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Genetic and Physiological Characterization of a Spontaneous Mutant of *Escherichia coli* B/r with Aberrant Control of Deoxyribonucleic Acid Replication

KYUNG-KOO CHOUNG, EVANGELINE ESTIVA, AND HANS BREMER*

The University of Texas at Dallas, Richardson, Texas 75080

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Strain TJK16, a low-thymine-requiring *thyA deoB* derivative of *Escherichia coli* B/r A, was found to have an increased initiation mass due to a mutation in a gene affecting the control of initiation of deoxyribonucleic acid replication. In contrast to temperature-sensitive initiation mutants, initiation in TJK16 was not temperature sensitive. By phage P1 transduction, it was found that the mutation lies within a small region of the chromosome between *dnaA* and *gyrB*; this region includes *dnaN* and *recF*. Coumermycin-resistant derivatives of B/r and TJK16 had the same initiation mass as their coumermycin-sensitive parents, and TJK16 had the same sensitivity to coumermycin as the B/r parent, suggesting that the initiation mutation is not in *gyrB*.

Replication of the *Escherichia coli* chromosome is initiated when the cell mass per replication origin on the chromosome has reached a certain value, called initiation mass (7). In the accompanying paper (6), we have determined the initiation mass for *E. coli* B/r A and for a low-thymine-requiring (*thyA deoB*) derivative of this strain, TJK16. Unexpectedly, we found that the initiation mass of TJK16 was 60 to 80% greater than that of the parent (wild type) B/r, suggesting that TJK16 has an altered control of initiation of replication. Here we have analyzed this altered control genetically and physiologically and found that it is due to a mutation in the *dnaA* region of the chromosome. Most likely it is in *dnaA*.

Independent of the exact location of the mutation, strain TJK16 is useful for studies of the effects of DNA replication control on the bacterial physiology, e.g., on cell division (cell size) and transcription (altered DNA concentration). Although these effects can also be observed with temperature-sensitive DNA initiation mutants grown at intermediate temperatures, it is difficult, with temperature-sensitive strains, to separate the effects of temperature on cell physiology from the initiation effects.

MATERIALS AND METHODS

Bacterial and phage strains. Table 1 lists the bacterial strains used and their origins. Phage P1 *kc* (4) was used for transduction, carried out according to Miller (15). TJK16 and LEE103, both *thyA deoB*, were obtained by the trimethoprim selection technique (15). TJK16, RY1C, and PLH301 were obtained from Jesse Kwoh, Ryland Young, and Priscilla Holmans, respectively; other strains were constructed by standard techniques (15). Coumermycin-resistant (*gyrB* [18]) strains were selected by their ability to give macroscopic colonies within 2 days of incubation at 37°C on coumermycin plates (2 μg/ml). With freshly prepared medium, no strains were found which were resistant to higher levels (>5 μg/ml) of the drug. Because coumermycin is unstable at 37°C, background growth of coumermycin-sensitive cells occurs after longer incubation.

Media. The following media were used: minimal medium C (11) supplemented with 0.2% glucose, thymine, threonine, and phenylalanine as indicated or with 0.6% Casamino Acids (Difco Laboratories); and LB medium (15). Minimal agar plates and LB agar plates were prepared as described by Miller (15). Coumermycin (a gift from Bristol Laboratories, Syracuse, N.Y.) was added from stock solutions (4 mg/ml) in dimethyl sulfoxide, stored at −20°C.

Determination of cell mass, cell number, and DNA. The concentration of cell mass in bacterial cultures was determined as optical density at 460 nm (OD_{460}) (1-cm light path). Cell number was determined with an electronic particle counter (Coulter Electronics, Inc., model B, with 20-μm orifice) equipped with a 100-channel size distribution analyzer (Coulter Channelyzer). Protein and DNA were determined colorimetrically as described previously (3). The average mass per cell ($\bar{M}$) was obtained as OD_{460} of culture divided by the number of cells per milliliter of culture; the average amount of DNA per cell ($\bar{G}$) was obtained as DNA (measured in genome equivalents) per OD_{460} divided by cells per OD_{460}.

RESULTS

Initiation mass and cell size of *E. coli* B/r and TJK16. The initiation mass, M_o, is defined as OD_{460} units per origin of replication (6,

TABLE 1. *E. coli strains used*

Strain	Genotype	Phenotype			Origin
		Thy[a]	Temp sensitivity	Cell size[a]	
B/r A		+	−	S	ATCC 12407
TJK16	*thyA deoB*		+	L	Spontaneous mutant of B/r A (J. Kwoh, Ph.D. thesis, University of Texas at Dallas, 1978)
LEE18	*thyA*$^+$ *deoB*$^+$	+	ND[b]	M	Spontaneous mutant (2 steps) of TJK16
LEE71	*thyA deoB*	−	−	L	Spontaneous mutant of TJK16
LEE72	*thyA deoB strB*	−	−	L	Spontaneous mutant of LEE71
LEE73	*thyA deoB strB bglR dnaA5*	−	+	LL	P1 transduction from PLH301 into LEE72
LEE77	*thyA deoB strB bglR*$^+$ *dnaA*$^+$	−	−	M	P1 transduction from B/r A into LEE73
LEE80	*thyA*$^+$ *deoB strB bglR*$^+$ *dnaA*$^+$	+	−	S	Spontaneous mutant of LEE77
RY1C	*phe*(Am) *thr*(Am)	+	ND	S	Spontaneous mutant of B/r A
LEE103	*phe*(Am) *thr*(Am) *thyA deoB*	−	−	M	Spontaneous mutant (2 steps) of RY1C
PC5	*leu-6 thyA47 deoC3 str-153 dnaA5*	−	+	LL	*E. coli* K-12 (9)
PLH300	*leu-6 thyA47 deoC3 str-153 dnaA5 bglR*	−	+	LL	Spontaneous mutant of PC5
PLH301	*phe*(Am) *thr*(Am) *bglR dnaA5*	−	+	LL	P1 transduction from PLH300 into RY1C
LEE86	*thyA deoB gyrB*	−	+	L	Spontaneous mutant of TJK16
LEE87	*thyA deoB gyrB bglR*	−	+	L	Spontaneous mutant of LEE86

[a] S, Small; M, medium; L, large; LL, very large.
[b] ND, Not determined.

7); it was obtained from measurements of DNA and OD_{460} in exponential cultures, and from measurements of the increase in DNA after inhibition of initiation, taken from a previous study of the replication in *E. coli* B/r and TJK16 (5). From each culture, DNA samples were taken between $OD_{460} = 0.3$ and $OD_{460} = 0.6$; such measurements were repeated several times, each time starting from a fresh overnight culture. Table 2 gives average results and variations for the different strains used. Under the conditions used, the initiation mass for TJK16 (see above) was 1.6-fold greater than the initiation mass for B/r, in agreement with previous results (6).

Since changes in the initiation mass produced similar changes in the average cell mass ($\bar{M}$ = OD_{460} per cell) and cell volume (volume distributions observed with an electronic size distribution analyzer), these mass and volume changes could be used as an indicator for changes in the initiation mass. With the exception of RY1C [the *thr*(Am) *phe*(Am) derivative of B/r], all strains used in this study had an increased average cell mass relative to initiation mass in comparison with wild-type B/r (Table 2; relative value of $\bar{M}/M_o$ is greater than 1.0). At a given growth rate and replication velocity (i.e., at a given C/τ ratio), the ratio of $\bar{M}/M_o$ is a measure of the duration of the D period (2). Thus, the increased $\bar{M}/M_o$ values suggest that the mutations used here also affected the D period by causing a delayed division after termination of a round of replication. These effects depended on thymine metabolism rather than initiation control, since in pairs of strains isogenic except in the *thyA* and *deoB* loci, the strain with a defect in *thyA* always had larger cells (Fig. 1; compare LEE103 with RY1C, or TJK16 with LEE18). Also, the presence of thymine or threonine affected the average cell mass and thus cell division (Table 3). Therefore, all cultures in the experiment in Table 2 were grown in the same thymine-, threonine-, and phenylalanine-supplemented medium, whether the supplements were required or not, to make the results comparable.

Some low-thymine-requiring strains of *E. coli* have been reported to have a longer doubling time of the cell number than of the cell mass, such that the mass of an average bacterium increases during culture growth (14). These changes in average cell mass might also reflect effects of cell division rather than on initiation (M_o) or DNA chain elongation (C), since DNA accumulated with the same doubling time as culture mass (14). In TJK16, mass and number doubling time agreed (Table 4; OD_{460} per cell remained constant during culture growth).

TABLE 2. *Parameters related to initiation of replication in E. coli B/r and its derivatives measured during exponential growth*[a]

Strain[b]	Doubling time (min)	$\bar{M}$	G/M	$\bar{G}$	M_o[c]	$\bar{M}/M_o$
B/r	45.1 ± 1.1[d]	0.29 ± 0.04	0.96 ± 0.10	2.6 ± 0.4	0.76 ± 0.08	
	(1.00)[e]	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
TJK16	47.4 ± 1.9	0.59 ± 0.10	0.60 ± 0.07	3.4 ± 0.4	1.21 ± 0.15	
	(1.05 ± 0.03)	(2.06 ± 0.20)	(0.61 ± 0.03)	(1.27 ± 0.15)	(1.64 ± 0.10)	(1.27 ± 0.15)
LEE18	47.5 ± 1.8	0.55 ± 0.6	0.60 ± 0.06	3.1 ± 0.3	1.21 ± 0.13	
	(1.05 ± 0.03)	(1.92 ± 0.14)	(0.63 ± 0.04)	(1.20 ± 0.11)	(1.60 ± 0.11)	(1.20 ± 0.11)
RY1C	44.8 ± 0.9	0.30 ± 0.5	0.97 ± 0.08	2.8 ± 0.4	0.74 ± 0.06	
	(1.00 ± 0.03)	(1.03 ± 0.04)	(1.02 ± 0.04)	(1.06 ± 0.06)	(0.98 ± 0.04)	(1.06 ± 0.06)
LEE103	44.1 ± 1.3	0.39 ± 0.6	0.87 ± 0.05	3.3 ± 0.4	0.83 ± 0.05	
	(0.99 ± 0.03)	(1.36 ± 0.10)	(1.00 ± 0.07)	(1.29 ± 0.07)	(1.06 ± 0.06)	(1.30 ± 0.09)
LEE72	60.3 ± 0.5	0.58 ± 0.3	0.56 ± 0.02	3.2 ± 0.3	1.35 ± 0.06	
	(1.31 ± 0.01)	(2.06 ± 0.15)	(0.64 ± 0.04)	(1.30 ± 0.11)	(1.59 ± 0.09)	(1.30 ± 0.11)
LEE77	57.3 ± 1.8	0.35 ± 0.2	0.92 ± 0.06	3.2 ± 0.2	0.82 ± 0.05	
	(1.26 ± 0.04)	(1.31 ± 0.06)	(1.03 ± 0.03)	(1.35 ± 0.08)	(0.97 ± 0.03)	(1.35 ± 0.08)
LEE80	57.5 ± 1.5	0.30 ± 0.1	1.07 ± 0.04	3.2 ± 0.2	0.71 ± 0.02	
	(1.26 ± 0.04)	(1.10 ± 0.06)	(1.19 ± 0.05)	(1.29 ± 0.03)	(0.85 ± 0.03)	(1.29 ± 0.04)

[a] Medium C was supplemented with glucose, thymine, threonine, and phenylalanine; growth was at 37°C. Units of measurement were: $\bar{M}$ in 10^{-9} OD_{460} units per average cell; G/M in 10^9 genome equivalents per OD_{460} unit of cells; $\bar{G}$ in genome equivalents per average cell; M_o in 10^{-9} OD_{460} units per origin of replication.

[b] See Table 1 for genotype of strains.

[c] Initiation mass, defined as OD_{460} units per origin of replication = $1/[(G/M)\cdot(O/G)]$. O/G is origins per genome, equivalent to increase in DNA, ΔG, after inhibition of initiation: $\Delta G = 1.4$ for B/r, RY1C, LEE103, TJK16, and LEE18, which grow with approximately 45-min doubling time; $\Delta G = 1.33$ for LEE72, LEE77, and LEE88, which grow with approximately 60-min doubling time (from references 5, 6).

[d] Values given are averages from 10 different experiments and the standard deviation. In each experiment all strains were tested on the same day; from each culture, four DNA samples and two cell number samples were taken between $OD_{460} = 0.3$ and 0.6. These were averaged to give one value for that culture on that day. The standard deviation given refers to the day-to-day variations of these averages. The day-to-day variations are two to three times greater than the sample-to-sample variations.

[e] Values in parentheses represent values relative to B/r; for each experiment, the B/r value was set at 1.0, and the values from the other strains obtained on the same day were normalized to this value.

***thyA deoB* mutations have no effect on initiation.** A thymine-prototrophic double revertant of TJK16, LEE18 (*thyA*$^+$ *deoB*$^+$), was found to have retained the increased initiation mass; also, an independently isolated low-thymine-requiring derivative of *E. coli* B/r A, LEE103, had the normal initiation mass of the B/r parent (Table 2). These observations show that the mutation affecting initiation in TJK16 is independent of the mutations affecting thymine metabolism.

Effect of temperature on initiation in TJK16. In liquid culture, growth of TJK16 at 42°C was identical to that of B/r (followed to stationary phase), but on solid medium, growth of TJK16 at 42°C stopped at a stage when microcolonies (~0.1 mm in diameter) had formed. These arrested microcolonies contained viable cells that could be replica plated at 30°C. In the genetic experiments described below, the temperature sensitivity was cotransduced with the initiation control gene of TJK16. Different temperature-resistant derivatives of TJK16 had different, usually intermediate, cell sizes, suggesting that they were (perhaps intragenic) suppressor mutations that simultaneously suppressed the temperature sensitivity and the initiation defect. One temperature-resistant derivative (LEE71; see Table 1) was identical to

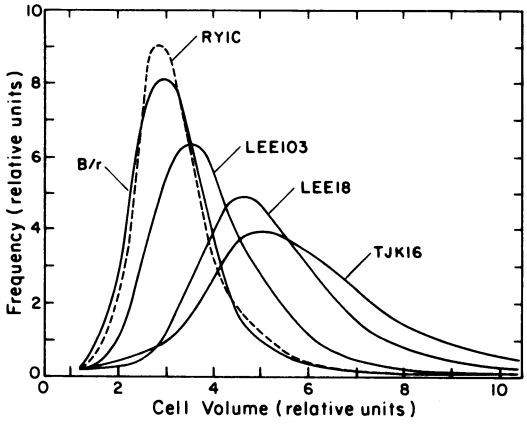

FIG. 1. *Cell volume distributions for E. coli B/r and RY1C (both Thy*$^+$*, normal initiation mass), LEE103 (Thy*$^-$*, normal initiation mass), LEE18 (Thy*$^+$*, increased initiation mass), and TJK16 (Thy*$^-$*, increased initiation mass).*

TABLE 3. *Effect of growth medium on the average cell mass of TJK16 and LEE18 (= thyA⁺ deoB⁺ double revertant of TJK16)*

Strain	Medium supplements[a]	Doubling time (min)	Mass density (OD_{460})	$\bar{M}$[b]		$\bar{M}/M_o$[c] (relative)	ΔD[c] (min)
				OD_{460}/cell	Relatives		
TJK16	Gluc, Thy	46	0.30	0.48×10^{-8}	1.92	1.20	+12
			0.60	0.41	1.64	1.02	+1
	Glu, Thy, Thr, Phe	48	0.29	0.52	2.08	1.30	+18
			0.60	0.54	2.16	1.35	+21
LEE18	Gluc	45	0.29	0.41	1.64	1.02	+1
			0.62	0.39	1.56	0.98	−1
	Gluc, Thy	45	0.32	0.44	1.76	1.10	+6
			0.61	0.43	1.72	1.08	+5
	Gluc, Thy, Thr, Phe	49	0.28	0.50	2.00	1.25	+16
			0.59	0.50	2.00	1.25	+16

[a] Gluc, 0.2% glucose; Thy, 20 μg of thymine per ml; Phe, 20 μg of phenylalanine per ml; thr, 60 μg of threonine per ml.

[b] For cell number counts, duplicate samples were taken when the culture reached $OD_{460} = 0.3$ and 0.6; they were then diluted 1:200 and 1:400, respectively, into counting solution. No correction for coincidence was necessary, and background was negligible. Relative values were normalized to a B/r value of 0.25×10^{-8} OD_{460} units/cell (see Table 4).

[c] Calculated from relative M_o values (Tables 2: 1.0 for B/r and 1.6 for TJK16 and LEE18). The relative $\bar{M}/M_o$ values are a measure for the increased D period (ΔD) compared with B/r: relative $\bar{M}/M_o = 2^{\Delta D/\tau}$, or increase in $D = \Delta D$ (in minutes) $= \ln(\bar{M}/M_o) \cdot \tau/\ln 2$; e.g., $\Delta D = +12$ means that the D period is 12 min longer than in B/r.

TABLE 4. *Average cell mass ($\bar{M}$) as a function of mass density (OD_{460}) of the culture and increase in the duration of the D period (ΔD) in comparison with B/r, of TJK16 and LEE18 growing in glucose minimal medium supplemented with thymine (20 μg/ml)*

Strain	Doubling time (min)	Mass density (OD_{460})	$\bar{M}$[a]		$\bar{M}/M_o$ (relative)	ΔD (min)
			OD_{460}/cell	Relative		
B/r	45	0.29	0.260×10^{-8}	1.00	1.0	0
		0.60	0.247			
		1.00	0.245			
TJK16	45	0.37	0.552	2.17	1.35	+19
		0.68	0.540			
		1.00	0.540			
LEE18	45	0.31	0.450	1.80	1.12	+7
		0.56	0.440			
		1.00	0.465			

[a] Equal to OD_{460} of culture per cells/milliliter, or OD_{460} units/cell.

TJK16 in all other respects, including the increased initiation mass (Table 2 shows M_o of LEE72, a streptomycin-resistant derivative of LEE71). Derivatives of LEE71 were used in the genetic experiments described below.

To see how temperature affects initiation in TJK16, the amounts of protein and mass per genome were measured as a function of temperature (Fig. 2). These are a measure of P_o and M_o (protein and mass per origin) if the C/τ ratio remains constant (see below). In B/r, mass per genome (and thus M_o) and protein per genome (and thus P_o) increased with temperature above 30°C (Fig. 2). A similar increase in initiation mass with temperature has been observed previously with *E. coli* K-12 (J. Frey, Ph.D. thesis, University of Geneva, Geneva, Switzerland, 1980). In TJK16, the temperature dependencies of M_o and P_o were qualitatively different from those observed with B/r, but at high temperatures the initiation mass of TJK16 decreased in relation to B/r, which is the opposite of the effect seen in temperature-sensitive initiation mutants.

The replication velocity (reciprocal of C) was estimated from the initial slope of the DNA accumulation curves in temperature shift experiments (Fig. 3); i.e., it was assumed that the

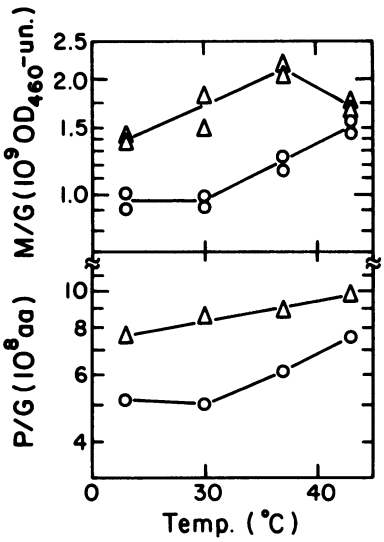

FIG. 2. *Mass per genome (M/G) and protein per genome (P/G) for E. coli B/r (○) and TJK16 (△) as a function of growth temperature. Cultures were grown exponentially at different temperatures in glucose minimal medium with 20 μg of thymine per ml. Duplicate samples for protein and DNA were taken at $OD_{460} = 0.4 \pm 0.1$, to give DNA/OD_{460} and protein/OD_{460}. (Top) The average for the reciprocal of DNA/OD_{460}, i.e., M/G is plotted for two experiments (all lower values of each pair of measuring points are from one experiment; the higher values are from a second experiment). (Bottom) P/G obtained as protein/OD_{460} divided by DNA/OD_{460} from one of the two experiments in the top panel (with the higher M/G values).*

immediate change in the rate of DNA synthesis after the temperature shift reflected only the effect of temperature on the velocity of the replication forks. The replication velocity and the growth rate had the same temperature dependence in B/r and TJK16 (Fig. 4); i.e., C/τ did not significantly change with temperature for either strain. In a shift from 37 to 43°C, the rate of DNA synthesis changed identically in the two strains (Fig. 3), giving no indication of a special temperature sensitivity of initiation in TJK16. Thus, the temperature sensitivity of TJK16 reflected a pleiotropic effect of the mutation in the initiation control gene, but it was not directly related to initiation control.

Initiation mutation of TJK16 is linked to *dnaA*. A *dnaA*(Ts) marker from PLH301, a B/r derivative which also has a mutation in the *bglR* gene that maps near *dnaA* (1) and that allows PLH301 to utilize salicin as a carbon source (16), was transduced, using phage P1, into LEE72, a streptomycin-resistant and temperature-resistant derivative of TJK16 that had retained the increased initiation mass (Table 2). (LEE72 had a somewhat longer doubling time [Table 2] due to its ribosomal protein [streptomycin resistance] mutation.) A *dnaA* transductant (LEE73) was obtained by selecting for the ability to grow on salicin at 30°C (*bglR* transductants), followed by replica plating and screening for inability to grow at 42°C (*dnaA* cotransductants). Figure 5 shows an experiment in which DNA accumulation was followed during a shift from 30 to 42°C in the donor (PLH301), the recipient (LEE72), and *dnaA*(Ts) transductant (LEE73). The transductant showed the delayed cessation of DNA synthesis typical for DNA initiation mutants. Since the initiation mass in *dnaA*(Ts) mutants is increased even at permissive temperatures (Frey, Ph.D. thesis, 1980), and also since data obtained at 30°C were not comparable to those obtained at 37°C, LEE73 was not included in the experiments of Table 2. Rather, to see whether the initiation mutation of TJK16 maps close to *dnaA*, a $dnaA^+$ allele from B/r was transduced into LEE73, selecting for the ability to grow at 42°C. One of the $dnaA^+$ transductants was named LEE77. LEE77 had, in fact, regained the wild-type initiation mass (Table 2); i.e., it had lost the initiation mutation of TJK16, indicating that this mutation maps close to *dnaA*. Of several other transductants, only the cell size was measured. All temperature-resistant transductants, including LEE77, had regained the smaller cell size (see $\bar{M}$ values in Table 2) of B/r in comparison with TJK16 or LEE72.

Initiation control mutation in TJK16 maps between *gyrB* and *bglR*. It is not known whether replacement of the mutant initiation control gene of TJK16 occurred with the transduction of *dnaA*(Ts) into LEE72 (construction of LEE73) or with the transduction of $dnaA^+$ into LEE73 (construction of LEE77). Therefore, the cotransduction frequency of $dnaA^+$ and the initiation marker of TJK16 could not be used for exact mapping of the initiation marker.

To more accurately estimate the map location of the initiation mutation in TJK16, we made TJK16 *bglR* and coumermycin resistant (*gyrB*; construction of LEE87 [Table 1]) and transduced these markers with phage P1 into B/r, selecting for *bglR* (growth on salicin) and checking for cotransduction of *gyrB* (replica plating on coumermycin plates) and cell size (Fig. 6; cell volume distributions).

The results showed that 369 of 489 (75%) *bglR* transductants (selected on salicin plates) had simultaneously cotransduced *gyrB*; of these, 30 of 30 tested also had increased cell size (e.g., no. 56 of Fig. 6). Of the other 120 *bglR* transductants which were coumermycin sensitive, 79 were tested for their cell size. Of these, 72 had the

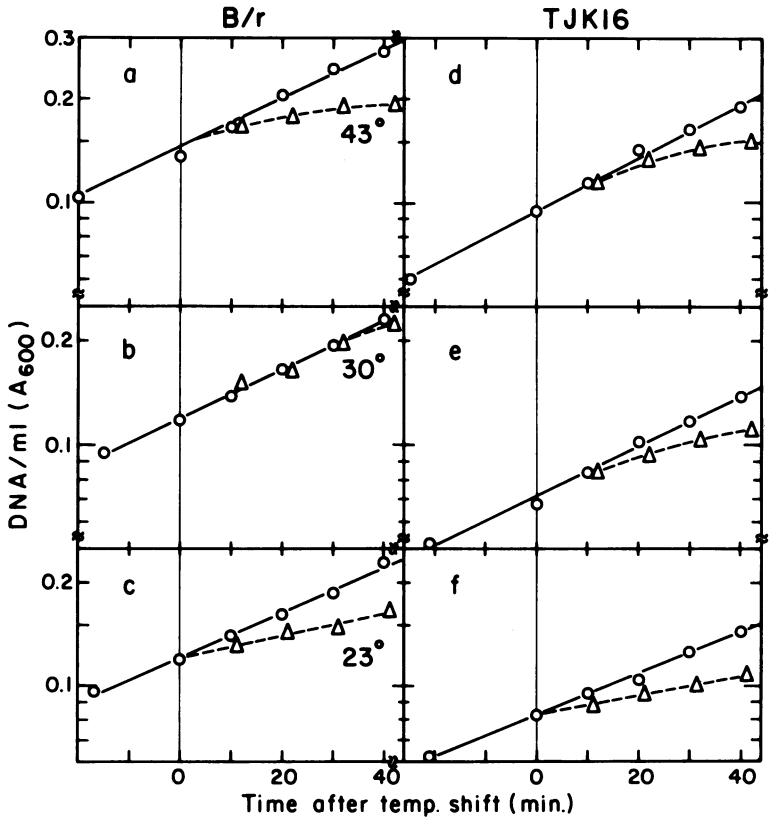

FIG. 3. *DNA accumulation in E. coli B/r (a-c) and TJK16 (d-f) after a shift in temperature from 37 to 43, 30, or 23°C as indicated. Culture medium was supplemented with glucose and 20 μg of thymine per ml. Symbols: (△) shifted portion; (○) unshifted control. Absorbency at 600 nm (A_{600}), colorimetric assay for DNA; an A_{600} of 1.0 represents a DNA concentration of 2.84 genome equivalents per ml of culture.*

small (B/r-) size (e.g., no. 7, 10, 20, 21, 34, 64, and 68 in Fig. 6); the remaining seven had an increased size (e.g., no. 78 in Fig. 6). This indicates that the initiation control mutation mapped between *gyrB* and *bglR*, closer to *gyrB* (see Discussion).

TJK16 and B/r have the same sensitivity to coumermycin. The preceding mapping experiments showed that the initiation mutation in TJK16 was separable from a mutation in the *gyrB* gene that resulted in coumermycin resistance. The genetic distance between the initiation mutation and the coumermycin resistance mutation was about 0.06 map unit (see Discussion). This is consistent with the shortest distance between *gyrB* and *dnaA* (i.e., the initiation mutation of TJK16 may lie in the *dnaA* gene) and with the longest distance within *gyrB* (see Discussion); i.e., the initiation mutation of TJK16 may also lie within *gyrB*. This possibility had to be considered, since Orr et al. (16) reported that a *gyrB* mutation, resistant to coumermycin, had an increased initiation mass but the same replication velocity as the coumermycin-sensitive parent strain; i.e., it had a phenotype similar to that of TJK16. Since genetic mapping could not be refined much further because of the necessity to screen cell volumes, we compared the sensitivity to coumermycin of TJK16 and B/r. In liquid culture (LB medium), coumermycin caused reductions in the rate of mass accumulation (to 12, 5, and 3% at 2, 5, and 10 μg of coumermycin per ml, respectively, determined between 2 and 4 h after the addition of the antibiotic) which were identical in B/r and TJK16 (Fig. 7). Similar results were obtained with glucose minimal medium (data not shown). Also, the colony-forming ability at different concentrations of coumermycin was essentially the same for TJK16 and B/r. Thus, the mutation in TJK16 was different from the mutation described by Orr et al., since our experiments gave no indication of a functionally altered gyrase in TJK16. This does not exclude the possibility that the mutation is in *gyrB*.

Selective advantage of TJK16 over B/r.

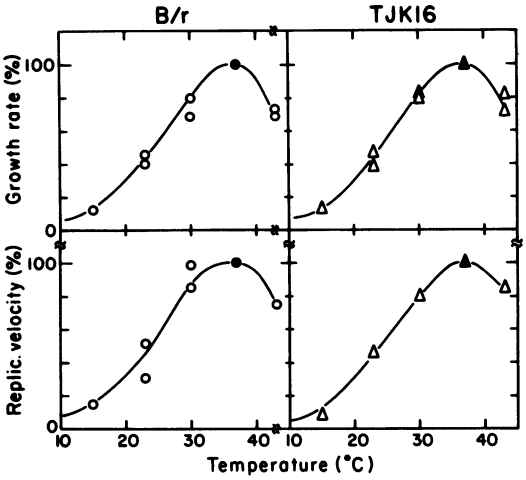

FIG. 4. *Relative growth rate and replication velocity of E. coli B/r and TJK16 as a function of growth temperature. Cultures were grown in glucose minimal medium supplemented with thymine (20 μg/ml). (Top) Relative growth rate in two experiments (same as in Fig. 2, top panel); 100% corresponds to a doubling time of 44 ± 1 min (growth rate is proportional to the reciprocal of the doubling time). (Bottom) Relative replication velocity corresponds to the slope of the DNA curves in Fig. 3, immediately after the temperature shift (average between 0 and 20 min). The graph includes an additional experiment at 15°C, not shown in Fig. 3, and a repeat of the 23 and 30°C experiment with B/r. Solid symbols are reference points, with value at 37°C set at 100%.*

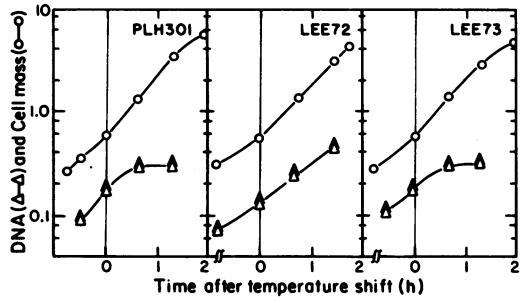

FIG. 5. *Accumulation of cell mass (OD_{460}) and DNA (absorbency at 600 nm [A_{600}]) in PLH301 [donor of dnaA(Ts)], LEE72 [recipient for dnaA(Ts)], and LEE73 [P1 transductant, having received the dnaA(Ts) allele] growing in glucose-Casamino Acids medium after a temperature shift from 30 to 42°C.*

The acquisition of a mutation in an initiation gene during the isolation of TJK16 would be an extremely unlikely coincidence, unless this mutation would give TJK16 a selective advantage over its competitors (lacking the initiation mutation) under some condition of growth during the isolation procedures. Since these involved partial thymine starvation and growth in the presence of trimethoprim, we compared the growth characteristics of TJK16 and LEE103 under such conditions. (LEE103 has, in addition to its *thyA* and *deoB* mutations, two mutations to amino acid auxotrophy, which allowed quantitation of cells from both strains in a mixed culture by plating on minimal and on amino acid-supplemented media.) Neither growth in the presence of trimethoprim nor thymine starvation gave TJK16 a selective advantage. However, in mixed cultures of LEE103 and TJK16, with TJK16 in the minority, an enrichment of TJK16 was found in repeated cycles of overnight growth and culture dilution (Fig. 8). Once

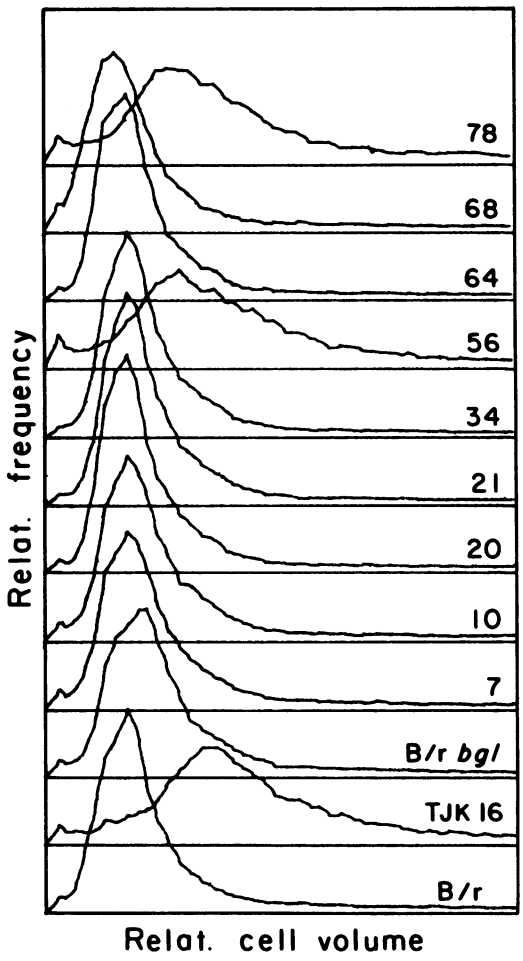

FIG. 6. *Cell volume distributions of a sample of P1 transductants: donor, TJK16 bglR gyrB; recipient, E. coli B/r. Transductants were selected for growth on salicin (bglR) and tested for cotransduction of gyrB by replicating on coumermycin plates. In this sample, only no. 56 is a bglR gyrB cotransductant. The lower three distributions are shown for comparison.*

TJK16 and LEE103 were in about equal concentrations, no further enrichment (i.e., over 50% TJK16) occurred. These observations suggest that the initiation control mutation gives TJK16 a selective advantage over its competitors under certain conditions of growth, e.g., in an overnight culture, which might have led to the isolation of the initiation mutation in TJK16.

DISCUSSION

Mapping of the initiation control mutation in TJK16. Seven of 79 *bgl* transductants from TJK16 (donor) into B/r (recipient) received the cell size marker of TJK16 but not *gyrB*; i.e., recombination must have occurred between *gyrB* and the cell size marker. The cell size marker cannot lie outside *gyrB* and *bglR*, since this would require a total of four recombi-

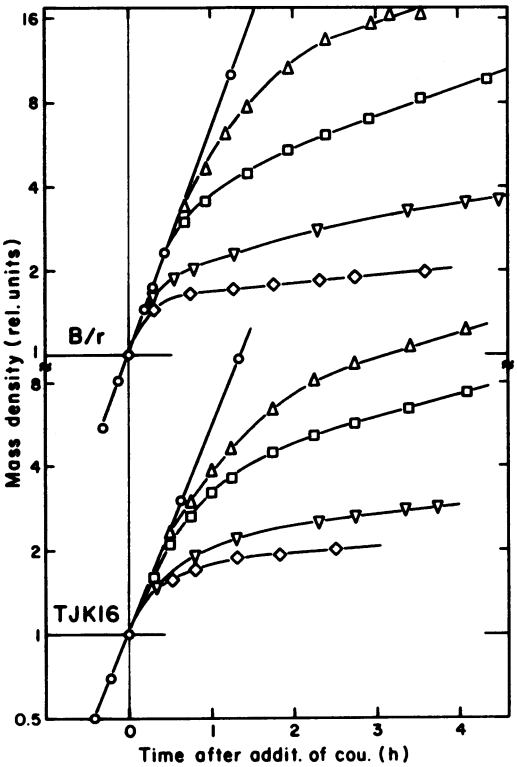

FIG. 7. *Growth of E. coli B/r and TJK16 at various concentrations of coumermycin. Cultures were grown in LB medium to an* OD_{650} *ot 0.5 (±10%); then they were divided into five portions to which 0 (○), 1 (△), 2 (□), 5 (▽), or 10 (◇) μg of coumermycin, respectively, was added per ml. Monitoring of growth was continued as indicated (the* OD_{650} *rather than* OD_{460} *was measured to reduce the blank value of the LB medium). The data were normalized to 1.0 at t = 0. Doubling times of the untreated cultures were 22 (B/r) and 24 (TJK16) min. At all concentrations (>0), the bacteria became filamentous; i.e., cell division was inhibited.*

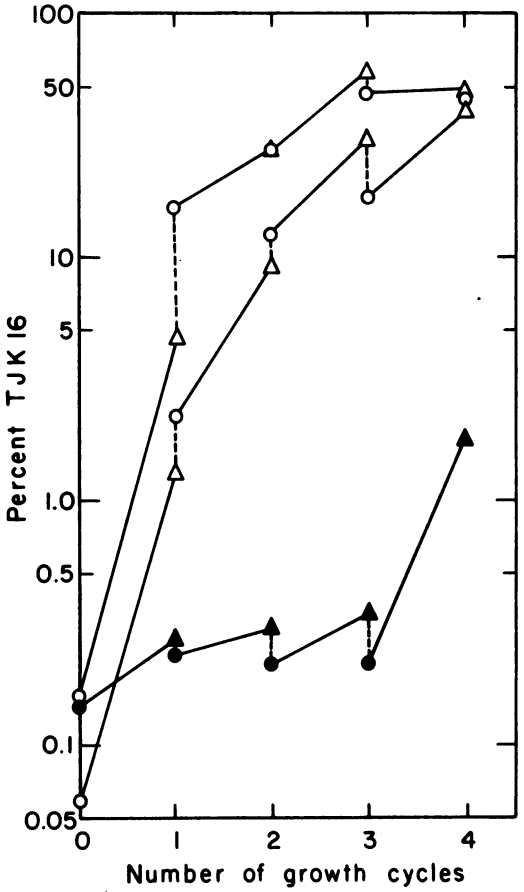

FIG. 8. *Selective advantage of TJK16 during growth with LEE103 in mixed cultures. The growth medium was supplemented with glucose, phenylalanine, threonine, and thymine. The graph shows three repeats of the same experiment: an exponential culture of TJK16 was diluted into an exponential culture of LEE103 (*OD_{460} *= 0.2) such that the fraction of TJK16 cells was about 0.1% of the total number of cells; the fraction was determined immediately by plating on minimal (plus thymine) plates (only TJK16 grows) and on LB plates (all cells grow). After overnight growth to stationary phase, the mixed culture was plated as before (percentage of TJK16 plotted; triangular symbols at abscissa value 1.0), diluted 1:300 into fresh medium, and grown again (beginning of a new growth cycle). At* OD_{460} *= 0.2, the exponential culture was plated (circular symbol at abscissa value 1.0), and growth was continued overnight. The above procedures were repeated until completion of the fourth cycle on the 4th day. (Open symbols) Two experiments with similar results; (closed symbols) no significant enrichment of TJK16 occurred initially, but when the overnight cultures of the first three cycles were plated again after several days of storage at room temperature, each showed an about 100-fold enrichment of TJK16. Each of the three experiments shown was done in duplicate (not shown) with a 10-fold-higher input ratio of TJK16 to B/r. The enrichment observed during the first cycle was always very similar in the duplicates.*

nation events to get the size marker and *bglR*, but not *gyrB*, integrated into the recipient chromosome. The approximate frequency, 7 of 79 (~10%), corresponds to the distance between the size marker and *gyrB* relative to the distance between *gyrB* and *bglR*. Assuming the latter to be equal to 0.6 map units and assuming 1 map unit to correspond to 40,000 base pairs (1), we estimate a distance of 0.06 map units, corresponding to 2,400 base pairs, between the *gyrB* marker and the cell size marker. This is about equal to the minimum distance of *gyrB* and *dnaA*, but also about equal to the maximum distance within the *gyrB* gene (10). Thus, genetic mapping does not provide a clear-cut answer to the question of whether the initiation control mutation of TJK16 maps in the *gyrB* gene (close to the N-terminal end, whereas the *gyrB* mutation used maps close to the C-terminal end; for direction, see reference 10) or whether it maps outside of *gyrB*. Two genes, *recF* and *dnaN*, map between *gyrB* and *dnaA* (L. W. Ream, L. Margosian, A. J. Clark, F. G. Hansen, and K. von Meyenburg, Mol. Gen. Genet., in press). *dnaN* has a polypeptide product of 43 to 45 kilodaltons (10, 13); for *recF*, a polypeptide product has not been found (10; Ream et al., in press). Thus, genetic mapping locates the initiation control mutation in a small region of the chromosome that contains four genes, three of which are known to be involved in replication.

Initiation control. The *dnaA* gene product is known to be required for initiation (since initiation stops when a temperature-sensitive *dnaA* product is inactivated), but it has not been shown that normally, in a *dnaA*$^+$ strain, the *dnaA* product is limiting and thus controlling initiation (9). If it were, initiation of replication would be controlled positively, rather than negatively by a repressor (18).

Kimura et al. (13) constructed an *E. coli* strain with a temperature-sensitive amber suppressor and an amber mutation in *dnaN*. Under nonpermissive conditions, initiation continued for at least 1 h in this strain. This indicates that the product of *dnaN* was not limiting initiation, even under the conditions of amber suppression, and thus the *dnaN* protein is not a candidate for a positive control factor for initiation.

DNA gyrase has also been implicated in initiation, since a coumermycin-resistant *gyrB* mutant was shown to have an increased initiation mass (16). Our coumermycin-resistant B/r and TJK16 strains had the same initiation mass and cell size as their coumermycin-sensitive parents, in contrast to the coumermycin-resistant mutant described by Orr et al. (16), which had an increased initiation mass and size. This may be related to the fact that Orr et al. had selected their *gyrB* mutant strain for simultaneous temperature sensitivity and coumermycin resistance. However, mutation to coumermycin resistance occurred with an approximately two orders of magnitude lower frequency in TJK16 than in B/r. Similarly, mutations to nalidixic acid resistance could not be obtained from TJK16 but were readily obtained from B/r (P. Holmans, personal communication). This is consistent with the idea that gyrase is part of the initiation complex; i.e., most of the coumermycin-resistant *gyrB* mutations may be compatible only with the normal initiation complex of B/r, and not with the mutant complex in TJK16. Since TJK16 showed no significant difference in coumermycin sensitivity compared with B/r, it seems likely that TJK16 has a normal gyrase. Also, in wild-type bacteria, gyrase is not likely to be produced in limiting amounts for initiation since larger amounts of gyrase are presumably required at many transcription and replication sites.

The initiation mutation in TJK16 is not likely to be in the *recF* gene, since TJK16 does not have increased UV sensitivity, like *recF* (Ream et al., in press), nor does a *recF* strain have an increased initiation mass, like TJK16. (This was checked with an isogenic pair of *recF recF*$^+$ strains kindly provided to us by Walter Ream.)

The suggestion above, that the initiation protein of TJK16 interacts with DNA gyrase differently from the wild-type initiation protein, implies that TJK16 has a structurally altered initiation protein, as is also suggested by its temperature sensitivity. Thus, the cell size mutation in TJK16 is not likely to be only a control site (promoter or operator) mutation. The initiation protein (i.e., presumably *dnaA*) may have different functional regions, one involved in initiation and another involved in autoregulation. A mutation could affect the activity (initiation) or the synthesis (autoregulation) of the initiation protein. We cannot determine which of these possibilities applies to TJK16.

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