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Genetic and Morphological Characterization of an *Escherichia coli* Chromosome Segregation Mutant

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The temperature-sensitive nucleoid segregation mutant of *Escherichia coli*, PAT32, formerly described as a *parA* mutant, has been shown to carry a mutation near 66 min on the genetic map. Fine mapping with phages from the collection of Kohara et al. is consistent with its being a *parC* allele. Observation by fluorescence microscopy revealed the formation, at a nonpermissive temperature, of filaments containing one or two large nucleoids and of normal-size anucleate cells. There was also a significant loss of viability.

Prokaryotes, by definition, have no nuclear membrane. Their DNA is nevertheless maintained within a restricted region of the cytoplasm, generally called the nucleoid. Although much has been learned about the replication of the DNA within the nucleoid, little is known about the process of nucleoid partitioning. DNA gyrase is required to decatenate the newly replicated chromosomes (16), but it is not known what actually pulls the chromosomes apart, in the absence of any visible spindle apparatus.

The genetic approach to the problem of nucleoid partitioning in *Escherichia coli* has defined two types of mutant. The first class, called Par$^-$, are conditional lethal mutants which, under nonpermissive conditions, continue to synthesize DNA but do not segregate their nucleoids. These mutants generally form filaments with a single, large nucleoid, together with a certain number of small anucleate cells. Several such *par* mutants have been described elsewhere. Hirota et al. (4) reported the isolation of two *parA* mutants (genes near 95 min on the genetic map) and one *parB* mutant (gene near 66 min). Hussain et al. (5) later described a *parD* mutant (88 min), and Kato et al. isolated *parC* (9) and *parE* (7) mutants (66 min). The second type of partitioning defect is a class of nonlethal mutants, called *muk*, which exhibit a high rate of partitioning accidents resulting in the spontaneous formation of anucleate cells. Three such loci have been described previously (2, 3, 12).

The *par* mutants, although apparently good candidates for defining partitioning functions, have been disappointing. The mutation carried by original *parB* mutant (strain MFT110) was shown to be an allele of the *dnaG* gene (66 min), coding for DNA primase (13); its phenotype was thus due to a replication defect. The *parD* mutant was shown to have a mutation in the *gyrA* gene (50 min, coding for the DNA gyrase A subunit), presumably affecting decatenation (6). One of the original *parA* mutants, strain MFT100, has been shown to be a double mutant; the mutation responsible for the Par$^-$ phenotype is an allele of the *gyrB* gene (82 min, coding for the DNA gyrase B subunit), again affecting decatenation (8). The recently identified *parC* and *parE* genes also seem to code for a topoisomerase (7), suggesting that these mutants, too, may have a decatenation defect.

TABLE 1. Bacterial strains[a]

Strain	Relevant marker	Other markers	Construction or source
PAT32	*parC32* (formerly *parA*)	*thr leu trp his thyA argH lacY malA*(λ^r) *xyl mtl mel tonA rpsL*	Lab collection
C600		*thi thr leuB lacY tonA supE*	Lab collection
GC7442	Δ*tolC*::Tn*5*	*his* Δ*trpE5 recD*	Lab collection
GC3526	Δ*tolC*::Tn*5*	As C600	Km^r transductant of C600 with P1 grown on GC7442
NK6027	*metC162*::Tn*10*	HfrH λ$^-$ Δ(*gpt-lac*)*5 relA1 spoT1 thi-1*	P. Marlière
GC3528	Δ*tolC*::Tn*5*	As GC3526, *metC*::Tn*10*	Tc^r transductant of GC3526 with P1 grown on NK6027
GC3531	*parC32*	As C600	Tol$^+$ (temp sensitive, Km^s Tc^s) transductant of GC3528 with P1 grown on PAT32
GC2070	*tolC*	*thr leu pro his arg thi lac gal ara xyl mtl supE rpsL tsx*	Lab collection

[a] All strains are derivatives of *E. coli* K-12.

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TABLE 2. Three-point transductional crosses[a]

Cross[b]	Result of selection for:					
	Met$^+$ or Tc^r[c]			Tol$^+$ or Km^r[d]		
	par	*tolC*	No.	*metC*	*par*	No.
GC3528 + P1 grown on PAT32	+	−	50	−	+	42
	−	−	4	−	−	10
	−	+	40	+	−	15
	+	+	3	+	+	3
PAT32 + P1 grown on GC3528	−	+	30	+	−	33
	+	+	36	+	+	+
	+	−	37	−	+	41
	−	−	1	−	−	0

[a] P1 transduction was carried out according to the method of Miller (11). The selection for Met$^+$ was on solid M63 plates, and selection for Km^r and Tc^r was on solid LB plates (11) containing 50 μg of kanamycin per ml or 12.5 μg of tetracycline per ml, respectively. Tol$^+$ selection was on EMB plates (11), since strain PAT32 did not grow on 1% sodium dodecyl sulfate.

[b] GC3528 relevant genotype, *metC*::Tn*10*(Tc^r) *tolC*::Tn*5*(Km^r); PAT32 relevant genotype, *parC32*.

[c] For the cross GC3528 + P1 grown on PAT32, selection was for Met$^+$; for the cross PAT32 + P1 grown on GC3528, selection was for Tc^r.

[d] For the cross GC3528 + P1 grown on PAT32, selection was for Tol$^+$; for the cross PAT32 + P1 grown on GC3528, selection was for Km^r.

Thus, the only candidate left among the Par$^-$ mutants was the second *parA* mutant, strain PAT32. Furthermore, its mutation was mapped in the 94- to 97-min region of the map, where no other *par* mutations are known, since the 95-min-linked mutation in MFT100 has been shown not to be involved in the Par$^-$ phenotype (8, 10a). We therefore decided to reexamine the *parA* mutation in strain PAT32.

Mapping of the *parA* mutation in strain PAT32. The *parA* mutant PAT32 (Table 1) was obtained after mutagenesis of strain PA3092 and could thus carry more than one mutation. We therefore wished to transduce the *parA* mutation from PAT32 into another strain. The mutation was originally reported to lie between 91.5 and 97.5 min (13a). However, we were unable to recover temperature-resistant transductants of PAT32 with markers in this region: *malB*::Tn*9* at 91.5 min (0 of 104 transductants), *mel*$^+$ at 93.4 min (0 of 100), *zje*::Tn*10*(Tc^r) near 94 min (0 of 78), and *purA*$^+$ at 95 min (0 of 98; the *purA* mutation was introduced into PAT32 by cotransduction with *zje*::Tn*10*).

To confirm this result, we carried out a cross with HfrG6 (point of origin, 67 min, clockwise injection); essentially no temperature-resistant recombinants were found among Thr$^+$ Leu$^+$ recombinants. Similarly, few temperature-resistant recombinants were obtained in crosses with HfrH (point of origin, 97 min, clockwise injection) or HfrKL16 (point of origin, 62 min, counterclockwise injection). In contrast, many such recombinants were obtained with HfrPK 19 (point of origin, 43 min, clockwise injection). This suggests a location between the origins of HfrG6 and HfrKL16, i.e., between 62 and 67 min. Transductional crosses confirmed the mapping and showed that PAT32 carries a single temperature-sensitive lethal mutation 70% cotransducible with *metC*::Tn*10* (65 min) and 66% cotransducible with *tolC*::Tn*5* (66.4 min), and the order *metC-parA-tolC* was established (Table 2). Furthermore, the *parA* mutation conferred temperature sensitivity when transduced into the recipient strain GC3528. We show below that it also conferred the Par$^-$ phenotype of PAT32.

Very fine mapping was done with phages from the collection of Kohara et al. (10) carrying DNA inserts from the 66-min region. To carry out these tests, PAT32 was first transduced to Mal$^+$(λ^s) and lysogenized with wild-type λ. A lawn of this lysogen was then spotted with phages 504 to 509, which cover the kb 3190 to 3280 region on the map of Kohara et al. (10), corresponding to about 65 to 67 min on the genetic map (1). The plate was incubated for 2 h at 29°C and then transferred to 42°C overnight. Phages 505 and 506 gave rise to confluent growth, whereas the others gave no growth (Table 3). Putative double lysogens were isolated from the spots of phages 505 and 506, and the presence of the *parA* allele was confirmed by making P1 stocks on them and transducing a *tolC* strain to Tol$^+$. In both cases, some of the transductants became temperature sensitive. The *parA* mutation thus lies in the region common to phages 505 and 506, between kb 3216 and 3222 on the map of Kohara et al. (10), or at about 66.0 min on the genetic map (1). The fact that the *parA*(λ*par*$^+$) strains were temperature resistant shows that the *parA* mutation is recessive. The *parA* mutation lies in the same region as the recently described *parC* and *parE* genes, both carried by phage 506 (7). Furthermore, phage 505 carries the *parC*$^+$ gene but not *parE*$^+$, whereas phage 507 carries *parE*$^+$ but not *parC*$^+$ (14). It is therefore highly probable that PAT32 is a *parC* mutant; we propose calling the allele *parC32*.

Similar tests for *metC*$^+$ and *tolC*$^+$ confirmed the order *metC-parC-tolC* (Table 3).

The Par$^-$ phenotype of *parC32*. Nucleoids were visualized by fluorescent staining with 4′,6-diamidino-2-phenylindole. A modification of existing staining protocols (3) was employed, using agar-coated microscope slides. Slides were dipped into a jar of molten agar (0.6%) at 60 to 65°C, wiped on the back, and placed on a paper towel. As soon as the agar had gelled (about 1 min), the slides were transferred to a rack in a covered box containing a little water to prevent drying. Agar-coated slides were always freshly prepared on the day of use. The agar medium was the same as that in which the cultures were growing. A 10- to 14-μl sample of a

TABLE 3. Fine mapping of *parC32* and neighboring mutations

Strain	Relevant marker	Selection[a]	Complementing phage(s)	Presence of original mutation
NK6027(λ)	*metC*::Tn*10*	Met$^+$	504, 505	Tc^r
PAT32(λ)	*parC32*	Temp resistant at 42°C	505, 506	P1 cotransduction of temp sensitivity with Tol$^+$
GC3526(λ)	*tolC*::Tn*5*	Tol$^+$	507	Km^r
AB1157*tolC*(λ)	*tolC*	Tol$^+$	506,[b] 507	Not tested

[a] To test for the presence of the *tolC*$^+$ allele, phage were incubated with bacteria for 4 to 8 h at 29°C and then spotted on LB plates containing 1% sodium dodecyl sulfate; no growth was observed if the preincubation was omitted. The *metC*$^+$ allele was tested as for *tolC*$^+$, except that the bacteria were centrifuged at the end of the incubation period, resuspended in 0.01 M $MgSO_4$, and then spotted on minimal glucose plates lacking methionine.

[b] These colonies may arise by marker rescue.

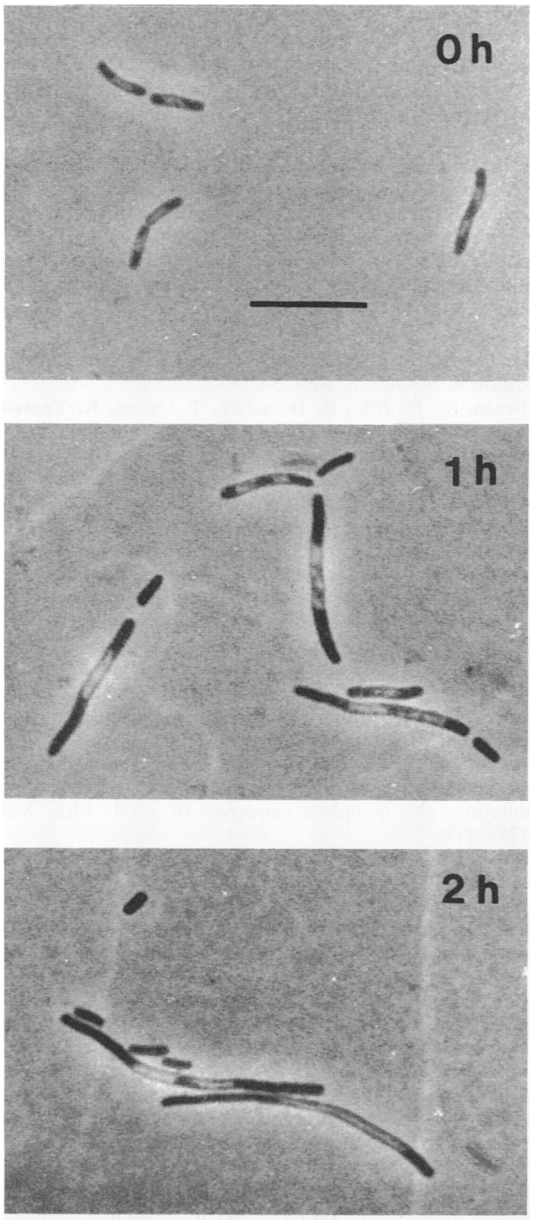

FIG. 1. Fluorescent staining of nucleoids in the *parC32* mutant. An exponential-phase culture of the *parC32* transductant GC3531 growing in LB_0 at 29°C was shifted to 42°C at time zero. At 0, 1, and 2 h, samples were prepared for fluorescence microscopy. Photomicrographs were taken at the lowest level of visible light with 400 ASA film and, typically, a 15-s exposure. The bar represents 10 μm.

culture at an optical density at 600 nm of between 0.05 and 0.1 was spread on the agar face of a coated slide with a pipette tip. The slide was allowed to dry for 1 h, then covered with methanol, and left to dry again for a second hour. A 10-μl drop of 4′,6-diamidino-2-phenylindole (5 μg/ml in saline) was placed on the slide, and a coverslip was added. Slides were examined in phase contrast in a Zeiss Jenamed microscope with simultaneous visible light and UV (410 nm) illumination. This technique produces especially clear images of bacterial nucleoids; apparent segregation defects in wild-type *E. coli* were observed less frequently than with other protocols, especially those employing chloramphenicol to condense nucleoids.

Nucleoid partitioning was examined in PAT32, the original *parC32* strain, and in the *parC32* transductant GC3531. After incubation for various times at 42°C in LB_0 broth (LB broth [11] containing no NaCl), both strains exhibited heterogeneous populations, including normal-size anucleate cells and nucleate filaments; the transductant is shown in Fig. 1. The DNA in the filaments was generally in a single, large nucleoid near the cell center, confirming the Par^- phenotype of the *parC32* mutant.

An additional, quantitative measure of chromosome segregation capacity was developed. Photomicrographs of bacteria that had been made to filament, either by the presence of a suitable mutation (*parC32*, *ftsZ84*, or *gyrB*) or by the addition of the antibiotic furazlocillin, were analyzed to determine the lengths and the number of nucleoids of individual cells. The ratio of cell length to the number of nucleoids provides a quantitative indication of the ability of the cell to segregate nucleoids. In the wild type (C600), this ratio is about 2 μm per nucleoid (Table 4). A similar value is found when the wild type is made to filament with furazlocillin or when an *ftsZ84* mutant is grown at a nonpermissive temperature. In contrast, after 1 h of growth at 42°C, C600 *gyrB* and C600 *parC32* both gave average values of about 7 μm per nucleoid. These cells are typically filaments 10 to 14 μm in length with one, two, or three distinct nucleoids.

A kinetic experiment was carried out with the *parC32* transductant GC3531. Cells were grown to exponential phase in LB_0 broth at 29°C and then shifted to 41.5°C. Periodically, the optical density at 600 nm, cell concentration, and cell volume were measured, and samples were stained with 4′,6-diamidino-2-phenylindole for observation of nucleoid location. A control culture of C600 was treated in parallel. Over a 3-h period, cell number increased 2-fold in the mutant culture, compared with 43-fold in the control (Fig. 2). Mass growth of the mutant, as measured by optical density determination, increased approximately fourfold in the first 2 h and then essentially ceased. Nucleoid partitioning in the mutant culture showed a clear defect.

TABLE 4. Average cell length per nucleoid

Strain	Treatment[a]	Avg cell length/nucleoid (μm ± SD)	Avg cell length (μm ± SD)	No. of cells analyzed
C600	37°C	2.10 ± 0.41	4.2 ± 0.9	30
C600	37°C, FZ, 40 min	2.18 ± 0.37	16.5 ± 3.6	30
C600 *parC32*	29°C, FZ, 120 min	2.80 ± 0.91	14.0 ± 3.3	18
C600 *parC32*	42°C, FZ, 60 min	7.24 ± 1.91	17.2 ± 3.8	20
C600 *ftsZ84*	42°C, 40 min	1.87 ± 0.33	16.6 ± 5.1	13
C600 *gyrB*	42°C, 60 min	6.89 ± 2.09	9.4 ± 3.0	25

[a] FZ, treatment with the antibiotic furazlocillin (1.5 μg/ml).

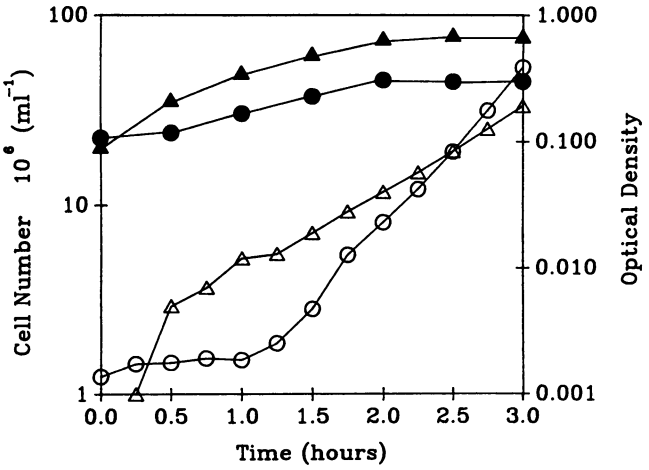

FIG. 2. Effect of a temperature shift on cell growth and division. Strains C600 and GC3531 were shifted from 29 to 41.5°C at time zero. The optical density of the culture and the particle concentration (as determined with a Coulter Counter) were measured at regular intervals after the shift. Symbols: △, C600 optical density; ○, C600 particle density; ▲, GC3531 optical density; ●, GC3531, particle density.

GC3531 (C600 *parC32*) lost viability rapidly after a shift to 42°C in LB_0 broth, showing about 10^{-3} survival after 1 h at the nonpermissive temperature. Even at 29°C, exponential LB_0 cultures of this strain contained approximately 15% nonviable cells (89 of 602), compared with 0.6% nonviable cells (8 of 1,296) for the wild type. On LB plates incubated overnight at 42°C, both GC3531 and PAT32 gave rise to colonies at a frequency of about 10^{-7}. To assess the number of nonviable cells in a culture, agar-coated slides were spread with 10 to 14 μl of culture (optical density at 600 nm, 0.020 to 0.025) and incubated in a humidified box at 37°C (29°C for thermosensitive strains) until microcolonies formed (3 to 4 h). Slides were examined at a magnification of ×400 in phase contrast without a coverslip. The fraction of nonviable cells was calculated by dividing the number of individual cells by the sum of individual cells plus microcolonies.

The viability of the mutant at the permissive temperature was also affected by the salt concentration of the medium. A culture growing in LB_0 broth gave about half the number of CFU when plated on LB broth (10 g of NaCl per liter) instead of LB_0 broth, and in LB broth the doubling time was 90 min, compared with about 71 min in LB_0 broth. Furthermore, the median volume of GC3531 cells was larger in LB broth than in LB_0 broth.

The *parC32* mutant characterized here is similar to other *metC*-linked partition mutants: the *clmF* mutants of *Salmonella typhimurium* (15) and the *parC* and *parE* mutants described by Kato et al. (7, 9). All of these mutants exhibit the classical Par^- phenotype of temperature-sensitive growth and formation of filaments containing centrally located nucleoids at a nonpermissive temperature. In the C600 genetic background, the *parC32* mutation caused a loss of viability at 42°C and anucleate cells were formed. At the permissive temperature, the *parC32* mutant exhibited sensitivity to high osmolarity and 15% of the cells were unable to grow. The *parC* and *parE* alleles affect GyrB- and GyrA-like proteins, respectively, as judged by sequence similarity (7). It is not known whether these proteins have gyrase activity nor why *E. coli* requires two DNA gyrases. In particular, it is not clear why both gyrases are needed for proper DNA partitioning. The physiological role of the *parC* and *parE* gene products, compared with that of the *gyrA-gyrB*-encoded gyrase, requires further experimentation.

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REFERENCES

1. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54**:130–197.
2. **Hiraga, S., H. Niki, R. Imamura, T. Ogura, K. Yamanaka, J. Feng, B. Ezaki, and A. Jaffé.** 1991. Mutants defective in chromosome partitioning in *E. coli*. Res. Microbiol. **142**:189–194.
3. **Hiraga, S., H. Niki, T. Ogura, C. Ichinose, H. Mori, B. Ezaki, and A. Jaffé.** 1989. Chromosome partitioning in *Escherichia coli*: novel mutants producing anucleate cells. J. Bacteriol. **171**:1496–1505.
4. **Hirota, Y., A. Ryter, and F. Jacob.** 1968. Thermosensitive mutants of *Escherichia coli* affected in the process of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. **33**:677–694.
5. **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 localisation in *Escherichia coli*. Mol. Microbiol. **1**:73–81.
6. **Hussain, K., E. J. Elliott, and G. P. C. Salmond.** 1987. The $ParD^-$ mutation of *Escherichia coli* also carries a $gyrA_{am}$ mutation: the complete sequence of *gyrA*. Mol. Microbiol. **1**:259–273.
7. **Kato, J., Y. Nishimura, R. Imamura, H. Niki, S. Hiraga, and H. Suzuki.** 1990. New topoisomerase essential for chromosome segregation in E. coli. Cell **63**:393–404.
8. **Kato, J., Y. Nishimura, and H. Suzuki.** 1989. *Escherichia coli parA* is an allele of the *gyrB* gene. Mol. Gen. Genet. **217**:178–181.
9. **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**:3967–3977.
10. **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.

10a. **Maguin, E., and R. D'Ari.** Unpublished observations.

11. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Niki, H., A. Jaffé, R. Imamura, T. Ogura, and S. Hiraga.** 1991. The new gene *mukB* codes for a 177 kd protein with coiled-coil domains involved in chromosome partitioning of *E. coli*. EMBO J. **10**:183–193.
13. **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.

13a. **Ricard, M.** 1972. Doctoral thesis. Université Paris 7, Paris.

14. **Rudd, D. E.** 1992. Alignment of *E. coli* DNA sequences to a revised, integrated genomic restriction map, p. 2.3–2.43. *In* J. Miller (ed.), A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
15. **Schmid, M. B.** 1990. A locus affecting nucleoid segregation in *Salmonella typhimurium*. J. Bacteriol. **172**:5416–5424.
16. **Steck, T. R., and K. Drlica.** 1984. Bacterial chromosome segregation: evidence for DNA gyrase involvement in decatenation. Cell **36**:1081–1088.