

## Chromosome Partitioning in *Escherichia coli*: Novel Mutants Producing Anucleate Cells

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To study the chromosomal partitioning mechanism in cell division, we have isolated a novel type of *Escherichia coli* mutants which formed anucleate cells, by using newly developed techniques. One of them, named *mukAI*, is not lethal and produces normal-sized anucleate cells at a frequency of 0.5 to 3% of total cells in exponentially growing populations but does not produce filamentous cells. Results suggest that the mutant is defective in the chromosome positioning at regular intracellular positions and fails frequently to partition the replicated daughter chromosomes into both daughter cells, resulting in production of one anucleate daughter cell and one with two chromosomes. The *mukAI* mutation causes pleiotropic effects: slow growth, hypersensitivity to sodium dodecyl sulfate, and tolerance to colicin E1 protein, in addition to anucleate cell formation. Cloning of the *mukA* gene indicates that the *mukAI* mutation is recessive and that the *mukA* gene is identical to the *tolC* gene coding for an outer membrane protein.

During the bacterial cell cycle, replicated chromosomal DNA molecules are spatially separated from one another prior to cell division and septation, creating two daughter cells; as a result, each daughter cell contains at least one copy of the chromosome. Several hypotheses have been formulated to explain the chromosome partitioning in bacteria (18, 22, 29, 36-38, 41); however, there is no decisive experimental evidence for these hypotheses.

Studies on the genetics of chromosomal segregation in *Escherichia coli* have used conditionally lethal mutants which are capable of DNA synthesis and septation for some time after transfer to nonpermissive temperature, but which have lost control over the spatial location of the septa. Examples of this type of mutant include the temperature-sensitive mutants *parA*, *parB* (15), *parD* (16), *parC* (21), and *gyrB* (35), and also a cold-sensitive *pcsA* mutant (24, 25). At nonpermissive temperatures, these mutants form elongated cells with a large nucleoid or nucleoids. Abnormal cell division occurs near the ends of elongated cells, producing anucleate cells. This type of mutant has so far been considered to be defective in some factor(s) involved in chromosome partitioning. However, the *parB* mutant was found to be an allele of the *dnaG* gene, which codes for primase, essential for DNA replication, and the phenotype of elongated cells observed at nonpermissive temperature reflects the inhibition of cell division associated with perturbed DNA synthesis (33). At nonpermissive temperature, cells of the *pcsA* mutant elongate and the RecA protein is induced, suggesting the induction of the SOS response (our unpublished data). In the temperature-sensitive *gyrB* mutant of the B subunit of DNA gyrase, a catenate of replicated daughter chromosomal DNA molecules is produced at nonpermissive temperature (39). Therefore, the daughter chromosomes cannot be physically separated. The *parD* mutant strain also carries a *gyrA* amber mutation, and the phenotype of elongated cells, in fact, is probably due to the *gyrA* mutation rather than to the *parD* mutation itself (17).

On the other hand, we have found that the mini-F plasmid has its own genetically controlled mechanism for partitioning of plasmid DNA molecules into daughter cells. This mechanism depends on two gene products, the SopA and SopB proteins, and a *cis*-acting site, *sopC*, of the plasmid (13, 26, 31, 34). The *cis*-acting site cloned on another replication type of plasmid exhibits incompatibility against a coexisting mini-F plasmid. Moreover, we have found that *oriC* plasmids or minichromosomes, which replicate by using the replication origin of the *E. coli* chromosome (*oriC*), do not have a partition mechanism, so that *oriC* plasmid DNA molecules are partitioned essentially at random into daughter cells. An *oriC* plasmid carrying the *sop* genes of F plasmid is stably maintained even in nonselective media. When the *oriC* plasmid loses one of the *sop* genes, the plasmid becomes unstable and plasmid-free segregants appear at high frequency. Moreover, the *oriC* plasmid has no detectable effects on the replication and equipartition of the host chromosome. These results suggest that the chromosomal segment of *oriC* plasmids, including *oriC* and its flanking regions, does not determine the function of partition (34).

The decatenation of replicated chromosomal DNA molecules and the equipartition of these separated DNA molecules into daughter cells may be different steps. The temperature-sensitive *gyrB* mutant described above is defective in the former step. We thought that a new type of mutant defective in the latter step might be isolated by new genetic techniques. This type of hypothetical mutant affected in the equipartition mechanism may be nonlethal. In such mutants, cell division would be expected to occur essentially normally, and replicated daughter chromosomes would be decatenated by each other. However, these decatenated daughter chromosomes would not be certain to be partitioned into both daughter cells at cell division. As a result, one anucleate daughter cell and one with two chromosomes should be produced at a nonnegligible frequency in the ways shown in Fig. 1. This model is based on the following four assumptions. (i) Two copies of replicated daughter chromosomes exist in a cell at cell division under conditions in

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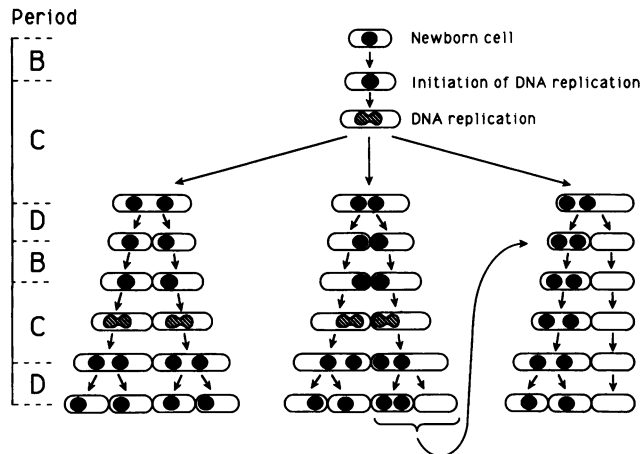


FIG. 1. A hypothetical model for nonlethal bacterial mutants defective in chromosome partitioning. A hypothetical mutant is nonlethal and defective in a factor involved in the putative partitioning mechanism of the bacterial chromosome. Replicated daughter chromosomes are not guaranteed to be partitioned into both daughter cells during cell division; as a result, one anucleate daughter cell and another daughter cell having two daughter chromosomes are produced at a certain frequency in populations by the ways shown in this figure.

which the doubling time of bacteria is more than 60 min at 37°C (5, 12). (ii) Chromosomal replication occurs once during the next generation in a daughter cell which received one daughter chromosome. (iii) Chromosomal replication is arrested for one generation in a daughter cell which received two daughter chromosomes, until the next cell division occurs. (iv) The anucleate cells produced remain stably in the culture without lysis. As shown in Fig. 1, in one type of case, replicated and decatenated chromosomes are located near one of the cell poles before septation and both chromosomes are partitioned into only one of the daughter cells in cell division. In another type of case, both replicated and decatenated chromosomes are located in the center of cell before septation and septum formation in the center produces two daughter cells having one daughter chromosome which is located near the new pole of the cell. If the chromosome is replicated near the new pole and replicated

chromosomes stay there until the next septation occurs, one daughter cell with two chromosomes and one anucleate daughter cell are produced (Fig. 1). Cultures of this type of mutant should therefore contain anucleate cells whose size is similar to that of newborn nucleate cells. In contrast to plasmids which show random segregation (32), the frequency of anucleate cell production is expected to be lower in hypothetical mutants defective in the bacterial chromosome partitioning, since nucleoids are very large. The chance that both nucleoids are located in one half of a dividing cell may be lower than the theoretical one in random segregation of plasmid DNA molecules into daughter cells.

In accordance with this working hypothesis, we sought to isolate a new type of nonlethal mutant defective in the putative chromosome-partitioning mechanism. For the purpose, we developed techniques for isolating anucleate cell forming nonlethal mutants and isolated a number of such mutants. In this paper, we describe the new techniques and a mutant that appears to be defective in chromosome positioning and partitioning. We will strictly use the term "partitioning" or "equipartition of chromosomes (nucleoids)" only for divided daughter cells or septated chains of cells. If cell division or septation did not occur, we will use the term "intracellular localization of chromosomes (nucleoids)."

## MATERIALS AND METHODS

**Bacterial strains, bacteriophages, and plasmids.** All bacterial strains used were derivatives of *E. coli* K-12 (Table 1). The cosmid vector pHSG262 (3) was obtained from the Japanese Cancer Research Resources Bank and used to prepare a library of chromosomal DNA segments of W3110 strain. Vector phage EMBL4 (8) was used for a library of chromosomal DNA segments of SH3252. The plasmid vector pACYC184 (4) was used for subcloning chromosomal DNA segments. Phage P1vir was used for general transduction of chromosomal markers to map a mutated gene. A temperature-sensitive runaway replication plasmid, pOU82 (9), was kindly provided by Søren Molin. Plasmid pXX747 was constructed by joining the *Bam*HI DNA segment containing the *lacZ* gene of *E. coli* and a *Bam*HI DNA segment of pOU82. Mini-F plasmid pXX325 was described previously (20).

**Media.** The following media were used for bacterial

TABLE 1. Bacterial strains

Strain	Genotype	Source, reference, or construction
W3110	Prototroph	Y. Kohara
SH2	$\Delta trpE5 his$	Derivative of W3110 14
SH317	<i>thyA pro rpsL</i> (ColE1)	A. Maeda
SH392	<i>met hsdR sfiC</i>	20
SH3208	$\Delta trpE5 his$ ( $\lambda$ )	SH2
SH3210	$\Delta trpE5 his$ ( $\lambda$ ) (pXX747)	SH3208
SH3211	$\Delta trpE5 his mukA1$ ( $\lambda$ ) (pXX747)	SH3210
SH3243	<i>minB1 zcf-117::Tn10</i>	6
SH3246	$\Delta trpE5 his zcf-117::Tn10 minB1$ ( $\lambda$ ) (pXX747)	P1vir(SH3243) $\rightarrow$ SH3208
SH3247	$\Delta trpE5 his zcf-117::Tn10$ ( $\lambda$ ) (pXX747)	P1vir(SH3243) $\rightarrow$ SH3208
SH3252	$\Delta trpE5 his mukA1$ ( $\lambda$ )	SH3211
SH3255	$\Delta trpE5 his zcf-117::Tn10 minB1$ ( $\lambda$ )	SH3246
SH3256	$\Delta trpE5 his zcf-117::Tn10$ ( $\lambda$ )	SH3247
SH3257	<i>thyA leu dnaG rpsL tonB</i> ( $\lambda$ ) (pXX747)	This paper
NM539	<i>supF hsdR</i> (P2 <i>cox3</i> )	8
VCS257	Derivative of DP50 <i>supF</i>	Gigapack kit

growth. L medium consists of 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% yeast extract, and 0.5% NaCl (pH 7.4). P medium consists of 1% polypeptone and 0.5% NaCl (pH 7.4). Minimal E medium (40) was supplemented with 0.5% glucose or glycerol, 50  $\mu$ g of L-tryptophan per ml, and 50  $\mu$ g of L-histidine per ml or 0.4% Casamino Acids (Difco). To test for the expression of  $\beta$ -galactosidase, agar plates of P or L medium containing 40  $\mu$ g of X-Gal (5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside) were used. To prepare agar plates, 1.4% (wt/vol) agar was added to media. For the X-Gal-containing plates, we used a specific agar, Agarose-I (Dojindo Laboratory, Kumamoto, Japan), to get good colony color. To select antibiotic-resistant transformants, antibiotics were added to media at the following concentrations: ampicillin, 25  $\mu$ g/ml; tetracycline, 15  $\mu$ g/ml; kanamycin, 20  $\mu$ g/ml; chloramphenicol, 20  $\mu$ g/ml.

**Isolation of anucleate cell-forming mutants.** Growing cells of SH3210 were treated with the mutagen ethyl methanesulfonate (5%) for 20 min. The cells were washed, suspended in L medium containing ampicillin, divided into 60 tubes, and incubated overnight at 30°C. A small portion of each culture was diluted and spread on P agar plates containing X-Gal to obtain about 1,000 colonies on a plate. After 15 h of incubation at 42°C, blue mutant colonies were picked and purified on P agar plates containing X-Gal at 42°C.

**Cloning of the *mukA* gene.** A library of chromosomal DNA extracted from the wild-type strain W3110 was constructed by using the cosmid vector pHSG262. The chromosomal DNA was partially digested with *Sau3AI* and ligated with *Bam*HI-digested pHSG262 DNA. The DNA sample was packaged in vitro into  $\lambda$  phage particles by using a Gigapack kit (Stratagene, San Diego, Calif.) and used to infect the *mukA* mutant harboring pXX747 (SH3211). Kanamycin and ampicillin resistant colonies were selected on P agar plates containing X-Gal, kanamycin, and ampicillin at 42°C. Large white colonies were isolated and purified. Recombinant plasmid DNA was extracted, analyzed, and used for subcloning.

A library of chromosomal DNA extracted from the *mukA* mutant SH3252 was constructed by using the EMBL4 bacteriophage  $\lambda$  cloning vector. *Sau3AI* partial digest of DNA and *Bam*HI-digested EMBL4 phage DNA were ligated and packaged in vitro. The library was infected to NM539 which is permissive only to recombinant phages. Screening by plaque hybridization techniques (28) was performed by using a biotinylated DNA probe of the *mukA*<sup>+</sup>-carrying pAX614 plasmid labeled by the BluGENE nonradioactive nucleic acid detection system (Bethesda Research Laboratories, Gaithersburg, Md.).

The chromosomal DNA segments were subcloned by using the plasmid vector pACYC184 and host strain SH392. Constructed plasmids harboring a chloramphenicol resistance determinant were introduced into the pXX747-carrying strains SH3210 and SH3211 by transformation. Chloramphenicol- and ampicillin-resistant transformants were isolated, streaked on L agar plates containing X-Gal, and incubated at 42°C for 15 h to observe the color of single colonies.

**Microscope observation of cells and nucleoids.** We developed a new method to observe clearly the shape of cells and nucleoids simultaneously. In this method, nucleoids (chromosomal DNA) shine brightly with light blue fluorescence, other cytoplasmic portions of cells are dark blue, and the background is orange. Anucleate cells are thus clearly detected as dark blue or black cells, when they are present in the sample. Moreover, when "ghost" cells, which have lost

cellular components, are present, they are nearly transparent, not dark blue, and can therefore be clearly distinguished from nucleate and anucleate cells.

Cells of a culture were collected by centrifugation (19,000  $\times g$ , 5 min) and washed once with saline (0.84% sterilized NaCl solution). The washed cells were suspended in an appropriate volume of saline. A 5- to 10- $\mu$ l portion of the sample was spread on a clean slide glass and dried at room temperature. Alternatively, a colony grown on a plate was picked with a platinum loop or a clean plastic tip and suspended in 5 to 10  $\mu$ l of saline on a clean glass slide. The sample was dried and fixed completely with drops of methanol for 5 min. If any cells remained unfixated by the methanol, they did not stain strongly with the fluorescence compound in a later step. After a 5-min fixation with methanol, the slide was washed six times with tap water (not distilled water or deionized water) in a large beaker. After washing, the slide was dried at room temperature, and then 10  $\mu$ l of poly-L-lysine (5  $\mu$ g/ml of distilled water) was spread over the sample with a plastic tip. The slide was dried again at room temperature to fix all cells tightly to it. DAPI solution (4',6-diamino-2-phenyl-indole) (10  $\mu$ l; 5  $\mu$ g/ml of saline), which binds specifically to DNA, was dropped on the sample. A clean glass cover slip washed with ethanol immediately before use was put on the drop.

After immersion oil was put between the cover slip and an objective (a Plan 100DL objective lens for phase contrast, 100X), the cells were observed through a Optiphot microscope (Nikon), combining the phase-contrast system and the fluorescence system (an XF-EFD apparatus and a DM400 dichroic mirror for UV excitation). The two systems were used simultaneously in a dark room without color filters in the microscope. When the light of a halogen lamp was reduced to an appropriate level, the fluorescent nucleoids and cell shape were clearly visible at the same time. The microscope was also equipped with a camera and an autoexposure apparatus. Color film was used for photographs. This method was called "Hiraga's fluo-phase combined method."

Wooden toothpicks should not be used to pick and/or suspend cells, because they are sometimes contaminated with dried mycoplasmas, which form visible structures never stained with DAPI, even after methanol fixation.

**Graphic analysis of the amount of DNA per cell.** Bacterial cells were observed and photographed by Hiraga's fluo-phase combined method and by the fluorescence method under UV light only using reversal color film for slides (ASA 400; Fuji or Eastman Kodak Co., Rochester, N.Y.). With the fluorescence method, samples were photographed for an appropriate exposure time by hand. Color slides obtained by the fluorescence method were analyzed with a graphic analyzer, a two-dimensional/one-dimensional UV-VIS video densitometer with the Image Analyzer II Program (Biomed Instruments, Inc., Fullerton, Calif.), to measure the fluorescence intensity of each cell, i.e., the amount of DNA stained with DAPI in each cell.

## RESULTS

**Strategy of isolation of anucleate cell-forming mutants.** We have developed techniques for isolating nonlethal mutants which form frequently anucleate cells. We constructed plasmid pXX747, which is a runaway replication plasmid carrying the *lacZ* gene and its operator-promoter region of the *E. coli* chromosome (Fig. 2). We introduced this plasmid by transformation into a bacterial strain lysogenic for phage  $\lambda$ . The copy number of this plasmid is low in  $\lambda$  lysogens since

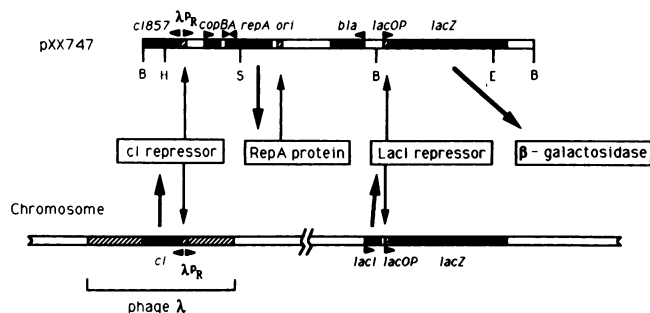


FIG. 2. Strategy of isolation of nonlethal bacterial mutants producing anucleate cells. A bacterial strain lysogenic for phage  $\lambda$  and harboring plasmid pXX747 was used as the parental strain to isolate anucleate cell-forming nonlethal mutants. The upper bar represents the plasmid pXX747 carrying the *lacZ* gene coding for  $\beta$ -galactosidase. The lower bar represents host bacterial chromosome carrying phage  $\lambda$  at the  $\lambda$  attachment site (*att* $\lambda$ ) located on the chromosome. Arrows associated with the bars represent promoter sites and the direction of transcription. *bla*,  $\beta$ -Lactamase gene conferring ampicillin resistance. The *repA* gene codes for RepA protein, which is essential for initiation of plasmid replication from the replication origin *ori*. The *cI* and *cI857* genes code for wild-type and temperature-sensitive altered *cI* repressors, respectively. Transcription from the  $\lambda$  *p<sub>R</sub>* promoter is regulated by the *cI* repressor. *copA* and *copB* concern the regulation of plasmid copy number (9). Restriction enzyme sites H, S, E and B in the upper bar represent *Hind*III, *Sal*I, *Eco*RI, and *Bam*HI, respectively. For details, see the text.

synthesis of the RepA protein, which is essential for initiation of plasmid replication, is under control of the  $\lambda$  *p<sub>R</sub>* promoter, which is repressed to low levels by the  $\lambda$  *cI* repressor (9). Under these conditions, the *lac* operon is also repressed since the chromosome is *lacI*<sup>+</sup>. If the plasmid pXX747 segregates into an anucleate cell,  $\beta$ -galactosidase will be synthesized at a high level at 42°C. This is because anucleate cells have lost the chromosome in which the *cI* and *lacI* genes are located, and they can synthesize neither the normal *cI* repressor nor the *lacI* repressor. The *cI* repressor synthesized before the cell division creating the anucleate cell is gradually degraded in the anucleate cell, and then the expression of the *repA* gene is derepressed at 42°C (since the temperature-sensitive altered *cI* repressor coded by the mutated *cI857* gene located on pXX747 is inactive at 42°C). As a result, the *repA* gene product is overproduced as described by Gerdes et al. (9). Overproduction of the RepA protein will cause an increase in the plasmid copy number in anucleate cells. Thus the copy number of the *lac* operator will increase without concomitant synthesis of *lacI* repressor. This will result in derepression of  $\beta$ -galactosidase synthesis in anucleate cells carrying pXX747. We can therefore expect that in a strain carrying pXX747, anucleate cell-forming mutants will make blue colonies at 42°C on plates containing X-Gal, in contrast to white colonies of the pXX747-carrying parental strain.

To test this strategy, we performed preliminary experiments in a *minB1* mutant strain, which produces anucleate cells at high frequencies (1, 6, 19). The plasmid pXX747 was introduced into a *minB1*  $\lambda$  lysogen (SH3246) by transformation, and ampicillin-resistant transformants were tested for the color of colonies grown at 42°C on P agar plates containing X-Gal. As shown in Table 2, the pXX747-harboring *minB1* mutant (SH3246) formed blue and white colonies at 42°C. The blue colonies were resistant to ampicillin, suggesting pXX747-carrying cells. White colonies were sensitive to this antibiotic, suggesting pXX747-free segregants.

TABLE 2. Preliminary experiments in a minicell-forming mutant, *minB1*<sup>a</sup>

Strain	Relevant genetic character and plasmid	Color of colonies	
		Amp <sup>r</sup>	Amp <sup>s</sup>
SH3246	<i>minB1</i> ( $\lambda$ ) (pXX747)	Blue	White
SH3247	<i>min</i> <sup>+</sup> ( $\lambda$ ) (pXX747)	White	White
SH3255	<i>minB1</i> ( $\lambda$ )		White
SH3256	<i>min</i> <sup>+</sup> ( $\lambda$ )		White

<sup>a</sup> Cultures of bacterial strains were spread on P agar plates containing X-Gal and incubated at 42°C for 15 h to observe the color of single colonies. Fifty colonies grown on the plates were picked and tested for the sensitivity to ampicillin to determine whether each clone carried plasmid pXX747. Amp<sup>r</sup> and Amp<sup>s</sup>, Ampicillin resistant and sensitive, respectively.

In contrast, a pXX747-carrying *min*<sup>+</sup> isogenic strain (SH3247) formed only white colonies, resistant or sensitive to ampicillin. Plasmid-free *minB1* and *min*<sup>+</sup> strains (SH3255 and SH3256) also formed only white colonies under the same conditions (Table 2). These results indicate that the strategy is effective for isolating anucleate cell-forming nonlethal mutants. Although about 50% of the total cells are anucleate in the cultures of the *minB1* mutant, the mass of anucleate cells is about 5% of total cell mass, because minicells are very small, whereas nucleate cells are large and elongated, often having many nucleoids (19). This suggests that the technique is quite sensitive for detecting anucleate cell-forming nonlethal mutants.

**Isolation of anucleate cell-forming mutants.** Strain SH3210, a  $\lambda$  lysogen harboring pXX747, was chosen as the parental strain to isolate anucleate cell-forming mutants. Cells of SH3210 were treated with the mutagen ethyl methanesulfonate as described in Materials and Methods. Blue mutant colonies grown in P agar plates containing X-Gal at 42°C were isolated and purified. Among these, one can expect  $\beta$ -galactosidase constitutive mutants [*lacI* and *lacO*(Con)]. To discard this type of mutant in the next step, we restreaked a purified blue colony from each mutant clone on a P agar plate containing X-Gal and incubated for 15 h at 42°C. Since pXX747 is somewhat unstable, the plate will include some plasmid-free segregants. From 20 to 50% of pXX747-free segregants were in single colonies grown in nonselective plates at 42°C. If the clone made only blue colonies on the plate, independently of the presence or absence of pXX747, it was discarded as a constitutive mutant for expression of the chromosomal *lacZ* gene. If, on the other hand, the clone made blue and white colonies, a white colony, which is a plasmid-free segregant sensitive to ampicillin, was isolated, spread on a P agar plate lacking X-Gal, and incubated at 42°C. One of the colonies grown on the plate was picked and tested for cell shape, nucleoids, and anucleate cells by the newly developed microscopic technique described in Materials and Methods.

Of 500 mutants isolated, about half formed only blue colonies, suggesting constitutive mutants for the expression of  $\beta$ -galactosidase. The remaining half of the mutants formed blue colonies in the presence of pXX747 but white colonies in its absence. Among these we distinguished five morphological classes, mutants which form (i) anucleate normal-sized cells, (ii) anucleate minicells, (iii) rounded cells, (iv) filamentous cells, and (v) normal-sized nucleate cells. The second class of mutants produced minicells lacking chromosomal DNA, independently of temperature. These mutations were located in or near the *minB* gene by P1vir transduction (19). In the third class of mutants, cells formed nearly normal rods at 30°C and rounded or spherical forms of large volume



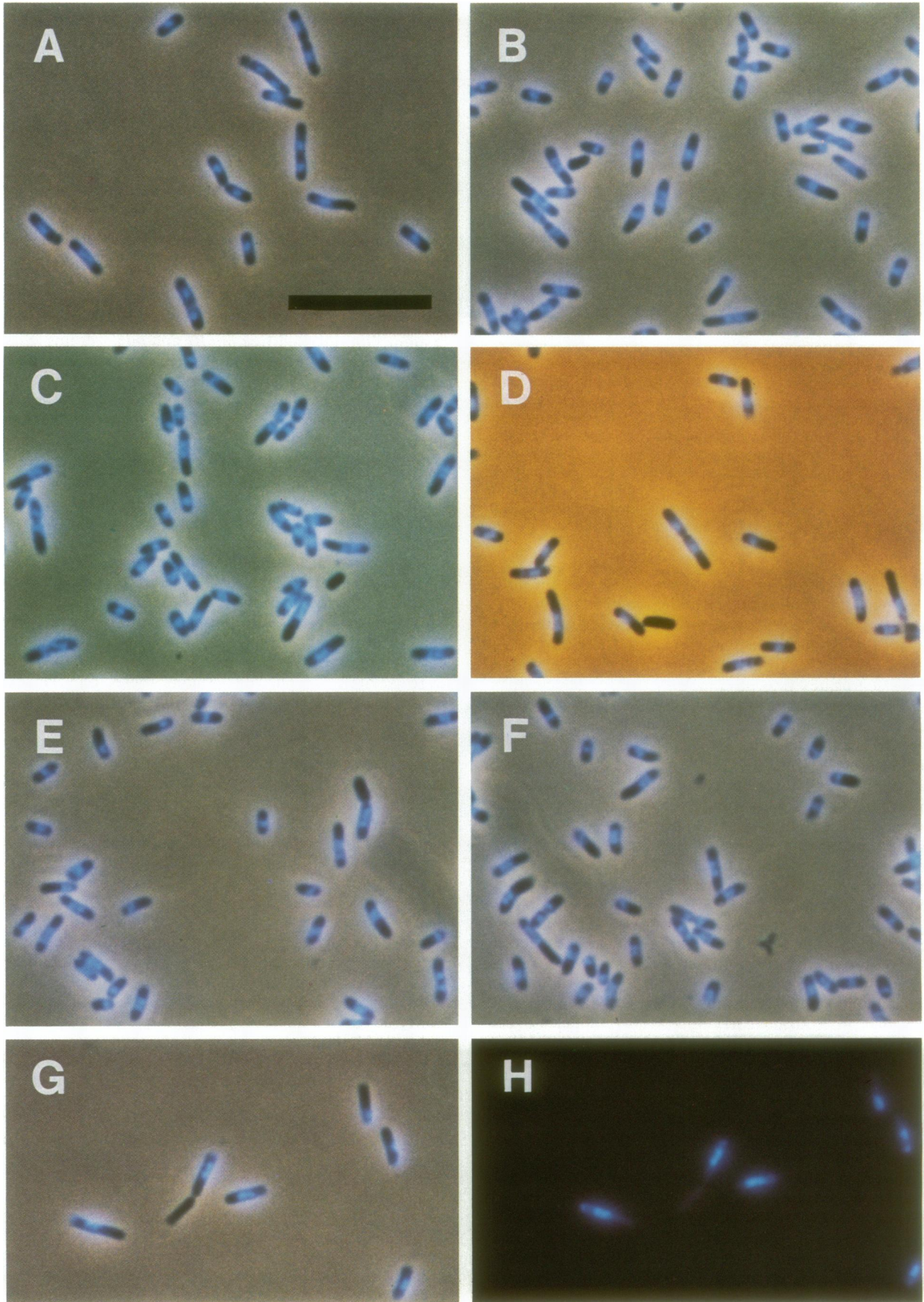


TABLE 3. Frequencies of anucleate cells in bacterial cultures grown in various media<sup>a</sup>

Strain	<i>mukA</i> gene	Medium	Doubling time at 37°C (min)	Ratio of anucleate cells to total cells (%) at temp:	
				37°C	30°C
SH3208	<i>muk</i> <sup>+</sup>	L	35	<0.03	<0.03
		ME, glucose, Trp, CA	45	<0.03	<0.03
		ME, glucose, Trp, His	80	<0.03	<0.03
		ME, glycerol, Trp, His	120	<0.03	<0.03
SH3252	<i>mukA1</i>	L	40	0.5	NT
		ME, glucose, Trp, CA	60	0.5	NT
		ME, glucose, Trp, His	150	1	1
		ME, glycerol, Trp, His	175	2	3

<sup>a</sup> Bacterial cells were grown in L medium and minimal medium E (ME) supplemented with 0.5% glucose or glycerol; Trp, tryptophan (50 µg/ml); His, histidine (50 µg/ml); CA, Casamino Acids (0.4 %; Difco). Anucleate cells in the cultures were detected by Hiraga's fluo-phase combined method. NT, Not tested.

at 42°C; spherical ghost cells were also observed in cultures of these mutants at 42°C. In cultures of the fourth class of mutants, filamentous cells with many nucleoids and normal- or nearly normal-sized anucleate cells were observed in addition to normal-size rod-shaped cells. The fifth class of mutants were probably *lacO*(Con) mutants located on the plasmid, or leaky mutants of the *lacI* gene located on the chromosome.

The first class of mutants produced anucleate normal-sized cells at low frequencies, independently of temperature, in addition to normal nucleate cells. One of these, named *mukA1* (*muk* stands for the Japanese word "mukaku," meaning "anucleate"), was chosen for further analysis.

**Properties of the *mukA1* mutant.** The *mukA1* strain SH3252, which had lost pXX747, was tested by the newly developed microscopic technique, Hiraga's fluo-phase combined method, to evaluate anucleate cell formation. As shown in microscopic color photographs (Fig. 3), the majority of cells had one or two nucleoids that shone brightly with light blue fluorescence. However, low frequencies of anucleate cells were clearly detected as black or dark blue cells lacking chromosomal DNA (Fig. 3B, C, and D). The frequencies of anucleate cells in cultures in various media are shown in Table 3. Anucleate cells were always present at 0.5 to 3% of total cells in the *mukA1* strain, with higher frequencies in poor media than in rich ones. In contrast, anucleate cells were less than 0.03% of total cells in the *muk*<sup>+</sup> control strain SH3208 (Table 3). The doubling time of the *mukA1* strain was longer than that of the *mukA*<sup>+</sup> strain. In the *mukA1* mutant, the majority of anucleate cells were rods of a length corresponding to that of newly divided nucleate cells.

Distribution of the cell length of the *muk*<sup>+</sup> strain and the *mukA1* mutant growing in minimal glucose medium is shown in Fig. 4A and B. The average cell length of *mukA1* was

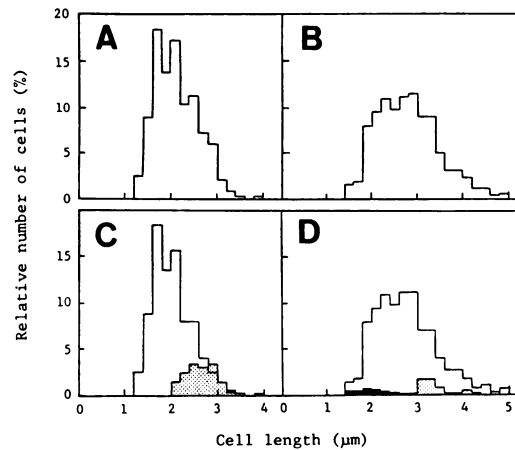


FIG. 4. Distribution of cell length in *muk*<sup>+</sup> and *mukA1* strains. Cells growing exponentially in minimal glucose medium containing tryptophan and histidine at 37°C were fixed, stained with DAPI, and photographed. (A and C) SH3208 (*muk*<sup>+</sup>). (B and D), SH3252 (*mukA1*). Panels A and B show total cells. In panels C and D, open area represents cells with one nucleoid mass (DNA content corresponds to 1 to 2 copies of the chromosome), dotted area represents cells with two separated nucleoid masses, and solid area represents anucleate cells. Total cell numbers measured are 326 and 336 in *muk*<sup>+</sup> and *mukA1*, respectively.

slightly longer than that of the *muk*<sup>+</sup> strain. Figure 4C and D shows the distribution of cell length of cells with one nucleoid mass of which the DNA content corresponds to 1 to 2 copies of the chromosome, cells with two separated nucleoid masses, and anucleate cells. The number of cells with two separated nucleoid masses was only 7% of the total cell number in *mukA1* and 17% in the parental strain. The number of anucleate cells was 2.6% of the total cells in *mukA1*.

Figure 5 shows intracellular localization of nucleoid masses in cells with one nucleoid mass (the DNA content is 1 to 2 chromosomes) and cells with two nucleoid masses. Intracellular position of nucleoid masses distributed more widely in the *mukA1* mutant than the *muk*<sup>+</sup> strain.

**DNA content of abnormal pairs of anucleate and a nucleate cell.** In addition to anucleate cells, there were pairs of cells: one anucleate cell of unit cell length and one nucleate cell of 1 to 2 units. Samples of such pairs are shown in Fig. 3E, F, G, and H. Using a graphic analyzer, we measured the relative amount of DNA in this type of cell compared with that in other nucleate cells. The nucleate in a pair of this type had about twice as much DNA as newborn nucleate cells did, although the nucleate in this type of pairs had various lengths from 2 to 4 µm (Fig. 6). This is consistent with the early speculation that chromosome replication is arrested for one generation in a daughter cell which received two daughter chromosomes, until the next cell division occurs (Fig. 1). It is unlikely that anucleate cell formation in the *mukA1* mutant is caused by a defect in chromosomal replication.

FIG. 3. Photographs of cells of the *mukA1* mutant and the wild-type strain. Cells were grown at 37°C in minimal glucose medium containing tryptophan and histidine. Cells were observed by Hiraga's fluo-phase combined method or by the fluorescence method as described in Materials and Methods. (A) SH3208 (*muk*<sup>+</sup>). (B through H) SH3252 (*mukA1*). All pictures except for panel H were taken by Hiraga's fluo-phase combined method. Panel H was taken by the fluorescence method under UV light only, without the light of a halogen lamp. Panels G and H are a pair of pictures of the same sample. A dispersion filter (Nikon D) and a pale blue filter (Nikon NCB10) were used for the light of a halogen lamp in panels A through C and E through G, but the pale blue filter was omitted in panel D. All pictures are enlarged to the same magnification. Bar in panel A, 10 µm.

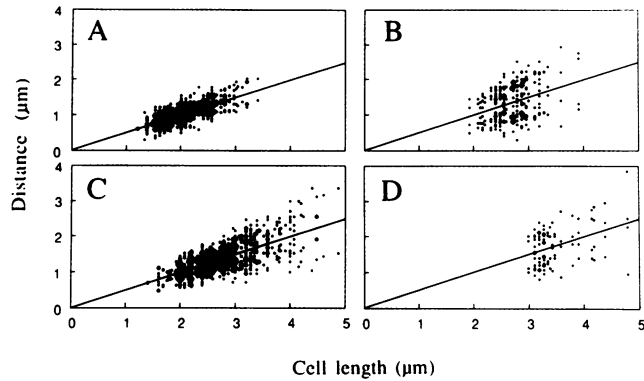


FIG. 5. Intracellular localization of nucleoid masses in *muk*<sup>+</sup> and *mukA1* strains. Distances from the center of each nucleoid mass to two poles of the cell are plotted. (A and B), SH3208 (*muk*<sup>+</sup>). (C and D), SH3252 (*mukA1*). Panels A and C show cells with one nucleoid mass (the DNA content is 1 to 2 copies of the chromosome). Panels B and D show cells with two separated nucleoid masses.

**Stability of mini-F plasmid in *mukA1*.** Mini-F plasmid pXX325 was stably inherited in the *mukA1* mutant (SH3252), indicating that the *mukA1* mutation did not affect replication, decatenation, and partition of the plasmid (data not shown).

**Spontaneous induction of prophage λ.** The level of spontaneous induction of λ prophage was the same in both the *muk*<sup>+</sup> and *mukA1* strains growing exponentially at 37°C in minimal glucose medium containing tryptophan and histidine. This suggests that the *mukA1* mutation does not cause DNA damage which induces the SOS response, such as induction of phage λ and of the SfiA protein, which is an inhibitor of cell division (for a review, see reference 27).

**Cloning of the chromosomal DNA segment complementing the *mukA1* mutation.** To clone the *mukA*<sup>+</sup> gene, we prepared a chromosomal DNA library of the wild-type strain W3110 by using the cosmid vector pHSG262 (kanamycin resistant). After in vitro packaging of DNA into phage particles, the *mukA1* cells harboring pXX747 (SH3211) were infected. Colonies resistant to both kanamycin and ampicillin were isolated and tested for the Muk phenotype as described above. Some cosmid clones were found to complement the *mukA1* mutation. These cosmids were analyzed for the

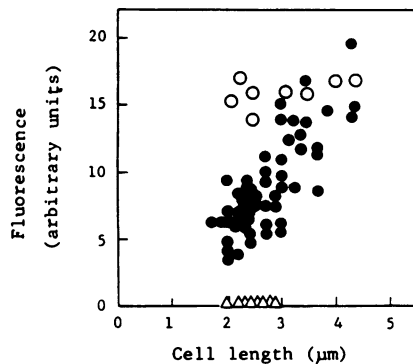


FIG. 6. DNA content of *mukA1* cells. Exponentially growing cells of SH3252 (*mukA1*) were fixed, stained, and photographed in two ways as described in Materials and Methods. Intensity of fluorescence of each cell was analyzed with a graphic analyzer. ○ and △, Nucleate cells (○) and anucleate cells (△) in eight abnormal pairs; ●, 60 single nucleate cells.

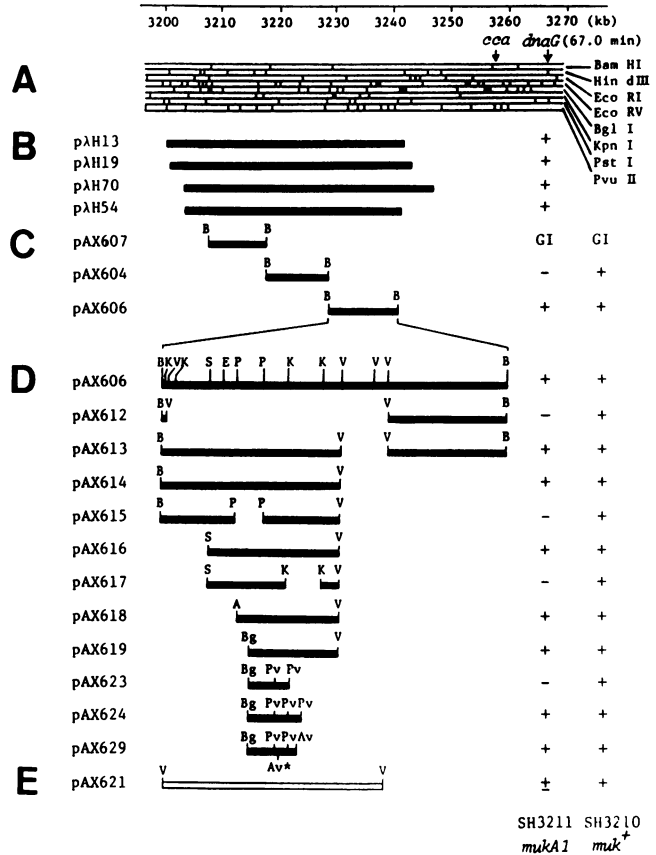


FIG. 7. Cloning of the *mukA*<sup>+</sup> gene and the *mukA1* mutated gene. (A) Upper lines represent part of the cleavage map of the chromosome of *E. coli* W3110 described by Kohara et al. (23). Scales are expressed in kilobase coordinates in which the position of *thr* at 0 min on the genetic map is taken as 0 kb. (B) rλH clones (kanamycin resistant) are cosmids carrying chromosomal DNA segments of the wild-type strain W3110 (■). (C) Plasmids pAX607, pAX604, and pAX606 (chloramphenicol resistant; pACYC184 vector) carry *Bam*HI subsegments of the cosmid rλH54. (D) Deletion derivatives of pAX606. (E) pAX621 carries the *Eco*RV chromosomal segment (□) of the *mukA1* mutant (SH3252). Each cosmid or plasmid was introduced into pXX747-harboring strains SH3210 (*muk*<sup>+</sup>) and SH3211 (*mukA1*). Resulting transformants were tested for their Muk phenotype on L agar plates containing X-Gal at 42°C. Results of the complementation test for the Muk phenotype are shown on the right side of the figure. + and −, Muk<sup>+</sup> and Muk<sup>−</sup> phenotypes, respectively. GI denotes partial inhibition of host cell growth by the indicated plasmid (Muk phenotype was not clear). A, *Ava*I; Av, *Ava*II; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; V, *Eco*RV. An asterisked *Ava*II site shown in pAX629 was methylated by *dam* methylase.

cleavage map of restriction enzymes. As shown in Fig. 7, four clones tested, rλH13, rλH19, rλH54, and rλH70, had a common DNA segment of the *E. coli* chromosome. We searched the whole restriction map of the *E. coli* chromosome (23) for this common segment, and found it located at about 65.5 min, near the *dnaG* gene (Fig. 7). To confirm the location of the *mukA1* mutation, we performed transduction with phage P1vir. A temperature-sensitive *dnaG* mutant (SH3257), which was lysogenic for λ and harbored pXX747, was infected with P1vir phage prepared in the *mukA1* mutant (SH3252). Temperature-resistant transductants were purified and tested for the color of colonies on L agar plates containing X-Gal at 42°C. Of 100 transductants, 54 clones showed the Muk<sup>−</sup> phenotype; i.e., they formed blue colo-



nies at 42°C in the presence of pXX747 and white colonies in the absence of pXX747. The *mukA* gene is therefore 54% cotransducible with *dnaG*. Furthermore, this result indicates that the *mukA1* mutation can be introduced into other strains by transduction, suggesting that a single mutation is sufficient to confer the Muk<sup>-</sup> phenotype on L agar plates containing X-Gal at 42°C.

To locate the *mukA* gene precisely, we prepared plasmids carrying chromosomal *Bam*HI subsegments of pλH54 by using pACYC184 as a vector (chloramphenicol resistant). The *Bam*HI site at the right-hand end of the chromosome segment carried by pλH54 was presumably created by the joining of a *Sau*3AI site of the chromosome segment to the *Bam*HI site of pHSG262. As shown in Fig. 7, pAX606 complemented the *mukA1* mutant (SH3211), whereas pAX604 did not. Deletion derivatives were isolated from pAX606 and tested for their ability to complement the *mukA1* mutation. pAX613, pAX614, pAX616, pAX618, pAX619, pAX624, and pAX629 complemented the mutation, whereas pAX612, pAX615, pAX617, and pAX623 did not. These results locate the *mukA* gene within the 1.9-kilobase (kb) *Bgl*III-*Ava*II DNA segment carried by pAX629 (Fig. 7).

**Cloning of the *mukA1* mutated gene.** To clone the chromosomal DNA segment carrying the *mukA1* mutated gene, we prepared a library of chromosomal DNA segments obtained from the *mukA1* mutant (SH3252) by using the EMBL4 bacteriophage λ cloning vector. Phages of the library were infected to NM539, and the plaques were tested by plaque hybridization techniques using pAX614 DNA labeled with BluGENE as a probe. Four positive clones were isolated and analyzed for the cleavage map of restriction enzymes. All clones tested had a common chromosomal DNA segment corresponding to the W3110 DNA segment containing the *mukA*<sup>+</sup> gene. Subcloning was done from one of these clones by using pACYC184 as vector. As shown in Fig. 7, pAX621, carrying the 6.5-kb *Eco*RV segment of the SH3252 chromosome, complemented weakly the *mukA1* mutation. This complementation can be explained by large amounts of altered protein coded by high copies of the *mukA1* gene.

***trans*-acting function of the *mukA* gene.** To test whether the *mukA1* mutation was complemented in *trans* by pAX619 or in *cis* by the plasmid integrated into the *E. coli* chromosome, we tried to eliminate the plasmid from the *mukA1* strain and tested the Muk phenotype. The vector plasmid pACYC184, which carries determinants of tetracycline and chloramphenicol resistance, was introduced into the *mukA1* mutant harboring both pXX747 (ampicillin resistance) and pAX619 (chloramphenicol resistance) by transformation. Tetracycline resistant transformants were purified and grown in L medium containing tetracycline and ampicillin but not chloramphenicol. Under these conditions, cells carrying both pACYC184 and pXX747 but not pAX619 were easily selected. During incubation in this medium, pAX619 was eliminated by incompatibility with the homologous plasmid pACYC184. Ten purified colonies were tested for colony color on L agar plates containing X-Gal and chloramphenicol at 42°C. All the tested pAX619-free segregants which harbored both pXX747 and pACYC184 showed the Muk<sup>-</sup> phenotype. This suggests that a *trans*-acting factor coded by pAX619 complements the *mukA1* mutation located on the chromosome.

**Pleiotropic effects of the *mukA1* mutation.** The *tolC*, *ribB*, and *attP1 attP7* markers are located in the 65.5-min region of the *E. coli* chromosome (for a review, see reference 2). Mutants of the *tolC* gene have pleiotropic phenotypes, tolerance to colicin E1, and hypersensitivities to dyes,

detergents, and antibiotics (7). We found that the *mukA1* mutant (SH3252) was tolerant to killing by colicin E1 protein and hypersensitive to sodium dodecyl sulfate (SDS), whereas the *muk*<sup>+</sup> strain SH3208 was sensitive to the colicin and resistant to SDS. Three phenotypes, production of anucleate cells, tolerance to colicin E1, and hypersensitivity to SDS, of *mukA1* were complemented by pAX624 and pAX629 but not pAX623.

We have isolated independently nine spontaneous mutants resistant to colicin E1 protein from a *muk*<sup>+</sup> strain harboring pXX747 (SH3210) and tested these mutants for their Muk phenotypes on a plate containing X-Gal at 42°C. One among them showed the Muk<sup>-</sup> phenotype on the plate. The mutation was therefore named *muk-2*. Plasmid-free segregants from the *muk-2* mutant were isolated and tested for anucleate cell formation by Hiraga's fluo-phase combined method. This mutant frequently produced anucleate cells, like the *mukA1* mutant. The *muk-2* mutant was also hypersensitive to SDS. The colicin tolerance of the *muk-2* mutation, but not those of the other tolerant mutants, was complemented by pAX624 and pAX629.

## DISCUSSION

The new type of nonlethal mutant (*mukA1*) producing anucleate cells was characterized. Filamentous cells, which can produce anucleate cells from their extremities, were not observed in cultures of the *mukA1* mutant, in contrast to conditionally lethal mutants, such as temperature-sensitive *gyrB* (35), *parB* (*dnaG*) (15, 33), and *parC* (21) mutants at nonpermissive temperature. As shown in Fig. 4, cells having one nucleoid mass in which the DNA content corresponds to 1 to 2 copies of the chromosome make up 93% of the total cell number in *mukA1* populations and 83% in *muk*<sup>+</sup> populations. This difference between *mukA1* and *muk*<sup>+</sup> can be explained by the difference of time from cell division to initiation of DNA replication (B period), since doubling time in turbidity is longer in *mukA1* than *muk*<sup>+</sup> in the minimal glucose medium (Table 3). However, it is also possible that replicated chromosomes show a tendency to be positioned near each other in *mukA1*. It should be noticed that among 3 to 5 μm of *mukA1* cells, 81% of cells have one nucleoid mass and only 19% of cells have two separated nucleoid masses (Fig. 4D). About 4 μm of *mukA1* cells had 2 copies of the replicated chromosomes (Fig. 6). These results suggest that there was a large number of cells with 2 unit length in which replicated chromosomes were located near each other and observed as one nucleoid mass in *mukA1*. As shown in Fig. 5A, in *muk*<sup>+</sup> cells with one nucleoid mass, a nucleoid mass shows a tendency to be positioned in the center of the cell. In *muk*<sup>+</sup> cells with two separated nucleoid masses, nucleoid masses show a tendency to be located in positions one-fourth of cell length from cell poles (Fig. 5B). By contrast, intracellular positions of nucleoid masses were distributed widely in *mukA1* cells (Fig. 5C). As shown in Fig. 6, the content of DNA was constant and corresponded to two copies of the chromosome in the nucleate cell of a pair of anucleate and nucleate sisters, although the cell length of the nucleate cell ranged from 2 to 4 μm. This is consistent with the model shown in Fig. 1. In this model, we speculated that newborn cells of unit length which received two daughter chromosomes do not perform chromosome replication for one generation until the next cell division occurs, because it is well known that the initiation of chromosome replication in *E. coli* is controlled to keep constant the ratio of DNA to cell mass.



If the *mukA1* mutant should be partially defective in chromosome replication and miss occasionally chromosome replication, pairs of a unit length anucleate sister and a unit length nucleate sister having one copy of the unreplicated chromosome would be observed in cultures of *mukA1*. Since the chromosome must replicate in the next generation, the DNA content in the nucleate cells of pairs of this type would be in proportion as the cell length. The possibility of a defect in replication is, however, inconsistent with the result shown in Fig. 6.

Mini-F plasmid pXX325 was stably inherited in the *mukA1* mutant as well as the *muk*<sup>+</sup> strain. This plasmid carries the *sop* genes, but lacks the *ccd* genes and the *fcr* sequence for site-specific resolution. The *mukA1* mutant is not defective in replication, decatenation, and partition of the plasmid. It is therefore unlikely that the *mukA1* mutant is partially defective in decatenation of replicated daughter chromosomes as temperature-sensitive *gyrB* mutants. The results presented in this paper suggest that the *mukA1* mutant is defective in the cellular mechanism which acts to locate replicated and decatenated chromosomes in normal intracellular positions, but not defective in chromosome replication and decatenation.

The plasmid pAX629 carrying the 1.9-kb *Bgl*II-*Ava*II chromosomal segment complemented the *mukA1* mutation. The result of our recent DNA sequencing (unpublished data) indicates that this segment has the sole open reading frame homologous with the *tolC* gene described by Hackett and Reeves (11), although there are some differences in the sequences. This shows that the *mukA* gene is identical to the *tolC* gene. We found some mistakes in the DNA sequence of the *tolC* gene described by Hackett and Reeves (11) and also found mistakes of cleavage sites of *Pvu*II in the region of the restriction maps of the W3110 chromosome described by Kohara et al. (23). The *parC* gene (21) is located in the *Bam*HI DNA segment which is carried by pXX604. The *tolC* mutants are known to prevent the expression of three outer membrane proteins, OmpF, NmpC, and protein 2. Most of this effect is likely to reflect posttranscriptional control (30). Hackett et al. (10) and Hackett and Reeves (11) described the cloning and sequencing of a chromosomal DNA segment which complements *tolC* mutations, and they found that this cloned gene codes for an outer membrane protein. The *mukA1* and *muk-2* mutations were hypersensitive to SDS and tolerant to colicin E1. We isolated recently SDS-resistant revertants from the *mukA1* mutant. Some among them showed the Muk<sup>+</sup> phenotype and colicin sensitivity and others showed the Muk<sup>-</sup> phenotype and colicin sensitivity. Therefore, the Muk<sup>-</sup> phenotype does not correlate with the hypersensitivity to SDS and the tolerance to colicin E1. We also characterized and mapped other *muk* mutants which were located at other loci different from *mukA1* on the *E. coli* chromosome (unpublished data).

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#### LITERATURE CITED

- Adler, H. I., W. Fisher, A. Cohen, and A. Hardigree. 1967. Miniature *E. coli* cells deficient in DNA. *Proc. Natl. Acad. Sci. USA* **57**:321-326.
- Bachmann, B. J. 1983. Linkage map of *Escherichia coli*, edition 7. *Microbiol. Rev.* **47**:180-230.
- Brady, G., H. M. Jantzen, H. U. Bernard, R. Brown, G. Schutz, and T. Hashimoto-Gotoh. 1984. New cosmid vectors developed for eukaryotic DNA cloning. *Gene* **27**:223-232.
- Chang, A. C. Y., and S. N. Cohen. 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. *J. Bacteriol.* **134**:1141-1156.
- Cooper, S., and C. E. Helmstetter. 1968. Chromosome replication and the division cycle of *Escherichia coli* B/r. *J. Mol. Biol.* **31**:519-540.
- Davie, E., K. Sydnor, and L. I. Rothfield. 1984. Genetic basis of minicell formation in *Escherichia coli* K-12. *J. Bacteriol.* **158**:1012-1023.
- Davies, J. K., and P. Reeves. 1975. Genetics of resistance among colicins in *Escherichia coli* K-12: cross-resistance among colicins of group A. *J. Bacteriol.* **123**:102-117.
- Fischauf, A. M., H. Lehrach, A. Poustka, and N. Murray. 1983. Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.* **170**:827-842.
- Gerdes, K., J. E. L. Larsen, and S. Molin. 1985. Stable inheritance of plasmid R1 requires two different loci. *J. Bacteriol.* **161**:292-298.
- Hackett, J., R. Misra, and P. Reeves. 1983. The TolC protein of *Escherichia coli* K12 is synthesized in a precursor form. *FEBS Lett.* **156**:307-310.
- Hackett, J., and P. Reeves. 1983. Primary structure of the *tolC* gene that codes for an outer membrane protein of *Escherichia coli* K12. *Nucleic Acids Res.* **11**:6487-6495.
- Helmstetter, C. E., and S. Cooper. 1968. DNA synthesis during the division cycle of rapidly growing *Escherichia coli* B/r. *J. Mol. Biol.* **31**:507-518.
- Hiraga, S., T. Ogura, H. Mori, and M. Tanaka. 1985. Mechanisms essential for stable inheritance of mini-F plasmid, p. 469-487. In D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), *Plasmids in bacteria*. Plenum Publishing Corp., New York.
- Hiraga, S., and C. Yanofsky. 1972. Normal repression in a deletion mutant lacking almost the entire operator-proximal gene of the tryptophan operon in *E. coli*. *Nature (London)* **237**:47-49.
- Hirota, Y., M. Ricard, and B. Shapiro. 1971. The use of thermosensitive mutants of *E. coli* in the analysis of cell division p. 13-31. In L. A. Manson (ed.), *Biomembranes*. Plenum Publishing Corp., New York.
- Hussain, K., K. J. Begg, G. P. C. Salmond, and W. D. Donachie. 1987. *ParD*: a new gene coding for a protein required for chromosome partitioning and septum localization in *Escherichia coli*. *Mol. Microbiol.* **1**:73-81.
- Hussain, K., E. J. Elliott, and G. P. C. Salmond. 1987. The *parD*<sup>-</sup> mutant of *Escherichia coli* also carries a *gyrA*<sub>am</sub> mutation. The complete sequence of *gyrA*. *Mol. Microbiol.* **1**:259-273.
- 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.
- Jaffé, A., R. D'Ari, and S. Hiraga. 1988. Minicell-forming mutants in *Escherichia coli*: production of minicells and anucleate rods. *J. Bacteriol.* **170**:3094-3101.
- Jaffé, A., T. Ogura, and S. Hiraga. 1985. Effects of the *ccd* function of the F plasmid on bacterial growth. *J. Bacteriol.* **163**:841-849.
- Kato, J., Y. Nishimura, M. Yamada, H. Suzuki, and Y. Hirota. 1988. Gene organization in the region containing a new gene involved in chromosome partition in *Escherichia coli*. *J. Bacteriol.* **170**:3967-3977.
- Koch, A. L., H. L. T. Mobley, R. J. Doyle, and U. N. Streips. 1981. The coupling of wall growth and chromosome replication

- in gram-positive rods. FEMS Microbiol. Lett. 12:201-208.
23. Kohara, Y., K. Akiyama, and K. Isono. 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell 50:495-508.
  24. Kudo, T., K. Nagai, and G. Tamura. 1976. Characterization of a cold-sensitive chromosome segregation mutant of *Escherichia coli*. Agric. Biol. Chem. 41:89-95.
  25. Kudo, T., K. Nagai, and G. Tamura. 1976. Genetic analysis of a cold-sensitive chromosome segregation mutant of *Escherichia coli* K12. Agric. Biol. Chem. 41:607-608.
  26. Kusakawa, N., H. Mori, A. Kondo, and S. Hiraga. 1987. Partitioning of the F plasmid: overproduction of an essential protein for partition inhibits plasmid maintenance. Mol. Gen. Genet. 208:365-372.
  27. Little, J. M., and D. W. Mount. 1982. The SOS regulatory system of *Escherichia coli*. Cell 29:11-12.
  28. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
  29. Mendelson, N. H. 1985. A model of bacterial DNA segregation based upon helical geometry. J. Theor. Biol. 112:25-39.
  30. Morana, R., and P. Reeves. 1982. The *tolC* locus of *Escherichia coli* affects the expression of three major outer membrane proteins. J. Bacteriol. 150:1016-1023.
  31. 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.
  32. Nordström, K., S. Molin, and H. Aagaard-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.
  33. Norris, V., T. Alliotte, A. Jaffé, 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.
  34. 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.
  35. Orr, E., N. F. Fairweather, I. B. Holland, and R. H. Pritchard. 1979. Isolation and characterization of a strain carrying a conditional lethal mutation in the *cou* gene of *Escherichia coli* K12. Mol. Gen. Genet. 177:103-112.
  36. Ryter, A., Y. Hirota, and F. Jacob. 1968. DNA-membrane complex and nuclear segregation in bacteria. Cold Spring Harbor Symp. Quant. Biol. 33:669-676.
  37. Sargent, M. G. 1975. Control of cell length in *Bacillus subtilis*. J. Bacteriol. 123:7-19.
  38. Schlaeppli, J.-M., and D. Karamata. 1982. Cosegregation of cell wall and DNA in *Bacillus subtilis*. J. Bacteriol. 152:1231-1240.
  39. Steck, T. R., and K. Drlica. 1984. Bacterial chromosome segregation: evidence for DNA gyrase involvement in decatenation. Cell 36:1081-1088.
  40. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. 218:97-106.
  41. Woldringh, C. L., and N. Nanninga. 1985. Structure of nucleoid and cytoplasm in the intact cell, p. 161-197. In N. Nanninga (ed.), Molecular cytology of *Escherichia coli*. Academic Press, Inc., New York.