3*(Ind$^-$) mutation did not alter significantly the morphology of *sfiA11 hupA hupB* mutant bacteria (Fig. 5), indicating that induction of LexA-controlled genes is not required for growth of bacteria that lack HU protein.

Cellular levels of LexA repressor in *lexA*$^+$ and *lexA3*(Ind$^-$) derivatives. To test directly for the possibility that the *lexA* regulon might, however, be induced gratuitously in *hupA hupB* mutant bacteria, the cellular levels of LexA repressor in *sfiA11 lexA*$^+$ and *sfiA11 lexA3*(Ind$^-$) bacteria carrying or not carrying the *hupA* and *hupB* mutations were measured by immunoblotting techniques. The cellular level of RecA protein, measured directly by immunoblotting techniques, and the expression of the *sfiA* gene, measured by using phage λp*sfiA*::*lac* as a reporter, were also estimated in the same strains. Although the *sfiA* and *recA* genes are under the control of the LexA repressor, their regulations differ markedly in that the LexA repressor binds more tightly to the operator of the *sfiA* gene than to the operator of the *recA* gene, and following inactivation of the LexA protein, the derepression of the *sfiA* gene is at least 10 times stronger than that of the *recA* gene (33, 39). In other words, full induction of the *sfiA* gene as compared with that of the *recA* gene occurs at lower cellular levels of LexA repressor and is much stronger.

TABLE 2. Frequency of anucleate cells

Strain	Genotype		No. of anucleate cells/ total cells analyzed	Frequency (%)
	sfiA	*hup*		
AB1157	+	+	0/100	<1
ENZ257	+	*A,B*	73/711	10.3
ENZ258	*11*	+	0/100	<1
ENZ262	*11*	*A,B*	12/668	1.8

TABLE 3. Cellular levels of LexA and RecA proteins and expression of the *sfiA* gene in *sfiA11 hupA hupB* mutants

Expt	Genotype		Relative amt of protein		
	lexA	*hup*	LexA[a]	RecA[a]	SfiA[b]
A	+	+	1	1	1
	+	A,B	0.46	1.15	2.67
	3	+	1.22	0.50	0.41
	3	A,B	0.58	0.53	0.17
B	+	+	1 (0.49)[c]		
	+	A,B	0.52 (0.20)		

[a] Cellular levels of LexA and RecA proteins measured by immunoblotting techniques in ENZ258 (*sfiA11 hup*$^+$), ENZ262 (*sfiA11 hupA hupB*), ENZ283 (*sfiA11 lexA3 hup*$^+$), and ENZ298 (*sfiA11 lexA3 hupA hupB*). Values are relative to ENZ258 and are the averages of two independent experiments.

[b] Expression of *sfiA-lacZ* operon fusion measured by assay of β-galactosidase in λp*sfiA*::*lac* lysogens ENZ347 (*sfiA11 hup*$^+$), ENZ353 (*sfiA11 hupA hupB*), ENZ356 (*sfiA11 lexA3 hup*$^+$), and ENZ357 (*sfiA11 lexA3 hupA hupB*). Values are relative to ENZ347 and are the averages of four independent experiments.

[c] In experiment B, cellular levels of LexA protein measured by immunoblotting techniques in ENZ258 (*sfiA11 hup*$^+$) and ENZ262 (*sfiA11 hupA hupB*). Values in parentheses correspond to the levels of LexA measured after 1 h of incubation at 37°C in the presence of 100 μg of rifampin per ml. Values are relative to bacteria of strain ENZ258 not exposed to rifampin and are the averages of two independent experiments.

We found that the cellular level of LexA repressor was twofold lower in *hupA hupB* mutant bacteria than in *hup*$^+$ parent bacteria whether the LexA repressor was susceptible to cleavage (Ind$^+$) or not (Ind$^-$) (Fig. 6; Table 3). This therefore suggested that the decrease in LexA protein levels observed in *hupA hupB* bacteria was independent of the specific cleavage of the LexA repressor and hence of the activation state of the RecA protein. This conclusion was supported by two other observations. First, we found that the half-life of the LexA repressor, measured by its stability while protein synthesis was inhibited by rifampin, was identical in *hup*$^+$ and *hupA hupB* bacteria (Table 3). In contrast, it has been shown that the half-life of the LexA protein measured under similar conditions was shortened in *uvrA6* mutant bacteria, presumably because of a higher basal level of activated RecA protein in this repair-deficient strain (47). Second, we observed that the rates of DNA synthesis were identical in *hup*$^+$ and *hupA hupB* bacteria, ruling out the hypothesis that DNA replication may be severely affected in bacteria lacking HU protein (data not shown).

If not due to a high rate of cleavage, the low cellular levels of LexA repressor found in *hupA hupB* mutant bacteria might be simply explained by a stronger repression of the *lexA* gene by the LexA repressor. Such a hypothesis, which is in good agreement with the observation of Flashner and Gralla (14) that HU protein may facilitate or inhibit binding of repressors in vitro, may also help explain the poor induction of the *sfiA* and *recA* genes found in *lexA*$^+$ *hupA hupB* mutants (Table 3) (33, 39): derepression of the *sfiA* and *recA* genes, which should result from the low cellular level of LexA repressor present in *hupA hupB* mutants, might be partly alleviated by a tighter binding of the repressor. This

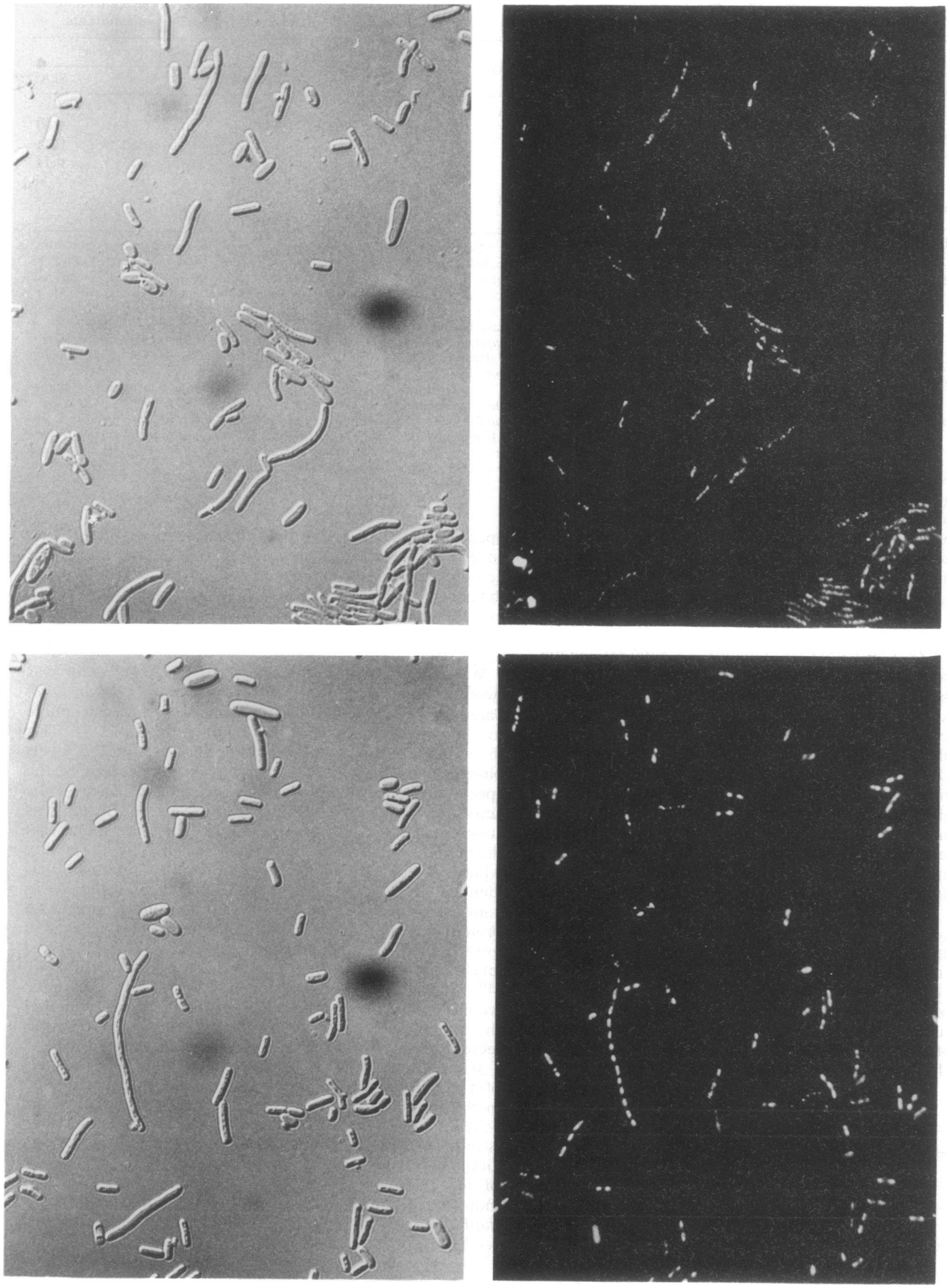

FIG. 7. Mutation *ΔrecA306* does not suppress the effect of an *sfiA11* mutation. ENZ280 (*sfiA11 ΔrecA306 hup*$^+$) (left panels) and ENZ281 (*sfiA11 ΔrecA306 hupA hupB*) (right panels) bacteria were observed by light and fluorescence microscopy. Bar, 10 μm.

notion was further supported by the results found in *lexA3* mutants, in which cellular levels of LexA repressor were higher than in the corresponding *lexA*$^+$ derivatives, probably because the LexA3 mutant repressor is resistant to the basal level of activated RecA protein in the cells (29). Indeed, in *lexA3 hupA hupB* mutant bacteria, induction of the *sfiA* gene, whose promoter intrinsically binds LexA repressor tightly, appeared to be totally overcome by repression (Table 3).

Introduction of the Δ*recA306* mutation did not prevent suppression of filamentation by *sfiA11*. Could the *sfiA11* mutation prevent cell filamentation even though the *sfiA* gene is induced weakly, if at all? Indeed, this situation has been reported in bacteria suffering from cell wall damage but, for unknown reasons, this suppression was dependent upon the expression of the *recA*$^+$ gene (8). That a similar process might be operating in bacteria lacking HU protein appeared unlikely. Although analysis in the *sfiA11* Δ*recA306* background was complicated by the fact that all strains produced elongated and numerous DNA-less cells, it was clear that introduction of the Δ*recA306* mutation in *sfiA11 hupA hupB* bacteria did not restore a morphology as filamentous as in *sfiA*$^+$ *hupA hupB* mutant bacteria (Fig. 7).

DISCUSSION

We show here that the *sfiA11* mutation reduces filamentation of bacteria lacking HU protein, even though overproduction of the SfiA division inhibitor is probably not the cause of cell filamentation. In fact, *lexA3*(Ind$^-$) and *sfiB* (*ftsZ*)*114* (Rsa) mutations, which normally counteract the overproduction of SfiA inhibitor by, respectively, preventing *sfiA* gene induction and the interaction of SfiA protein with its target, the FtsZ membrane protein, could not restore a normal morphology to *hupA hupB* mutant bacteria. Moreover, the presence of the *sfiB*(*ftsZ*)*114* (Rsa) mutation, along with the *hupA hupB* mutations, gave rise to a very abnormal cell morphology. Considering in particular the contrasting effects of *sfiA11* and *sfiB*(*ftsZ*)*114* mutations in *hupA hupB* mutant bacteria, we propose that the lack of HU protein might perturb the expression of the cell division machinery, hence triggering cell filamentation and production of anucleate cells.

The role of most of the cell division proteins is unknown, yet there is evidence that FtsI, FtsQ, FtsA, and FtsZ proteins form a complex which synthesizes the new septum during cell division (18). Unbalanced expression of these proteins leads to cell filamentation (27, 49, 54). Such imbalance in bacteria lacking HU protein might be compensated for by the presence of the SfiA11 protein (or rather by the absence of functional SfiA$^+$ protein), whereas it would be dramatically increased by the presence of the FstZ114 altered protein. In fact, the phenotype of *ftsZ114 hupA hupB* mutant bacteria is reminiscent of that of the new mutant *ftsZ3*(Rsa), whose FtsZ altered protein normally prevents cell growth (2).

Unbalanced expression of FtsI, FtsQ, FtsA, and FtsZ proteins may result from several causes, such as mutations in *fts* genes (18, 27), overproduction of FtsA protein (54), and defects in the localization of Fts proteins at the division site (17). Some of the proteins needed as a molecular chaperone to position the cell division proteins into the cell membrane apparently belong to the heat shock regulon (17). In fact, *dnaK* mutant bacteria share many common morphological features with bacteria lacking HU protein (6). However, we can exclude the possibility that the lack of HU protein alters significantly the expression of heat shock proteins such as DnaK and Lon (protease La) since (i) phage λ lysogenized and grew efficiently on *hupA hupB* mutants (unpublished data), whereas it does not grow on *dnaK* mutants (11) and it lysogenizes *lon* mutants poorly (16), and (ii) *hupA hupB* mutants did not have a mucoid morphology when grown on minimal medium at 28°C, in contrast to *lon* mutant bacteria (16). On the other hand, the notion that the expression of Fts proteins may be perturbed because of defects in gene expression rather than in cellular localization is supported by the observation that the expression of the *ftsZ* gene is somewhat altered in *hupA hupB* mutants (unpublished data).

A clue as to how the lack of HU protein might perturb the synthesis of cell division proteins may be given by our finding that cellular levels of LexA protein in *hupA hupB* mutant bacteria were half of those in *hup*$^+$ parents. This apparent decrease was independent of the half-life of LexA repressor, and the same decrease was found in all *hupA hupB* mutants whether the repressor was sensitive to proteolytic cleavage (LexA Ind$^+$) or not (LexA3 Ind$^-$). These results therefore suggest that the expression of the *lexA* gene was hampered in *hupA hupB* mutant bacteria, although the mechanism whereby this occurs remains to be elucidated. However, since the *sfiA* and *recA* genes, which are under the negative control of the LexA repressor, also appeared to be underexpressed in *hupA hupB* mutant bacteria, a simple interpretation of these results is that expression of the genes which belong to the *lexA* regulon is lower than expected in *hupA hupB* mutants because of a stronger repression by the LexA repressor. In this light, cell filamentation in *hupA hupB* mutants might be simply explained by the hypothesis that the lack of HU protein also alters the expression of *fts* genes since it has been shown that a LexA-binding site resides in the *ftsI* region (27).

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