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Gene Organization in the Region Containing a New Gene Involved in Chromosome Partition in *Escherichia coli*

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A new mutation, *parC*, causing abnormal chromosome segregation was identified in two thermosensitive mutants of *Escherichia coli*. The thermosensitive growth of the mutants was corrected by pLC4-14 in the Clarke-Carbon collection. This plasmid carries a putative gene which can suppress the cell division defect due to *ftsI* (*pbpB*) and has hence been termed *sufI* (*sui*). The nearness of *parC* to *metC* was confirmed, and cotransduction frequency of *parC* was 59% with *metC* and 20% with *glc*. The *parC-sufI* region was analyzed by subcloning the chromosome region of pLC4-14. The *parC* and the *sufI* gene products were electrophoretically identified as proteins of 75 and 55 kilodaltons (kDa), respectively. The allelism of *parC*$^+$ on pLC4-14 to *parC1215* was confirmed by cloning *parC1215*. The *sufI* gene appeared to be dispensable for cell viability, and overproduction of its product caused suppression of *ftsI*. An essential gene coding for a 25-kDa protein was found between the *parC* and the *sufI* gene. These three genes were transcribed in the same direction and may be organized into an operon, with *parC* to the proximal side and with internal promoters at least for the distal genes. The localization of the gene products was examined in maxicells. The *sufI* protein was synthesized as a precursor which could be chased into a mature form. The major part of the mature form was found in the soluble fraction. The 25-kDa protein was found almost exclusively in the membrane fraction. The *parC* protein was associated with the membrane fraction in the presence of Mg^{2+} but found in the soluble fraction when Mg^{2+} was sequestered with EDTA.

A goal of cell division is segregational transmission of a genome to daughter cells. To prevent production of chromosomeless cells, the process of cell division in *Escherichia coli*, as well as in other organisms, is placed under elaborate control to couple it with replication and partition of chromosomes. For example, perturbation of chromosome replication arrests cell division by induction of a division inhibitor, the *sulA* gene product, targeting the *ftsZ* (*sulB*) gene product, whose function is essential for progression of the division process (13, 14, 19). Chromosome partition should be another process that regulates cell division. This process, although barely understood, seems to include two aspects: molecular separation (resolution of topological linkages) and topographical segregation of the replicated chromosomes. The latter aspect has been postulated to involve the attachment of replicons to the cell surface structure, the growth of which contributes to topographical segregation of the attached replicons (15).

The segregation mechanism of replicons has recently been investigated with plasmids that maintain themselves in one or two copies per chromosome and hence require certain partition functions for their stable inheritance through cell division cycles. Genetic loci responsible for plasmid partition have been identified on several plasmids and shown to function in *cis* or in *trans* (20, 21, 28, 30). One *cis*-acting and two *trans*-acting factors encoded in the *sop* region of an F plasmid are involved in the stable inheritance of the F replicon; the *cis*-acting factor is a DNA segment presumably forming a complex with the *trans*-acting gene products and then providing a site for membrane attachment (23, 30). The *oriC* plasmid can be stabilized for maintenance when joined with the *sop* region of an F plasmid (30).

Two kinds of *Escherichia coli* mutants defective in chromosome partition have been described as *parA* and *parB*. They are thermosensitive and reveal the distinctive morphology of centrally congregated nucleoids at the restrictive temperature (11, 12). The *par* mutations have recently been investigated for their relationship with the termination of DNA replication, and *parB* is most probably allelic to *dnaG* (for primase) and located at 67 min on the genetic map (29). Analyses of such mutations are indeed expected to provide clues to the mechanism of chromosome partition and its control over the cell division process.

We found another *par* mutant in the thermosensitive mutant stock described previously (33). The thermosensitive phenotype of the mutant was corrected by pLC4-14, which is known to cover the 65-min region on the *E. coli* chromosome map (6). The new *par* mutation is clearly different from either *parA* or *parB*, as described in the present report, and will be termed *parC*. The plasmid pLC4-14 also corrected the thermosensitive defect due to *ftsI* (*pbpB* at 2 min, for penicillin-binding protein 3 [PBP3]), and the putative gene that can suppress *ftsI* mutation was called at first *sui* (34) and then *sufI* (37). The latter term (*suf*, for suppression of Fts) comes from the finding that similar suppression phenomena are also seen in other combinations of different *fts* mutations and plasmids carrying a distinct chromosome region (27, 37), such as *ftsE* (76 min) and pLC19-48 (carrying the 3-min region).

The chromosomal region carried by pLC4-14 may include a cluster of genes essential for chromosome partition and its

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TABLE 1. Bacterial strains

Strain	Relevant markers	Other markers or derivation	Source or reference
JE11215	*parC1215*	From PA3092 (27)	
JE10572	*parC572*	From PA3092	
PL8-31	*glc-1 str serA25*	F⁻ *thr-1 leu-6 thi-1 metG87 hisC3 proA2 metK85 mtl xyl galK lacY supE*	Y. Komeda
JE6839	*metC*	F⁻ *argG his mtl malA gal lac str tsx*	GSRC[a]
JE8515	*parC1215*	Met⁺ transductant of JE6839	
JE7611	*ftsI*	F⁻ *thi met pro xyl str lacZ tonA tsx recA*	25
P4x8		Hfr *metB1*	GSRC
N1126	*polA12*	F⁻ *thi leu pro malA his thy cysC lacZ ara mtl xyl str spc*	35
TH1219	*minB recA*	F⁻ *rpsL tsr tar*	7
C600		F⁻ *thi thr leuB lacY tonA supE*	1
JM109	*recA1* (*lacI*^{q})	Δ(*lac-pro*) *endA1 gyrA96 thi-1 hsdR17 supE44 relA1* (F′ *traD36 proA lacI*^{q}*ZM15*)	38
EJ812	*parC1215*	Met⁺ transductant of C600 *met*::Tn*10* derivative	
NK6027	*metC162*::Tn*10*	HfrH Δ(*gpt-lac*)*5 relA1 spoT1 thi-1*	GSRC

[a] GSRC, Supplied by Genetic Stock Research Center of the National Institute of Genetics (Mishima, Japan).

linkage with cell division, and this motivated us to analyze the gene organization in this region. The analyses of the *parC-sufI* gene region disclosed another essential gene located between the *parC* and *sufI* genes. The products of these genes were identified, and localization of the gene products was investigated. The results suggested that the three genes may be organized into an operon with internal promoters and that the *parC* protein interacts with the membrane.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. Bacterial strains are as listed in Table 1. Plasmid pTR27 carried a *Bam*HI fragment carrying base sequences such that termination signals occur in any reading frame and the direction of translation (I. N. Maruyama, K. Horikoshi, M. Soma, and Y. Hirota, unpublished data). pLC4-14 is a synthetic ColE1 plasmid made by Clarke and Carbon (4). pYS8 and pYS10 carry a kanamycin resistance (Km^r) marker which can be removed as a *Pvu*II and a *Bam*HI fragment, respectively, and were kindly provided by Y. Suzuki. Derivatives of mini-F plasmids pSC138 (36) and pKO16E (35) and a plasmid with a *Bam*HI fragment containing the chloramphenicol resistance (Cm^r) gene, pJL3-4779, were kindly provided by K. Tanimoto. pACYC184 has been described (3).

Bacteria were grown routinely in LB broth or on LB plates (22). Thymine was added to a final concentration of 60 mg/liter when needed. S-broth contained (per liter): Bactotryptone, 5 g; yeast extract, 3 g; NaCl, 3.5 g; thymine, 60 mg; glucose, 3.6 g; and 20 ml of buffered salt solution (16). Transformants were selected on LB plates containing an appropriate drug at the following concentrations: tetracycline, 10 μg/ml; kanamycin, 40 μg/ml; ampicillin, 20 μg/ml; and chloramphenicol, 15 μg/ml. Transductants were selected on LB plates containing tetracycline (2.5 μg/ml) or kanamycin (40 μg/ml) or both.

Mapping of *parC*. JE11215 (*parC1215*) was mated with P4x8 to construct Hfr strains carrying *parC*. The resultant Hfr (*parC1215*) was crossed with PL8-31 and selected for SerA$^+$ and Str^r. Examination of thermosensitivity of the recombinants suggested the marker order *serA-glc-parC1215-str*. One of the recombinants (*glc parC*) was used as a donor for P1 transduction of *parC* into JE6839 (*metC*); the transductants were selected for MetC$^+$ and scored for the ParC and Glc phenotypes, and then *parC*$^+$ was transduced from JE6839 to one of the MetC$^+$ transductants that received *parC* and *glc* by selecting for Glc$^+$ and scored for the ParC and MetC phenotypes. The thermosensitive phenotype was shown to be ParC$^-$ by mapping.

Plasmid construction. To facilitate in vitro excision and insertion of a required DNA region, the *Bam*HI fragment containing a translation terminator from pTR27 or a Cm^r marker from pJL3-4779 was inserted into the *Bam*HI site on pUC8 to construct pJK270 and pJK272, respectively. The terminator in pJK270 and the Cm^r marker in pJK272 can be removed to any blunt-end site by excising a fragment with blunt ends.

The mini-F vectors pJK280, pJK282, and pJK284 were constructed as shown in Fig. 1. The *Hpa*I fragment of pSC138, which contained the essential region for replication and stable maintenance of a mini-F plasmid, was ligated with *Pvu*II fragments carrying the *lac* promoter-operator region along with a Tc^r gene or a Km^r gene for constructing pJK280 or pJK282, respectively. The *Pvu*II fragment for pJK280 was constructed by inserting the *Eco*RI fragment of pJK250 into the *Eco*RI site of pUC9, and that for pJK282 was constructed by inserting the *Pvu*II fragment of pYS8 into the *Hin*cII site of pUC8. To construct pJK284, the mini-F fragment of pJK282 was excised with *Eco*RI and *Hin*dIII nucleases at the site derived from pUC8 and ligated with the *Eco*RI-*Hin*dIII fragment carrying an Ap^r marker of pSC138.

The plasmids shown in Fig. 2 were constructed as follows. The *Sma*I fragment containing the *parC* gene was excised from pLC4-14; an *Eco*RI synthetic linker was attached to the ends; and the fragment was inserted into the *Eco*RI site on pBR322, pACYC184, pJK282, and pJK284 (Fig. 1) to produce pJK800, pJK805 (used to construct pJK806 below), pJK816, and pJK818, respectively. A translation terminator excised as a *Sma*I-*Hin*cII fragment from pJK270 was ligated with the linear fragments of pJK800 digested with *Pvu*II to obtain pJK809. The terminator obtained as a *Bam*HI fragment from pTR27 was similarly ligated with pJK800 digested with *Bam*HI to obtain pJK807. To construct pJK811, the *Hpa*I fragment containing the *parC* gene was cloned into a *Sca*I site on pBR322. To construct pJK815, the Cm^r gene on the *Sma*I-*Hin*cII fragment of pJK272 was inserted into the *Pvu*II site in the *parC* gene. pJK806 was obtained by deleting *Pst*I fragments from pJK805. pJK801 was constructed by the same procedure as used for the pJK800 construction (see above); pJK800 and pJK801 carry this *Eco*RI fragment in opposite orientations. pJK823 was constructed by deleting the small *Bam*HI fragment from pJK801. To construct pJK813, pJK823 was digested with exonuclease *Bal*31 from its unique *Bam*HI site, and the plasmid carrying an appropriate deletion was selected. pJK803 was obtained by inserting the *Sma*I fragment containing the *parC* region of pLC4-14 into the *Sma*I site on pUC9.

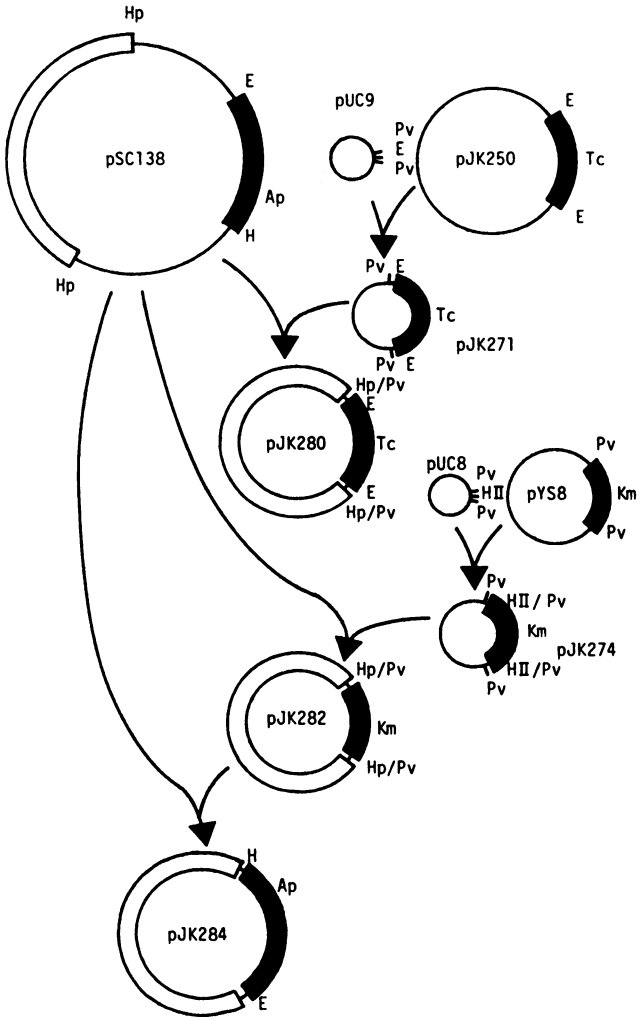

FIG. 1. Vectors derived from mini-F plasmids. Three mini-F vectors, pJK280 (Tc^r), pJK282 (Km^r), and pJK284 (Ap^r), were constructed from pSC138, a derivative of mini-F. The *Hpa*I fragment shown with an open bar contains all regions essential for replication and stable partition of a mini-F replicon. E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; HII, *Hin*cII; Pv, *Pvu*II.

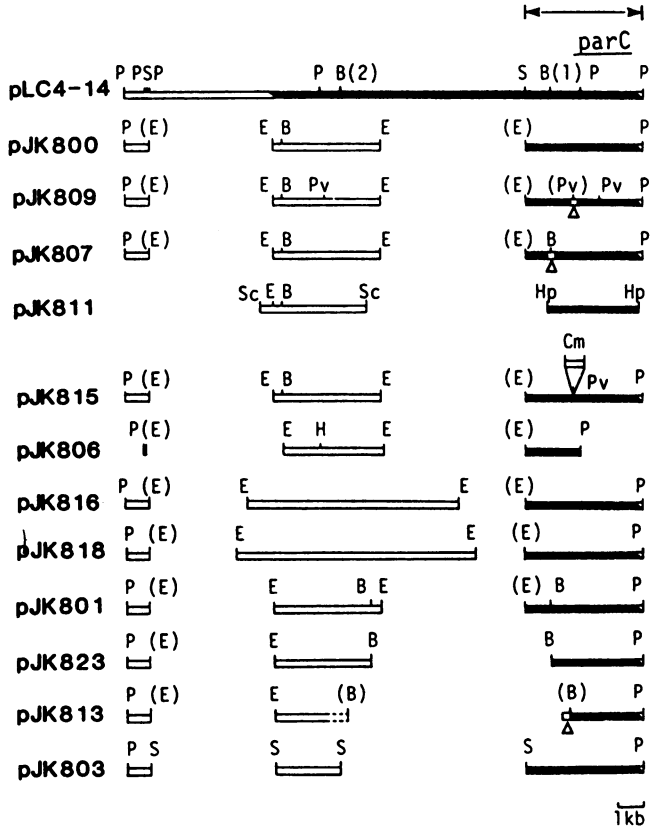

FIG. 2. Restriction map of plasmids carrying the *parC* region. Solid and open bars indicate regions derived from the chromosome and the plasmids, respectively, and the region containing the *parC* gene is indicated. Open squares with △ indicate insertion sites of translation terminators. The ability to correct the temperature-sensitive growth of JE11215 (*parC*) was used for cloning of the *parC* region. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; P, *Pst*I; Pv, *Pvu*II; S, *Sma*I; Sc, *Sca*I. Two *Bam*HI sites on pLC4-14 are shown as B(1) and B(2) on the map of pLC4-14 for convenience.

The plasmids shown in Fig. 3 were constructed as follows. To obtain pJK850, the *Sma*I-*Hin*cII terminator fragment was inserted into the *Sma*I site in the *Bam*HI fragment cloned on pMY310 or pMY311. To construct pJK853, first the *Sal*I-*Eco*RI fragment containing the *sufI* region of pHS132 was cloned into pSC138 by joining it with the *Sal*I-*Eco*RI fragment containing an Ap^r marker; the resultant plasmid was digested with *Eco*RI and partially with *Bam*HI to excise the *Eco*RI-*Bam*HI fragment containing both *sufI* and the Ap^r gene, and the excised fragment was ligated with the *Eco*RI-*Bam*HI fragment containing the F replicon of pJK280. pHS132 was constructed by inserting the *Bam*HI *sufI* fragment of pLC4-14 into the *Bam*HI site of pACYC184. To construct pJK877 and pJK878, the *Bam*HI fragment containing the *sufI* region of pLC4-14 was inserted into the *Bam*HI site of pUC8; the two plasmids received the *Bam*HI fragment in opposite orientations. To construct pJK875 and pJK876, the *Pst*I fragment containing the *sufI* region was similarly inserted into the *Pst*I site on pUC8; the two plasmids received the *Pst*I fragment in opposite orientations.

pJK855, pJK858, pJK863, and pJK864 were all derived from pLC4-14. To construct pJK855, first a Km^r marker was excised as an *Xho*I fragment from pKO16E and inserted into the unique *Sal*I site on pLC4-14; the resultant plasmid was subjected to excision of the small *Kpn*I fragment in the *sufI* region and replacement with the *Kpn*I fragment containing a Cm^r marker. The latter fragment was derived from the plasmid constructed by inserting the Cm^r marker as an *Sma*I-*Hin*cII fragment of pJK272 into the *Sma*I site in the *sufI* region of pMY311. To construct pJK858, first the *Bam*HI fragment containing the *sufI* region in pLC4-14 was replaced with the *Bam*HI fragment containing a Cm^r marker excised from pJL3-4779; the resultant plasmid received at its unique *Sma*I site the insertion of a Km^r marker contained in the *Pvu*II fragment of pYS8. To construct pJK863, first two derivatives of pLC4-14 were constructed: in one derivative, the *Bam*HI fragment containing a Cm^r marker of pJL3-4779 was inserted into the $BamHI_{(1)}$ site; and in the other, the *Bam*HI fragment containing a Km^r marker of pYS10 was inserted into the $BamHI_{(2)}$ site. Then, the *Sma*I fragment derived from the former and carrying the Cm^r marker was ligated with the *Sma*I fragment obtained by the partial digestion of the latter and carrying the Km^r marker. pJK864 was constructed by inserting the Km^r marker of pYS10 into

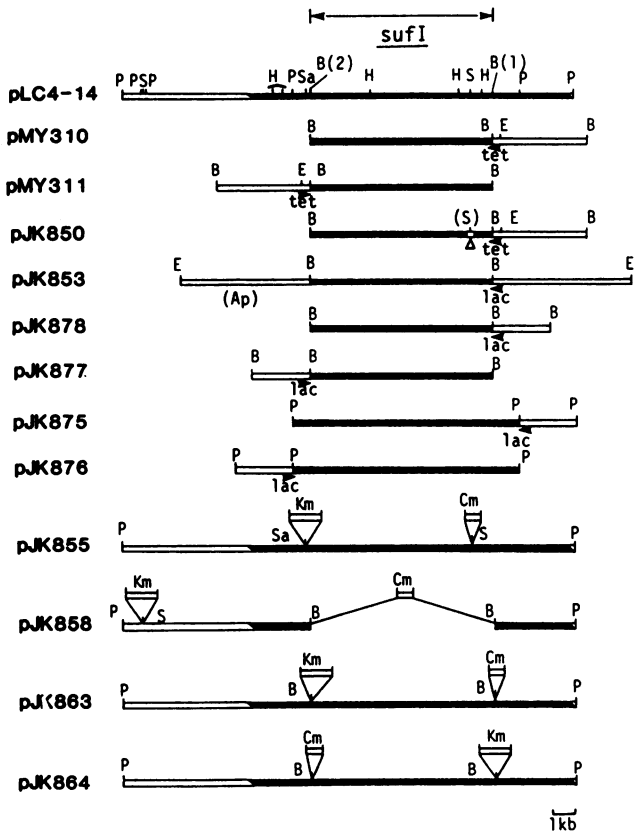

FIG. 3. Restriction map of plasmids carrying the *sufI* region. The restriction map was drawn as described in the legend to Fig. 2. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; P, *Pst*I; Pv, *Pvu*II; S, *Sma*I; Sa, *Sal*I; Sc, *Sca*I. Arrows with *tet* and *lac* represent the direction of transcription of the *tet* and *lac* promoters.

the $BamHI_{(1)}$ site and then the Cm^r marker of pJL3-4779 into the $BamHI_{(2)}$ site of pLC4-14; each marker was excised as a *Bam*HI fragment from the respective plasmid, and a partial digestion with *Bam*HI was applied to the second insertion.

Preparation and manipulation of DNA. The techniques for preparation of plasmid DNA, manipulation of DNA, and transformation were as described previously (16). To transform the *polA*(Ts) strain N1126 with a replacing plasmid (see Results), the procedure was modified as follows. The transformation mixtures were left at 0°C for 30 min. Heat shock was applied at 30°C for 2 min, and the mixtures were preincubated at 30°C for 2 h in LB broth. The cells were harvested and then incubated at 30°C for ca. 20 h in S-broth containing an appropriate drug. The drug-resistant recombinants were selected on LB plates containing no NaCl and tetracycline or kanamycin at 42°C. The acridine orange method (10) was used for curing of the mini-F derivatives. Chromosomal DNA was prepared by the procedure of Cosloy and Oishi (5).

Analyses of gene products. The preparation and labeling of minicells containing the appropriate plasmids and immunological separation were done as described previously (16). Minicells were prepared from stationary-phase cultures. Preparation and labeling of maxicells were carried out as described by Stoker et al. (32) with the *recA* strain JM109 or C600 *recA*. In vitro synthesis of protein was performed by a coupled transcription-translation system as described previously (25). The labeled gene products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% polyacrylamide gels, and fluorography was done as described previously (16).

Protein fractionation for gene product localization. Maxicells suspended in 300 μl of methionine assay medium (Difco) were labeled for 60 min at 37°C and divided into two parts. During the following procedure, 10 mM $MgCl_2$ was included in the buffer containing 10 mM Tris hydrochloride (pH 8.0) and 0.005% 2-mercaptoethanol, for one part; 10 mM EDTA was added to the other. The cells in each part were harvested, washed, and resuspended in 300 μl of the buffer described above. The cells were disrupted three times by sonication at 100 W for 30 s each with an Ohtake 520-2D sonicator. Each 150 μl of sonicated preparation was stored on ice and analyzed as the total fraction. The residual 150-μl portions were centrifuged at 42,000 rpm for 30 min with a Beckman LP42Ti rotor. The supernatant was reserved as the soluble fraction. The precipitate was suspended in 150 μl of the buffer and regarded as the insoluble (membrane) fraction.

Observation of nucleoids. Nucleoids were stained either with ethidium bromide (EtBr) (18) or with 4′,6-diamidino-2-phenylindole (DAPI) (26). For EtBr staining, cells washed with M9 medium were suspended in a solution containing 10 mM Tris hydrochloride (pH 7.4), 5 mM EDTA, 0.05% 2-mercaptoethanol, 0.05% chloramphenicol, and 0.8% ethanol and then mixed with an equal volume of 0.01% EtBr solution. For DAPI staining, the cells in 1 ml of culture were fixed at harvest by adding 1 ml of 5% glutaraldehyde containing 10 mM EDTA and left at 4°C for 30 min. The fixed cells were collected by centrifugation, suspended in 1 ml of a buffer containing 10 mM Tris hydrochloride (pH 7.7), 1 mM EDTA, 0.5 mM spermidine, and 1 mM dithiothreitol and supplied with 1 ml of 5% glutaraldehyde. After centrifugation, the cells were suspended in an appropriate amount of the above buffer and combined with an equal volume of DAPI solution (2 μg/ml). The cells stained for nucleoids were observed and photographed with an incident fluorescence microscope equipped with phase-contrast optics (Olympus AHB-RFL-LB).

RESULTS

Location of a new *par* mutation and the *sufI* gene. Among the mutants in the thermosensitive mutant stock (33), two strains were found that showed temperature-insensitive growth when cross-streaked against JA200(pLC4-14). These strains, JE11215 and JE10572, were identified as *par* mutants by the morphology of nucleoids in cells incubated at the restrictive temperature. Figure 4 shows the morphological Par phenotype revealed with EtBr-stained nucleoids of JE11215 incubated at the restrictive temperature. The mutated gene leading to this Par phenotype was distinct from *parB* as the *parB* locus was distant from the chromosome region covered by pLC4-14 and also seemed to be distinct from *parA* inasmuch as the morphological Par phenotype of *parA* mutant MFT110 (isolated from PA3092 by M. Ricard and Y. Hirota) was not corrected by a plasmid equivalent to pLC4-14. The new *par* mutation will be referred to as *parC*.

The chromosome region carried by pLC4-14 covers *metC* (6), and therefore the *par* mutation in the mutants should be located at ca. 65 min. on the *E. coli* chromosome map. This was confirmed by mating and transduction experiments for *parC1215* in JE11215, as described in Materials and Methods. The cotransduction frequency of *parC1215* was 59% (29 of 49) with *metC* and 20% (5 of 25) with *glc*. This places *parC* at 65.3 min if *metC* is assumed to lie at 65.0 min.

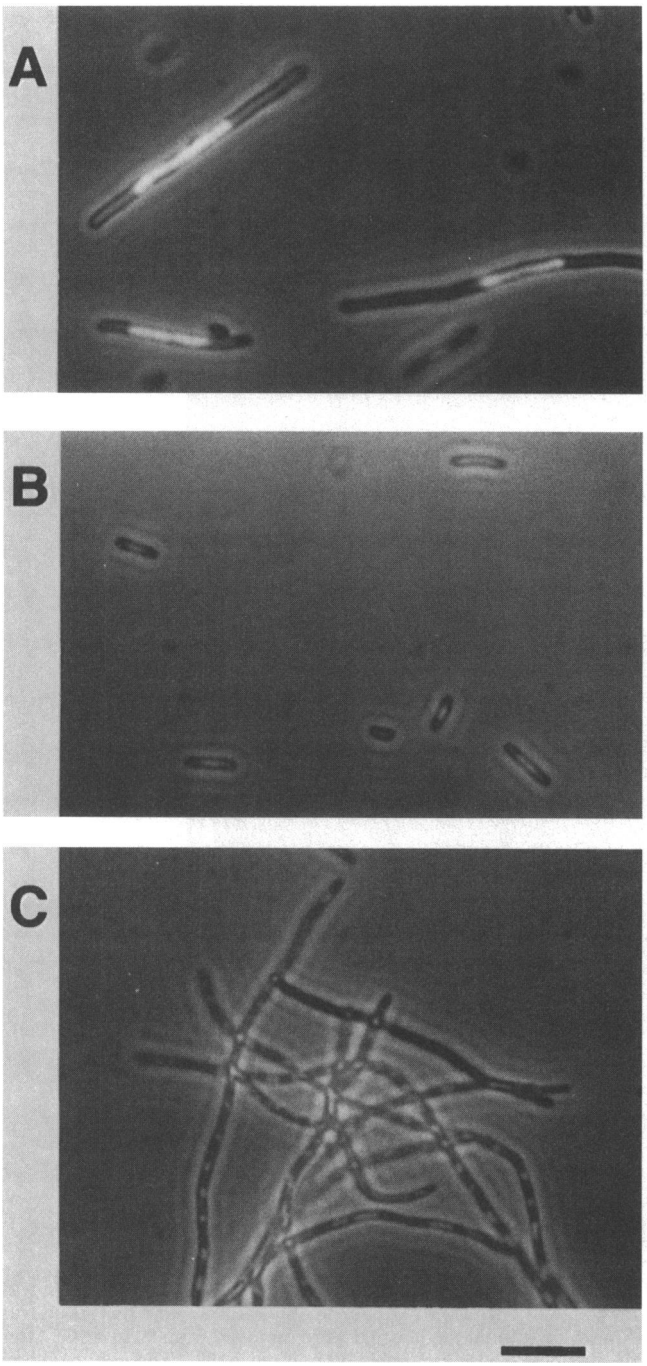

FIG. 4. Abnormal pattern of chromosome partition in the *parC* mutant. Cells grown overnight in S-broth at 30°C were diluted 1:100 into fresh S-broth. The diluted cells of JE11215 (*parC*) were grown for 3 h at 40 or 30°C, diluted again to 1:100, and grown for another 3 h at the same temperature. The diluted cells of JE7611 (*ftsI730*) were grown for 3 h at 40°C. The cells were collected by low-speed centrifugation and treated as described in Materials and Methods. (A) JE11215 (*parC*) grown at 40°C; (B) JE11215 (*parC*) grown at 30°C; (C) JE7611 (*ftsI730*) grown at 42°C. Bar, 10 μm.

Plasmid pLC4-14 carries the *sufI* (*sui*) gene, which can suppress the thermosensitive defect due to *ftsI* (see Introduction). Thus, the chromosome region of pLC4-14 was analyzed for the *parC* and *sufI* loci by subcloning parts of this region. The *Sma*I and the *Bam*HI fragment of pLC4-14 were cloned into pBR322 as shown in Fig. 2 and 3 to obtain pJK800, pMY310, and pMY311. While pJK800 corrected the thermosensitive defect in JE11215, pMY310 and pMY311 suppressed the *ftsI* phenotype. The latter two plasmids received the same *Bam*HI fragment from pLC4-14 but in opposite orientations, and the suppression of FtsI was more distinct in pMY310 than in pMY311. The *parC* gene should be located between the *Pst*I and the *Sma*I sites according to the restriction map shown in Fig. 2, and the *sufI* gene lies in the *Bam*HI fragment, probably oriented with transcription in the same direction as the Tc^r gene in pMY310. The thermosensitivity of a *parC* transductant, JE8515, was corrected when the *parC* region but not the *sufI* region was introduced on a plasmid.

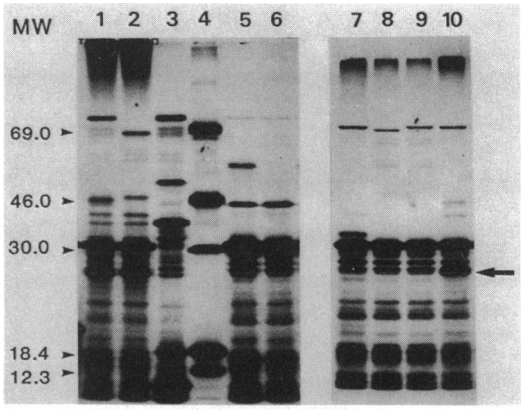

FIG. 5. Identification of the gene products. Proteins were synthesized in vitro with a ^{14}C-amino acid mixture (lanes 1 to 6) or [^{35}S]methionine under the direction of (lanes 1 and 10) pJK800, (lane 2) pJK809, (lane 3) pJK811, (lane 5) pMY310, (lane 6) pJK850, (lane 7) pJK823, (lane 8) pJK813, and (lane 9) pJK807. Lane 4 shows mass markers. The 25K protein produced with pJK800 is indicated with an arrow on lane 10. This protein should be present in lanes 1 and 2 and absent in lanes 5 and 6, but this is not entirely confirmative because these lanes were overexposed.

Identification of the *parC* product. To identify the *parC* product, a translation terminator (Materials and Methods) (17) was inserted into one of three *Pvu*II sites on pJK800 to construct pJK809, as shown in Fig. 2. The *Pvu*II site on pJK809 that received the terminator should be located in the region essential for expression of the *parC* gene because pJK809 did not correct the temperature-sensitive growth defect of JE11215. The proteins synthesized in vitro under the direction of these plasmids were analyzed by PAGE. The synthesis of a protein with an apparent mass of 75 kilodaltons (kDa) was detected for pJK800 but not for pJK809 (Fig. 5, lanes 1 and 2); pJK809 directed instead the synthesis of a protein migrating at the position of about 69 kDa (Fig. 5, lane 2). This 69,000-M_r (69K) product was not observed for pJK800. The pattern of the other protein products was much the same for both plasmids. The smaller protein produced with pJK809 may be a truncated polypeptide of the 75K protein. The 75K protein could also be detected in the products directed by pJK811 (Fig. 2) carrying the *Hpa*I fragment, a chromosomal region smaller than that carried by pJK800 (Fig. 5, lane 3). These results suggest that the 75K protein is a *parC* gene product. It cannot be ruled out that a polar effect might be developed in pJK809 by the terminator insertion, preventing the expression of a *parC* gene located in the region distal to the gene for the 75K protein. However, as described in the later section, no polypeptide was over-

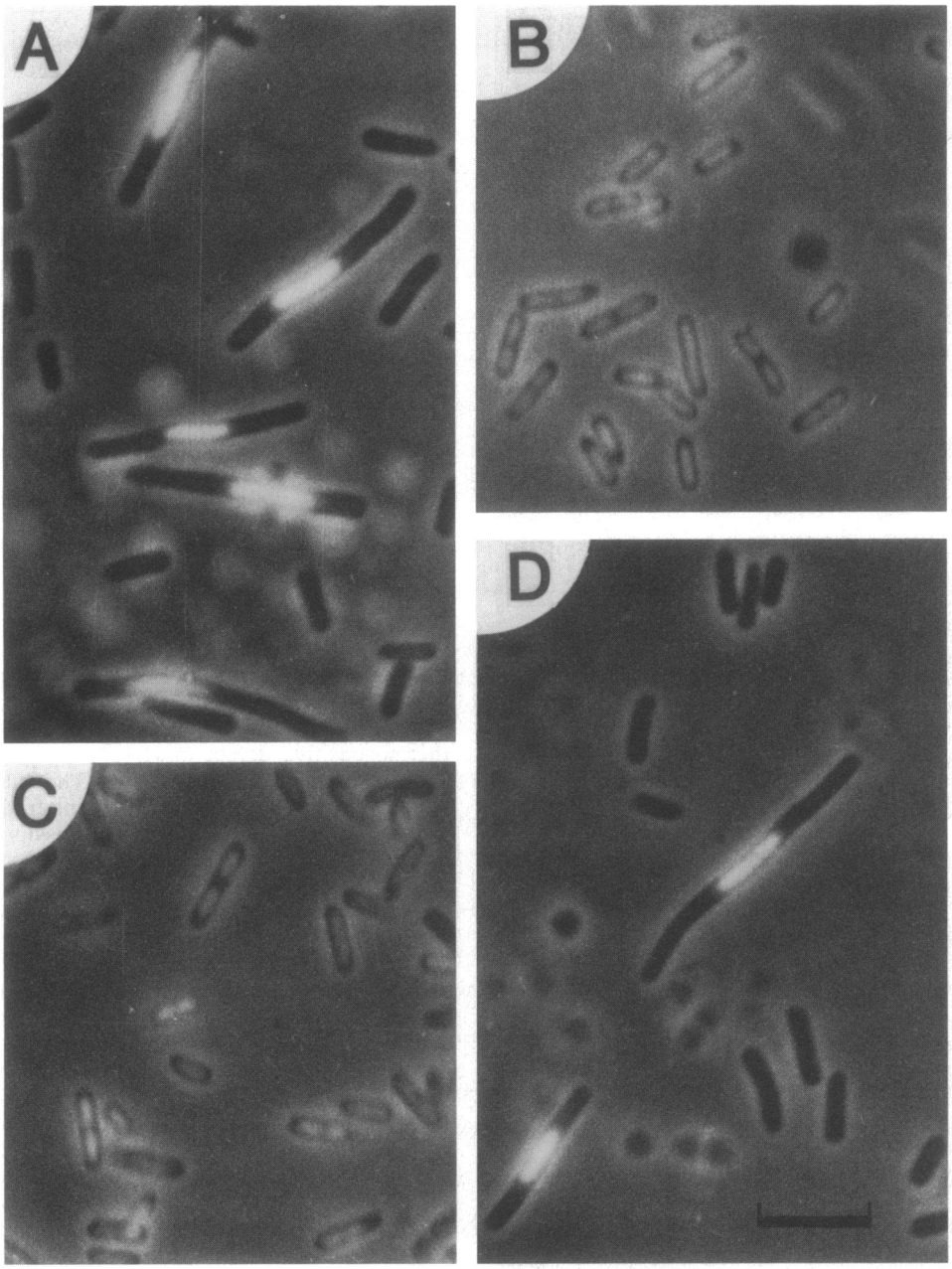

FIG. 6. Correction of the Par phenotype by *parC*$^+$ plasmids. To confirm the morphology resulting from the *parC* mutation, *parC1215* was cotransduced with *metC*$^+$ into C600 made Met$^-$ in advance by introduction of *metC*::Tn*10* from NK6027 to obtain EJ812, and this strain was transformed with plasmids carrying the *parC* region. Growing cells of EJ812 with or without the plasmid were removed from 30 to 42°C. After 2 h of incubation at 42°C, chloramphenicol was added at 250 μg/ml and the incubation was continued 30 min more (39). The cultures were treated for DAPI staining as described in Materials and Methods to observe morphology. (A) Without plasmid; (B) with pJK800 (*parC*$^+$); (C) with pJK811 (*parC*$^+$); (D) with pJK809 (*parC* interrupted by a translational terminator). See Fig. 3 for the *parC* regions in the plasmids. Note the prominent production of nucleoidless cells in A and D. Bar, 5 μm.

produced by increasing the transcription through the DNA region coding for the C-terminal portion of the 75K protein. To confirm the correction of ParC morphology by the plasmids that directed synthesis of the 75K protein, possible interference with ParC by unknown mutations was eliminated by constructing EJ812 as a *parC* transductant from a derivative of C600. EJ812 failed to grow on LB plates at 42°C and surprisingly produced a considerable fraction of nucleoidless cells along with manifestation of ParC in cells retaining nucleoids during a 2-h incubation at 42°C (Fig. 6A). The *parC* phenotype manifested by EJ812 was corrected by pJK800 and pJK811 (Fig. 6B and C) but not by pJK809, which is unable to direct synthesis of the 75K protein owing to the introduction of a translation terminator (Fig. 6D).

Cloning of the *parC* mutant gene. In order to confirm that the *parC* gene was carried by pLC4-14 and inactivated by the interruption at the *Pvu*II site, cloning of the mutant gene carrying *parC1215* was undertaken. This was accomplished

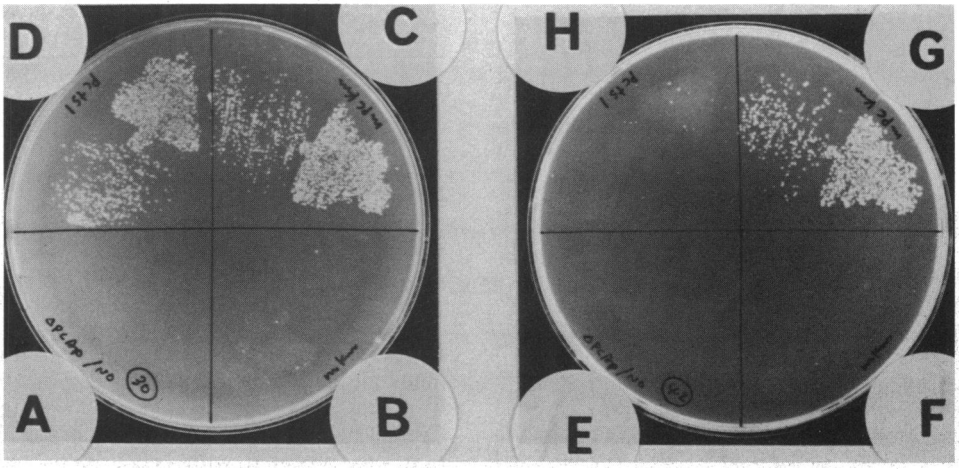

FIG. 7. Growth of cells transformed with plasmids carrying *parC*(Ts). N1126 (*parC*::*cat*)(pJK818 [Ap^r]) was subjected to transformation treatment (A and E) without plasmid, (B and F) with pJK282 (Km^r), (C and G) with pJK816 (Km^r *parC*$^+$), or (D and H) with pJK820 [Km^r *parC*(Ts)] and plated on LB plates containing kanamycin at 30°C (A–D) or 42°C (E–H).

by application of the compensating-replacing plasmid principle (17) as follows. A strain was constructed in which the *parC* gene on the chromosome was inactivated in the presence of a mini-F plasmid having *parC*$^+$ to compensate for the loss of *parC* function (a compensating plasmid). Cloning of *parC* was then performed by using a mini-F vector that displaces the compensating plasmid. N1126 (*polA12*) was transformed with pJK816 (Km^r *parC*$^+$), which served as a compensating F-replicon plasmid, and then with pJK815 (Cm^r Tc^r Ap^r), constructed by inserting a Cm^r gene into the *Pvu*II site within the *parC* gene (Fig. 2). The latter plasmid served as a replacing plasmid to replace the *parC* gene on the chromosome with the inactivated *parC* gene on the plasmid through genetic recombination. This strain was grown first at 30°C and then propagated at 42°C to cure the population of the replacing plasmid, which cannot replicate due to the *polA*(Ts) mutation. In this population, some cells were Cm^r Tc^s Ap^s. Some of these should have acquired the Cm^r insertion in the chromosomal *parC* locus by exchange with the plasmid-carried region prior to loss of the plasmid. One such isolate was designated N1126(*parC*::*cat*)(pJK816 [Km^r]). Its structure was confirmed by the following experiments. N1126(*parC*::*cat*)(pJK816 [Km^r]) was made Km^s by replacing pJK816 with pJK818 (Ap^r), having the same replicon and compensating capacity as pJK816. The resultant Km^s strain was then transformed with pJK282 (Km^r) or pJK816 (Km^r). The transformants developed with pJK816 at 42°C as well as at 30°C, but not with pJK282 at either temperature (Fig. 7B, C, F, and G). This shows that N1126(*parC*::*cat*)(pJK818 [Ap^r]) is viable only in the presence of the compensating plasmid. Thus, the insertion is present in the chromosome rather than in the original compensating locus. This strain was then used as a host for transformation with plasmids made by ligation of pJK282 (Km^r) with the *Bam*HI fragments of JE11215 chromosomal DNA. Transformants were selected for Km^r at 30°C. These had lost pJK818 (Ap^r) by displacement. The plasmid pJK820 (Km^r) was isolated as a candidate for containing *parC1215*. When N1126(*parC*::*cat*)(pJK818 [Ap^r]) was transformed with pJK820 and selected for Km^r, colonies of Km^r transformants developed normally at 30°C but scarcely at all at 42°C (Fig. 7D and H). This indicated that the DNA region containing the *parC1215* locus was cloned on this plasmid. From this it follows that the ability of pLC4-14 to correct the *parC*(Ts) defect is ascribable to the *parC* gene on this plasmid.

Identification of the *sufI* gene product. In studying the nature of the *sufI* gene in more detail, the effect of this gene on the growth of the *ftsI* mutant was first examined. The suppression was rather weak with pMY310 and still weaker with pMY311. The readthrough transcription from the *tet* promoter may have contributed to the *sufI* expression in pMY310, and the elevated level of the *sufI* gene product may be crucial for the suppression, as suggested previously (34). The suppression would be observable only in the induced state when the *Bam*HI fragment was inserted next to the inducible promoter-operator in the correct orientation when using a low-copy-number plasmid. This was examined with pJK853 (Fig. 3), in which the replicon was mini-F and the *Bam*HI fragment was joined to the *lac* promoter-operator region of pJK280 (Fig. 1) in such an orientation that the transcription from the *lac* promoter may proceed into this fragment from the *tet* promoter side of the *Bam*HI fragment in pMY310. The *ftsI* mutant carrying pJK853 showed thermoresistance only under induction by isopropyl-β-D-thiogalactopyranoside (IPTG), to the same extent as that shown by the strain carrying pMY311. Thus, the suppression of the *ftsI* mutation by the *sufI* gene results from the increase in the amount of a *sufI* gene product.

The *sufI* product was identified by inserting the terminator fragment into the region essential for *sufI* activity and analyzing the products synthesized in vitro. A 55K polypeptide was present in the products synthesized in vitro with pMY310 but absent in those synthesized with pJK850, constructed by inserting the terminator fragment into the *Sma*I site within the *Bam*HI fragment cloned on pMY310 (Fig. 5, lanes 5 and 6). The *ftsI* mutant carrying pJK850 remained thermosensitive. The *Bam*HI fragment was inserted into the *Bam*HI site on pUC8 to construct pJK877 and pJK878 (Fig. 3). The latter plasmid had the *Bam*HI fragment in the same orientation as that in pJK853 with respect to the *lac* promotor. A remarkable overproduction of the 55K protein was observed in in vitro (Fig. 8A, lanes 1 and 2) and maxicell protein synthesis (Fig. 8B, lanes 3 to 6) with pJK878 but not with pJK877. These results strongly support the interpretation that the 55K protein is encoded in the *sufI* gene. The maxicell experiments shown in Fig. 8B revealed an extra band that migrated slightly faster than the 55K

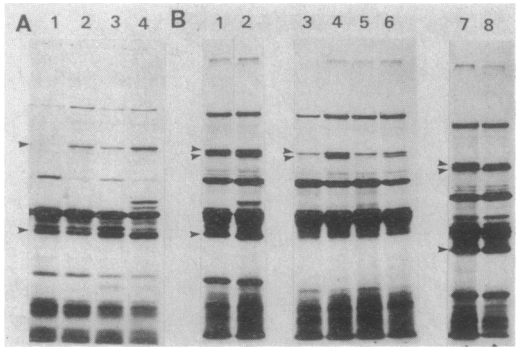

FIG. 8. Overproduction of the *sufI* and 25K proteins. [^{35}S] methionine-labeled proteins were synthesized in vitro (A) or in maxicells (B) under the direction of (lanes A-1, B-5, and B-6) pJK877, (lanes A-2, B-3, and B-4) pJK878, (lanes A-3 and B-1) pJK876, or (lanes A-4, B-2, B-7, and B-8) pJK875. The labeling procedures are described in Materials and Methods. (B) Odd-numbered lanes show the proteins synthesized with the maxicells incubated in the presence of glucose, and even-numbered lanes show those labeled after induction of the *lac* promoter by IPTG in the maxicells incubated in the absence of glucose. Lanes 7 and 8 show the patterns before and after a 30-min chase that followed 10 min of labeling. Arrowheads in panel A indicate the *sufI* product (top) and 25K protein. Arrowheads to the left of the lanes B-1, B-3, and B-7 indicate the precursor (top) and mature (middle) forms of the *sufI* product and 25K protein (bottom), if present. The *sufI* product synthesized in vitro corresponds to the precursor form synthesized in maxicells as judged by its electrophoretic mobility. The band indicated as the 25K protein, rather than the band immediately above it, agreed in relative mobility with the 25K protein band shown in Fig. 5, although the upper band was also intensified in lane A-3. The cause for the increase in the upper-band protein in lane A-3 is unknown. (The conclusion for the reading direction of the gene for the 25K protein was consistent with our unpublished sequence data.)

protein band. The faster-migrating protein was not observed in the in vitro products. The maxicells synthesizing the 55K protein were labeled for 10 min and chased for 30 min. The intensity of the faster-migrating band increased and the 55K protein band decreased after the chase, as demonstrated by the pattern obtained with pJK875 (Fig. 8B, lanes 7 and 8). This suggests that the *sufI* product undergoes processing.

To examine whether the *sufI* gene was dispensable for growth, the replacing-plasmid method was applied to isolate a mutant that had lost *sufI* gene function (see preceding section). Strain N1126 was transformed with pJK855 (Cm^r Km^r), in which the *sufI* region was interrupted by insertion of the *cat* gene (Fig. 3), and selected for Cm^r at the restrictive temperature for PolA function. Six of 100 Cm^r transformants were Km^s. When the Cm^r gene was inserted into the *parC* gene, no Cm^r Tc^s transformant was obtained by examining similar numbers of cells unless a compensating plasmid was present. Therefore, the isolation of the Cm^r Km^s transformants by using pJK855 suggests the dispensability of the *sufI* gene function for cell viability, although further analyses are required for a definite conclusion.

Identification of an essential gene between *parC* and *sufI*. Construction of a *sufI* deletion strain was attempted to establish the dispensability of the *sufI* gene for cell viability. The *Bam*HI fragment containing the *sufI* gene in pLC4-14 was replaced with the Cm^r gene, and the Km^r gene was inserted into the *Sma*I site in the ColE1 region of pLC4-14 to produce pJK858 (Fig. 3). Cells of N1126 were transformed with pJK858 and plated at 42°C. Contrary to expectation, no

TABLE 2. Plating efficiency of cells cured of pJK818 (Ap^r) by acridine orange

Host strain	Expt no.	No. of Ap^s colonies/100 colonies examined at acridine orange concn:	
		0 μg/ml	25 μg/ml
N1126	I	1	63
	II	1	62
N1126 Cm^r Km^s	I	0	0
	II	0	0

Km^s clone was found among 200 Cm^r transformants examined, although several percent of the Cm^r transformants were Km^s in the parallel experiment with pJK855, which had Cm^r and Km^r markers, as shown in Fig. 3. The transformation was repeated with N1126 cells having pJK853 as a compensating plasmid. This plasmid carried the same *Bam*HI fragment as that lost in pJK858 and led to overproduction of the *sufI* gene product as described in the above section. The result was the same (no Cm^r Km^s cells were found) even in the presence of the compensating plasmid, suggesting that the function of an essential DNA region was destroyed by deletion of the *Bam*HI fragment and could not be supplied by the compensating plasmid because it spanned one of the two interrupted *Bam*HI sites [*Bam*HI$_{(1)}$ and *Bam*HI$_{(2)}$ in Fig. 3]. Two replacing plasmids, pJK863 and pJK864, were constructed. Both had two drug resistance genes; pJK863 had the Cm^r gene at the *Bam*HI$_{(1)}$ site and the Km^r gene at the *Bam*HI$_{(2)}$ site, whereas pJK864 had the Km^r gene at the *Bam*HI$_{(1)}$ site and the Cm^r gene at the *Bam*HI$_{(2)}$ site (Fig. 3). When transformation and selection were performed by using N1126 cells as described above, Km^s colonies were obtained among the Cm^r transformants isolated with pJK864 but not with pJK863. The result suggested the existence of an essential gene that lost its function by the interruption at the *Bam*HI$_{(1)}$ site. Since pJK807 (Fig. 2), having an insertion of the translational terminator at the *Bam*HI$_{(1)}$ site, rendered JE11215 (*parC*) thermoresistant, the gene inactivated by the interruption at the *Bam*HI$_{(1)}$ site seems not to be the *parC* gene.

Isolation of the Cm^r Km^s strain was then carried out by using pJK863 as a replacing plasmid in the presence of a compensating plasmid, pJK818 (Ap^r), which is a mini-F derivative and carries the *Sma*I fragment covering the *Bam*HI$_{(1)}$ site (Fig. 2). In the presence of this compensating plasmid, six Km^s clones were obtained among 100 Cm^r transformants. One of the Cm^r Km^s clones was investigated for its viability by scoring the plating efficiency after curing of the compensating plasmid (pJK818) with acridine orange. As shown in Table 2, no Ap^s colony was obtained from the Cm^r Km^s recombinant, whereas the parental strain carrying pJK818 gave rise to Ap^s colonies at a frequency of about 60%. The results strongly suggest that the gene or the operon covering the *Bam*HI$_{(1)}$ site is indispensable for cell viability.

The gene product affected by the interruption at the *Bam*HI$_{(1)}$ site was investigated by SDS-PAGE analyses of the in vitro products with pJK807 and with pJK800. These plasmids contained the same chromosome region as that carried by pJK818 except for the insertion of a translation terminator at the *Bam*HI$_{(1)}$ site on pJK807 (Fig. 2). A ca. 25K protein band was intensified in the lane for pJK800 compared with that for pJK807 (Fig. 5, lanes 9 and 10, arrow). It is inferred, by the following considerations, that the increased band intensity is due to the synthesis of the

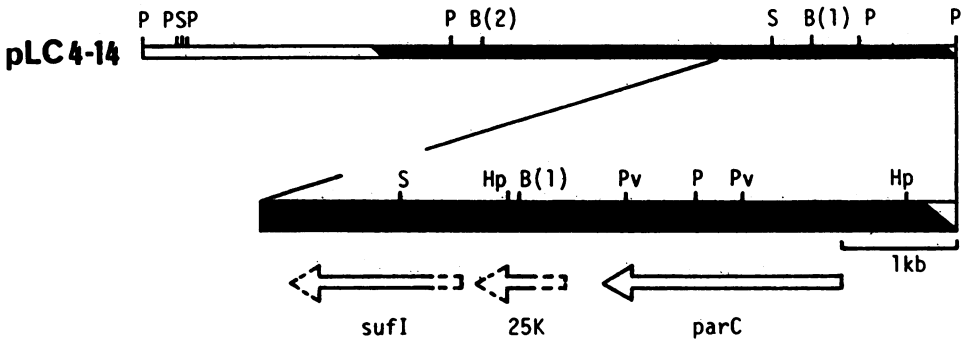

FIG. 9. Possible location of the genes for *parC*, *sufI*, and the 25K protein. The arrows below the map indicate the roughly estimated location and direction of transcription of the *parC* gene, the *sufI* gene, and the gene for the 25K protein. The ends of the *sufI* gene and the gene for the 25K protein could not be inferred in the present work and are shown as dotted lines. Two *Bam*HI sites on pLC4-14 are described as $B_{(1)}$ and $B_{(2)}$ on the map of pLC4-14 for convenience. B, *Bam*HI; Hp, *Hpa*I; P, *Pst*I; Pv, *Pvu*II; S, *Sma*I. The symbols below the map, sufI, 25K, and parC, represent the *sufI* gene, the gene for the 25K protein, and the *parC* gene, respectively.

25K protein encoded in the gene that covers the *Bam*HI$_{(1)}$ site and that the protein band seen at the same position in pJK807 is not related to this 25K protein. The protein band at the 25K position (background 25K band), such as in pJK807, was invariably far less dense in the plasmids carrying the region cut or interrupted at the *Bam*HI$_{(1)}$, site except for pJK811, when observed after short exposures. The level of the background 25K band was essentially unaffected whether the *Bam*HI$_{(1)}$ site was cut (e.g., pJK823) or interrupted by insertion of a translation terminator (pJK807); by deletion of either side of the *Bam*HI$_{(1)}$ site (e.g., pMY310 and pJK823 or pJK813); or by insertion orientation of the *Bam*HI fragment within a Tc^r gene, whereby expression of the *sufI* gene on this fragment was affected (pMY310 and pMY311). A similar 25K band was also present in the pattern of pJK811, but it was even thinner than in pMY310, suggesting that the essential gene for the 25K protein is inactive in pJK811. In this plasmid the *Bam*HI$_{(1)}$ site was present but the chromosome region was cut at the *Hpa*I site (Fig. 2). Thus, it is concluded that the 25K protein identified in pJK800 is the product of the essential gene containing the *Bam*HI$_{(1)}$ site and also, probably, the *Hpa*I site. (This conclusion was consistent with our unpublished sequence data.)

Possible operon formation by the *parC*, *sufI*, and 25K protein genes. The insertion of a translation terminator into the *Pvu*II site located closer to the *Bam*HI$_{(1)}$ site (pJK809) resulted in production of a smaller *parC* protein, suggesting that this *Pvu*II site was located toward the distal end of the *parC* coding region. Since the *Bam*HI$_{(1)}$ site is located outside of the *parC* gene, the *parC* coding region is expected to end between the *Pvu*II and the *Bam*HI$_{(1)}$ sites. Therefore, a short deletion extending from the *Bam*HI$_{(1)}$ site toward the *Pvu*II site was introduced, and its effect on the protein product was examined. For this purpose, pJK813 was constructed by appropriate digestion with exonuclease *Bal*31 at the *Bam*HI site of pJK823 and subsequent insertion of a translation terminator into the digested site (Fig. 2). The deletion created at the *Bam*HI site in pJK813 shortened the *Bam*HI$_{(1)}$-*Pvu*II region by 0.7 kilobases (kb) from the *Bam*HI$_{(1)}$ site. The in vitro protein synthesis directed by pJK813 showed the production of a protein smaller in apparent size than the 75K protein regarded as the *parC* product but larger than the 69K protein resulting from the terminator insertion at the *Pvu*II site in pJK809 (Fig. 5, lanes 7 and 8, 1 and 2). The result is consistent with the interpretation that the 69K protein represents a truncated *parC* protein and that the *parC* gene is transcribed in the direction from the *Pvu*II to the *Bam*HI$_{(1)}$ site. Considering the coding capacity required for the 75K protein and the distance of 0.6 kb between the *Pst*I site and either of the *Pvu*II sites, the *parC* coding region should cover the region containing the *Pst*I site and the rightward *Pvu*II site.

To investigate the effect of transcription that may proceed through the *parC* gene on the expression of the gene for the 25K protein and the *sufI* gene, pJK875 and pJK876 were constructed. These two plasmids received the *Pst*I fragment in opposite orientations (Fig. 3); in pJK875 the direction of *parC* transcription matches that of the *lac* promoter. The products of the in vitro protein synthesis directed by these plasmids were analyzed. The 25K protein and the *sufI* product synthesized with pJK875 were higher in level than those synthesized with pJK876 (Fig. 8A, lanes 3 and 4). This result suggests that the gene for the 25K protein, the *sufI* gene, and the *parC* gene are all transcribed in the same direction. The gene products were also examined in maxicells. Under the IPTG-induced condition, the maxicells harboring pJK875 synthesized larger amounts of the 25K protein and the *sufI* product than those harboring pJK876 (Fig. 8B, lanes 1 and 2). These results suggest that an operon exists, containing the *parC* gene, the gene for the 25K protein, and the *sufI* gene in that order from the proximal side. However, the gene for the 25K protein and the *sufI* gene were significantly expressed in the DNA fragment connected in the opposite orientation to the *lac* promoter; e.g., in pJK876 for these two genes and in pJK877 for the *sufI* gene. Thus, each of these genes may also have its own promoter. Based on the results in this section, the coding regions and the direction of transcription for the three genes are schematically represented in Fig. 9.

Localization of the gene products in cells. The location of the three gene products was investigated in maxicells labeled with [^{35}S]methionine under the direction of a plasmid containing the corresponding gene(s). The plasmids used were pJK803 carrying the *parC* gene and the gene for the 25K protein, pJK878 carrying the *sufI* gene, and pJK878 carrying the three relevant genes. The maxicells were lysed by sonication and fractionated by centrifugation in the presence of either 10 mM $MgCl_2$ or 10 mM EDTA, and the soluble and insoluble fractions were analyzed by SDS-PAGE after precipitating proteins with trichloroacetic acid (TCA).

The 25K protein was found almost exclusively in the insoluble fraction, and the distribution was not affected by the presence or absence of Mg^{2+}, whereas the *parC* protein

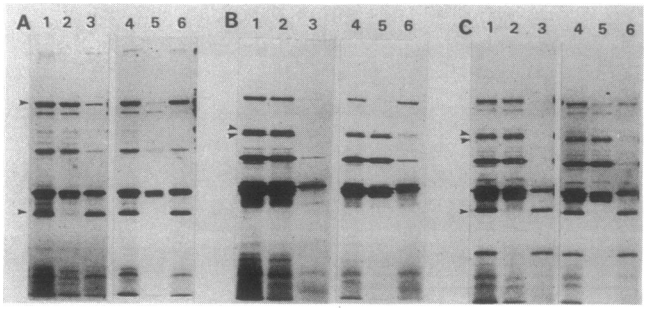

FIG. 10. Localization of the *parC* and *sufI* gene products and the 25K protein. [^{35}S]methionine-labeled proteins were synthesized in maxicells of JM109 under the direction of plasmids pJK803 (A), pJK878 (B), and pJK875 (C) and fractionated as described in Materials and Methods. The fractionated protins were precipitated by addition of 20 μl of 50% TCA and analyzed by SDS-PAGE as described in Materials and Methods. Lanes 1 and 4 show the total fraction, lanes 2 and 5 show the soluble fraction, and lanes 3 and 6 show the insoluble fraction. Lanes 1 to 3 show the fractions prepared in the presence of EDTA, and lanes 4 to 6 show those prepared in the presence of Mg^{2+}. Arrowheads to the left of lane 1 indicate (from the top) the *parC* product and the 25K protein (A), the precursor and mature form of the *sufI* product (B), and the precursor and mature forms of the *sufI* product and the 25K protein (C).

was detected in the soluble fraction in the absence of Mg^{2+} and in the insoluble fraction in the presence of Mg^{2+} (Fig. 10A). The 25K protein is most probably localized in the membrane, while the *parC* product appears not to be incorporated into the membrane and its attachment to membrane depends on Mg^{2+}. The majority of the *sufI* products, both the precursor and the mature form, were present in the soluble fraction irrespective of the presence or absence of Mg^{2+}. However, a small amount of the precursor could be detected in the insoluble fraction in the presence of Mg^{2+} (Fig. 10B and C). The precursor of β-lactamase associated with the insoluble materials was increased a little in the presence of Mg^{2+} in all the preparations. Although it might be suspected that the mature form of the *sufI* protein is released into the periplasm, the localization of this protein is not obvious at present.

DISCUSSION

A new mutation that affects chromosome partition in *E. coli* was discovered and termed *parC*, mapped at 65.3 min by P1 transduction. The *parC* gene product was identified as a 75K protein by SDS-PAGE. Proteins of similar size have been seen in *oriC*-membrane complexes (9, 24) and F partition-specific complexes (8).

The process of chromosome partition presumably involves interaction of the chromosome with the cell surface structure, and the origin complex may be a structure for this interaction. The 75K protein found in the F partition-specific complex was assumed to originate from the background synthesis in minicells. The *parC* product might be identical to this protein and be shared by the chromosome and mini-F for their partitioning. A genetic analysis for these proteins would be an effective approach to the problem, and analyses of *par* mutants may help in understanding the roles of the proteins contained in the DNA-membrane complex.

The *parC* product was found to be retained in the membrane fraction in the presence of Mg^{2+} but distributed in the soluble fraction in the absence of Mg^{2+} (although the present approach does not exclude possible effects of overproduction on localization of the gene products). The same Mg^{2+}-dependent localization has been found for the *sopB* protein involved in partition of a mini-F plasmid (K. Nagai, S. Inamoto, S. Hiraga, T. Ogura, and G. Tamura, Annu. Meet. Mol. Biol. Soc. Jpn. 1984, abstr. no. 7:B-17). The similarity in Mg^{s+} dependence may suggest a property common to the proteins engaged in partitioning of replicons. Alternatively, it may suggest the involvement of the *parC* protein in the interaction of the *sopB* product with mini-F. In this case, the *parC* protein would form a complex with the *sopB* product and show the same localization. The basic mechanism of partition might be the same as for the mini-F and the chromosome, as postulated by Jacob and others (15).

The *sufI* product was identified as a 55K protein and found to be synthesized in a precursor form. The *sufI* products were found largely in the soluble fraction by localization experiments. The *sufI* gene was originally assumed to suppress *ftsI* by overproduction of its gene products (34). This was confirmed by the present study. Overproduction was required for the correction of the temperature-sensitive defect due to *ftsI* and still the correction was generally imperfect. The function of the *sufI* product in a cell is not obvious but it appears to be dispensable for cell viability. However, it might have a vital function that is redundant due to the existence of another protein with equivalent function. The mechanism of suppression of *ftsI* is even less understood. The *ftsI* product is PBP3, which participates in septal murein synthesis (2, 31, 33), and the active center of PBP3 must exist in a periplasmic space where murein lies. Considering this and the existence of a precursor form, the *sufI* products may exist in a periplasmic space and help to stabilize the mutant PBP3, presumably through weak interactions only attained by overproduction of the *sufI* protein.

Analyses of the *parC-sufI* region elucidated another gene indispensable for cell viability. The three genes, *parC*, the gene for the 25K protein, and *sufI*, probably constitute an operon (or part of one). However, at least the latter two genes appear to have their own promoters. It will be interesting to clarify the function of the 25K protein, which is indispensable for cell viability, and to investigate the possible overlapped regulation for 25K protein synthesis. The isolation of thermosensitive mutants defective in the 25K protein would be a useful step. It could be facilitated by application of the compensating-replacing plasmid principle utilized for defining an essential gene as described in the present and a previous report (17). With this approach, mutants for the 25K protein have been isolated by displacing the compensating plasmid with mutagenized ones. These mutants are now under investigation.

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LITERATURE CITED

1. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39**:440–452.
2. **Botta, G. A., and J. T. Park.** 1981. Evidence for involvement of penicillin-binding protein 3 in murein synthesis during septation but not during cell elongation. J. Bacteriol. **145**:333–340.
3. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic mini plasmid. J. Bacteriol. **134**:

1141–1156.
4. **Clarke, L., and J. Carbon.** 1975. Biochemical construction and selection of hybrid plasmids containing specific segments of *E. coli* genome. Proc. Natl. Acad. Sci. USA **72**:4361–4365.
5. **Cosloy, S. D., and M. Oishi.** 1973. The nature of the transformation process in *Escherichia coli* K12. Mol. Gen. Genet. **124**: 1–10.
6. **Dwivedi, C. M., R. C. Ragin, and J. R. Uren.** 1982. Cloning, purification, and characterization of β-cystathionase from *Escherichia coli*. Biochemistry **21**:3064–3069.
7. **Harayama, S., and G. L. Hazelbauer.** 1982. A gene coding for a periplasmic protein is located near the locus for termination of chromosome replication in *Escherichia coli*. J. Bacteriol. **151**: 1391–1396.
8. **Hayakawa, Y., T. Murotsu, and K. Matsubara.** 1985. Mini-F protein that binds to a unique region for partition of mini-F. J. Bacteriol. **163**:349–354.
9. **Hendrickson, W. G., T. Kusano, H. Yamaki, R. Balakrishnan, M. King, J. Murchie, and M. Schaechter.** 1982. Binding of the origin of replication of *Escherichia coli* to the outer membrane. Cell **30**:915–923.
10. **Hirota, Y.** 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **46**:57–64.
11. **Hirota, Y., A. Ryter, and F. Jacob.** 1968. Thermosensitive mutants of *E. coli* affected in the processes of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. **33**:677–693.
12. **Hirota, Y., M. Ricard, and B. Shapiro.** 1971. The use of thermosensitive mutants of *E. coli* in the analysis of cell division. Biomembranes **2**:13–31.
13. **Huisman, O., R. D'Ari, and J. George.** 1980. Further characterization of *sfiA* and *sfiB* mutations in *Escherichia coli*. J. Bacteriol. **44**:185–191.
14. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell-division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81**:4490–4494.
15. **Jacob, F., S. Brenner, and F. Cuzin.** 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**:329–348.
16. **Kato, J., H. Suzuki, and Y. Hirota.** 1984. Overlapping of the coding regions for α and γ components of penicillin-binding protein 1b in *Escherichia coli*. Mol. Gen. Genet. **196**:449–457.
17. **Kato, J., H. Suzuki, and Y. Hirota.** 1985. Dispensability of either penicillin-binding protein-1a or -1b involved in the essential process for cell elongation in *Escherichia coli*. Mol. Gen. Genet. **200**:272–277.
18. **Kuroiwa, T., S. Nishibayashi, S. Kawano, and T. Suzuki.** 1981. Visualization of DNA in various phages (T4, χ, T7, φ29) by ethidium bromide epifluorescent microscopy. Experimenta **37**: 969–970.
19. **Lutkenhaus, J. F.** 1983. Coupling of DNA replication and cell division: *sulB* is an allele of *ftsZ*. J. Bacteriol. **154**:1339–1346.
20. **Meacock, P. A., and S. N. Cohen.** 1980. Partitioning of bacterial plasmids during cell division: a cis-acting locus that accomplishes stable plasmid inheritance in cell populations. Cell **20**: 529–542.
21. **Miki, T., A. M. Easton, and R. H. Rownd.** 1980. Cloning of replication, incompatibility, and stability functions of R plasmid NR1. J. Bacteriol. **141**:87–99.
22. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Mori, H., A. Kondo, A. Ohshima, T. Ogura, and S. Hiraga.** 1986. Structure and function of the F plasmid genes essential for partitioning. J. Mol. Biol. **192**:1–15.
24. **Nagai, K., W. Hendrickson, R. Balakrishnan, H. Yamaki, D. Boyd, and M. Schaechter.** 1980. Isolation of a replication origin complex from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77**: 262–266.
25. **Nakamura, M., I. N. Mauayama, M. Soma, J. Kato, H. Suzuki, and Y. Hirota.** 1983. On the process of cellular division in *Escherichia coli*: nucleotide sequence of the gene for penicillin-binding protein 3. Mol. Gen. Genet. **191**:1–9.
26. **Nishibayashi, S., and T. Kuroiwa.** 1982. Behavior of leucoplast nucleoids in the epidermal cell of onion (*Allium cepa*) bulb. Protoplasma **110**:177–184.
27. **Nishimura, Y., Y. Takeda, A. Nishimura, H. Suzuki, M. Inoue, and Y. Hirota.** 1977. Synthetic ColE1 plasmids carrying genes for cell division in *Escherichia coli*. Plasmid **1**:67–77.
28. **Nordstrom, K., S. Molin, and H. Aagard-Hansen.** 1980. Partitioning of plasmid R1 in *Escherichia coli*. I. Kinetics of loss of plasmid derivatives deleted of the *par* region. Plasmid **4**:215–227.
29. **Norris, V., T. Alliotte, A. Jaffe, and R. D'Ari.** 1986. DNA replication termination in *Escherichia coli parB* (a *dnaG* allele), *parA*, and *gyrB* mutants affected in DNA distribution. J. Bacteriol. **168**:494–504.
30. **Ogura, T., and S. Hiraga.** 1983. Partition mechanism of F plasmid: two plasmid gene-encoded products and a cis-acting region are involved in partition. Cell **32**:351–360.
31. **Spratt, B. G.** 1975. Distinct penicillin-binding proteins involved in the cell division, elongation, and shape of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **72**:2999–3003.
32. **Stoker, N. G., J. M. Pratt, and I. B. Holland.** 1984. *In vitro* gene expression systems in prokaryotes, p. 153–178. *In* B. D. Hames and S. J. Higgins (ed.), Transcription and translation. IRL, Oxford.
33. **Suzuki, H., Y. Nishimura, and Y. Hirota.** 1978. On the process of cellular division in *Escherichia coli*: a series of mutants of *E. coli* altered in the penicillin-binding proteins. Proc. Natl. Acad. Sci. USA **75**:664–668.
34. **Takeda, Y., A. Nishimura, Y. Nishimura, M. Yamada, S. Yasuda, H. Suzuki, and Y. Hirota.** 1981. Synthetic ColE1 plasmids carrying genes for penicillin-binding proteins in *Escherichia coli*. Plasmid **6**:86–98.
35. **Tanimoto, K., and T. Iino.** 1984. An essential gene for replication of the mini-F plasmid from origin I. Mol. Gen. Genet. **196**: 59–63.
36. **Timmis, K., F. Cabello, and S. Cohen.** 1975. Cloning, isolation, and characterization of replication region of complex plasmid genomes. Proc. Natl. Acad. Sci. USA **72**:2242–2246.
37. **Yamada, M., and Y. Hirota.** 1981. Isogenic and allogenic suppression of *fts* mutation of *Escherichia coli*. Annu. Rep. Natl. Inst. Genet. Jpn. **31**:33–34.
38. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
39. **Zusman, D. R., A. Carbonell, and J. Y. Haga.** 1973. Nucleoid condensation and cell division in *Escherichia coli* MX74T2ts52 after inhibition of protein synthesis. J. Bacteriol. **115**:1167–1178.