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Chromosome Replication and Cell Division in Plasmid-Containing *Escherichia coli* B/r

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The kinetics of chromosome replication and cell division have been examined in *recA* mutants of *Escherichia coli* B/r containing F′ plasmids of various sizes. Plasmid-mediated alterations in growth properties were detected only with the presence of the larger F′ plasmids, and were reflected in decreased mean cell sizes and growth rates. The lengths of *C* and *D* in all plasmid-containing strains were in accord with the values for plasmid-free parental strains growing with similar generations times. The findings were consistent with an absence of competition between the chromosomal and extrachromosomal replicons for rate-limiting components involved in the initiation of deoxyribonucleic acid synthesis or in the elongation of deoxyribonucleic acid chains.

Bacterial plasmids are extrachromosomal replicons whose replication and maintenance are autonomous of analogous chromosomal events (5). This autonomy does not imply an independence of plasmid-related functions from cellular growth and division. For instance, the presence of plasmids such as Rts1 (22) or certain mutants of ColVB*trp* (13) which are temperature sensitive for replication can cause inhibition of cell division in *Escherichia coli* at the nonpermissive temperature. In addition, the presence of UV-irradiated P1 plasmids in *E. coli* has been reported to block cell division (16). Only after the damaged replicon was repaired did cell division commence. A further indication of plasmid-mediated effects on cell growth is the altered morphology of some plasmid-containing strains. *E. coli* cells containing the R factor R1*drd-19*, and copy number mutants of this plasmid, have been reported to have an increased mean cell size (7). The changes in cell size were most pronounced in poorer growth media and with higher plasmid copy numbers, and were accompanied by a slight reduction in growth rate. This increase in cell size was interpreted to be the result of competition between chromosomal and plasmid replicons for rate-limiting components of the DNA synthetic apparatus. Both initiation of DNA synthesis and DNA chain elongation were assumed to be affected by this competition. The increase in mean cell size was thought to be a reflection of skipped cell divisions in those cells in which plasmid replication precluded chromosome replication. Increases in mean cell sizes have also been observed in some strains of *E. coli* containing F′*1ac* plasmids (6), but not in others (1, 8).

To examine the premise that competition between chromosomal and extrachromosomal replicons for components of the DNA synthesis apparatus might be an intrinsic aspect of plasmid maintenance, a series of F′ plasmids containing DNA homologous with different portions of the chromosomal region between 94 and 13 min of the genetic map of *E. coli* (2) were introduced into *E. coli* B/r F. A study of cell cycle parameters (i.e., *C*, *D*, and τ) in these strains revealed that the DNA synthetic capacity of the host cell was in excess of the demands placed on it by the need to replicate both chromosome and plasmid.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Derivatives of *E. coli* B/r F (10) were used in these studies and are listed in Table 1. F′ plasmids were introduced into strain B/r F44 by mating with appropriate F′-containing *E. coli* K-12 strains. Selection of plasmid-containing stocks of B/r F44 was based on auxotrophic complementation of chromosomal markers. The extrachromosomal nature of this complementation was ascertained by curing with acridine orange (11) and by isolation of covalently closed circular molecules from the plasmid-containing strains. Maintenance of F′ plasmids in B/r F44 was monitored by demonstrating that unselected markers could be transferred from the plasmid-containing strains to appropriate recipient cells, e.g., the presence of the distal *purE* marker was confirmed by mating B/r F44/F′144 and B/r F44/F′209 with a *his*$^+$ derivative of *E. coli* AT3143 (*purE*) (21) and selecting for adenine-independent recombinants.

Minimal salts medium contained (per liter of distilled, deionized water): 2 g of NH_4Cl, 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 3 g of NaCl, and 0.25 g of $MgSO_4$. Glucose and glycerol were used as carbon sources at

TABLE 1. *Bacterial strains*

Strain	F′	Ancestral Hfr	Genotype of chromosome	Source
B/r F			*his*	This laboratory
B/r F41			*his thr pro thy*A *rpsL*	This laboratory
B/r F44			*his thr pro rec*A *rpsL*	Mating F41 with KL16-99[a]
B/r F44/F′101	F′101	HfrH	*his thr pro rec*A *rpsL*	Mating F44 with KLF1/AB2463[a]
B/r F44/F′104	F′104	HfrH	*his thr pro rec*A *rpsL*	Mating F44 with KLF4/AB2463[a]
B/r F44/F′121	F′121	HfrH	*his thr pro rec*A *rpsL*	Mating F44 with KLF21/KL132[a]
B/r F44/F′144	F′144	HfrH	*his thr pro rec*A *rpsL*	Mating F44 with KLF44/KL251[a]
B/r F44/F′209	F′209	P4X	*his thr pro rec*A *rpsL*	Mating F44 with ORF209/X709[b]

[a] The *E. coli* Genetic Stock Center, Barbara Bachmann, Curator.
[b] Obtained from R. Curtiss.

0.1% final concentrations. When necessary, growth media were supplemented with 50 μg of L-amino acids per ml and 10 μg of thymine per ml.

Analytical procedures. Chromosome replication patterns of plasmid-containing cells were determined as described previously (10). Estimates of average cell mass, expressed as absorbance at 450 nm per cell × 10^8, were computed on the basis of measurements of absorbance obtained with a Zeiss PMQII spectrophotometer at 450 nm in a 1-cm light-path cuvette and particle number obtained with a model ZB Coulter Counter. Absorbance, cell concentrations, and viabilities were monitored for two to three generations after cultures grown overnight had reached approximately 5 × 10^7 cells per ml. To obtain representative experimental values for absorbance per cell and relative viability in a given culture, these parameters were computed at A_{450} = 0.2. Size distributions were obtained with a Coulter Counter Channelyzer. To insure uniformity of Channelyzer scans, the diluent used in these studies was composed of 0.9% NaCl, 0.05 M sodium phosphate (pH 7.0), and 20% formaldehyde (3).

RESULTS

Patterns of chromosome replication in plasmid-containing cells. A series of F′ plasmids of various sizes, and possessing overlapping chromosomal homologies, were introduced into *E. coli* B/r F44 to assess the extent of F′-mediated interference in host chromosome replication and cell division, and the dependence of any such interference on plasmid size or merogenomic region. The sizes and regions of homology of these plasmids are shown schematically in Fig. 1. F′ plasmids 101, 104, 121, and 144 were all derived from HfrH, and F′209 was derived independently from P4X (15).

The effects of plasmid content on chromosome replication and cell division were analyzed with the membrane-elution technique (9). Briefly, exponential-phase cultures were pulse labeled with [^{14}C]thymidine, bound to the surface of a nitrocellulose membrane filter, and flushed continuously with growth medium to elute newborn cells from the membrane-bound population. Representative measurements of the

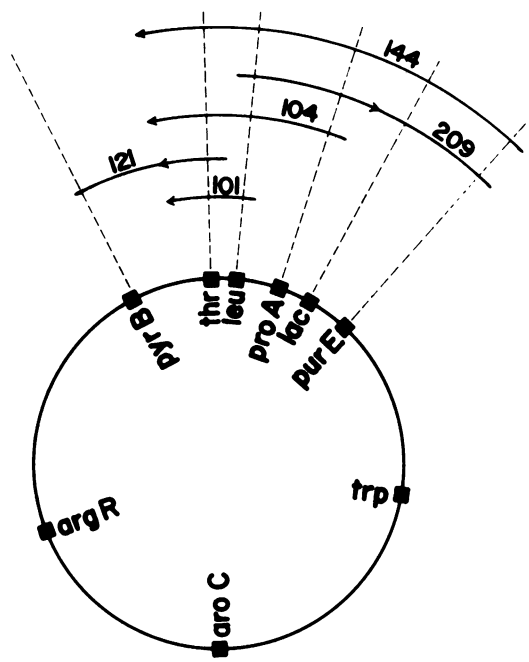

FIG. 1. *Schematic representation of the F′ plasmids employed. The regions of homology with the host chromosome are shown. The position and orientation of the F factor in each plasmid is designated by an arrow.*

radioactivity in newborn, glycerol-grown cells are shown in Fig. 2. Vertical interrupted lines indicate generations of membrane-bound growth. The rate of DNA synthesis during the division cycle is read from right to left in each generation. Figures 2a and b show the results with *E. coli* B/r F (*rec*$^+$) and B/r F44 (*recA*), respectively. The *recA* mutant possessed a slightly increased (ca. 10%) membrane-bound generation time (τ_{mem}) and $C + D$ interval, which is the time interval between initiation of chromosome replication (an abrupt decrease in radioactivity per newborn cell) and the subsequent cell division (the preceding vertical line). The properties of B/r F44/F'104 (Fig. 2c) were very similar to those of its plasmid-free parent (Fig. 2b). However, the presence of F'144 in B/r F44 caused an elongation of τ_{mem} with only a minimal increase in the value of $C + D$ (Fig. 2d). The elongation of τ_{mem} resulted in the appearance of a period devoid of DNA synthesis during the beginning of the division cycle. The altered cell cycle properties of B/r F44/F'144 were due solely to the presence of the plasmid since cultures cured of the F'144 plasmid with acridine orange were restored to parental characteristics (data not shown).

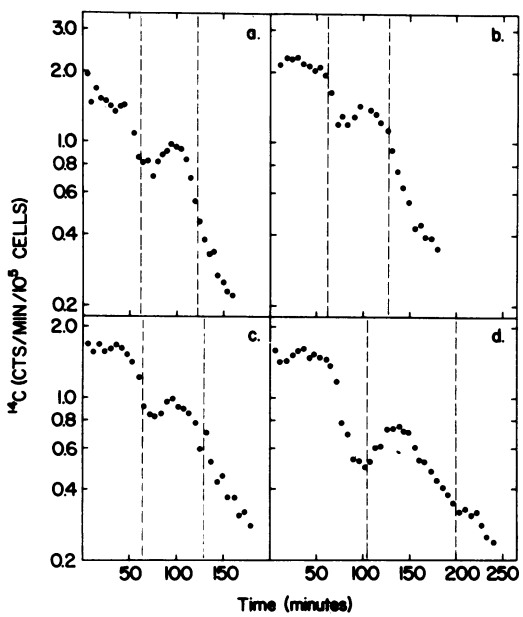

FIG. 2. *Radioactivity per cell in the effluent from glycerol-grown membrane-bound cultures of (a) E. coli B/r F, (b) E. coli B/r F44, (c) E. coli B/r F44/F'104 and (d) E. coli B/r F44/F'144. Exponential-phase cultures were exposed to 0.01 μCi of [^{14}C]thymidine per ml for 0.1 generation and then bound to the surface of a membrane filter. Generations of elution are indicated by the interrupted lines.*

Measurements of the lengths of C, D, and τ_{mem} in relation to plasmid size in glycerol-grown cells are summarized in Fig. 3. The generation times of cells possessing F'101, 104, or 121 were essentially the same as the τ_{mem} of the plasmid-free parental B/r F44. τ_{mem} was increased somewhat with F'209 and significantly extended with F'144. Values for C, D, and $C + D$ varied little between strains. Values of C and $C + D$ in strains containing F'101, 104, 121, or 209 were clustered around 45 and 62 min, respectively. In B/r F44/F'144, C was longer, about 54 min on the average, but this was not unexpected in light of the generation time of this strain (10).

Average values for all cell cycle parameters examined in glucose- and glycerol-grown cells are shown in Table 2. In both glucose- and glycerol-grown cultures, the presence of the *recA* lesion resulted in detectable increases in generation times. In plasmid-containing, glucose-grown cells, $C + D$ was found to be equal to, or slightly shorter than, the value for the plasmid-free parental strain. The increase in τ_{mem} in

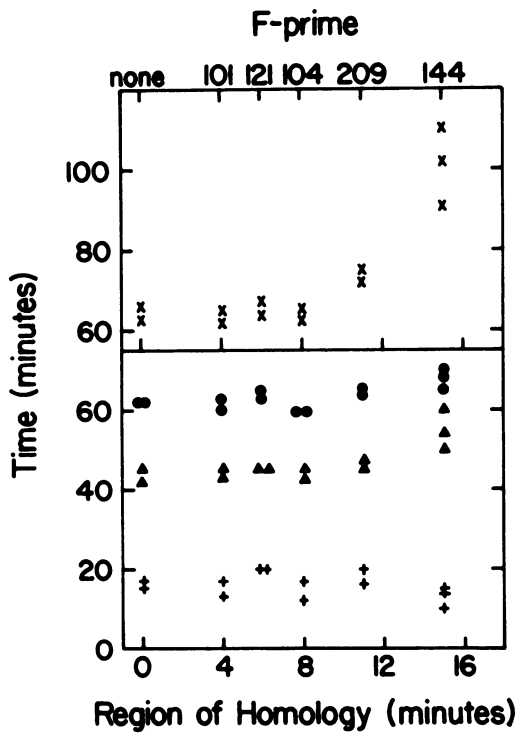

FIG. 3. *Values of C, D, and τ_{mem} in glycerol-grown cultures. Data from membrane-elution experiments of the type shown in Fig. 2 are shown with respect to the extent of homology each F' had with the chromosome. Experimental values for zero homology are from plasmid-free cultures of B/r F44. Symbols: +, D period; ▲, C period; ●, C + D; ×, τ_{mem}.*

TABLE 2. *Cell cycle and growth parameters of F'-containing cells*[a]

Strain	Region of homology (min)[b]	Glucose					Glycerol				
		Viability[c,e]	τ_{mem}[d] (min)	τ_{exp}[c] (min)	C + D[d] (min)	Abs/cell × 10^{8}[c,e]	Viability[c]	τ_{mem}[d] (min)	τ_{exp}[c] (min)	C + D[d] (min)	Abs/cell × 10^{8}[c,e]
B/r F	—	ND	46	ND	60	ND	ND	60	ND	60	ND
F41	—	1.00	47	45	62	0.201	1.00	61	61	63	0.140
F44	—	0.87	52	55	63	0.192	0.62	65	76	62	0.141
F44/101	98-2	0.78	55	65	57	0.190	0.38	64	96	61	0.146
F44/104	98-6	ND	52	ND	60	ND	ND	64	ND	60	ND
F44/121	94-0	ND	51	ND	62	ND	ND	66	ND	64	ND
F44/144	98-13	0.96	60	65	60	0.165	1.20	103	113	68	0.125
F44/209	2-13	ND	57	59	63	0.178	ND	74	91	65	0.130

[a] All values presented are the average of the results of at least four separate determinations. ND, Not determined.

[b] Regions of chromosomal homology for each F-prime are based on genetic characterization of the plasmids (14) and the recalibrated linkage map of *E. coli* K-12 (2). —, F .

[c] Viability, τ_{exp}, and absorbance per cell were based on measurements of cultures growing exponentially.

[d] τ_{mem} and $C + D$ are from membrane-elution experiments.

[e] Abs/cell × 10^{8} and viability were computed on the basis of particle number $Abs_{450} = 0.2$.

glycerol-grown cells containing F'144 was less evident in glucose-grown cells.

In summary, clear alterations in cell cycle properties were only evident in strains which contained the larger plasmids (i.e., F'144 and F'209) and were most pronounced in glycerol-grown cultures.

Size of plasmid-containing cells. If a competition existed between plasmid and chromosome for rate-limiting components involved in the initiation of DNA synthesis, it should be reflected by an increase in the average cell size of plasmid-containing cells. That is, if the length of $C + D$ remained unchanged, and initiation of chromosome replication was selectively delayed due to the competition, mean cell size at initiation, and consequently mean cell size in an exponential-phase culture, would be increased. To examine this possibility, the average cellular mass of some of the plasmid-containing strains was measured and compared to the mass of plasmid-free parental cells. To accomplish this, particle concentration, cellular viability, and absorbance at 450 nm were monitored for two to three generations of exponential growth, as shown in Fig. 4 for strain B/r F44/F'101. A summary of generation times determined from particle concentrations (τ_{exp}) and the results of measurements of viability and absorbance per 10^{8} cells are shown in Table 2. The values of τ_{exp} were generally larger than the corresponding τ_{mem}. Contributing to this phenomenon were differences in the method of measurement (9) and the presence of the *recA* lesion in these strains. Cultures of *recA* mutants of *E. coli* K-12 are known to have altered growth properties and a significant percentage of nonviable cells (4). The

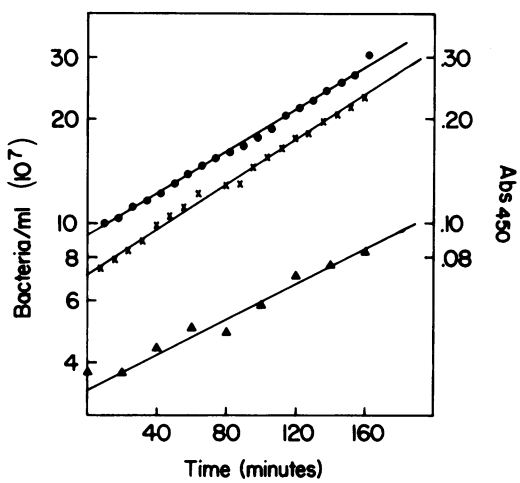

FIG. 4. *Growth characteristics of E. coli B/r F44/F'101 in glycerol-minimal medium. An exponentially growing culture was sampled for measurement of particle concentration (×), viable number (▲), and absorbance at 450 nm (●). Glucose-minimal agar plates supplemented with 0.2% Casamino Acids were used for determination of viability. Sampling for absorbance measurements was conducted as described previously (18).*

viabilities of the B/r *recA* strains (Table 2) varied between approximately 80 and 100% of the parental *rec*$^{+}$ strain in glucose-grown cultures, but were lower in glycerol-grown cultures, particularly in the case of B/r F44/F'101. The higher viabilities of B/r F44/F'144 are unexplained, except that the presence of cells of smaller volume in the cultures may have resulted in underestimates of particle concentra-

tions. The *recA*-mediated effects were more pronounced in measurements of generation times made on batch cultures than on membrane-bound cultures, because values determined in the latter experiments were not affected by membrane-bound cells incapable of dividing. Consistent with its reduced viability, values of τ_{exp} for B/r F44/F'101 in glucose- and glycerol-minimal media were appreciably longer than the corresponding values of τ_{mem}, which may reflect properties unique to this strain.

Measurements of absorbance per cell indicated that the mean cell masses of B/r F41 (*rec*$^+$), B/r F44 (*recA*), and B/r F44/F'101 were essentially the same (Table 2). In B/r F44/F'144 and B/r F44/F'209, mean cell masses were lower. The decreased mean cell masses were most likely reflections of the decreased growth rates of these strains (20).

To further analyze the differences in cell sizes detected by absorbance measurements, particle size distributions were measured in populations of plasmid-containing and plasmid-free cells. Particle size distributions of B/r F44 and B/r F44/F'144 are shown in Fig. 5. Consistent with the conclusions based on absorbance measurements, cells of strain B/r F44/F'144 were smaller than cells of the plasmid-free parental strain in both glucose- and glycerol-minimal media. It is noteworthy that the plasmid-containing strain did not have a higher proportion of larger, filamentous cells relative to its plasmid-free parent. Since plasmid-containing cells were not larger than their plasmid-free parents, the possibility of rate-limiting competition between chromosome and F' plasmid for components involved in the initiation of DNA synthesis appears unlikely.

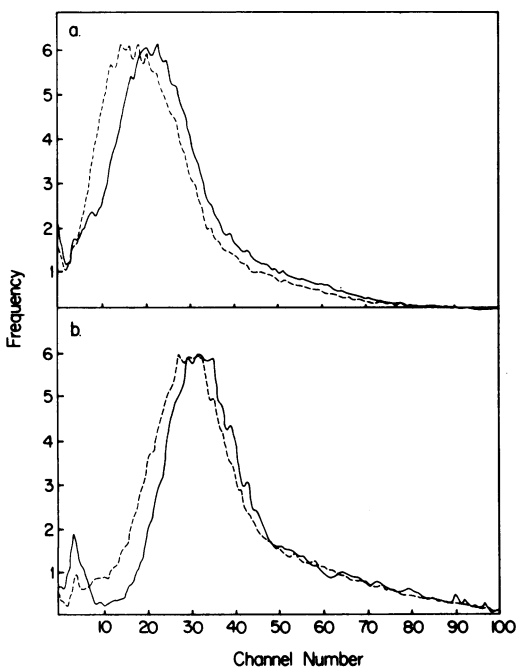

FIG. 5. *Cell size distributions in cultures of E. coli B/r F44 and B/r F44/F'144. Shown are glycerol- (a) and glucose- (b) grown cultures. Symbols: ——, F44; ·····, F44/F'144.*

DISCUSSION

We have shown that the stable maintainance of F' plasmids can result in perturbations of normal cell growth in a manner which differs from previous findings with regard to some R and F'*lac* plasmids (6, 7). Engberg et al. (7) reported that the R factor R1*drd-19*, and copy number mutants of this plasmid, perturbed cell growth by increasing mean cell size with only a minimal change in generation time. They suggested that these aberrations in growth properties could have been due to an uncoupling of chromosome replication and cell division due to competition between plasmid and chromosomal replicons for rate-limiting factors involved in both initiation of DNA synthesis and DNA chain elongation. In our experiments, when growth perturbations were observed in cells containing the larger plasmids, cell sizes and growth rates were both decreased. These findings, and the absence of a generalized plasmid-induced increase in the *C* and *D* periods, are not consistent with such competitive interactions. If the competition existed at the level of initiation of chromosome replication, an increase in mean cell size (or cell mass/DNA ratio) would be anticipated in all plasmid-containing strains. An increase in mean cell size was not a characteristic of the F'-containing cells. In our case, the responses of glycerol-grown cells to the presence of the larger F' plasmids (F'144 and to a lesser extent F'209) were similar to the responses observed when the plasmid-free parental cells were grown in poorer carbon sources, such as sodium succinate or sodium acetate (10). If the competition existed at the level of DNA chain elongation, it would result in an elongation of the *C* period. The anticipated extent of elongation of *C* would depend upon the possibe mode of competitive interaction. If the competition were for precursor molecules and depended on the ratio of molecular weights between the plasmid and the chromosome, then a small increase in C would be anticipated, i.e., approximately 6 min for F'144. However, the competition might not be so straightforward and could depend on plasmid copy number, the sequence of replication of plas-

mid molecules, and steric hindrance of replication forks if there were physical interaction between the replicons (12). In some experiments, the *C* periods were slightly longer as a consequence of introduction of a plasmid (e.g., in glycerol-grown B/r F44/F'144), but the *C* periods were not in excess of those expected for plasmid-free cells growing at similar rates (10).

The host cell used in these studies, B/r F44, contained a *recA* allele. The presence of this mutation precluded *recA*-dependent recombination between plasmid and chromosome (14). Since the presence of the *recA* allele altered cell growth properties, e.g., resulted in reduced viability, care must be taken in assessing the patterns of growth of *recA*, plasmid-containing cells. Membrane-elution analysis, because it was based on the release of newborn cells from a membrane-bound culture, tended to minimize the effects of viability differences since nondividing cells were excluded from analysis. Measurements of *C*, *D*, and τ_{mem} in membrane-elution experiments were therefore representative of the average values of these parameters in the dividing portion of the populations. This probably accounts, in part, for the shorter generation times determined for membrane-bound cultures compared to batch cultures (Table 2). The differences were particularly striking with B/r F44/F'101 which had generation times similar to B/r F44 in membrane-elution experiments, but significantly longer generation times in batch cultures.

In the course of these investigations, the presence of plasmids and their extents of chromosomal homologies were defined genetically. Plasmid-containing strains were repeatedly cloned and tested for their ability to transfer the unselected markers *leu, lac, pyrB,* and *purE*. This genetic definition is in keeping with the characterization of these plasmids, although it does not preclude loss of other regions of chromosomal DNA. Four of the F' plasmids examined (F'101, 104, 121, and 144) shared a common origin, whereas F'209 was derived independently. In agreement with the observations of Ou and Anderson (19), differences have been observed between F'209 and the other F' plasmids examined. The HfrH-derived F' plasmids were maintained in the cells during growth in nonselective nutritional conditions, whereas F'209 was present in only 2% of the population after eight mass doublings in Luria broth. Therefore, direct comparison of the physiological consequences of maintenance of F'209 and the other plasmids may be inappropriate. However, comparisons between the F' plasmids derived from HfrH are less problematical since they are assumed to differ only in the extent of chromosomal homology.

In general, the larger plasmids produced greater perturbations of cell growth than the smaller plasmids examined. All plasmids contained overlapping regions of chromosomal DNA, but the region between *pro* and *purE* was unique to the plasmids which were found to have the most pronounced effects. There is precedent for a region of localized meropolyploidy leading to poor growth. When the dosage of the *unc* region of the chromosome in *E. coli* was increased, cell growth and morphology were profoundly altered (17). Our attempts to determine whether plasmid size or a localized alteration in gene dosage was responsible for the observed effects have not been successful. In this regard, B/r F44 containing F'128 (a plasmid with homology to the *lac-pro* region of the chromosome [15]) was examined and found to grow at a reduced rate ($\tau_{mem} = C + D = 65$ min at 37°C in glucose-minimal medium). The significance of this observation with regard to the relationship between growth perturbation and plasmid genetic composition was questioned when it was found that the presence of F'128 rendered the cells temperature sensitive for growth, i.e., incapable of colony formation at 42°C. In contrast to the temperature-sensitive response of B/r F44/F'128, the generation times of B/r F44/F'144 and B/r F44 were in the same proportion during growth at 25, 37, and 42°C. Furthermore, growth perturbations were not detected in *E. coli* B/r strains containing different F'*lac* plasmids (1,8).

The observations presented and discussed here lead to an interesting possibility regarding interactions between plasmid maintenance and cell division. Previously, conditions had been described under which the presence of some plasmids resulted in the inhibition of cell division and concomitant filament formation (13, 16, 22). Such effects were observed when plasmid replication or maintenance were defective. It is alluring to suggest that plasmid-mediated filament formation, and concomitant increases in average cell size, may be attributable to a defective (or marginally defective) plasmid replication system and that the introduction of a large, stringent plasmid lacking defects in replication or maintenance leads not to an increased cell size, but to the opposite response described in this paper.

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