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Modulation of Cell Wall Synthesis by DNA Replication in *Escherichia coli* during Initiation of Cell Growth

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Resting cells of *Escherichia coli* are able to initiate growth and murein biosynthesis in the presence of β-lactam antibiotics binding to penicillin-binding proteins (PBPs) 1a and 1b (E. J. de la Rosa, M. A. de Pedro, and D. Vázquez, Proc. Natl. Acad. Sci. USA 82:5632–5635, 1985). Under these conditions, cells elongate normally until they approach the first doubling in mass, the time at which cell lysis starts. Assuming that coupling between DNA replication and cell division both in cells starting growth and in growing cells is essentially similar, triggering of the lytic response in the β-lactam-treated cells coincides with the termination of the first round of DNA replication. This coincidence suggests that both events are interrelated. We investigated this possibility by studying the initiation of growth in cultures of wild-type strains and in cell division mutants treated with β-lactams inhibiting PBPs 1a and 1b and with the DNA replication inhibitor nalidixic acid. Addition of nalidixic acid, even late in the first cell cycle, prevented the lytic response of the cells to the blockade of PBPs 1a and 1b. The effect of nalidixic acid is more likely due to its action on DNA replication itself than to its indirect inhibitory effect on cell division or to its ability to induce the SOS system of the cell. These observations favor the idea that the cell wall biosynthetic machinery might be modulated by DNA replication at precise periods during cell growth.

Penicillin-binding proteins (PBPs) are a set of bacterial membrane proteins crucially involved in inserting new precursors into the murein and in shaping and dividing the growing cell (23, 25). PBPs are characterized by their ability to interact with β-lactam antibiotics specifically at the active center, forming a covalent, enzymatically inactive complex (31).

In *Escherichia coli*, seven genetically independent PBPs have been identified (26). It is generally agreed that in exponentially growing cells, PBPs 1a and 1b are involved in cell wall elongation; PBP 2 is required for correct shaping; PBP 3 mediates septum formation during cell division; PBP 4 participates in postinsertional modifications of new murein; and PBPs 5 and 6 regulate the number of pentapeptide side chains in murein (5, 23–25).

We are presently investigating the physiology of the PBPs of *E. coli* during initiation of murein synthesis, when resting cells start growing. The results so far suggest that the function of PBPs 1 and 2 at least differs considerably in exponentially growing cells and in cells initiating growth (22; A. G. Pisabarro, F. G. del Portillo, E. J. de la Rosa, and M. A. de Pedro, FEMS Microbiol. Lett., in press).

PBPs 1a and 1b are bifunctional enzymes which have been described as the main DD-transpeptidase/transglycosylase activities of the cell. Both proteins use undecaprenyl-disaccharide-pentapeptide as a substrate, catalyzing the insertion and elongation of new peptidoglycan chains into the sacculus, which is concomitantly enlarged (6, 19, 25). The activity of PBPs 1a and 1b is essential for the survival of the growing cell. Although inactivation of only one of them does not compromise cell viability or cell growth, simultaneous inhibition of both proteins promotes the lytic response of the cell (19, 23, 26). In fact, in growing cultures of *E. coli*, cell lysis is efficiently triggered by the addition of β-lactams that specifically bind to PBPs 1a and 1b, such as cefsulodin (3, 17).

PBP 2 is also a bifunctional enzyme with DD-transpeptidase/transglycosylase activity, having the same substrate specificity as PBPs 1 (13). It is an essential protein whose inactivation in exponentially growing cells results in inhibition of cell division and formation of rounded cells, which eventually lose their viability (23, 26).

The response of *E. coli* cells initiating growth to β-lactams binding to PBPs 1 differs greatly from that stated above for actively growing cells. Resting cells are able to initiate growth in the presence of high concentrations of β-lactams that bind to PBPs 1, such as cefsulodin and cefmetazole. Under these conditions, provided that PBP 2 remains active, β-lactam-treated cells nearly double their mass, maintaining a normal morphology and synthesizing proteins and murein at rates similar to those in control cultures, until cell lysis is triggered shortly (10 to 15 min) before the onset of cell division (3; Pisabarro et al., in press).

In exponentially growing cells, DNA replication and cell division are tightly coupled processes. Impairment of DNA replication leads to inhibition of septation and filamentation (18, 32). Inhibition of division when DNA replication is altered is primarily mediated by the SOS system, induced by activation of the *recA* gene product, although additional coupling mechanisms have been described (15, 27). Balanced, exponentially growing cells divide at a constant time after completion of the corresponding round of chromosome replication (2, 16). This period of time, the D period, lasts about 20 min for most *E. coli* strains growing with generation times shorter than 60 min (10).

Assuming that during initiation of growth, termination of a round of chromosome replication is required for the first division to occur and the length of the D period is not much increased, the expected time for the termination of DNA replication matches the time when the activity of PBPs 1

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TABLE 1. Bacterial strains

E. coli strain	Genetic characteristics	Source or reference
W7EA	*dapA lysA thyA*	This work[a]
D3	*ilv his trp*(Am) *thyA* (*deo*) *ara*(Am) *lac-125*(Am) *galU42*(Am) *galE tsx*(Am) *tyrT* [*supFA81*(Ts)], *ftsA3*(Ts)	28
AX655	*thr leu thi arg proA his ftsI* [*sep2158*(Ts)]	30
JM12	*thr leu pro his arg lac gal rpsL recA441* (*tif*)	1
JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* Δ(*lac-proAB*) (F′ *traD36 proAB lacI*^{q}*ZM15*)	33

[a] Spontaneous *thy* derivative of *E. coli* W7 (9) obtained by selection with trimethoprim (20).

becomes essential for the cell to divide normally. Unless it were purely fortuitous, this coincidence suggests a regulatory interrelationship between DNA replication and murein biosynthesis. To further document our hypothesis, we studied the response of wild-type strains and cell division mutants of *E. coli* to inhibition of PBPs 1 under permissive and restrictive conditions for DNA replication. The experimental results obtained favor the hypothesis that the activity of PBPs 1 might be modulated by DNA replication during the first round of cell division after resumption of growth.

MATERIALS AND METHODS

Strains and growth conditions. The bacterial strains used are listed in Table 1. Cultures were grown aerobically at 30 or 37°C in MC medium (29) supplemented with 2,6-*meso*-diaminopimelic acid (10 μg/ml), thymine (50 μg/ml), thiamine (2 μg/ml), glucose (2 mg/ml), and the amino acids required by each strain at 200 μg/ml. Under these conditions cells enter stationary phase at an OD_{550} of 2.0.

In experiments with cells initiating active growth, cultures were kept in stationary phase (with aeration) at 37°C (30°C for thermosensitive strains). Initiation of growth was allowed by diluting the cultures 1:5 with fresh, prewarmed medium of identical composition. Growth was followed by monitoring the OD of the cultures at 550 nm. Particle number was measured in a Coulter counter ZM particle analyzer (Coulter Electronics Ltd., Lutton, U.K.) after fixation of the cells with 0.2% (vol/vol) Formalin and 0.9% (wt/vol) NaCl.

Antibiotics. Nalidixic acid (NAL) and norfloxacin were purchased from Sigma Chemical Co. (St. Louis, Mo.) and Merck Sharp & Dohme de España, S.A. (Madrid, Spain), respectively. Cefsulodin was a generous gift from Takeda Chemical Industries Ltd. (Osaka, Japan).

Antibiotic treatment of cultures initiating growth. When cultures were treated with antibiotics, the drugs were added to the respective subcultures 30 min before they were diluted 1:5 with prewarmed medium containing the respective drugs at the same concentrations, unless otherwise stated.

In experiments with thermosensitive strains, cultures were grown and kept in stationary phase for 24 h at the permissive temperature (30°C) and then divided into the required number of subcultures and further incubated for 30 min at either the restrictive (42°C) or the permissive temperature before being diluted 1:5 with fresh medium prewarmed to the corresponding temperature.

Measurement of DNA synthesis. DNA synthesis in cells initiating growth was measured by following the incorporation of [*methyl*-^{3}H]thymine (Amersham Internationl) into cells uniformly labeled with the same precursor. Cultures were grown for 10 generations in the presence of the radioactive precursor (0.1 μCi/μg, 50 μg/ml) and kept for 24 h in stationary phase under the same conditions. To initiate growth, cultures were diluted 1:5 with prewarmed medium supplemented with the radioactive precursor at the same specific activity and concentration as above. Duplicate samples of 1 ml were removed at regular intervals and precipitated with 1 ml of 10% (wt/vol) ice-cold trichloroacetic acid. Precipitates were collected on Whatman GF/C glass fiber filters and washes with cold 5% (wt/vol) trichloroacetic acid and ethanol; after drying, radioactivity was measured by liquid scintillation.

Analysis of peptidoglycan by HPLC. To specifically analyze peptidoglycan synthesized during initiation of growth, the specific radioactive precursor *meso*-[3,4,5-^{3}H]diaminopimelic acid (0.1 μCi/μg, 10 μg/ml) (Service des Molécules Marquées, Comisariat à l'Energie Atomique, Gif-sur-Yvette, France) was added to the cultures at the time of dilution. To stop incorporation, cultures were mixed 1:1 with a boiling solution of 8% (wt/vol) sodium dodecyl sulfate.

Peptidoglycan was purified as described by Höltje et al. (12), and the muropeptide composition was determined by high-pressure liquid chromatography (HPLC) by the method of Glauner and Schwarz (7).

The muropeptides were resolved on a LiChrosorb RP18 column (250 by 4 mm, 5-μm particle size; E. Merck AG., Darmstadt, Federal Republic of Germany [FRG]) connected to a Berthold LB505 radioactivity detector (Laboratorium Prof. Dr. Berthold, Wildbad, FRG). The muropeptide composition was determined by computer integration of the areas under the corresponding peaks in the radioactivity profile. Individual muropeptides were grouped as described previously (22).

RESULTS

Initiation of DNA replication and cell division in cells starting active growth. When a 24-h-old stationary-phase culture of *E. coli* W7EA was allowed to reinitiate growth, the OD began to increase immediately, while the cell number started to rise only when the OD had nearly doubled, about 120 min later (Fig. 1).

To determine when DNA replication begins in cells initiating growth, a culture of *E. coli* W7EA was grown with [^{3}H]thymine (0.1 μCi/μg, 50 μg/ml) for 10 generations to uniformly label the chromosome. After 24 h in stationary phase, the cultures were diluted in new medium supplemented with the radioactive precursor, and samples were withdrawn periodically to measure the radioactivity incorporated into the DNA. The results (Fig. 1) indicate that DNA synthesis started approximately 50 to 60 min after dilution of the cultures, that is, about 60 min before initiation of cell division.

Inhibition of cell division by NAL in cells initiating growth. In exponentially growing cells, inhibition of DNA replication leads to blockade of the subsequent round of cell division (2, 16, 27). To confirm the validity of this observation for cells initiating growth, the effect of NAL on the first round of cell division was studied.

A 24-h-old stationary-phase culture of *E. coli* W7EA was diluted in new, prewarmed medium and divided into a number of parallel subcultures. NAL (40 μg/ml) was added to the corresponding subcultures after increasing periods of time, and samples were withdrawn to measure the variation

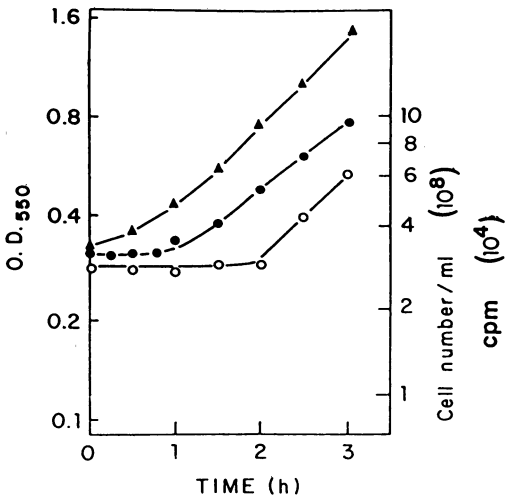

FIG. 1. Initiation of cell growth and DNA replication by *E. coli* W7EA. A 24-h-old stationary-phase culture of *E. coli* W7EA, uniformly labeled with [^{3}H]thymine (0.1 μCi/μg, 50 μg/ml), was diluted 1:5 (vol/vol) with new, prewarmed medium supplemented with the radioactive precursor at the same specific activity and concentration. Samples were periodically withdrawn to measure incorporation of [^{3}H]thymine (●), particle number (○), and OD_{550} (▲). Generation time of cultures growing exponentially under otherwise identical conditions was 45 min.

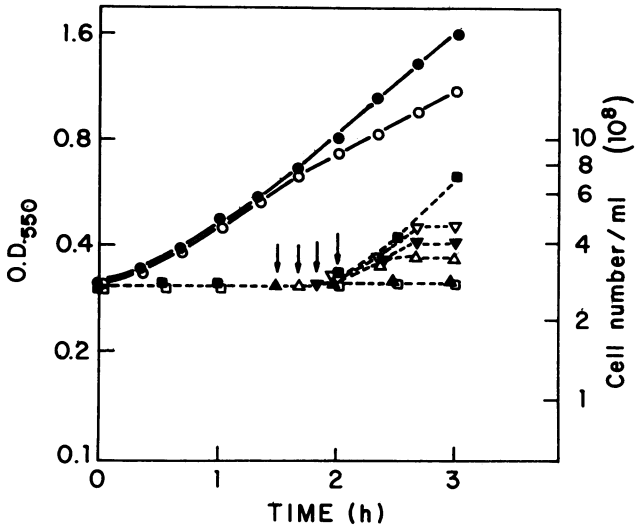

FIG. 2. Effect of NAL on the first round of cell division after initiation of growth. A 24-h-old stationary-phase culture of *E. coli* W7EA was diluted 1:5 in new, prewarmed medium and divided into parallel subcultures. NAL, at a final concentration of 40 μg/ml, was added to the corresponding subcultures after increasing periods of time, and samples were periodically removed to measure the OD_{550} (——) and particle concentration (– – – –). For the sake of clarity, only OD_{550} curves for the untreated control (●) and the subculture treated with NAL from 0 min (○) are represented. Particle concentration was measured in subcultures to which NAL was added at 0 min (□), 90 min (▲), 100 min (△), 110 min (▼), and 120 min (▽) and in an untreated control (■). The arrows indicate the times of NAL addition.

in cell number and OD. At the chosen concentration of NAL (40 μg/ml), incorporation of [^{3}H]thymine into the DNA of exponentially growing cells of *E. coli* W7EA was stopped in less than 5 min. Cell division was inhibited whenever the drug was added earlier than 10 to 20 min before the time due for cell division (Fig. 2). Therefore, completion of a round of chromosome replication is apparently required for the first round of cell division to occur in cells initiating growth.

Effect of NAL on induction of cell lysis in cultures initiating growth in the presence of cefsulodin. To test the hypothetical modulation of the activity of PBPs by DNA replication, we studied the effect of NAL on the initiation of growth of *E. coli* W7EA treated with cefsulodin. This drug binds specifically to PBPs 1a and 1b at the concentration used in our experiments (150 μg/ml) (21).

Figure 3 shows the results obtained when DNA replication was inhibited with NAL (40 μg/ml). While cells initiating growth in the presence of cefsulodin alone doubled their OD and lysed afterwards, the subculture treated with NAL plus cefsulodin reached a plateau once its initial OD was doubled, maintaining a stable value for at least 2 h.

Somewhat surprising was the relatively small decrease in OD observed in the subculture treated with cefsulodin. In fact, the OD leveled off at about half the maximum value reached in the culture. This could indicate that only half of the cells lysed. However, observation of the samples by phase-contrast microscopy showed that after 4 h, more than 90% of the cells had lysed, although they were not as badly damaged as exponentially growing cells treated in a similar way.

Determination of particle number and mean cell volume throughout the experiment indicated that in the subculture treated with NAL plus cefsulodin, the cell number was constant from time zero up to the initiation of cell lysis (4 h), while mean cell volume increased from 1.06 to 1.65 μm^3 at the beginning of the plateau time (2 h), remaining constant from this time onwards (mean cell volume at 4 h was 1.69

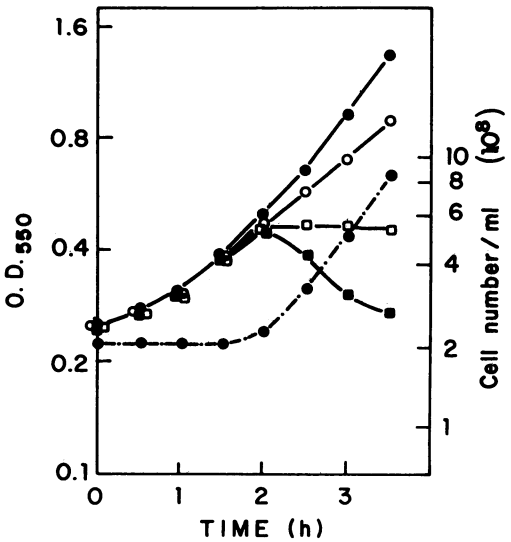

FIG. 3. Initiation of growth in cultures of *E. coli* W7EA treated with cefsulodin and NAL. A 24-h-old stationary-phase culture of *E. coli* W7EA was divided into four equal subcultures, which were treated with NAL (40 μg/ml) (○), cefsulodin (150 μg/ml) (■), a combination of NAL and cefsulodin at the concentrations indicated (□), or not treated (●). After 30 min, the subcultures were diluted 1:5 in new, prewarmed medium containing the same antibiotics at the same concentrations. Samples were removed periodically to measure the OD_{550} (——) and, in the control subculture, the particle concentration (– · – · –).

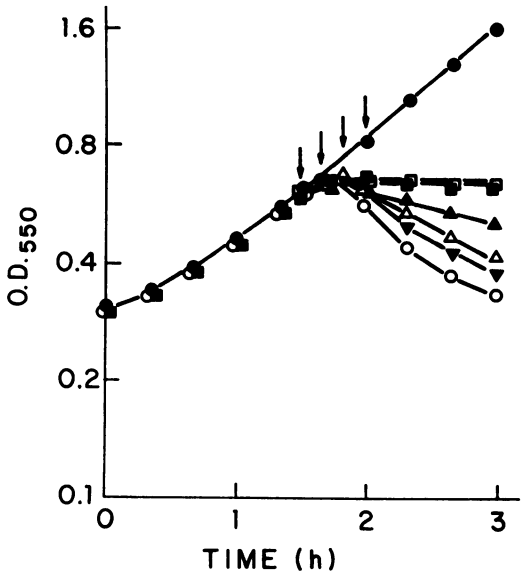

FIG. 4. Prevention of NAL of cell lysis in *E. coli* W7EA initiating growth in the presence of cefsulodin. A 24-h-old stationary-phase culture of *E. coli* W7EA was divided into a number of subcultures, and cefsulodin (150 μg/ml) was added to all but one of them (control subculture) (●). After 30 min, subcultures were diluted 1:5 with new, prewarmed medium containing cefsulodin (150 μg/ml), except for the control, which was diluted in a medium without antibiotic, and NAL was added (arrows) after 0 min (■), 90 min (□), 100 min (▲), 110 min (△), or 120 min (▼). An additional subculture (○) was treated only with cefsulodin as a positive control for lysis.

μm^3). These data indicate that the constancy in OD at the plateau was due to inhibition of both cell lysis and cell growth in the subculture treated with NAL plus cefsulodin, not to the possible alternative of overlapping lysis and filamentation.

Essentially identical results were obtained when DNA replication was inhibited with norfloxacin (0.3 μg/ml) or when cefmetazole (a β-lactam that binds to all PBPs except PBP 2) (20 μg/ml) was used instead of cefsulodin.

The behavior of two other strains of *E. coli*, MC6 (4) and JM83 (33), was also studied to rule out a possible strain-specific effect. The results in all instances were similar to those described for *E. coli* W7EA (data not shown).

To determine whether DNA replication inhibitors had to be present from the very beginning to elicit their protective action, an experiment was performed in which NAL was added at different times to cultures initiating growth in the presence of cefsulodin. Addition of NAL prevented cell lysis even when it was added late in the first cell cycle, up to 20 min before initiation of cell division (Fig. 4).

Induction of cell lysis by cefsulodin in cultures of cell division mutants initiating growth. Hoffman et al. (11) showed that growing cells of *E. coli* became particularly sensitive to the bacteriolytic action of β-lactams at the time of septation. Therefore, prevention of lysis by NAL could be attributed to its inhibitory effect on cell division rather than to its action on DNA replication. If this were the case, then cell division mutants able to replicate their chromosome normally should be resistant to the induction of cell lysis by cefsulodin when initiating growth at the restrictive temperature (42°C).

Figure 5 shows the results obtained with three mutants impaired in cell division at different levels. Clearly none of the mutations checked (*ftsA*, *ftsI*, and *tif*) was able to prevent or delay the bacteriolytic action of cefsulodin. These observations suggest that the protective action of inhibitors of DNA replication is related to the replication process itself.

Inhibition of DNA replication prevents the lytic action of cefsulodin in *recA* mutants initiating growth. Disruption of DNA replication triggers the *recA*-mediated SOS response of the cell (18). This consideration suggests that the preventive action of inhibitors of DNA replication discussed here could be a hitherto unknown phenotypic feature of the SOS system. This possibility was checked by studying the initiation of growth in *recA* mutants under different conditions. Due to the *gyrA* character of the *recA* strains we had access to, norfloxacin (1 μg/ml) was used instead of NAL. Although norfloxacin binds to the A subunit of DNA gyrase, most NAL-resistant *gyrA* mutants are sensitive to norfloxacin (14). In fact, *E. coli* JM109 was sensitive to low concentrations of norfloxacin (1 μg/ml) in our experiments.

After inhibition of DNA replication in growing cultures of *recA* mutants, cell division is not as efficiently prevented as in wild-type strains, leading to a characteristic production of DNA-less cells (15). This kind of atypical division could obscure the interpretation of our experiments with *recA* mutants, because cells that can bypass the DNA replication-cell division coupling mechanisms could also most likely bypass the mechanism preventing induction of cell lysis when DNA replication is inhibited in cells initiating growth. As a matter of fact, *E. coli* JM109 divided considerably when initiating growth in the presence of norfloxacin, increasing in cell number from 2.1×10^8 particles per ml at time zero to 3.3×10^8 particles per ml 180 min later. Equivalent figures for *E. coli* W7EA (*recA*$^+$) were 1.75×10^8 and 1.9×10^8 particles per ml, respectively.

As *recA* mutants are unable to elicit the SOS response after inhibition of DNA replication (18, 32), norfloxacin should not modify the bacteriolytic action of cefsulodin if its protective effect is mediated by the SOS system. However, the results (Fig. 6) clearly showed that norfloxacin was able to prevent bacteriolysis in *E. coli* JM109 initiating growth in the presence of cefsulodin, although not as efficiently as NAL in *recA*$^+$ strains (compare Fig. 3 and 6), probably because of the atypical division of JM109 cells treated with DNA replication inhibitors commented on above.

Structure of peptidoglycan synthesized by cells initiating growth in the presence of NAL. The preventive action of inhibitors of DNA replication could be attributed to alterations in the structure of murein, making it less susceptible to murein hydrolases (8). This possibility was tested by studying the muropeptide composition of the murein synthesized during the first 2 h after dilution by cells initiating growth in the presence of NAL (40 μg/ml) after remaining in stationary phase for 24 h. The experiment was performed as described in Materials and Methods. The results (Table 2) clearly indicate that NAL had no major effect on the muropeptide composition of murein synthesized by cells initiating growth.

DISCUSSION

Stationary-phase cultures of *E. coli* were able to initiate growth and murein synthesis in the presence of β-lactams that bind to PBPs 1 (3; Pisabarro et al., in press). Under these conditions, growth of control and β-lactam-treated cells was comparable for about one doubling in cell mass, when cell lysis started in the β-lactam-treated culture, coin-

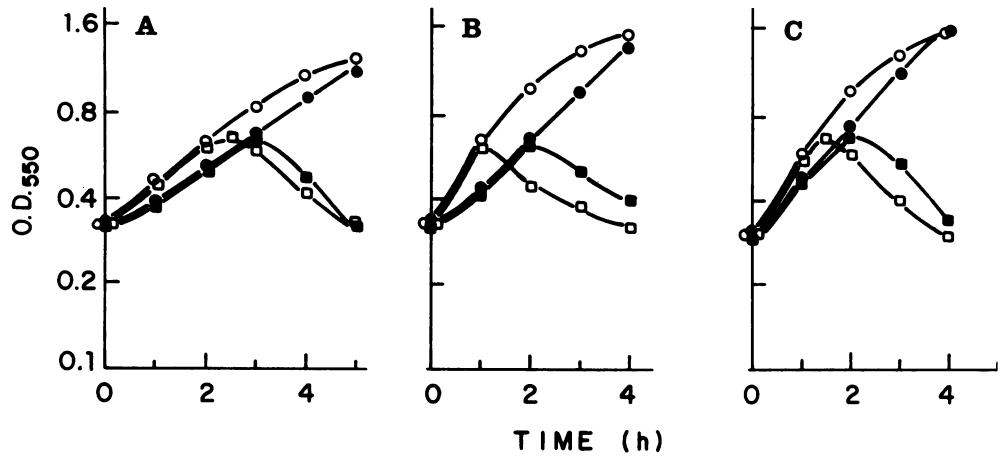

FIG. 5. Induction of cell lysis in cultures of thermosensitive cell division mutants of *E. coli* initiating growth in the presence of cefsulodin. Stationary-phase, 24-h-old cultures of strains D3 (*ftsA*) (A), AX655 (*ftsI*) (B), and JM12 (*tif*) (C) grown at 30°C were divided into four equal subcultures, and two subcultures of each strain were transferred to 42°C. Adenine (100 μg/ml) was added simultaneously to the subcultures of *E. coli* JM12 transferred to 42°C to further enhance the expression of the SOS system. After 5 min, cefsulodin (150 μg/ml) was added to one subculture of each pair, and 30 min later the subcultures were diluted 1:5 in new medium, prewarmed to the corresponding temperature and supplemented with cefsulodin (150 μg/ml) for subcultures preincubated with this antibiotic. Symbols: ●, 30°C; ■, 30°C plus cefsulodin; ○, 42°C; □, 42°C plus cefsulodin.

ciding with the initiation of cell division in the control culture. This coincidence suggested that a change should occur at this time in the murein biosynthetic machinery, making the activity of PBPs 1 essential for cell survival. An appealing hypothesis was to assume that the activity of PBPs 1 could be modulated by or coordinated with DNA replication.

To investigate this proposal, we first had to confirm that the main features of the coupling mechanisms between DNA replication and cell division established for exponentially growing cells could be extrapolated to cells initiating growth.

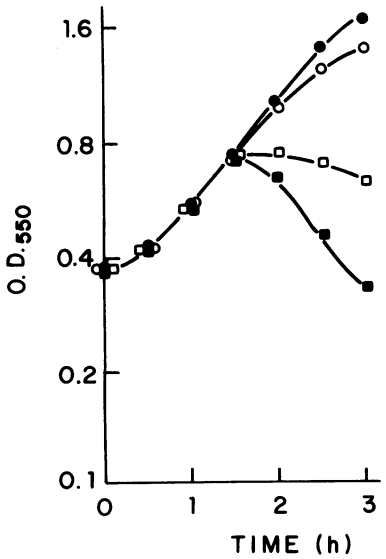

FIG. 6. Initiation of growth in cultures of *E. coli* JM109 treated with cefsulodin and norfloxacin. The experiment was performed exactly as described in the legend to Fig. 3, except that DNA replication was inhibited with norfloxacin (1 μg/ml) instead of NAL. Cefsulodin was added at a final concentration of 150 μg/ml. Symbols: ●, untreated culture; ○, plus norfloxacin; ■, plus cefsulodin; □, plus norfloxacin and cefsulodin.

According to our results, the first round of cell division after resumption of growth was, as expected, tightly coupled to a late event in chromosome replication (Fig. 2), probably to its termination. The fact that *recA* mutants initiating growth under restrictive conditions for DNA replication divided to a considerable extent (57% increase in particle number) indicates that tight coupling between DNA replication and cell division was dependent on the activation of the SOS system, as in exponentially growing cells (15). Initiation of the first round of DNA replication in *E. coli* W7EA apparently occurred 50 to 60 min before the cells started to divide (Fig. 1). Assuming that the first cells to divide were those initiating DNA replication earlier, it follows that the first round of chromosome replication takes less than 60 min, a value rather close to the minimum time (41 min) required by *E. coli* to duplicate its chromosome under optimal conditions (10). This observation suggests that the rate of DNA replication in cells initiating growth or growing exponentially should not differ substantially. Together, these results indicate that coordination between DNA replication and cell division is not drastically modified during initiation of cell growth.

The hypothetical modulation of the activity of PBPs 1 by DNA replication was studied by following the effect of DNA replication inhibitors on the bacteriolytic activity of cefsulodin, a β-lactam antibiotic specifically binding to PBPs 1a and 1b.

Addition of NAL to cells initiating growth in the presence of cefsulodin prevented the bacteriolytic response normally elicited by this β-lactam when cells double their mass (Fig. 3). The fact that different combinations of β-lactams and inhibitors of DNA replication had the same effect on a number of unrelated *E. coli* strains supports the generality of this phenomenon. Moreover, the results obtained with different cell division mutants (Fig. 5) suggest that the lysis-preventing effect of DNA replication inhibitors was the result of interference with the DNA replication process, not of an indirect inhibitory effect on cell division. The observation that NAL was effective in preventing cell lysis even when added late during the first cell cycle (Fig. 4) indicates that termination of chromosome replication might trigger the

TABLE 2. Composition of the murein synthesized by *E. coli* W7EA initiating growth in the presence of NAL

Sample	Muropeptide composition[a] (molar fraction × 100)[b]					
	Monomers	Dimers	Trimers	DD muropeptides	Lipoproteinmuropeptides	Anhydromuropeptides
Control (no NAL)	70.8	28.2	0.9	3.6	3.7	1.2
NAL (40 μg/ml)	72.8	26.3	0.8	3.8	2.4	1.5

[a] Muropeptides have been grouped as described previously (22). Monomers, disaccharide peptides; dimers, bisdisaccharide peptides; trimers, cross-linked tridisaccharide peptides; DD muropeptides, cross-linked muropeptides with a diaminopimelyl-diaminopimelic acid bridge in their molecule; lipoprotein muropeptides, muropeptides with a covalently bound molecule of lipoprotein; anhydromuropeptides, muropeptides with a (1-6)-anhydromuramyl residue in their molecule.

[b] Calculated from the distribution of *meso*-[^{3}H]diaminopimelic acid after fractionation by HPLC.

change in the murein biosynthetic machinery which renders the cell sensitive to induction of lysis after inhibition of PBPs 1.

The failure of NAL to affect the composition of murein synthesized during initiation of growth (Table 2) suggests that its protective action was most likely exerted through an alteration in the functional status of PBPs 1 rather than by modifications induced in the structure of murein, making it resistant to hydrolytic activities.

NAL is known to be a good inducer of the SOS system of the cell. Therefore, the possible involvement of the SOS system in the lysis-preventing action of DNA replication inhibitors was checked by studying the behavior of *recA* mutants. The results obtained apparently ruled out this hypothesis. The mutant strain responded to the different treatments very much like the wild-type strains (Fig. 6). A possible alternative, under consideration at present, is that this is a phenotypic manifestation of the recently described TER pathway (27), an additional regulatory system ensuring coordination between termination of DNA replication and cell division.

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