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A Locus Affecting Nucleoid Segregation in *Salmonella typhimurium*

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Thirteen temperature-sensitive lethal mutations of *Salmonella typhimurium* map near *metC* at 65 min and form the *clmF* (conditional lethal mutation) locus. The mutations in this region were ordered by three-point transduction crosses. After a shift to the nonpermissive temperature, many of these *clmF* mutants failed to complete the segregation of nucleoids into daughter cells; daughter nucleoids appeared incompletely separated and asymmetrically positioned within cells. Some *clmF* mutants showed instability of F′ episomes at permissive growth temperatures yet showed no detectable defect with smaller multicopy plasmids such as pSC101 or pBR322. In addition, many of the *clmF* mutants rapidly lost viability yet continued DNA replication at the nonpermissive temperature. These results suggest that the *clmF* locus encodes at least one indispensable gene product that is required for faithful partitioning of the bacterial nucleoid and F-plasmid replicons.

Bacteria achieve faithful segregation of daughter genomes at each cell division by a mechanism that is poorly understood. Jacob et al. (12) proposed that the replicating genomes attach to the membrane or wall structure and that growth of the cell wall at the midpoint of the cell between the daughter nucleoids pushes the two genomes apart, passively achieving faithful segregation. This passive segregation model requires localization of cell wall addition between the two nucleoids as well as attachment of the DNA to the membrane or wall. Cell wall addition is achieved by two independent systems, one of which causes elongation of the cell (PBP 2-dependent cell wall addition), whereas the other accomplishes formation of the septum (PBP 3-dependent addition) (22, 29). The location(s) of cell wall elongation is controversial, since some results suggest peptidoglycan addition at the cell midpoint (25), but other results suggest diffuse addition at over 100 cellular locations (4, 5, 33). Thus, at this time, the location(s) of cell wall elongation can neither support nor refute the passive model of nucleoid segregation.

The passive segregation model also requires attachment of the DNA to the membrane or wall structure. In *Bacillus subtilis*, attachment of the origin of replication to the membrane is required for the initiation of replication (34). In *Escherichia coli*, Schaecter and co-workers have demonstrated specific attachment of a DNA fragment from the *oriC* region with an outer membrane fraction in vitro (9, 16). Within the *oriC* segment, many *dam*-modified GATC sites exist, and hemimethylation of the *oriC* fragment (as would occur just after DNA replication) causes increased affinity for membrane sites in vitro (20). This finding suggests that the *oriC* fragment may associate with the membrane just after replication and then dissociate from it later in the cell cycle (20). Two proteins of 55 and 75 kilodaltons (kDa) may aid the *oriC*-membrane association (9). It is unclear whether DNA regions other than *oriC* show specific affinity for the membrane or aid nucleoid partitioning.

Mutations in genes affecting nucleoid segregation have been identified among conditional lethal mutations that form long filaments at the nonpermissive temperature (10a, 23). In some of these filamenting mutants, DNA replication continues at the nonpermissive temperature, but cytological examination reveals that daughter nucleoids are not correctly partitioned. Two unlinked genes, *parA* and *parB*, were identified by this combined genetic and cytological method (10a). *parB* was recently shown to be allelic with *dnaG* (DNA primase) at 67 min (19), but the role of this gene product in nucleoid segregation is unclear. The *parA* locus has recently been identified as an allele of *gyrB* (13), and some alleles of *gyrA* (11) and *gyrB* (18, 30) show a partitioning defect. After DNA replication, the two circular genomes must decatenate before full separation of the nucleoids can occur; the DNA gyrase enzyme, encoded by the *gyrA* and *gyrB* genes, is thought to cause this decatenation (30). Other filamenting conditional lethal mutants show regularly spaced nucleoids within the filament. These mutants, having no apparent defect in nucleoid partitioning, show that the completion of septation is not required for faithful partitioning of nucleoids.

Recently, Kato et al. (14) described two new temperature-sensitive lethal mutations of *E. coli* that show linkage with *metC* and are defective in nucleoid partitioning. Recombinant DNA experiments show that the mutations in a gene they call *parC* are complemented by a plasmid encoding a 75-kDa membrane-associated protein (14). In addition, a second essential gene is closely linked to *parC* and encodes a 25-kDa protein that has no known function.

A collection of 440 temperature-sensitive lethal mutants of *Salmonella typhimurium* has recently been described (27). This collection of mutants has undergone extensive genetic and cytological analysis, and results suggest that most chromosomal genes mutable to a temperature-sensitive lethal phenotype are represented in this collection. Two regions of the chromosome show an extraordinary number of conditional lethal mutations. One of these regions (called the *clmF* locus) is closely linked to *metC*; 13 temperature-sensitive lethal mutations in the collection map in this region. Genetic and cytological characteristics of the *clmF* mutants suggest that the genes affected are important for correct partitioning of daughter nucleoids.

MATERIALS AND METHODS

Bacterial strains. All strains of *S. typhimurum* used are derivatives of *S. typhimurium* LT2. The temperature-sensitive mutants were isolated in either a wild-type LT2 background (SE5026 through SE5261) or a *leu-485* background

TABLE 1. Bacterial strains

Strain	Description
Used as recipients for fine structure mapping (mutagenized background)	
SE5171	*clmF171*
SE5174	*clmF174*
SE5202	*clmF202*
SE5206	*clmF206*
SE5213	*clmF213*
SE5219	*clmF219*
SE5229	*clmF229*
SE5232	*clmF232*
SE5309	*clmF279 leu-485*
SE5311	*clmF281 leu-485*
SE5359	*clmF361 leu-485*
SE5372	*clmF374 leu-485*
SE5375	*clmF377 leu-485*
Used as donors for fine structure mapping (mutagenized background)	
SE7735	*zge-2393*::Tn*10 clmF202*
SE7739	*zge-2393*::Tn*10 clmF229*
SE7740	*zge-2393*::Tn*10 clmF232*
SE7742	*zge-2393*::Tn*10 clmF279 leu-485*
SE7745	*zge-2393*::Tn*10 clmF377 leu-485*
SE7770	*zge-2393*::Tn*10 clmF171*
SE7771	*zge-2393*::Tn*10 clmF174*
SE7773	*zge-2393*::Tn*10 clmF213*
SE7774	*zge-2393*::Tn*10 clmF219*
SE7775	*zge-2393*::Tn*10 clmF281 leu-485*
SE7776	*zge-2393*::Tn*10 clmF374 leu-485*
SE8020	*zge-2393*::Tn*10 clmF361 leu-485*
SE8030	*zge-2393*::Tn*10 clmF206*
clmF in isogenic background	
SE7763	*clmF377 leu-485 zge-2393*::Tn*10*
SE7765	*clmF229 leu-485 zge-2393*::Tn*10*
SE7767	*clmF202 leu-485 zge-2393*::Tn*10*
SE7769	*clmF279 leu-485 zge-2393*::Tn*10*
SE7778	*clmF232 leu-485 zge-2393*::Tn*10*
SE7781	*clmF213 leu-485 zge-2393*::Tn*10*
SE7782	*clmF219 leu-485 zge-2393*::Tn*10*
SE7784	*clmF281 leu-485 zge-2393*::Tn*10*
SE7815	*clmF171 leu-485 zge-2393*::Tn*10*
SE7818	*clmF174 leu-485 zge-2393*::Tn*10*
SE7985	*clmF374 leu-485 zge-2393*::Tn*10*
Miscellaneous	
SE5743	F'_{254} Lac$^+$ *his-644*
SE7146	pBR322 *his-203*
SE7147	pSC101 *his-203*
SE7319	*zge-2393*::Tn*10*
SE7321	*zge-2395*::Tn*10*
SE7992	*clmF361 zge-2395*::Tn*10 leu-485* (mutagenized background)

[a] All are derived from *S. typhimurium* LT2. The original temperature-sensitive *clmF* alleles were obtained by diethyl sulfate mutagenesis; the strains derived from mutagenized strains are indicated.

(SE5292 through SE5493). The temperature-sensitive lethal mutants were identified among cells mutagenized by diethyl sulfate (24) by replica plating onto LB plates at 21 and 44°C (27). *E. coli* strains were obtained from B. Bachmann and the *E. coli* Genetic Stock Center. The strains used are described in Table 1. All *clmF* mutations were moved into a *leu-485* (SE5017) unmutagenized background by P22-mediated generalized transduction, using the linked insertion *zge-2393*::Tn*10*.

Transductional methods. Generalized transduction of *S. typhimurium* strains was accomplished by the use of P22 HT201 *int*, a high-frequency transducing mutant (28) made integration defective by G. Roberts and J. Roth. All transductions were performed at multiplicities of infection of between 0.1 and 5 bacteriophage per cell.

Tn*10* insertions near the *clmF* mutations were identified from a pool of 2,000 independent Tn*10*-containing strains by cotransduction of Tn*10* and temperature-independent growth. The methods for the generation of the pool of Tn*10* strains and identification of nearby Tn*10* insertions are described by Davis et al. (7).

The map locations of the *clmF* mutations were identified by using the library of mini-Tn*10* (Tn*10*Δ16Δ17) insertions described by Kukral et al. (15). Many of the Tn*10*Δ16Δ17 insertions in this library are genetically mapped, and all insertions reside in DNA that is cloned in a corresponding λ library (15).

Cytological methods. Temperature-sensitive mutants were observed by phase and fluorescence microscopy, using a Zeiss Photomicroscope III. Cultures of the temperature-sensitive mutants were made in liquid LB at 30°C with shaking. At an optical density at 600 nm of between 0.2 and 0.4, the culture temperature was shifted to 44°C, where the cultures were left, with shaking, for 2.0 to 2.5 h. After this time, a sample of cells was treated with 3% toluene at 37°C for 15 min, and then 4′,6-diamidino-1-phenylindole (DAPI) was added to 0.1 μg/ml. After 5 min of incubation at room temperature, cells were immobilized on polylysine-treated glass microscope slides. Photomicrographs visualizing both phase and fluorescence images were achieved by decreasing the phase image intensity through the use of the neutral density (0.5) filter and the green (VG-9) filter. Black and white TMAX400 film was developed with Diafine developer. Before use, 20 μl of 1% polylysine was spread onto a glass microscope slide with the side of a plastic repeat pipettor tip and allowed to air dry at room temperature; the slide was then flooded with water, drained, and allowed to air dry.

Media. Complex medium was LB (5 g of yeast extract, 10 g of tryptone [Difco Laboratories], 5 g of NaCl, and 15 g of agar per liter); liquid medium was identical but without the agar. Green plates (6) served as phage sensitivity indicator plates. MacConkey medium (Difco) was prepared as recommended by Difco and was supplemented with lactose for the experiments shown in Table 4.

Minimal medium was the E medium of Vogel and Bonner (32) supplemented with 0.2% glucose. Supplements to E medium were made at the concentrations given by Davis et al. (7). Growth of all *S. typhimurium* LT2 strains on E medium at 44°C requires addition of methionine. Selection for growth on lactose as carbon source was performed on the NCE medium of Berkowitz et al. (3) supplemented with 0.2% lactose.

Conjugation methods. Conjugation crosses, either between two *Salmonella* strains or between an *E. coli* donor and a *Salmonella* recipient, were performed by mixing samples of freshly grown overnight cultures and then incubating the mixtures at room temperature for 30 min before plating them on selective medium. Generally, the donor/recipient ratio was 1:10.

Plasmid stability. The F'_{254} replicon was introduced into the *clmF* mutants by selecting Lac$^+$ (*S. typhimurium* strains are naturally Lac$^-$). The exconjugants were purified on MacConkey lactose plates. Lac$^+$ (pink) colonies were chosen and repurified on the same medium. In this second purification, 30 to 300 colonies were examined for the Lac$^+$

TABLE 2. *clmF-metC* linkage[a]

Recipient strain	% Linkage with given donor strain			
	AK3213 (*zge-3213*)	AK3246 (*zge-3246*)	SE7319 (*zge-2393*)	SE7321 (*zge-2395*)
SE5174 (*clmF174*)	18 (9/52)	72 (36/52)	80 (40/50)	ND
SE5311 (*clmF281*)	8 (4/51)	50 (25/52)	64 (18/28)	ND
SE5219 (*clmF219*)	12 (6/51)	46 (23/52)	69 (27/39)	ND
SE5202 (*clmF202*)	6 (3/52)	62 (31/52)	70 (35/50)	ND
SE5213 (*clmF213*)	18 (9/52)	76 (38/52)	78 (39/50)	ND
SE5359 (*clmF361*)	15 (3/20)	50 (10/20)	35 (17/48)	37 (15/41)
SE5372 (*clmF374*)	2 (1/52)	16 (8/52)	32 (10/31)	50 (15/30)
SE5206 (*clmF206*)	<2 (0/52)	28 (14/52)	20 (10/50)	70 (21/30)
metC30	65 (13/20)	5 (1/20)	11 (5/46)	<4 (0/26)

[a] P22-mediated generalized transduction crosses between phage carrying Tn*10* insertions linked to *clmF*$^+$ and temperature-sensitive *clmF* alleles. Selection was made for growth on LB-tetracycline plates at 30°C, after which the ability of Tet transductants to grow at 44°C was assayed. Numbers in parentheses represent number of transductants growing at 44/number of Tet^r transductants tested. In the last line, linkage of the Tn*10* insertions to *metC* is shown. In this cross, selection was for Tet^r, after which the percentage of Met$^+$ transductants was determined. The *clmF* alleles are listed in the order determined by the crosses of Fig. 1 and Table 3. The Tn*10* insertions present in strains SE7319 and SE7321 are full-sized Tn*10* insertions; the insertions present in AK3213 and AK3246 are Tn*10*Δ16Δ17 (15). ND, Not determined.

or Lac$^-$ phenotype to obtain an estimate of the stability of F'_{254} in these strains. To test the stability of pSC101 and pBR322, the plasmids were introduced into *clmF*-carrying strains by generalized transduction, using phage P22 grown on an *S. typhimurium* strain carrying one of these two plasmids (SE7146 or SE7147) and selecting for Tet^r transductants. The transductants were purified once on LB-tetracycline plates and then purified a second time on LB plates to allow segregation of the plasmid. In this case, 25 to 200 colonies were picked from the LB plates and tested for maintenance of tetracycline resistance. The growth on LB plates allows approximately 20 generations of nonselective growth, which was sufficient for identification of the *cis*-acting *par* sites on pSC101 by Meacock and Cohen (17).

Radioactive labeling. Cultures of *clmF* mutants were grown in LB broth at 30°C with shaking to an optical density at 600 nm of approximately 0.1. At this time, [^{3}H]thymidine (10 μCi/ml; 76.7 Ci/mmol; Dupont, NEN Research Products) was added. Incubation at 30°C was continued for 50 min, during which time 0.25-ml samples were periodically removed and placed into 1 ml of cold 10% trichloroacetic acid and set on ice. The temperature of the culture was shifted to 44°C, and further samples were taken in the same manner. The samples were filtered through GF/A filters, washed with 3 ml of cold 10% trichloroacetic acid, dried with ethanol, and then counted in Eco-Scint (National Diagnostics) scintillation cocktail.

RESULTS

Mapping the *clmF* mutations to the *metC* region. Thirteen temperature-sensitive lethal mutations that show transductional linkage to the *zge-2393*::Tn*10* insertion (27) collectively form the *clmF* group of mutations. Two mini-Tn*10* insertions of known map location, *zge-3213*::Tn*10*Δ16Δ17 and *zge-3246*::Tn*10*Δ16Δ17 (15), were found to be linked to the *clmF* mutations (Table 2) as well as to *metC* alleles at 65 min (Table 2; 15). Most of the temperature-sensitive *clmF* alleles showed weak linkage to *metC*; the *clmF281* mutation showed 25% linkage to the *metC30* point mutation (data not shown).

TABLE 3. Three-point crosses to order *clmF* mutations[a]

Recipient strain	% Tet^r with seven donor strain												
	SE7771 (*clmF174*)	SE7775 (*clmF281*)	SE7770 (*clmF171*)	SE7774 (*clmF219*)	SE7739 (*clmF229*)	SE7735 (*clmF202*)	SE7773 (*clmF213*)	SE8020 (*clmF361*)	SE7742 (*clmF279*)	SE7745 (*clmF377*)	SE7740 (*clmF232*)	SE7776 (*clm374*)	SE8030 (*clmF206*)	SE7992 (*clmF361*)
SE5174 (*clmF174*)		40	45	25	30	65	45	ND	50	70	50	85	83	3
SE5311 (*clmF281*)	0		50	ND	40	30	35	28	30	30	45	55	40	ND
SE5171 (*clmF171*)	2	0		10	35	40	25	43	45	55	60	95	55	9
SE5219 (*clmF219*)	0	0	8		40	25	25	ND	25	35	50	45	70	ND
SE5229 (*clmF229*)	5	10	2	0		45	20	27	50	70	70	90	70	0
SE5202 (*clmF202*)	2	2	0	10	15		5	16	20	55	55	60	48	13
SE5213 (*clmF213*)	2	0	10	0	0	10		27	5	40	35	ND	50	ND
SE5359 (*clmF361*)	5	2	5	0	2	15	10		ND	70	60	75	38	ND
SE5309 (*clmF279*)	ND	ND	ND	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
SE5375 (*clmF377*)	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	ND	ND	ND
SE5232 (*clmF232*)	3	3	0	0	3	10	0	0	3	6		46	37	ND
SE5372 (*clmF374*)	5	2	0	0	5	0	0	0	5	15	10		43	55
SE5206 (*clmF206*)	0	2	0	0	0	0	5	3	0	15	0	0		50

[a] P22-mediated transduction between phage grown on the indicated donor and recipient strains. The donor strains carry *zge-2393*::Tn*10* as well as the indicated *clmF* allele except for strain SE7992, which carries *zge-2395*::Tn*10*). Selection was made for growth on LB at 44°C, after which the percentage of Tet^r transductants was determined from testing 20 to 60 transductants. ND, No data.

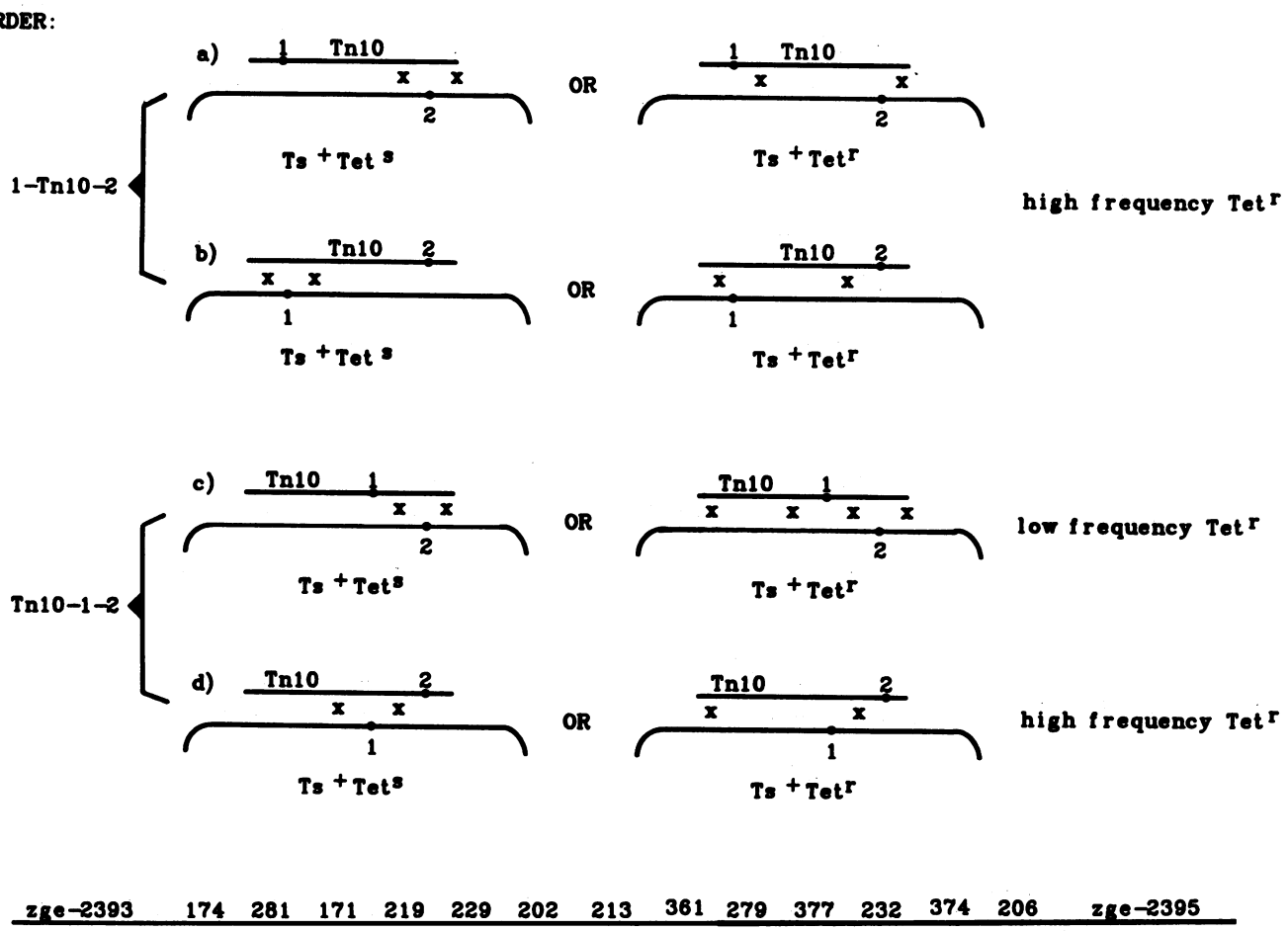

FIG. 1. Fine structure mapping of the *clmF* alleles by transduction crosses. The donor in each cross is shown above by a straight line; the recipient is below. For each cross, the two recombinant types that can arise are shown side by side. The numbers 1 and 2 are used to represent two different *clmF* alleles. The two crosses diagrammed in a and b are reciprocal crosses, as are c and d. Two potential orders of the *zge-2393*::Tn*10* and *clmF* alleles yield different results in reciprocal crosses. a and b show the results if Tn*10* lies between two *clmF* alleles; c and d show the results if Tn*10* lies on one side of two *clmF* alleles. The deduced order of *clmF* alleles is given below. Ambiguities exist in the relative order of mutations *clmF202*, *clmF213*, *clmF361*, and *clmF279*.

Fine structure map of *clmF* mutations. Three-point transduction crosses between pairs of *clmF* mutations and the *zge-2393*::Tn*10* insertion revealed the relative order of the *clmF* mutations. Strains were constructed that carry *zge-2393*::Tn*10* and each of the *clmF* temperature-sensitive lethal alleles (Table 1). Each of these strains was used as a transduction donor in a cross with each of the original *clmF* temperature-sensitive lethal alleles. Selection was made for growth at high temperature (44°C), which demands a recombination event between the two *clmF* markers. Subsequently, the fraction of transductants inheriting Tet^r was scored (Table 3).

The *zge-2393*::Tn*10* insertion lies to the left of all *clmF* markers (Fig. 1). Reciprocal crosses, switching the alleles present in the donor and recipient, provided the data to support the position of this insertion. If *zge-2393*::Tn*10* resided between two *clmF* alleles, reciprocal crosses between these alleles would both yield relatively high cotransduction of Tet^r, since the Tn*10* insertion could be inherited without requiring multiple crossover events in either cross (Fig. 1a and b). There were no such pairs of reciprocal crosses (Table 3). All reciprocal crosses showed that one cross generated a high frequency of Tet^r transductants, whereas the other cross had a low frequency of Tet^r transductants. These data are consistent with the position of *zge-2393*::Tn*10* on one side of all *clmF* alleles. The insertion was arbitrarily set on the left in the map of Fig. 1.

Each of the entries in Table 3 shows the frequency of coinheritance of Tet^r in a transduction cross and provides a relative order for the two *clmF* mutations on the basis of whether the frequency of Tet^r cotransduction is high or low. Cotransduction of the Tn*10* insertion will be infrequent (generally 0 to 15%) if the order of markers is *zge-2393*::Tn*10*–*clmF* (donor)–*clmF* (recipient), since inheritance of the Tn*10* insertion would require four crossover events (Fig. 1c). In contrast, cotransduction of the *zge-2393*::Tn*10* insertion will be frequent (generally 25 to 85%) if the order of markers is *zge-2393*::Tn*10*–*clmF* (recipient)–*clmF* (donor) (Fig. 1d). The reciprocal cross, found at the symmetrical position across the diagonal in Table 3, should give the same relative order of the two markers and thus provides a means to check the deduced order of the two markers. Since the reciprocal

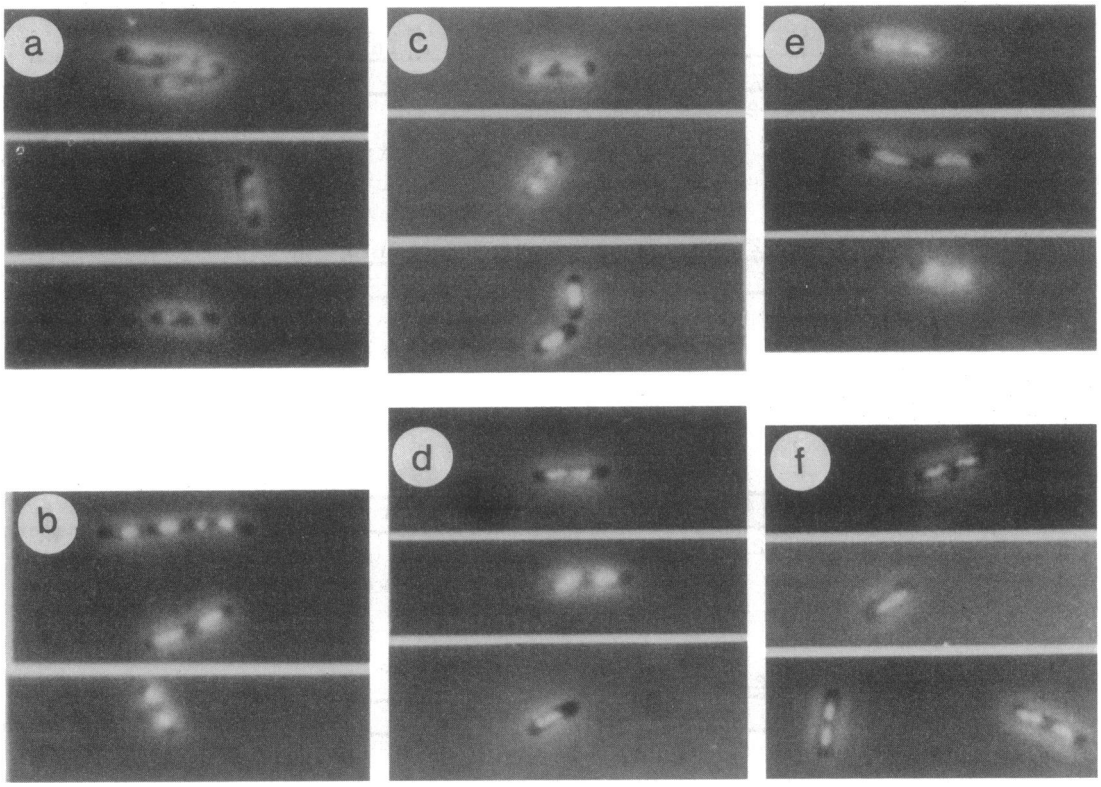

FIG. 2. Photomicrographs of *clmF* strains grown at 30°C. Cells were stained with DAPI, and both phase-contrast and fluorescence images were observed as described in Materials and Methods. (a) SE7784 (*clmF281*); (b) SE7815 (*clmF171*); (c) SE7782 (*clmF219*); (d) SE7765 (*clmF229*); (e) SE7769 (*clmF279*); (f) *leu-485*.

cross switches the donor and recipient alleles, a reversal in the frequency of Tet^r coinheritance is expected. From each row and column of data in Table 3, an allele can be positioned between sets of markers that lie to its right and to its left.

The data in Table 3 are arranged in the deduced order of the *clmF* markers. In this arrangement, all crosses above the diagonal should have high percentages of Tet^r transductants, and those below the diagonal should have low percentages of Tet^r transductants. An incorrect positioning of an allele will cause loss of this pattern. In the 133 crosses shown in Table 3, most crosses yield data fully consistent with the deduced order of markers. However, several of the crosses involving the four markers *clmF202*, *clmF213*, *clmF361*, and *clmF279* give data that prevent a consistent order of these four markers with respect to one another, although they are unambiguously ordered with respect to the other *clmF* alleles. For example, using *clmF202* as a donor and *clmF213* as a recipient, the low frequency of Tet^r transductants leads to the conclusion that *clmF202* lies to the left of *clmF213*. However, the reciprocal cross, using *clmF213* as a donor and *clmF202* as a recipient, leads to the conclusion that *clmF213* lies to the left of *clmF202*. These conflicts were not resolved, and these markers are enclosed in brackets on the genetic map (Fig. 1). Minor inconsistencies between closely linked markers have been observed previously in fine structure maps (31). The order shown for these mutations is least ambiguous. The mutations *clmF279* and *-377* were positioned only by their behavior as donors, since these mutations are leaky, which prevents their use as recipients in these transduction crosses. The two markers *clmF361* and *clmF279* were positioned next to one another, since the number of ClmF$^+$ recombinants that arose in crosses between these two markers was 10,000-fold lower than in unrestricted crosses using a *clmF*$^+$ donor. Crosses between other pairs of *clmF* alleles were reduced between 2- and 100-fold when compared with an unrestricted cross with a *clmF*$^+$ donor.

The insertions *zge-2393*::Tn*10* and *zge-2395*::Tn*10* must lie on opposite sides of the *clmF* cluster. Most of the data in Table 3 were obtained from donor strains that carry the *zge-2393*::Tn*10* insertion. However, strain SE79992 in Table 3 is a donor strain that carries *zge-2395*::Tn*10* and *clmF361*. When compared with the donor SE8020 (which carries *zge-2393*::Tn*10* and *clmF361*), this set of crosses showed a reversed pattern of coinheritance of Tet^r. These results would arise if the two insertions, *zge-2395*::Tn*10* and *zge-2395*::Tn*10*, lie on opposite sides of the *clmF* locus. Crosses using a donor carrying *clmF206* and *zge-2395*::Tn*10* also lead to this conclusion (data not shown).

Nucleoid cytology of the *clmF* mutants. Daughter nucleoids failed to fully separate after *clmF* mutants were shifted to the nonpermissive temperature. Cultures of strains carrying the *clmF* mutations were grown in LB broth at 30°C and then shifted to 44°C for 2 h. Cells were prepared for microscopy (see Materials and Methods) before and after the temperature shift and stained with the DNA-binding fluorescent dye DAPI. The photomicrographs in Figure 2 show cells from the wild-type strain and strains *clmF171*, *-219*, *-229*, *-279*, and *-281* after growth at 30°C. Cells appeared quite normal in size and morphology. To demonstrate the nucleoid partitioning stage, cells with two nucleoids are shown in Fig. 2. Only

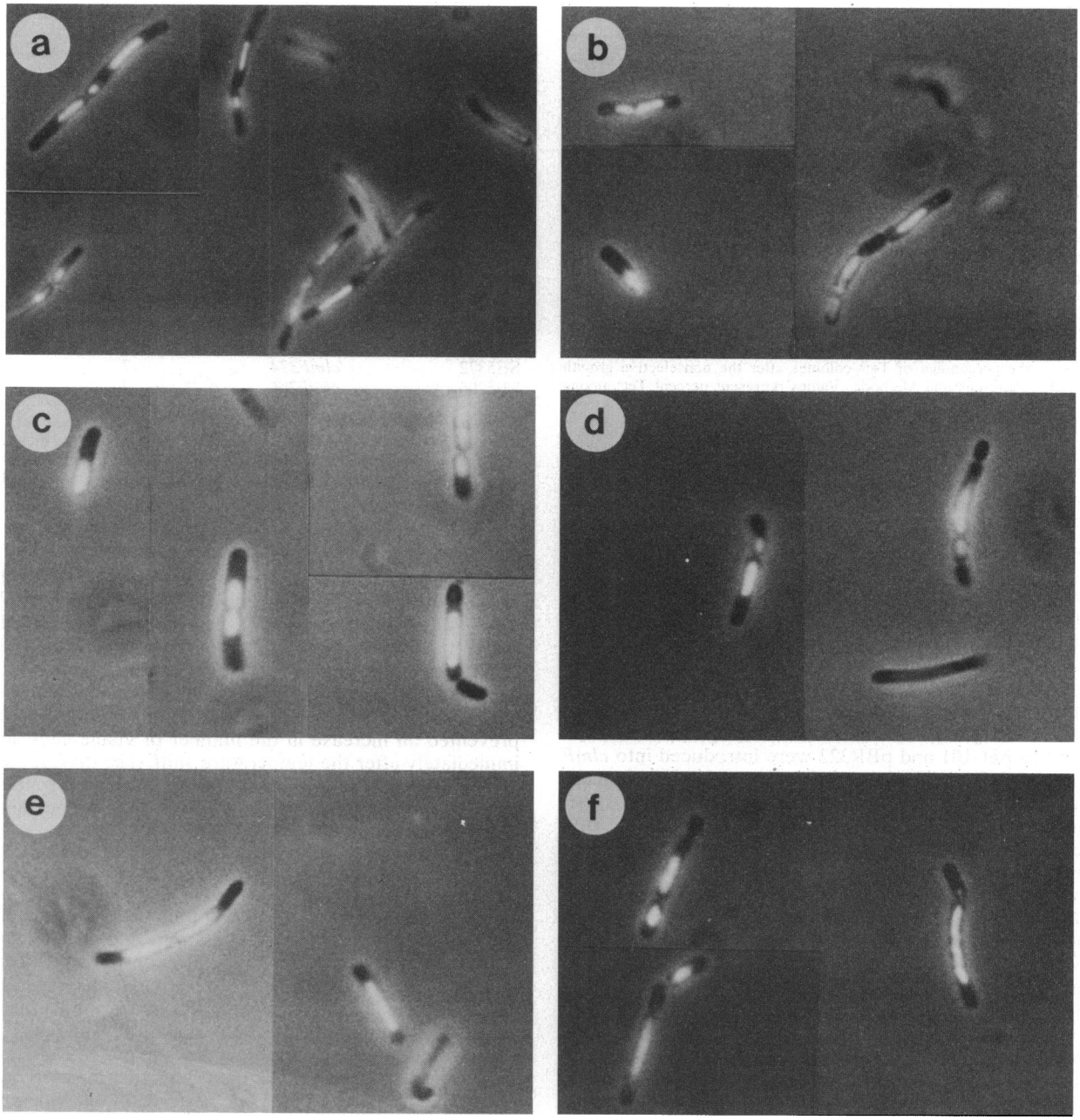

FIG. 3. Photomicrographs of *clmF* strains incubated at 44°C for 2 h. Cells were stained with DAPI, and both phase-contrast and fluorescence images were observed as described in Materials and Methods. (a) SE7784 (*clmF281*); (b) SE7815 (*clmF171*); (c) SE7782 (*clmF219*); (d) SE7765 (*clmF229*); (e) SE7769 (*clmF279*); (f) SE7763 (*clmF377*).

a small fraction of *clmF* cells grown at 30°C showed doublet or incompletely separated nucleoids. In contrast, the photomicrographs in Fig. 3 show *clmF* strains after growth for 2 h at 44°C. In these cells, daughter nucleoids were incompletely separated, even when septation had obviously begun (Fig. 3a, c, and e) or even completed (Fig. 3b, c, and e). This led to individual cells with asymmetrically placed nucleoids (Fig. 3b, c, and e) as well as asymmetrically positioned nucleoids paired in adjacent cells of a chain (Fig. 3a, c, and e). In addition, septation could occur within inclusion of a visible nucleoid body, leading to DNA-less cells. These DNA-less cells were seen either as terminal cells or as internal cells in a chain (Fig. 3b, c, d, and f). No evidence of cell lysis was observed at the nonpermissive temperature.

Plasmid stability in *clmF* mutants. Mutations that alter the faithful partitioning of the bacterial nucleoid may also affect the stable inheritance of accessory plasmids. The stability of F'_{254}, pSC101, and pBR322 was measured in *clmF* mutants at 30°C. *S. typhimurium* strains are naturally Lac^- and thus white on MacConkey-lactose plates; strains that stably inherit the F'_{254} Lac^+ episome will grow as red colonies on MacConkey-lactose plates. The fraction of Lac^- (white) colonies in a population was determined after approximately 20 generations of nonselective growth on solid medium as

TABLE 4. Plasmid stability in *clmF* mutants[a]

Strain	Allele	% Loss of plasmid phenotype[b]		
		F'_{254}	pSC101	pBR322
SE7818	*clmF174*	<3	<4	<2
SE7815	*clmF171*	<2	<4	2
SE7782	*clmF213*	38	<3	0.5
SE7778	*clmF232*	13	<4	<4
leu-485	*clmF*$^+$	0.4	ND	1

[a] The F'_{254} episome was introduced into strains SE7818, SE7815, SE7782, SE7778, and *leu-485*, and the stability of the episome was tested by loss of the Lac$^+$ phenotype as described in Materials and Methods.

[b] Lac$^-$ colonies seen among four to six independent exconjugants in which 30 to 300 colonies were scored for each exconjugant. Plasmids pBR322 and pSC101 were introduced into strains SE5174, SE5171, SE5213, SE5232, and *leu-485* by P22 transduction, and stability of these plasmids was measured by determining the percentage of Tet^s colonies after the nonselective growth described in Materials and Methods. Values represent percent Tet^s among two to four independent transductants in which 25 to 200 colonies were scored for each transductant. ND, Not determined.

TABLE 5. Irreversible effects of 44°C incubation on *clmF* mutants[a]

Strain	Allele	% Survival	
		2 h	6 h
SE5174	*clmF174*	67	1
SE5311	*clmF281*	25	3
SE5171	*clmF171*	67	3
SE5219	*clmF219*	16	<0.3
SE5229	*clmF229*	67	1
SE5202	*clmF202*	25	<0.3
SE5359	*clmF361*	67	2
SE5213	*clmF213*	40	7
SE5375	*clmF377*	33	33
SE5232	*clmF232*	100	100
SE5309	*clmF279*	100	60
SE5372	*clmF374*	7	<0.3
SE5206	*clmF206*	7	<0.3

[a] Overnight cultures of the *clmF* strains were grown in LB at 30°C. The overnight cultures were diluted 10^6-fold, plated on LB plates, and incubated at 30°C (for total viable cells) or at 44°C for 2 h (or 6 h) and then at 30°C overnight. The percentage colonies surviving the high-temperature incubation was then determined.

described in Materials and Methods (Table 4). The mutations *clmF213* and *clmF232* caused instability of F'_{254} by this assay; other *clmF* alleles have been observed to be unstable with F'_{116} and F'_{16}. F′ plasmids encode a function coupling correct partitioning of the F′ plasmid with cell division (the *ccd* mechanism [18]), and cells that fail to inherit an F′ element are prevented from dividing. The strains that carried F'_{254} with *clmF213* or *clmF232* now filamented at 30°C (although without F'_{254} they had a normal length at 30°C); the strains that carried F'_{254} with *clmF171* or *clmF174* had a normal cell length at 30°C (data not shown).

Plasmids pSC101 and pBR322 were introduced into *clmF* mutants by P22-mediated generalized transduction. In these experiments, the plasmids were introduced into the Tet^s strains SE5174, SE5171, SE5213, and SE5232. After approximately 20 generations of nonselective growth at 30°C, the percentage of the population still carrying the plasmid was determined. These *clmF* mutants showed no increased instability of pBR322 or pSC101 at the permissive growth temperature (Table 4).

Irreversible effects of *clmF* mutations. Many of the *clmF* mutations caused permanent loss of viability upon a shift to the nonpermissive temperature. Overnight cultures of *clmF* mutants were diluted, plated on complex medium, and incubated at either 30°C, to obtain the total number of viable cells, or at 44°C for 2 or 6 h. After the short incubation at the nonpermissive temperature, the plates were incubated at 30°C. The number of colonies surviving the temperature shift was compared with the total number of viable cells plated (Table 5). The effects of three mutations (*clmF232*, *-279*, and *-377*) were fully reversible even after 6 h at the nonpermissive temperature, and these mutations map together in the genetic map (Fig. 1). A temperature shift in strains carrying the other *clmF* mutations caused permanent inviability in a large proportion of the population. The temperature-induced inviability occurred very quickly in some of these strains, as shown below.

DNA replication and cell viability of *clmF* mutants. The ability of *clmF* mutant strains to replicate DNA after a shift to the nonpermissive temperature was measured in liquid cultures and compared with the timing of the loss of viability in these strains. The incorporation of radioactive thymidine into a trichloroacetic acid-precipitable form continued in SE7818 (*clmF174*) for 1 h after the shift to the nonpermissive temperature, whereas viability of 60% of the cells was lost within 30 min after the temperature shift (Fig. 4). Mutants alleles *clmF219*, *-171*, *-206*, and *-377* (SE7782, -7815, -7772, and -7763) were also tested for their replication potential and viability; although they differed in the rate of viability loss, DNA replication continued for 1 h after the temperature shift in each of these strains, whereas the number of viable cells stayed constant or decreased. All of the *clmF* alleles tested prevented an increase in the number of viable cells almost immediately after the temperature shift. The optical density at 600 nm in these strains continued to increase after the temperature shift, suggesting that lysis does not account for the loss of viability.

DISCUSSION

The temperature-sensitive *clmF* mutations affect a gene or genes that map near *metC* at 65 min and that allow the separation and proper positioning of newly replicated genomes. These temperature-sensitive mutations cause a cytologically similar defect after a 2-h incubation at the nonpermissive temperature. Genetic and physiological data suggest that more than one gene product may be affected by the *clmF* mutations. Genetic data show that the *clmF* alleles are 20 to 80% linked to the *zge-2393*::Tn*10* insertions; on the basis of linkage, the *clmF174* and *clmF206* alleles should be about 12 kilobases apart (26). Three *clmF* mutations (*clmF232*, *-279*, and *-377*) cluster on the genetic map and have defects that are fully reversible after a 6-h incubation at the nonpermissive temperature, whereas the other *clmF* mutations cause irreversible defects after 2- or 6-h incubation at the nonpermissive temperature. Several *clmF* mutations on the left on the genetic map (Fig. 1) show stable F′ inheritance, whereas others on the right lose F′ plasmids. Further work will be required to determine whether the genetic distance accurately reflects the physical distance separating these mutations and whether the differences in phenotypes reflect lesions in different gene products. Until definitive experiments divide these mutations into different genes, the mutations will all retain the *clmF* designation.

Kato et al. described temperature-sensitive mutants linked to *metC* that have a nucleoid-partitioning defect (14). Their *parC* mutations are probably similar to some of the *clmF*

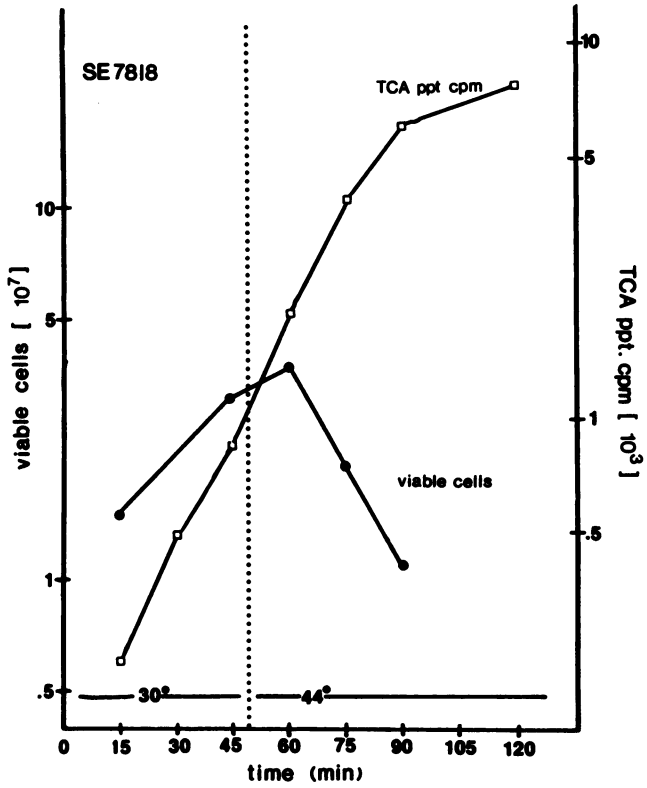

FIG. 4. Effect of temperature shift on DNA replication and cell viability. Strain SE7818 (*clmF174*) was grown at 30°C and labeled with [^{3}H]thymidine, and samples were taken as described in Materials and Methods. The temperature was shifted to 44°C, and sampling was continued. The number of viable counts was determined by plating on LB plates at 30°C. TCA ppt cpm, Trichloroacetic acid-precipitable counts per minute.

mutations described here, as determined from the genetic linkage to *metC* and the cytologically observed partitioning defect. The *mukA* gene was identified by nonconditional mutations that cause the accumulation of anucleate cells (10). This gene is allelic with *tolC*, which encodes a nonessential outer membrane protein and which also maps near *metC*. However, according to the current genetic map position of *tolC* (2, 26) and the nonlethal phenotype of mutants, it is unlikely that the *tolC* (*mukA*) mutations are allelic with *parC* (*clmF*).

The cytological studies on the *clmF* mutants suggest why these very common conditional lethal mutants were not identified in previous extensive searches for cell division and partitioning mutants (23, 25). The *clmF* mutants show about a two- to fourfold increase in cell length after a 2-h shift to the nonpermissive temperature, but many of the cells in the population have septated to a relatively normal size. The mutants identified by Ricard and Hirota were first subject to a filtration step to retain mutants forming long filamentous cells (23). While these filamentous temperature-sensitive (*fts*) mutants have clearly provided important information in deducing the steps of cell division, some of the missing links in nucleoid partitioning may be identified through mutations in genes such as *clmF*, which do not cause a long filamentous phenotype.

Some *clmF* mutations cause instability of the F′$_{254}$ episome. These results provide genetic data to support the hypothesis that the F′ replicon shares at least part of its partitioning machinery with the chromosome. Two F plasmid-encoded proteins, *sopA* and *sopB*, bind to a site *sopC* to cause faithful partitioning of mini-F plasmids (1, 21). In addition, two host proteins of 33 and 75 kDa are bound at the *sopC* site (8). The 75-kDa host protein bound to the *sopC* site may be the same as the 75-kDa protein encoded by *parC* (14). The F-plasmid replicon encodes a mechanism to couple cellular division (*ccd*) with faithful partitioning of F plasmid by preventing cell division in cells that have mispartitioned F plasmid (18). Thus, the rate of F′ plasmid loss may be partially masked by inhibition of cell division. Since the loss of F′$_{254}$ occurs at permissive growth temperatures, the rate of F′ plasmid loss will also be affected by the degree of mutant phenotype remaining at the permissive growth temperature. The role of the *clmF* gene product(s) in F-plasmid partitioning requires further investigation. None of the *clmF* mutations appear to cause instability of the small multicopy plasmids pSC101 and pBR322, although the multicopy nature of these plasmids may prevent the observation of minor instability caused by the *clmF* mutations.

The *clmF* (*parC*) gene(s) seems to provide an essential function required for the faithful segregation of daughter replicons in enteric bacteria. Loss of the function results in mispositioned nucleoids but does not prevent formation of the septum between the mispositioned nucleoids. The wild-type *clmF* gene product(s), by correctly positioning the newly replicated nucleoids, helps to ensure faithful partitioning at cell division.

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LITERATURE CITED

1. **Austin, S., and A. Abeles.** 1983. The partition of unit-copy miniplasmids to daughter cells. II. The partition region of miniplasmid P1 encodes as essential protein and a centromere-like site at which it acts. J. Mol. Biol. **169**:373–387.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
3. **Berkowitz, D., J. M. Hushon, H. J. Whitfield, J. R. Roth, and B. N. Ames.** 1968. Procedure for identifying nonsense mutations. J. Bacteriol. **96**:215–219.
4. **Burman, L. G., J. Raichler, and J. T. Park.** 1983. Evidence for diffuse growth of the cylindrical portion of the *Escherichia coli* murein sacculus. J. Bacteriol. **155**:983–988.
5. **Burman, L. G., J. Raichler, and J. T. Park.** 1983. Evidence for multi-site growth of *Escherichia coli* murein involving concomitant endopeptidase and transpeptidase activities. J. Bacteriol. **156**:386–392.
6. **Chan, R. K., D. Botstein, T. Watanabe, and Y. Ogata.** 1972. Specialized transduction of tetracycline resistance by phage P22 in *Salmonella typhimurium*. II. Properties of a high frequency transducing lysate. Virology **50**:883–898.
7. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Hayakawa, Y., T. Murotsu, and K. Matsubara.** 1985. Mini-F protein that binds to a unique region for partition of mini-F plasmid DNA. J. Bacteriol. **163**:349–354.
9. **Hendrickson, W. G., T. Kusano, H. Yamaki, R. Balakvishnan, 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. **Hiraga, S., H. Niki, T. Ogura, C. Ichinose, H. Mori, B. Ezaki, and A. Jaffe.** 1989. Chromosome partitioning in *Escherichia*

coli: novel mutants producing anucleate cells. J. Bacteriol. **171:** 1496–1505.

10a. **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.

11. **Hussain, K., E. J. Elliot, and G. P. C. Salmond.** 1987. The parD mutants of Escherichia coli also carries a gyrA(am) mutation. The complete sequence of gyrA. Mol. Microbiol. **1:**259–273.

12. **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.

13. **Kato, J., Y. Nishimura, and H. Suzuki.** 1989. *Escherichia coli parA* is an allele of the *gyrB* gene. Mol. Gen. Genet. **217:** 178–181.

14. **Kato, J.-I., 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:**3967–77.

15. **Kukral, A. M., K. L. Strauch, R. A. Maurer, and C. G. Miller.** 1987. Genetic analysis in *Salmonella typhimurium* spaced insertions of transposon Tn*10*Δ16Δ17. J. Bacteriol. **169:**1687–1793.

16. **Kusano, T., D. Steinmetz, W. G. Hendrickson, J. Murchie, M. King, A. Benson, and M. Schaechter.** 1984. Direct evidence for specific binding of the replicative origin of the *Escherichia coli* chromosome to the membrane. J. Bacteriol. **158:**313–316.

17. **Meacock, P. A., and S. N. Cohen.** 1980. Partitioning of bacterial plasmids during cell division: a cis-acting locus that accomplishes stable plasmid inheritance. Cell **20:**529–542.

18. **Miki, T., K. Yoshioka, and T. Horiuchi.** 1984. Control of cell division by sex factor F in Escherichia coli. J. Mol. Biol. **174:**605–625.

19. **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.

20. **Ogden, G. B., M. J. Pratt, and M. Schaechter.** 1988. The replicative origin of the E. coli chromosome binds to cell membranes only when hemi-methylated. Cell **54:**127–135.

21. **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.

22. **Park, J. T.** 1987. The murein sacculus, p. 23–30. *In* F. C. Neidhardt, J. L. Ingraham, B. Magasanik, K. B. Low, M. Schaechter, and H. E. Umbarger (eds.), *Escherichia coli and Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.

23. **Ricard, M., and Y. Hirota.** 1973. Process of cellular division in *Escherichia coli*: physiological study on thermosensitive mutants defective in cell division. J. Bacteriol. **116:**314–322.

24. **Roth, J. R.** 1970. Genetic techniques in studies of bacterial metabolism. Methods Enzymol. **17:**3–35.

25. **Ryter, A., Y. Hirota, and U. Schwarz.** 1973. Process of cellular division in *Escherichia coli*: growth pattern of *E. coli* murein. J. Mol. Biol. **78:**185–195.

26. **Sanderson, K. E., and J. R. Roth.** 1988. Linkage map of *Salmonella typhimurium*, edition VII. Microbiol. Rev. **52:** 485–532.

27. **Schmid, M. B., N. Kapur, D. R. Isaacson, P. Lindroos, and C. Sharpe.** 1989. Genetic analysis of temperature-sensitive lethal mutants of *Salmonella typhimurium*. Genetics **123:**625–633.

28. **Schmieger, H.** 1972. Phage P22 mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119:**75–88.

29. **Spratt, B. G., and A. Pardee.** 1975. Penicillin-binding proteins and the cell shape in *E. coli*. Nature (London) **254:**516–517.

30. **Steck, R. T., and K. Drlica.** 1984. Bacterial chromosome segregation: evidence for DNA gyrase involvement in decatenation. Cell **36:**1081–1088.

31. **Syvanen, J. M., and J. R. Roth.** 1973. Structural genes for catalytic and regulatory subunits of aspartate transcarbamylase. J. Mol. Biol. **76:**363–378.

32. **Vogel, H., and D. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.

33. **Wientjes, F. B., and N. Nanninga.** 1989. Rate and topography of peptidoglycan synthesis during cell division in *Escherichia coli*: the concept of a leading edge. J. Bacteriol. **171:**3412–3419.

34. **Winston, S., and N. Sueoka.** 1980. DNA-membrane association is necessary for initiation of chromosomal and plasmid replication in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **77:**2834–2838.