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Involvement of FtsZ Protein in Shift-Up-Induced Division Delay in *Escherichia coli*

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A nutritional shift-up from glucose minimal medium to LB broth was previously shown to cause a division delay of about 20 min in synchronized cultures of *Escherichia coli*, and a similar delay was observed after a nutritional pulse (a shift-up followed rapidly by a return to glucose minimal medium). Using synchronized cultures, we show here that the pulse-induced division delay does not require protein synthesis during the period in LB broth, suggesting that a nonprotein signal is generated by the shift-up and transmitted to the cell division machinery. The cell division protein FtsZ, target of the SOS-associated division inhibitor SfiA (or SulA), seems to be involved in the postshift division delay. Mutants in which the FtsZ-SfiA interaction is reduced, either *sfiA* (loss of SfiA) or *ftsZ*(SfiB) (modification of FtsZ), have a 50- to 60-min division delay after a shift-up. Furthermore, after a nutritional pulse, the *ftsZ*(SfiB) mutant had only a 10- to 16-min delay. These results suggest that the FtsZ protein is the target element of the cell division machinery to which the shift-up signal is transmitted.

When enterobacteria are shifted from a nutritionally poor medium to a rich medium, the rate of mass increase quickly reaches its final postshift value (1, 14, 18). In contrast, the cell division pattern after a nutritional shift-up, as reflected by the rate of increase in cell number, follows complex kinetics. Early experiments on exponentially growing cultures suggested that the rate of cell division remained at its preshift value for 60 to 70 min ("rate maintenance"), then increased abruptly to its final postshift level (14, 18). Although slight deviations from this pattern were observed, the data seemed compatible with an elegant model of the bacterial cell cycle (4): in both rich and poor media the rate of DNA synthesis is determined solely by the frequency of initiation, and (at doubling times less than 70 min) replication termination follows 40 (or C) min after initiation, triggering division 20 (or D) min later. Thus, according to this model, an increase in initiation frequency could not affect the division rate for 60 (or $C + D$) min. During this time, the average cell mass increases to its final steady-state value.

Later work has shown that when the preshift growth rate is very slow (doubling time greater than 115 min), rate maintenance is not observed. One set of experiments indicated that in exponential cultures of *Escherichia coli* 15 T$^-$, B/r, or K-12 after a shift-up from a very poor medium, the division rate increased immediately to a rate intermediate between the pre-shift and postshift steady-state rates (23). On the other hand, studies with exponential and synchronized cultures of *E. coli* B/r showed that after shifts from very slow growth the division rate actually slowed down for 60 min, the extent of this perturbation depending on the strain used as well as on the nature of the shift-up (16).

In a recent report (13), postponement of division after nutritional shift-ups was confirmed in exponential and synchronized cultures of *E. coli* B/r, K-12, and ML strains, even for shifts from intermediate growth rates (60-min doubling time). The analysis suggested that the preshift division rate is not maintained after shift-up. A postponement of division was also observed after nutritional pulses—shift-ups followed rapidly by return to the original poor medium (13). The delay in the first division was essentially independent of cell age at the time of the pulse and of the length of the pulse (from 1 to 15 min), and it was similar to the division delay observed after a simple shift-up. For cells growing at a doubling time of 60 min, transient exposure to nutrient broth delayed the following division by about 20 min, even if the nutritional pulse was terminated 50 min before the expected division (and 70 min before the observed one). The cells thus possess a fairly long-lived memory of their brief passage in rich medium, and the decision of when to trigger division is clearly responsive to this information. The molecular basis of the memory and the elements that transmit the information to the division machinery are an intriguing puzzle.

In the present study we used synchronized cultures to show that the memory of a brief passage in rich medium can be established in the absence of protein synthesis. We also present evidence suggesting that the cell division protein FtsZ is involved in shift-up-induced postponement of division. Of as yet unknown molecular activity, the FtsZ protein is thought to play a key role in septation (5), and it has been shown to be the target of action of the SOS-associated division inhibitor SfiA (10). We show here that a mutant FtsZ protein which is unable to interact with SfiA causes an abnormally long cell division delay after a nutritional shift-up and significantly shortens the delay after a nutritional pulse.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The bacteria used were *E. coli* K-12 AB1157 (F$^-$ *thr-1 leu-6 proA2 his-4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 rpsL31 sup-37*) and two derivatives constructed by P1 *vir*-mediated transduction, GC2606 (AB1157 *sfiA100*::Tn*5*) and GC2638 [AB1157 *leu*$^+$ *ftsZ114*(SfiB)]. Bacteria were grown aerobically at 37°C.

Minimal medium for synchronization contained (per liter of distilled water): 10 g of 4-morpholinepropane sulfonate (MOPS; Calbiochem-Behring, La Jolla, Calif.), 4.6 g of

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NaCl, 1.5 g of KCl, 2 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4$, 1 mg of $FeSO_4 \cdot 7H_2O$, 20 mg of KH_2PO_4, supplemented with 4 g of glucose, 1 mg of thiamine, and 0.1 g of each required amino acid, and adjusted to pH 7.4 with KOH; for free (unlimited) growth, the KH_2PO_4 concentration was 140 mg/liter. LB broth contained (per liter of distilled water): 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract (Difco), and 5 g of NaCl, adjusted to pH 7.4 with NaOH.

Synchronization. Cultures were synchronized by 16 brief phosphate starvations, each followed by a twofold dilution and 85 min of incubation in the same phosphate-limited medium, as previously described (12). Synchronized cultures were diluted 12- to 25-fold in the same medium supplemented with a nonlimiting concentration of phosphate and grown as batch cultures.

Nutritional shifts and pulses. Shift-up experiments were done by diluting part of the culture four- to sixfold into prewarmed LB broth. A nutritional pulse was a shift-up followed 8 min later by filtration on membrane filters (HA, 0.45-μm pore size; Millipore Corp., Bedford, Mass.), washing without drying, and resuspension in prewarmed minimal medium; this operation took about 1 min and caused a 2- to 4-min delay in cell growth.

Cell counting and volume measurements. Bacterial concentrations were determined with a Coulter Counter (model ZB; Coultronics, S.A., Margency, France) equipped with a 30-μm orifice; volumes were measured with a C-1000 Channelyzer (Coulter Electronics, Ltd., Luton, England). Samples were diluted 100- to 1,000-fold in filtered saline-Formol (3).

Optical density measurements. Optical density was measured at 600 nm in a Zeiss PM6 spectrophotometer.

RESULTS

Nutritional pulse in the absence of protein synthesis. We first tested whether the transient division inhibition observed after a nutritional pulse requires de novo protein synthesis during the brief exposure to rich medium. *E. coli* K-12 AB1157 was synchronized in glucose minimal medium. During the second generation of free growth, a portion of the culture was supplemented with chloramphenicol (100 μg/ml); after 2 min it was diluted into prewarmed LB broth containing chloramphenicol and then 8 min later filtered and returned to glucose minimal medium without chloramphenicol. Under these conditions, the incorporation of [^{3}H]leucine into trichloroacetic acid-insoluble material during the 8 min in LB broth was less than 1.3% of that observed in the absence of chloramphenicol. Controls included a subculture subjected to an 8-min nutritional pulse in the absence of chloramphenicol and a subculture treated with chloramphenicol for 10 min but not exposed to LB broth. Cell number and optical density were monitored in all cultures. The results (Fig. 1) show that the nutritional pulse in the presence of chloramphenicol caused a 30-min postponement of the following cell division, compared with a 23-min delay after the pulse in the absence of chloramphenicol. The chloramphenicol treatment alone, without a nutritional shift, delayed the following division by 8 min. When this chloramphenicol effect is corrected for, it is clear that the absence of bulk protein synthesis during a nutritional pulse does not affect the ensuing postponement of cell division.

Shift-up in *sfiA* and *ftsZ*(SfiB) mutants. *E. coli* is known to code for at least one endogenous division inhibitor, the SfiA (or SulA) protein (8), whose synthesis is induced as part of

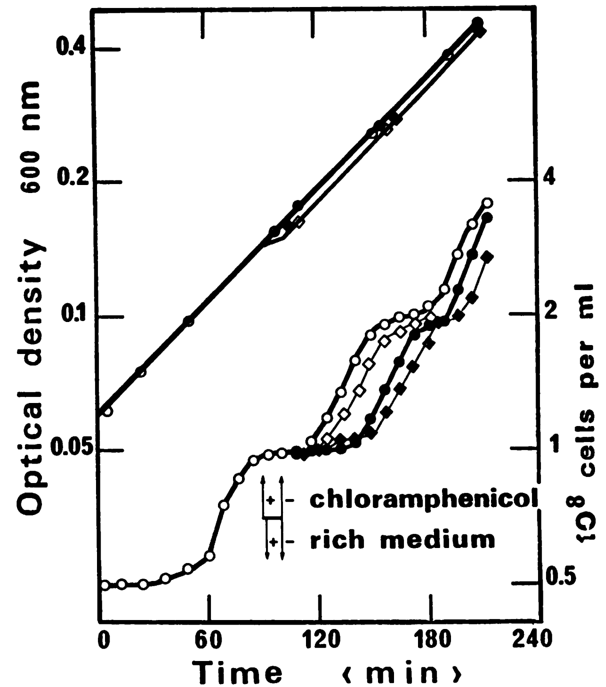

FIG. 1. Division delay after a nutritional pulse in the presence of chloramphenicol. Strain AB1157 was synchronized in glucose minimal medium and then at time zero was diluted into the same medium with nonlimiting phosphate (○). At the times indicated by the arrows, two portions of the culture were submitted to an 8-min nutritional pulse, in the absence (●) or presence (◆) of chloramphenicol (100 μg/ml, added 2 min before the pulse), and one portion of the culture was treated with chloramphenicol alone (◇). Cell number (lower curves) and optical density (upper curves) were monitored in all cultures.

the SOS response whenever DNA synthesis is perturbed (6). The SfiA protein is highly unstable, with a 1.2-min half-life (20), and although its synthesis is not induced after a nutritional shift-up (9), it is conceivable that the protein is stabilized under these conditions. This would lead to increased accumulation of SfiA after a shift-up, which could in turn contribute to the division delay observed.

Two types of mutation have been shown to abolish SfiA-dependent division inhibition. The first are simply *sfiA* (or *sulA*) mutations, including insertions and deletions. The second, originally called *sfiB* (or *sulB*) mutations, have been shown to lie in the *ftsZ* gene (11, 17) and result in an altered FtsZ protein (17). The FtsZ protein thus seems to be the target of action of the SfiA division inhibitor, and a direct FtsZ-SfiA interaction is suggested by the observation that the SfiA protein is stabilized in the presence of an excess of FtsZ protein, but not in the presence of an excess of FtsZ(SfiB) mutant protein (10).

To test a possible role of the SfiA division inhibitor in the shift-up-induced division delay, we wished to compare the effect of a glucose-to-LB broth shift-up in synchronized cultures of AB1157 and isogenic *sfiA* or *ftsZ*(SfiB) derivatives. To validate the experiment, we first verified that during steady-state growth in rich or poor medium the three strains were the same size. Cell volume measurements (Table 1) confirmed previous data (with different strains, media, and techniques [9]), indicating that the *sfiA* and *ftsZ*(SfiB) mutations have little effect on cell size.

The shift-up experiments were done with synchronized

TABLE 1. Cell volume of *sfi*$^+$, *sfiA*, and *ftsZ*(SfiB) strains

Strain	Vol[a] (μm^3) in:	
	Glucose	LB broth
sfi$^+$	0.676 ± 0.010	2.06 ± 0.014
sfiA	0.666 ± 0.018	2.27 ± 0.071
ftsZ(SfiB)	0.661 ± 0.010	1.73 ± 0.042

[a] Volumes were measured with the Coulter Counter for each strain during steady-state growth in glucose minimal medium or LB broth.

cultures of the three strains at different stages of the second or third cycle of free growth. In all cases the mass doubling time, evaluated by optical density, increased from ≈64 min in glucose minimal medium to ≈25 min in LB broth with an extrapolated lag of 5 to 8 min (Fig. 2). The first postshift division was postponed 20 to 25 min in the wild-type strain and 50 to 60 min in the *sfiA* and *ftsZ*(SfiB) mutants, with little or no dependence on cell age at the time of the shift-up (Fig. 2). Thus, the *sfiA* and *ftsZ*(SfiB) cells reached their new steady-state mass at the time of the first postshift division, whereas the wild-type cells did not reach it until the second postshift division.

These results suggest that the SfiA and FtsZ proteins are indeed involved in determining the shift-up-induced division delay, but in a division-promoting capacity rather than in their previously characterized role as a division inhibition system.

Nutritional pulses in *sfiA* and *ftsZ*(SfiB) mutants. Nutritional pulses generally cause a division delay similar to that induced by a simple shift-up without a return to poor medium (13). Since the *sfiA* and *ftsZ*(SfiB) mutations significantly lengthened this delay after a shift-up, we tested their effect on the division delay after a nutritional pulse. Synchronized cultures of AB1157 and its isogenic *sfiA* and *ftsZ*(SfiB) derivatives in glucose minimal medium were diluted into LB broth for 8 min at various times during the third cycle of free growth and then filtered and returned to glucose minimal medium. The first postshift division was postponed 20 to 25 min in the *sfi*$^+$ strain, 18 to 25 min in the *sfiA* mutant, and 10 to 16 min in the *ftsZ*(SfiB) mutant (Fig. 3). These delays were essentially independent of cell age at the time of the nutritional pulse. As a control, a simple shift-up was also done with each strain at the time of the second pulse; the division delays were the same as those shown in Fig. 2 (data not shown).

The above results indicate that the striking lengthening of the shift-up-induced division delay observed in *sfiA* and *ftsZ*(SfiB) mutants requires that the cells remain in rich medium; the division delay after an 8-min nutritional pulse was the same in the *sfiA* mutant and the parental strain and significantly shorter in the *ftsZ*(SfiB) mutant.

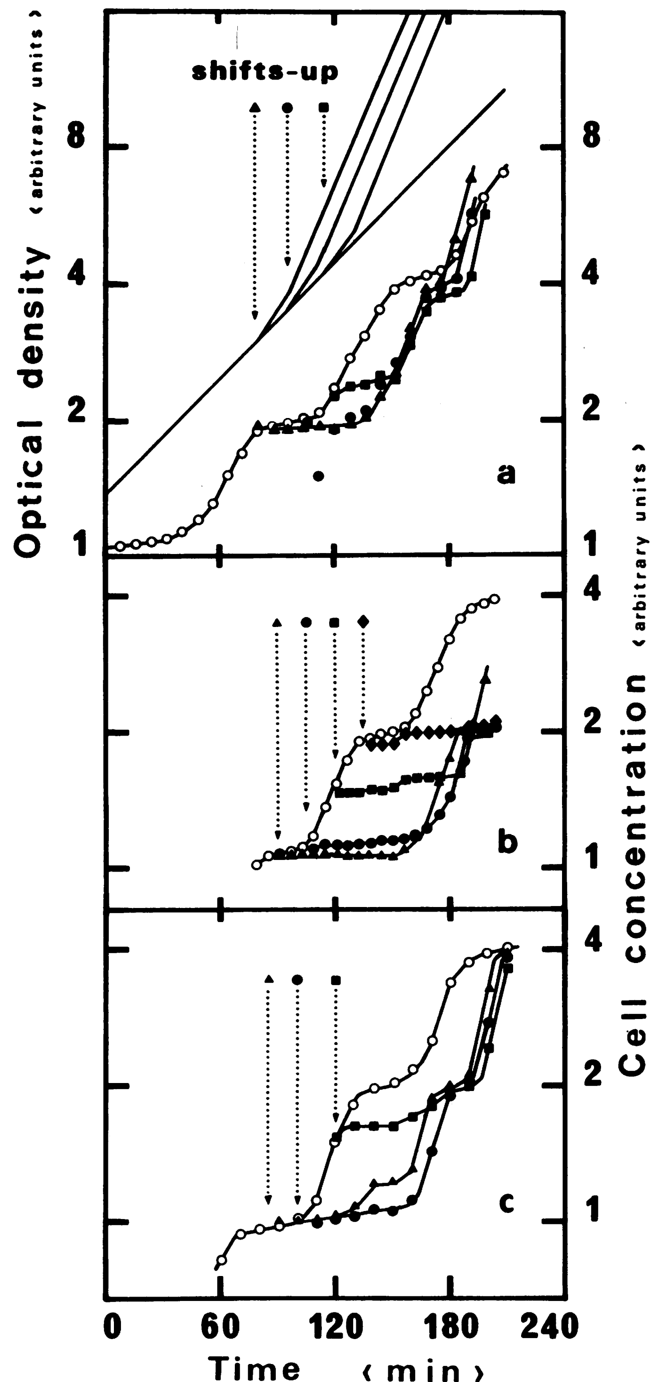

FIG. 2. Division delay after a nutritional shift-up in *sfi*$^+$, *sfiA*, and *ftsZ*(SfiB) strains. Strain AB1157 (a) and its *sfiA* (b) and *ftsZ*(SfiB) (c) derivatives were synchronized in glucose minimal medium; then at time zero they were diluted into the same medium with nonlimiting phosphate (○). At the times indicated by the arrows, portions of each culture were diluted four- to sixfold into LB broth (closed symbols). Cell number and optical density were monitored in all cultures. The optical density curves, very similar for all three strains, are shown only for the *sfi*$^+$ strain (data points not shown) in the upper part of panel a.

DISCUSSION

The response of enterobacteria to a nutritional shift-up, first analyzed in 1958 (14), has been the subject of numerous studies. Two features of this response that have attracted considerable attention are the rate of stable RNA synthesis and the rate of cell division. The rate of synthesis of rRNA (which represents about 85% of the stable RNA) increases within minutes after a shift-up to near its final steady-state level in rich medium (1, 21); this constitutes the first response to a nutritional shift-up. The rate of cell division, on the other hand, does not reach its steady-state level until some 60 to 70 min after the shift-up (18), making it the

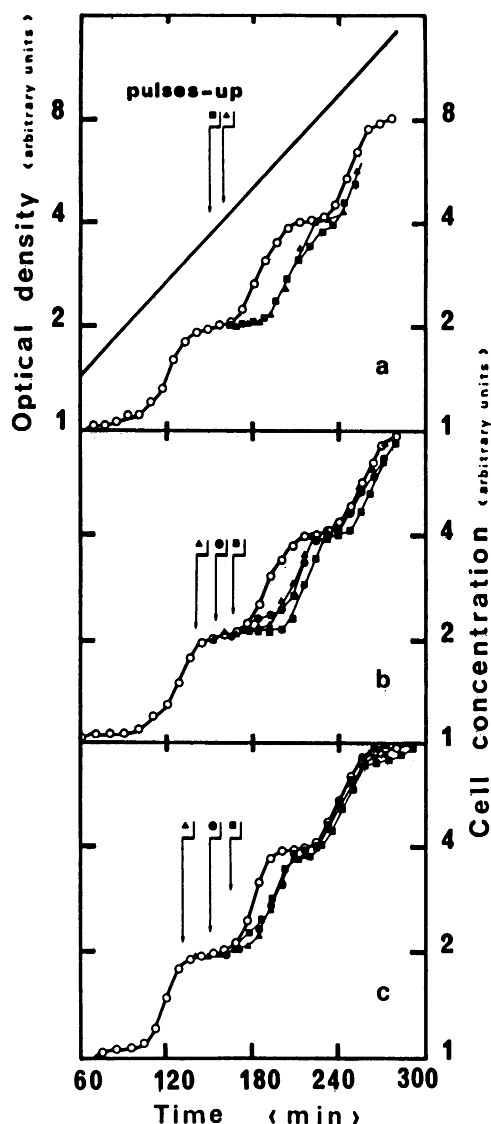

FIG. 3. Division delay after a nutritional pulse in *sfi*$^+$, *sfiA*, and *ftsZ*(SfiB) strains. Strain AB1157 (a) and its *sfiA* (b) and *ftsZ*(SfiB) (c) derivatives were synchronized in glucose minimal medium; then at time zero they were diluted into the same medium with nonlimiting phosphate (○). At the times indicated by the arrows, portions of each culture were diluted four- to sixfold into LB broth and, 8 min later, filtered, washed, and resuspended in preheated glucose minimal medium (closed symbols). Cell number and optical density were monitored in all cultures. The optical density curves, very similar for all three strains, are shown only for the *sfi*$^+$ strain (data points not shown) in the upper part of panel a; these curves were essentially unaffected by the nutritional pulses.

slowest growth parameter to adjust. However, the conclusion that during this period cell division continues at the preshift rate, based on studies with exponential cultures, should be revised in the light of more recent work with synchronized cultures showing that under many conditions the first postshift division is in fact postponed with respect to the unshifted culture (13, 16). A similar division delay is produced by a nutritional pulse, in which the cells remain only a short time in rich medium before being returned to poor medium, and the extent of the delay depends on the amplitude of the shift but not on cell age (13); the restoration of the normal (preshift) rhythm of division takes place over the following two cycles (13). These observations suggest (i) that the effect of a shift-up on cell division, i.e., the commitment to postpone the following division, is in fact as rapid and as quantitatively precise as the effect on the rate of rRNA synthesis and (ii) that the cell has some sort of molecular memory able to retain this commitment for up to 70 min after the return to poor medium.

Similar transient division postponement has been observed after low doses of UV irradiation (2), in which case it depended on the induction of the SOS division inhibitor SfiA and its interaction with the FtsZ protein. The nutritional shift-up and UV irradiation (or other DNA-damaging treatments) have one feature in common, namely, a drop in the DNA/mass ratio. Furthermore, in both conditions stable DNA replication is induced, a mode in which many rounds can be carried out in the absence of protein synthesis (15). SOS-associated division inhibition and stable DNA replication are both induced responses, dependent on de novo protein synthesis (7, 15). In the present study we analyzed the division postponement observed after a shift-up or nutritional pulse to see whether the response is inducible and whether the FtsZ-SfiA division inhibition system is involved.

When a culture growing in glucose minimal medium was transferred to LB broth for 8 min and then returned to minimal medium, the following division was postponed ≈20 min, whatever the cell age at the time of the pulse. The near total inhibition of protein synthesis in the rich medium (with LB broth plus chloramphenicol) did not reduce this division delay (Fig. 1). This strongly suggests that no inducible proteins are required to postpone division. The cell clearly detects the rich medium rapidly. It has been suggested that the cytoplasmic concentration of the nucleotide ppGpp, whose synthesis responds to the tRNA charge ratio, is the signal governing the shift-up response (22), but this is unlikely in the case of a nutritional pulse since the division delay occurs in poor medium. Other potential signals created by the nutritional pulse could be a modified protein, an RNA molecule, or internal metabolite pools. The latter would be expected to adjust rapidly to the rich medium, and *E. coli* might constantly evaluate the levels of certain key internal pools to assess the potential of the medium, analogous to its constant measurement of the external concentration of numerous metabolites to determine its chemotactic responses.

Our analysis of a possible role of the division inhibitor SfiA in the shift-up-induced postponement of division led to a surprising result: in an *sfiA* or *ftsZ*(SfiB) mutant, in which the normal FtsZ-SfiA interaction is abolished, the division delay was more than doubled (50 to 60 min compared with 20 to 25 min) (Fig. 2). On the other hand, the division delay observed after a nutritional pulse was not lengthened in the mutants; indeed, it was actually shorter in the *ftsZ*(SfiB) mutant than in the wild-type strain (Fig. 3).

The response of enterobacteria to a nutritional shift-up has been studied for 30 years, yet the bacterial functions and signals that trigger this response remain obscure. The above results implicate the FtsZ protein in the shift-up response, perhaps as the target element of the cell division apparatus to which the cell transmits information about growth conditions.

LITERATURE CITED

1. **Brunschede, H., T. L. Dove, and H. Bremer.** 1977. Establishment of exponential growth after a nutritional shift-up in *Escherichia coli* B/r: accumulation of deoxyribonucleic acid, ribonu-

cleic acid, and protein. J. Bacteriol. **129:**1020–1033.

2. **Burton, P., and I. B. Holland.** 1983. Two pathways of division inhibition in UV-irradiated *E. coli*. Mol. Gen. Genet. **190:** 309–314.
3. **D'Ari, R., and O. Huisman.** 1983. Novel mechanism of cell division inhibition associated with the SOS response in *Escherichia coli*. J. Bacteriol. **156:**243–250.
4. **Helmstetter, C. E., S. Cooper, O. Pierucci, and E. Revelas.** 1968. On the bacterial life sequence. Cold Spring Harbor Symp. Quant. Biol. **33:**809–822.
5. **Holland, I. B., and C. Jones.** 1985. The role of the FtsZ protein (SfiB) in UV-induced division inhibition and in the normal *Escherichia coli* cell division cycle. Ann. Microbiol. (Paris) **136A:**165–171.
6. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290:**797–799.
7. **Huisman, O., R. D'Ari, and J. George.** 1980. Inducible *sfi* dependent division inhibition in *Escherichia coli*. Mol. Gen. Genet. **177:**629–636.
8. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell-division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81:**4490–4494.
9. **Huisman, O., M. Jacques, R. D'Ari, and L. Caro.** 1983. Role of the *sfiA*-dependent division regulation system in *Escherichia coli*. J. Bacteriol. **153:**1072–1074.
10. **Jones, C., and I. B. Holland.** 1985. Role of the SulB (FtsZ) protein in division inhibition during the SOS response in *E. coli*: FtsZ stabilizes the inhibitor SulA in maxicells. Proc. Natl. Acad. Sci. USA **82:**6045–6049.
11. **Jones, C. A., and I. B. Holland.** 1984. Inactivation of essential division genes, *ftsA*, *ftsZ*, suppresses mutations at *sfiB*, a locus mediating division inhibition during the SOS response in *E. coli*. EMBO J. **3:**1181–1186.
12. **Kepes, F., and A. Kepes.** 1980. Synchronisation automatique de la croissance de *Escherichia coli*. Ann. Microbiol. **131A:**3–16.
13. **Kepes, F., and A. Kepes.** 1985. Postponement of cell division by nutritional shift-up in *Escherichia coli*. J. Gen. Microbiol. **131:**677–685.
14. **Kjeldgaard, N. O., O. Maaløe, and M. Schaechter.** 1958. The transition between different physiological states during balanced growth of *Salmonella typhimurium*. J. Gen. Microbiol. **19:**607–616.
15. **Kogoma, T., and K. G. Lark.** 1975. Characterization of the replication of *Escherichia coli* DNA in the absence of protein synthesis: stable DNA replication. J. Mol. Biol. **94:**243–256.
16. **Loeb, A., B. E. McGrath, J. M. Navre, and O. Pierucci.** 1978. Cell division during nutritional upshifts of *Escherichia coli*. J. Bacteriol. **136:**631–637.
17. **Lutkenhaus, J. F.** 1983. Coupling of DNA replication and cell division: *sulB* is an allele of *ftsZ*. J. Bacteriol. **154:**1339–1346.
18. **Maaløe, O., and N. O. Kjeldgaard.** 1966. Control of macromolecular synthesis, p. 97–129. W. A. Benjamin, Inc., New York.
19. **Maguin, E., H. Brody, C. W. Hill, and R. D'Ari.** 1986. SOS-associated division inhibition gene *sfiC* is part of excisable element e14 in *Escherichia coli*. J. Bacteriol. **168:**464–466.
20. **Mizusawa, S., and S. Gottesman.** 1983. Protein degradation in *Escherichia coli*: the *lon* gene controls the stability of SulA protein. Proc. Natl. Acad. Sci. USA **80:**358–362.
21. **Nierlich, D. P.** 1972. Regulation of ribonucleic acid synthesis in growing bacterial cells. I. Control over the total rate of RNA synthesis. J. Mol. Biol. **72:**751–764.
22. **Ryals, J., R. Little, and H. Bremer.** 1982. Control of rRNA and tRNA synthesis in *Escherichia coli* by guanosine tetraphosphate. J. Bacteriol. **151:**1261–1268.
23. **Sloan, J. B., and J. E. Urban.** 1976. Growth response of *Escherichia coli* to nutritional shift-up: immediate division stimulation in slow-growing cells. J. Bacteriol. **128:**302–308.