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Chromosomal Genes Essential for Stable Maintenance of the Mini-F Plasmid in *Escherichia coli*

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We have isolated mutants of *Escherichia coli* which do not support stable maintenance of mini-F plasmids (Δ*ccd rep*$^+$ *sop*$^+$). These host mutations, named *hop*, were classified into five linkage groups on the *E. coli* chromosome. Genetic analyses of these *hop* mutations by Hfr mating and P1 transduction showed their loci on the *E. coli* genetic map to be as follows: *hopA* in the *gyrB-tnaA* region, *hopB* in the *bglB-oriC* region, *hopD* between 8 and 15 min, and *hopE* in the *argA-thyA* region. Kinetics of stability of the *sop*$^+$ and Δ*sop* mini-F plasmids in these *hop* mutants suggest that the *hopA* mutants are defective in partitioning of mini-F rather than in plasmid replication. The *hopB*, *hopC*, and *hopD* mutants were partially defective in replication of mini-F. The physical structure of the plasmid DNA was normal in *hopA*, *B*, *C*, and *D* mutants. Large amounts of linear multimers of plasmid DNA accumulated in mutants of the fifth linkage group (*hopE*). None of the *hop* mutations in any linkage group affected the normal growth of cells.

The F and mini-F plasmids of *Escherichia coli* are stably maintained during cell division, despite the low copy number per cell (for review, see references 14, 16, and 20). Mini-F plasmids, which were constructed in vitro from the 9.6-kilobase (kb) *Eco*RI-generated f5 fragment of the F plasmid, have a partition mechanism which guarantees the exact partitioning of plasmid DNA molecules into both daughter cells at cell division. The partitioning is controlled by the products of the plasmid genes *sopA* and *sopB* and the DNA sequence of the *cis*-acting *sopC* region of the plasmid (13, 22, 29). The DNA sequence of the entire region involving *sopA*, *sopB*, and *sopC* has been described (27). In the *sopC* region, 12 direct repeats of the 43-base-pair (bp) motif were found (13, 27). The product of the *sopB* gene binds specifically to the *sopC* region (12, 21).

In the present paper, we describe the isolation and characterization of host chromosomal mutants which do not support stable maintenance of mini-F plasmids.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. Derivatives of *E. coli* K-12 used are shown in Table 1. Mini-F plasmids pXX325 and pXX326, which have the partition segment carrying the *sopA*, *sopB*, and *sopC* genes but not the *ccd* segment, were described previously (30). Mini-F plasmid pXX327, which lacks both the *sop* and *ccd* segments, was also described previously (15, 30). These mini-F plasmids carry the *bla* gene conferring ampicillin resistance. P1 plasmid λ-P1:5R *c*I857 *D*am Km^r was provided by Michael B. Yarmolinsky. This plasmid does not have the postsegregational killing mechanism of plasmid-free segregants. pUC13 (36), pSC101 (9, 10), pOU47 (11), and pKP1033 (25) were used. Phage λ *imm*^{21} *precA*$^+$ was obtained from Mutsuo Imai. Phage P1 *vir* was used for transduction.

Media. L medium (1% Bacto Tryptone [Difco Laboratories], 0.5% yeast extract, 0.5% NaCl, pH 7.4) and P medium (1% polypeptone, 0.5% NaCl, pH 7.4), which were supplemented with thymine (50 μg/ml), were used. In pyrimidine-requiring mutants, uracil (50 μg/ml) was added to P medium. A synthetic minimal medium, medium E (37), supplemented with 0.5% glucose and appropriate chemical compounds, was used to test requirements of cells. To test expression of β-galactosidase, X-gal plates (P medium containing 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside [X-gal] per ml) were used. For agar plates, 1.4% (wt/vol) agar was added. Antibiotics were used at the following concentrations: ampicillin, 25 μg/ml; tetracycline, 15 μg/ml; streptomycin, 100 μg/ml; kanamycin, 20 μg/ml.

Construction of plasmid pXX704. Mini-F plasmid pXX704, which carries the *lacI* gene of *E. coli*, was constructed by in vitro DNA recombination techniques (23) as shown in Fig. 1. First, the chromosomal DNA prepared from *E. coli* KN732 was digested with *Hin*dIII and fractionated by electrophoresis to isolate the DNA segment carrying the *lacI* gene and the *lacZYA* operon. DNA fragments of more than 5 kb were isolated, ligated with the *Hin*dIII-digested pUC13 DNA, and used for transformation of the Δ*lac* strain HI1006. Ampicillin-resistant dark blue colonies of transformants were selected on X-gal agar plates containing ampicillin (25 μg/ml). The resulting plasmid, named pXX701, was found to carry the *lacI* gene and the *lacZYA* operon. To subclone the segment containing the *lacI* gene but not the *lac* operon, pXX701 DNA was digested with *Hpa*I, and the 4.2-kb DNA segment containing the *lacI* gene was isolated and inserted into the *Sma*I site of pUC13. The resulting plasmid, named pXX703, was digested with *Eco*RI and *Bam*HI. The *Eco*RI-*Bam*HI segment containing the *lacI* gene was ligated with mini-F plasmid pXX326 DNA digested with *Eco*RI and *Bam*HI. The resulting plasmid, named pXX704, was used for this study. The pXX704 plasmid has the *sopA*, *sopB*, and *sopC* genes, which are essential for equipartitioning of plasmid DNA molecules into daughter cells in cell division. Since this plasmid lacks the *ccdA* and *ccdB* genes which control the postsegregational killing of plasmid-free segregants (15, 18), plasmid-free segregants are not killed by the *ccd* mechanism. These properties of pXX704 facilitate anal-

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TABLE 1. Bacterial strains

Strain	Genetic markers	Reference or source
KZ344	F⁻ *ilv thr trp metE tyr proA* (or *proB*) *rpsL lacI thy* (low) *recA1*	This laboratory
SH2700	F⁻; as KZ344 but *recA3639* (Rec⁺ spontaneous revertant)	This work
SH2722	F⁻; as KZ344 but *hopA18*	This laboratory
SH3014	F⁻; as SH2722 but *recA*⁺	This work
SH3018	F⁻; as SH2700 but *recA*⁺	This work
SH3192	F⁻ *pyrE41 zic-11*::Tn*10 uhp-2 tna-2 trpB*	This work
SH3194	F⁻ *hopA18 met gal lacY hsdR supE sfiC*	This work
SH3503	F⁻; as SH2700 but *hopB181 ts-1810*	This work
SH3504	F⁻; as SH3503 but *recA*⁺	This work
SH3506	F⁻; as SH2700 but *hopA383 ts-3830*	This work
SH3507	F⁻; as SH3506 but *recA*⁺ *srl*::Tn*10*	This work
SH3606	HfrC; as CSH61 but *lacI*	P1[SH2700] × CSH61,[a] LacI⁻ selection
SH3607	Hfr; as CSH74 but *lacI*	P1[SH2700] × CSH74, LacI⁻ selection
SH3650	F⁻; as SH2700 but *hopE319*	This work
SH3651	F⁻; as SH2700 but *hop-325*	This work
SH3652	F⁻; as SH2700 but *hopC351*	This work
SH3653	F⁻; as SH2700 but *hopE355*	This work
SH3654	F⁻; as SH2700 but *hopE365*	This work
SH3655	F⁻; as SH2700 but *hop-384*	This work
SH3656	F⁻; as SH2700 but *hopD410*	This work
SH3679	F⁻; as SH3651 but (λ *imm*^{21} *precA*⁺)	This work
SH3680	F⁻; as SH3652 but (λ *imm*^{21} *precA*⁺)	This work
SH3681	F⁻; as SH3653 but (λ *imm*^{21} *precA*⁺)	This work
SH3682	F⁻; as SH3654 but (λ *imm*^{21} *precA*⁺)	This work
SH3683	F⁻; as SH3655 but (λ *imm*^{21} *precA*⁺)	This work
SH3684	F⁻; as SH3656 but (λ *imm*^{21} *precA*⁺)	This work
CGSC4820	F⁻ *bglB bglR tna sup* λ^{r}	B. Bachmann
CGSC5015	Hfr (o-*tsx* . . . *lac*) *metB1 relA1 spoT1* λ^{r}	B. Bachmann
CSH55	F⁻ Δ(*lac-pro*) *supD nalA thi*	Cold Spring Harbor Laboratory
CSH61	HfrC (o-*purE* . . . *lip*) *trpR thi*	Cold Spring Harbor Laboratory
CSH74	Hfr (o-*lysA* . . . *argG*) *thi*	Cold Spring Harbor Laboratory
CSH78	Hfr (o-*malB* . . . *melB*) *sup-53 malB16* λ^{r}	Cold Spring Harbor Laboratory
HI1006	F⁻ *araD139* Δ(*ara-leu*)*7697* Δ(*lac*)*X74 galU galK rpsL thyA38*(Oc) *recA1*	Casadaban and Cohen (7)
KN732	F⁻ *dnaG2903 thr ilv his trpE9829*(Am) *tyr*(Am) *thyA sup-126*	T. Nagata

[a] P1[donor strain] × recipient strain.

ysis of plasmid maintenance. Plasmid DNA was extracted from cells by the alkaline lysis method (17).

Isolation of host chromosomal mutants defective in stable inheritance of the mini-F plasmids. The cells of strain SH2700 carrying plasmid pXX704 were grown exponentially in L medium containing ampicillin and treated with 5% ethylmethane sulfonate for 20 min at 37°C. The cells were collected by centrifugation and washed twice with saline to remove the mutagen. The washed cells were suspended in the same medium, diluted, divided into 100 tubes, and incubated overnight at 37°C. Each culture was diluted and spread on X-gal plates containing ampicillin. After overnight incubation of the plates, 1,200 pale blue mutant colonies were isolated and spread again on X-gal plates not containing ampicillin to confirm the color of colonies. One hundred clones were desired mutants which formed pale blue colonies having large numbers of blue sectors. Under these nonselective conditions, mutants form colonies in which plasmid-free segregants can be accumulated frequently in addition to plasmid-carrying cells; therefore, these colonies become pale blue and have a lot of blue sectors, since plasmid-free segregants produce β-galactosidase constitutively. Under the same conditions, the parental strain forms white colonies with only a few blue sectors and plasmid-free segregants form dark blue colonies.

Assay of plasmid stability. Maintenance of plasmids in host bacterial cells was measured in the absence of selective pressure as described by Ogura and Hiraga (29). L medium supplemented with 0.5% glucose and thymine (50 μg/ml) was used to test the kinetics of plasmid stability.

Mapping of mutations by Hfr mating. Mutant strains carrying plasmid pXX704 were used as recipients for Hfr mating. After matings with each Hfr strain, recombinants were selected. After purification, recombinants were tested for color of colonies grown on X-gal plates by the replica method. Recombinants showing the pale blue color were judged as being of the Hop⁻ phenotype. To confirm the Hop⁻ phenotype, the recombinant cells were purified on X-gal plates and the colonies were observed by a binocular zoom microscope (Olympus SZH-141, 7.5× to 64×). In some cases, white colonies of recombinants without blue sectors were also observed. These white clones may be due to integration of pXX704 into the *E. coli* chromosome at the homologous *lac* region, since circular plasmid DNA molecules could not be detected in these clones. Therefore, these white clones were not scored. Alternatively, in some Hfr crosses recombinants were judged for the Hop phenotype by the result of the kinetics of stability of the mini-F plasmid pXX325 in nonselective medium.

Mapping of mutations by transduction with phage P1 *vir*. Phage P1 *vir* grown in mutant strains was prepared and used for transduction. Bacterial strains carrying the mini-F plas-

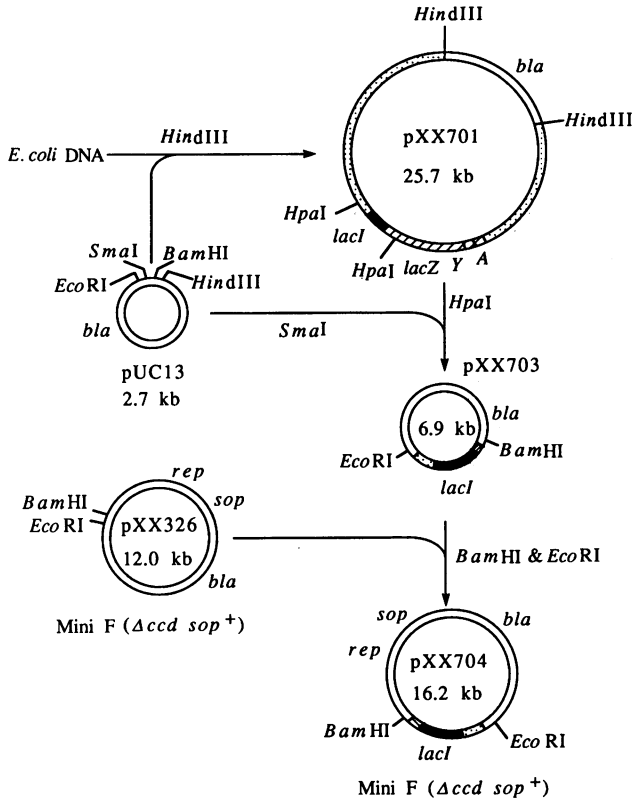

FIG. 1. Construction of the mini-F plasmid pXX704, carrying the *lacI* gene. Open bars, Vector DNA region; stippled bars, *E. coli* chromosome DNA region; hatched bars, *lac* operon region; solid bars, *lacI* gene.

mid pXX325 were used as recipients of transduction. Transductants were purified, analyzed for the stability of plasmid pXX325, and judged for the Hop phenotype.

Analysis of physical state of plasmid DNA. Bacterial cells harboring pXX325 grown exponentially at 37°C in L medium containing glucose (0.5%), thymine (50 μg/ml), and ampicillin (25 μg/ml) were collected by centrifugation, and total DNA was extracted by the method of Projan et al. (32). The DNA samples were analyzed by electrophoresis and Southern hybridization with ^{32}P-labeled DNA of plasmid pKP1033, which is a composite of pBR322 and mini-F (25).

Analysis of cell size distribution by flow cytometry. Flow cytometry procedures were described previously (18).

Microscopic observation of cells and nucleoids. Cells stained with 4',6-diamino-2-phenyl-indole were observed by a microscope.

RESULTS

Isolation of *E. coli* mutants defective in stable maintenance of mini-F plasmids. To define host functions essential for equipartition of plasmid DNA molecules into daughter cells during cell division, we isolated bacterial mutants which did not support stable maintenance of the mini-F plasmid pXX704 as described in Materials and Methods. We isolated 100 pale blue colonies having many blue sectors.

To test whether these mutants had a mutation on the *E. coli* chromosome or on pXX704, a plasmid-free segregant was isolated from each mutant and the mini-F plasmid pXX325, which had the *sop*$^+$ function, was introduced into the segregant and tested for plasmid stability in nonselective medium. Although pXX325 was stably maintained in the parental strain SH2700 (less than 2% plasmid loss over 12 h at 37°C), this plasmid was unstable in some mutants at various levels of stability. Mutants which showed only a slight reduction (less than 10% plasmid loss over 12 h) in plasmid stability were discarded. The remaining 11 host mutations were named *hop* (for *h*ost function *o*f *p*lasmid maintenance), and some of them were used for further experiments. The isolation and properties of the *hop-18* mutation were described previously (16).

Mapping of *hop* mutations by Hfr mating. To locate mutations in the *E. coli* chromosome, genetic analysis was performed by Hfr mating. *hop* mutants carrying pXX704 were used as the recipients in Hfr matings. Recombinants obtained by mating were purified and tested for the Hop$^-$ phenotype by observing the colony color on X-gal plates. The results are shown in Table 2. Mutation *hop-181* was located near the *ilv* operon (84 min). Mutation *hop-351* was located near the *thr* gene (0 min). Mutation *hop-410* was linked with the *proA* (or *proB*) gene (6 min) but not the *thr* gene. Mutations *hop-319*, *hop-355*, and *hop-365* were located near the *thyA* gene (61 min). Additional results in an Hfr cross indicate that *hop-410* was located between *lacI* (8 min) and the transfer origin of HfrC (15 min). Mutation *hop-325* was also located between 8 and 15 min. The *hop-18* and *hop-383* mutations were located near the *ilv* operon.

Fine mapping of *hop* mutations by transduction with phage P1 *vir*. *hop-181* and *hop-383* mutants showed remarked reduction of colony-forming ability at 42°C, 3×10^{-3} and $<10^{-3}$, respectively, on P agar plates. To test whether the temperature sensitivity and the Hop$^-$ phenotype were

TABLE 2. Mapping of *hop* mutations by Hfr mating

Recipient strain[a]	*hop* allele	Donor strain	No. of Hop$^+$ clones/no. tested that were also[b]:					
			Thy$^+$	Tyr$^+$	Trp$^+$	Pro$^+$	Thr$^+$	Ilv$^+$
SH3504	*hop-181*	SH3606	0/50	0/50	4/50	1/50	0/10	10/20
		CSH78	—[c]	—	—	—	—	58/200
SH3680	*hop-351*	SH3607	0/50	0/30	0/50	4/50	27/50	1/39
SH3684	*hop-410*	SH3607	0/49	1/100	1/50	35/40	1/24	—
SH3678	*hop-319*	SH3607	34/44	5/40	5/38	1/45	5/41	1/20
SH3681	*hop-355*	SH3607	41/48	—	6/42	—	—	—
SH3682	*hop-365*	SH3607	46/80	5/41	7/91	2/49	—	—
		CGSC5015	27/33	20/39	1/42	—	—	—

[a] The indicated strains harboring pXX704 were used as recipients in Hfr matings. Selective markers used are indicated.
[b] Counterselective markers were both streptomycin resistance and ampicillin resistance. Recombinants were purified and tested for the Hop phenotype on X-gal plates lacking ampicillin.
[c] —, Not done.

caused by a single mutation, we performed precise genetic analyses of these mutants by transduction with phage P1 *vir*. The Hop$^-$ phenotype and the temperature sensitivity of cell growth in the *hop-383* mutant cotransduced with the *ilv* marker; however, it was found that the two phenotypes were caused by different mutations, named *hop-383* and *ts-3830*, respectively. Both mutations were located on the counterclockwise side of *ilv* on the *E. coli* chromosome. The cotransduction frequency of *hop-383* and *ts-3830* with *ilv* was 9 and 52%, respectively.

To determine the locus of mutation *hop-181*, *hop-181* cells (SH3504) harboring pXX704 were infected with P1 *vir* phages prepared in *hop*$^+$ cells (CSH64), and 300 Ilv$^+$ transductants were isolated and tested for colony color on X-gal plates. Twenty-six transductants showed the Hop$^+$ phenotype (about 9% cotransduction frequency). However, all Ilv$^+$ transductants tested showed the Ts$^-$ phenotype, suggesting that the determinant of temperature sensitivity was located elsewhere than the *ilv* region. Furthermore, the *hop-181* cells (SH3504) harboring pXX325 were infected with P1 *vir* phage prepared in a *hop*$^+$ strain (CGSC4820). Ilv$^+$ transductants were isolated and tested for the BglR and MetE phenotypes. Among 10 Ilv$^+$ BglR$^+$ clones tested, 6 were Hop$^+$. However, all seven Ilv$^+$ Met$^+$ clones tested were Hop$^-$. The results suggest that *hop-181* is located counterclockwise of *ilv* but not between *ilv* and *metE*.

To know the locus of *hop-18*, the cells of a *hop*$^+$ *uhp* strain (SH3192) harboring pXX325 were infected with P1 *vir* phages prepared in the *hop-18* mutant (SH3194), and Uhp$^+$ transductants were selected and tested for the Hop phenotype by kinetics of stability of pXX325. The *hop-18* mutation was cotransduced with the *uhp* gene at a 3.5% cotransduction frequency. Furthermore, to know whether the *hop-18* mutation was located clockwise or counterclockwise to *uhp* on the *E. coli* chromosome, six Uhp$^+$ Tna$^+$ transductants were isolated and tested for the stability of pXX325. All Tna$^+$ Uhp$^+$ transductants showed the Hop$^-$ phenotype. This indicates that *hop-18* is located near *tnaA* (83 min), clockwise to *uhp*. The *hop-351* mutation was cotransduced with *thr* (0 min) at about 15%, but not with *pdxA* (1 min). On the other hand, *hop-319* was located between *argA* and *thyA* (60.5 to 61 min).

The results of genetic analyses of these mutants are summarized in Fig. 2. The *hop* mutants were classified into at least five linkage groups. We picked *hop-18*, *hop-181*, *hop-351*, *hop-410*, and *hop-319* from each linkage group and named them *hopA18*, *hopB181*, *hopC351*, *hopD410*, and *hopE319*, respectively. Mutation *hop-384* remains to be mapped.

Physical state of the pXX325 plasmid DNA in *hop* mutants. To test the physical state of plasmid DNA, the total DNA sample obtained from cells was analyzed by Southern hybridization. As shown in Fig. 3, most of the hybridizable pXX325 DNA was observed as supercoiled circular monomers in the parental *hop*$^+$ strain and the *hopA18*, *hopB181*, *hopC351*, *hop-325*, *hop-383*, *hop-384*, and *hopD410* mutants (data for *hop-325* are not shown), although bands corresponding to open circular monomers, linear monomers, and supercoiled circular dimers were also visible. In the other three mutants carrying *hopE319*, *hop-355*, and *hop-365*, a large amount of hybridizable DNA which appeared as a broad band of high molecular weight was observed, in addition to supercoiled circular monomers, open circular monomers, linear monomers, and supercoiled circular dimers (Fig. 3). The amount of circular monomers in lanes 8 and 9 was smaller than that in the other lanes in the gel

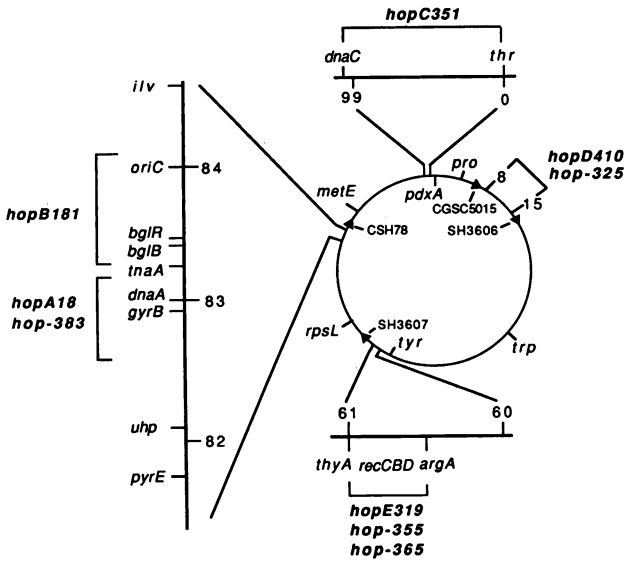

FIG. 2. Loci of the *hop* mutations on the *E. coli* chromosome. Loci of authentic genetic markers are according to Bachmann (5). The transfer origin and direction of the Hfr strains used are shown as arrowheads in the chromosome. Numbers indicate *E. coli* chromosome map locations in minutes.

shown in Fig. 3. However, this result is not sufficient to give the plasmid copy number per plasmid-carrying cell of these strains.

Stability of mini-F plasmids pXX325 and pXX327. If a host mutant is defective in a function essential for partitioning of the mini-F plasmid pXX325 but not for replication, this plasmid will become unstable to almost the same level as pXX327, which lacks the *sopABC* segment, and furthermore the stability of pXX327 will hardly be affected by the host mutation. Based on this hypothesis, we analyzed *hop* mutants for stabilities of pXX325 and pXX327 in nonselective medium. As shown in Table 3, the time required for 50% loss of pXX325 and pXX327 was more than 350 h and 8.8 ± 2.2 h (mean and standard deviation), respectively, in the *hop*$^+$

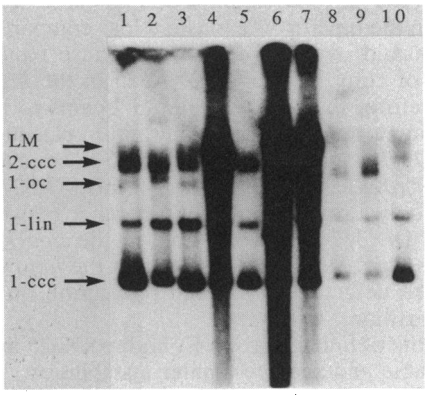

FIG. 3. Southern analysis of pXX325 plasmid DNA in the *hop*$^+$ parental strain and various *hop* mutants. Lanes: 1, SH2700 (*hop*$^+$); 2, SH2722 (*hopA18*); 3, SH3503 (*hopB181*); 4, SH3650 (*hopE319*); 5, SH3652 (*hopC351*); 6, SH3653 (*hop-355*); 7, SH3654 (*hop-365*); 8, SH3506 (*hop-383*); 9, SH3655 (*hop-384*); 10, SH3656 (*hopD410*). 1-ccc, Supercoiled closed circular monomers; 1-lin, linear monomers; 1-oc, open circle monomers; 2-ccc, supercoiled closed circular dimers; LM, linear multimers.

TABLE 3. Stability of the mini-F plasmids pXX325 and pXX327 in *hop* mutants[a]

Host strain	*hop* allele	Mean time required for 50% plasmid loss (h) ± SD	
		pXX325 (*sop*$^+$)	pXX327 (Δ*sop*)
SH2700	*hop*$^+$	>350	8.8 ± 2.2
SH2722	*hopA18*	7.6 ± 1.2	8.1 ± 0.66
SH3501	*hopB181*	2.1 ± 0.36	2.8 ± 0.65
SH3652	*hopC351*	20.0 ± 5.6	4.7 ± 0.99
SH3656	*hopD410*	4.4 ± 1.0	2.9 ± 0.31
SH3650	*hopE319*	16.5	16.0
SH3653	*hop-355*	14.0	11.0

[a] Bacterial cells harboring pXX325 or pXX327 were grown exponentially at 37°C in L medium supplemented with 0.5% glucose and analyzed for plasmid maintenance as described in Materials and Methods. The doubling time of SH2700 was about 40 min. Mutants also grew at similar doubling times. The mean and standard deviations were calculated for several experiments. Only a single experiment was carried out for the *hopE319* and *hop-355* mutants.

TABLE 4. Stability of a P1 plasmid, λ-P1:5R *c*I857 *D*am, in *hop* mutants[a]

Host strain	*hop* allele	Linkage group	Plasmid loss (%) over 20 h
SH2700	*hop*$^+$		3
SH2722	*hopA18*	A	0
SH3506	*hop-383*	A	1
SH3503	*hopB181*	B	1
SH3652	*hopC351*	C	3
SH3651	*hop-325*	D	3
SH3650	*hopE319*	E	13
SH3653	*hop-355*	E	16
SH3654	*hop-365*	E	18

[a] Bacterial strains carrying a P1 plasmid, λ-P1:5R *c*I857 *D*am, were grown at 30°C in L medium supplemented with 0.5% glucose, thymine (50 μg/ml), and kanamycin (20 μg/ml) and then diluted and incubated for 20 h at 30°C in medium lacking kanamycin.

parental strain. In the *hopA18* mutant, the time required for 50% loss of plasmids pXX325 and pXX327 was 7.6 ± 1.2 and 8.1 ± 0.66 h, respectively. Thus, there was no significant difference in stability between pXX325 and pXX327. In addition, the stability of pXX327 was not affected by the *hopA18* mutation. Similar results were also obtained with the *hop-383* mutant, which belonged to the same linkage group (A) as the *hopA18* mutant. These results suggest that the *hopA18* and *hop-383* mutants are defective in a host factor essential for partitioning of the mini-F plasmid but not for replication.

In the *hopB181* mutant, the time to 50% loss of plasmids pXX325 and pXX327 was 2.1 ± 0.36 and 2.8 ± 0.65 h, respectively. There was therefore no significant difference in stability between pXX325 and pXX327. The time to 50% loss of pXX327 in *hopB181* was about threefold less than that of pXX327 in the *hop*$^+$ strain. These results suggest that the *hopB181* mutant is partially defective in replication, but it is not clear whether it is also defective in partitioning.

By contrast, in the *hopC351* mutant, the time to 50% loss of plasmids pXX325 and pXX327 was 20.0 ± 5.6 and 4.7 ± 0.99 h, respectively. There was a large difference, about fourfold, between pXX325 and pXX327. In addition, pXX327 in the *hopC351* mutant was unstable: a ca. twofold decrease in the time to 50% plasmid loss compared with that of this plasmid in the *hop*$^+$ strain. These results suggest reduction of copy number of pXX325 in the *hopC351* mutant. Therefore, the *hopC351* mutant seems to be partially defective in plasmid replication but not in plasmid partition. In the *hopD410* mutant, the time to 50% plasmid loss was 4.4 ± 1.0 h for pXX325 and 2.9 ± 0.31 h for pXX327. Although there was only a slight significant difference between the two plasmids, pXX327 was less stable in the *hopD410* mutant than in the *hop*$^+$ strain, suggesting that the *hopD410* mutant may also be defective in plasmid replication rather than in plasmid partition.

In mutants of linkage group E, both pXX325 and pXX327 were unstable and showed similar instabilities. This can be explained by IncB, IncC, and IncD incompatibilities, which are caused by a large amount of linear multimer DNA, as described in the Discussion.

Stability of other plasmids in the *hop* mutants. The mini-P1 plasmid has its own genes controlling plasmid partitioning (1–4). To test for the stability of the mini-P1 plasmid in these *hop* mutants, bacterial cells carrying the P1 plasmid λ-P1:5R *c*I857 *D*am Km^r were analyzed. As shown in Table 4, the P1 plasmid was stable in all the *hop* mutants tested except for three mutants, *hopE319*, *hop-355*, and *hop-365*, of linkage group E. Stability of the plasmid pSC101 was also measured in the *hopA18*, *hopB181*, and *hop-383* strains. The plasmid was stable in these mutants. In addition, an R1 plasmid (pOU47) was also stable in the *hopB181* and *hop-383* strains.

Effect of *hop* mutations on host growth. To know whether these *hop* mutations affected cell growth, cell size distribution, and distribution of nucleoids, cells grown exponentially in L medium were analyzed by flow cytometer and microscope. No remarkable differences were observed between the parental strain and each *hop* mutant.

DISCUSSION

In this study, we have isolated host mutations causing defective stable maintenance of mini-F plasmids and classified them into five linkage groups on the chromosome. Experimental results suggest that the *hopA18* and *hop-383* mutants are defective in partitioning of the mini-F plasmid rather than in plasmid replication. Our recent results indicate that both the *hopA18* and *hop-383* mutations exist within the coding frame of the *gyrB* gene, which codes for subunit B of DNA gyrase (Ogura et al., unpublished data). As described in the present paper, the mini-F plasmid pXX325 is unstable, but the P1 plasmid, which has its own partition genes (1–4), is stable in the *hopA18* and *hop-383* mutants. Mutation *hopC351*, which seems to cause partially defective replication of mini-F, is located near the *thr* gene. The *seg* conditional mutants, which were defective in stable maintenance of F′ plasmids at 42°C, were also located near the *thr* gene (19). The *mafA* mutations causing defective stable maintenance of F′ plasmids were located at 1 min (38, 39). However, our recent data suggest that the *hopC351* mutation is located in a different locus from *seg* and *mafA* (Ogura et al., unpublished data). The mutation *hopD410*, which may also be partially defective in replication of mini-F, is located between the *lacI* gene (8 min) and the transfer origin of HfrC (15 min), where no mutations causing defective maintenance of the F plasmid have been described so far.

Three mutations, *hopE319*, *hop-355*, and *hop-365*, of the fifth linkage group, which were located between *argA* and *thyA*, caused abnormal replication of plasmid pXX325, resulting in linear double-stranded DNA multimers of plasmid pXX325. Similar linear multimer formation of plasmid DNA has been described for *recBC sbcB*, *recBC sbcA*, and *recD*

sbcB double mutants (8, 33). Biek and Cohen (6) isolated host mutants incapable of supporting stable maintenance of pSC101, which has the *cis*-acting *par* locus essential for its stable maintenance (24, 26, 35). The mutants had mutations in the *recD* gene, and multimerization of the plasmid DNA was observed (6). Our recent results indicate that the *hopE319*, *hop-355*, and *hop-365* mutations are also located in the *recD* gene (Niki et al., unpublished data). Although circular monomers of pXX325 were observed in the *hopE* (*recD*) mutants as in the *hop*$^+$ strain, pXX325 as well as pXX327 is unstable and there is no significant difference in stability between the two plasmids. The instability of pXX325 in these host mutants can be explained by IncB, IncC, and IncD incompatibilities. The IncB and IncC incompatibilities are exhibited by 19-bp repeated sequences, and the initiation of the θ-form replication of the mini-F plasmid was inhibited by these incompatibilities (28, 34). The IncD incompatibility is exhibited by the *sopC* site, which is essential for plasmid partitioning (22, 27, 29, 31). The initiation of θ-form replication of circular pXX325 DNA may be inhibited by the IncB and IncC incompatibilities of a large amount of linear multimer DNA of pXX325, and the partitioning of circular pXX325 DNA also may be inhibited by the IncD incompatibility. Circular monomers of pXX325 may be produced mainly from replicating σ-form intermediates.

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