JOURNAL OF BACTERIOLOGY, Jan. 1991, p. 1–5
0021-9193/91/010001-05$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 173, No. 1

Cell Cycle-Independent Lysis of *Escherichia coli* by Cefsulodin, an Inhibitor of Penicillin-Binding Proteins 1a and 1b

GERTRUDE H. JACOBY* AND KEVIN D. YOUNG

Department of Microbiology and Immunology, University of North Dakota School of Medicine, Grand Forks, North Dakota 58202

Received 14 May 1990/Accepted 1 October 1990

Cefsulodin lyses actively growing *Escherichia coli* by binding specifically to penicillin-binding proteins (PBPs) 1a and 1b. Recent findings (F. García del Portillo, M. A. de Pedro, D. Joseleau-Petit, and R. D'Ari, J. Bacteriol. 171:4217–4221, 1989) have linked cefsulodin-induced lysis to septation during the first division cycle after a nutritional shift-up or chromosome replication realignment. We synchronized cells by membrane filtration to determine whether cefsulodin-induced lysis depended on septation in normally growing cells. Populations of newly divided cells were allowed to grow for variable lengths of time. Cefsulodin was added to these synchronous cultures, which represented points in two to three rounds of the cell cycle. Since the cell numbers were small, a new lysis assay was developed that was based on the release of DNA measured by fluorometry. Lysis occurred at a constant time after addition of the antibiotic, regardless of the time in the cell cycle at which the addition was made. Thus, cefsulodin-induced lysis is not linked to septation or to any other cell cycle-related event.

The penicillin-binding proteins (PBPs) of *Escherichia coli* are inner membrane proteins involved in cell elongation and cell septation. The high-molecular-weight PBPs (PBPs 1, 2, and 3) catalyze transglycosylation and transpeptidation reactions during the final stages of peptidoglycan synthesis (24). It has been known for some time that PBPs 1a and 1b are specifically involved in the elongation of the sacculus. Mutants lacking either protein will elongate normally, but double mutants are lethal (26). PBP 2 is involved in the maintenance of cell shape, and PBP 3 is involved in the biosynthesis of the septum (17). Recently it has been reported that PBP 2 may also be involved in septation (14).

β-Lactams that bind PBPs 1a and 1b will lyse actively growing cells (10, 17, 18, 26). The mechanism by which β-lactam-induced lysis occurs is not fully understood but certainly involves reactions between murein hydrolases and the peptidoglycan (20). Cefsulodin is a β-lactam that lyses *E. coli* and binds specifically to PBPs 1a and 1b (3, 13). In a recent study (4), García del Portillo et al. analyzed cefsulodin lysis during the first division cycle after a nutritional shift-up or chromosome replication alignment. They concluded that lysis by cefsulodin was linked to cell division. Before this study, the activities of PBPs 1a and 1b had been believed to be confined only to cell elongation (15, 17). A new role for PBPs 1a and 1b in septation, a process as yet poorly understood, would be highly interesting and worth further investigation.

The correlation between cefsulodin action and cell septation depended on production of synchronously dividing cells by methods that may seriously alter the biochemistry of *E. coli*. The chemical composition of *E. coli* peptidoglycan changes with growth rate and in response to amino acid deprivation (16, 21, 23). These changes are directly related to a state of phenotypic tolerance, a resistance to antibiotic-induced lysis (22). Since such alterations could affect the cells' sensitivity to cefsulodin, they could confuse the interpretation of the relationship between lysis and division.

* Corresponding author.

We report here the timing of cefsulodin-induced lysis in cells synchronized by the membrane filtration method of Helmstetter (7), a method relatively free of biochemical perturbations. When cefsulodin was added to cell populations at different stages in the growth cycle, all lysed rapidly. Apparently, cell cycle-dependent events do not determine lysis sensitivity to this antibiotic.

MATERIALS AND METHODS

Strains and growth conditions. All studies were performed with *E. coli* B/r strain F26 (*his thy*), obtained from Charles E. Helmstetter. Cells were grown at 37°C in M9 minimal medium (12) supplemented with 0.1% glucose, glycerol, or succinate, plus thymine (40 μg/ml), thiamine (5 μg/ml), and vitamin-free Casamino Acids (0.1%; Difco Laboratories, Detroit, Mich.) or histidine (40 μg/ml).

Synchronization of cells. The synchronization procedure was based on that developed by Helmstetter (7). A GSWP 14250, 0.22-μm-pore-size membrane filter (Millipore Corp., Bedford, Mass.) was clamped in an acrylic filter holder (G. DuBuque, University of North Dakota) constructed according to the specifications described by Helmstetter (Fig. 8 in reference 7). Instead of multiple upper-membrane gaskets, we used a 1-inch (ca. 2.5-cm)-high acrylic ring and inserted a rubber gasket (same as the lower gasket) between the ring and the membrane.

Overnight cultures were diluted and allowed to divide at least three times to reach an A_{550} of 0.2. Then 100 ml of cells was filtered onto the membrane at low vacuum, leaving a small amount of liquid on the filter. The cells were washed with 100 ml of warm medium, again leaving some liquid. Excess medium was poured off, and the filter apparatus was inverted and placed on an acrylic holder (Fig. 8 in reference 7) in a 37°C chamber. The space above the membrane was filled with warm growth medium and connected to a pump, which was used to force warm medium through the membrane.

Loosely attached cells were flushed from the membrane for 2 min at a rate of 10 ml/min, after which the rate was

reduced to 2 ml/min. After flushing for an additional 2 min, samples were collected every 4 min into 50-ml Erlenmeyer flasks (8 ml per sample). Cells used in the synchronization experiments were harvested between 20 and 120 min of elution unless otherwise indicated. After each 4-min sample was collected, a 0.1-ml portion was removed immediately and placed into a tube containing 10 of ml saline-Formalin (0.9% NaCl, 0.11% formaldehyde; filtered through a 0.22-μm-pore-size filter) for cell counting. The remainder of each 8-ml sample was incubated with shaking in a 37°C water bath. After 25 samples had been collected, a 0.1-ml sample was removed from each incubating flask and was diluted into 10 ml of saline-Formalin for cell counting. This count defined the status of each culture in the cell cycle at the time of cefsulodin addition.

Cell counting procedure. Cells were counted in a Coulter Counter model ZB1 (Coulter Electronics, Inc., Hialeah, Fla.) fitted with a 30-μm-diameter aperture tube. Readings were made at the following settings: 1/amplification = 0.5, 1/current = 0.5, and sample volume = 0.1 ml.

Lysis with cefsulodin and DNA fluorometry. Cefsulodin (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 100 μg/ml of cells. Cell lysis was measured by an increase in free DNA, as assayed by the increase in fluorometry readings in the presence of the dye, Hoechst 33258 (Hoefer Scientific Instruments, San Francisco, Calif.). When cell numbers were great enough, lysis was monitored by a decrease in A_{550} readings. Every 5 min for approximately 60 min after cefsulodin addition, a 0.5-ml sample was removed from the incubating cells and diluted with 1.5 ml of filtered TNE buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.4) containing Hoechst 33258 dye to give a final dye concentration of 0.1 μg/ml. Fluorescence was measured in a TKO 100 Mini Fluorometer (Hoefer Scientific Instruments). For delayed readings, as in samples from synchronized cells, 0.5 ml of cells was placed in a small test tube prefilled with 1.4 ml of TNE buffer containing 0.07% (vol/vol) 40% formaldehyde. Immediately before assay, 0.1 ml of a 2-μg/ml dye solution was added.

RESULTS

Assay of cell lysis by DNA fluorometry. A disadvantage of the synchronization method of Helmstetter (7) is the small number of synchronized cells eluted from the membrane. There are too few cells to monitor lysis by a decrease in optical density. In searching among alternate ways of measuring lysis under these conditions, we found that increases in free DNA were directly related to cell lysis. Figure 1 shows lysis with 100 μg of cefsulodin per ml in a nonsynchronous population of F26 cells. Fluorometer readings of free DNA in control cell cultures remained stable, but the readings with cefsulodin rose exponentially after cefsulodin addition. This rise in the fluorometry readings preceded the observed drop in optical density. Immediate readings and those taken 2 h later in the presence of formaldehyde exhibited similar kinetics. It was thus possible to delay the fluorometer readings by sampling into a buffer containing formaldehyde. The readings remained stable up to 6 h after sampling.

In a separate experiment, duplicate samples were diluted into TNE buffer 60 and 70 min after cefsulodin addition. DNase I (bovine pancreas, type IV; Sigma) was added to a final concentration of 4 μg/ml to one sample, and the sample was incubated at 30°C for 15 min. The duplicate, lacking DNase, was read immediately. DNase treatment caused the

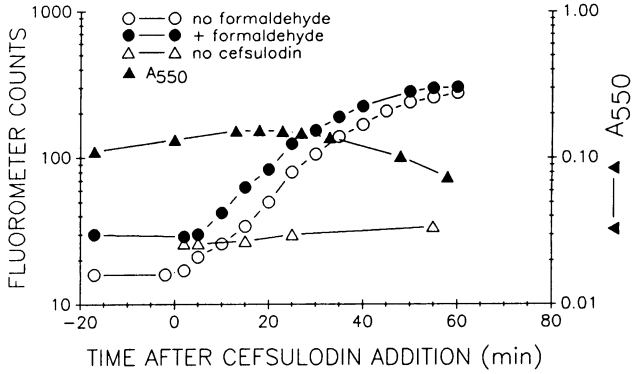

FIG. 1. Lysis of unsynchronized *E. coli* by cefsulodin. A fresh overnight culture of *E. coli* F26 was diluted and grown at 37°C in M9 medium plus glycerol (0.1%), histidine (40 μg/ml), and thymine (40 μg/ml). Cefsulodin was added to a final concentration of 100 μg/ml after the cells had doubled three times. Every 5 min, 0.5-ml samples were diluted into TNE-formaldehyde buffer for assay 2 h later and into TNE-dye buffer for immediate fluorometer readings. A reading of 100 corresponds to 100 ng of DNA standard per ml (TKO 104; Hoefer Scientific Instruments).

fluorometer counts to drop from 316 to 20 at 60 min and from 407 to 18 at 70 min. It therefore seemed clear that the assay was measuring DNA. The increase in fluorescence could be caused by DNA that leaked from cells into the medium or by the penetration of the dye into permeabilized cells, where it combined with DNA. Which of these two alternatives occurred has not been determined, though the sensitivity of the readings to DNase would seem to argue for the release of DNA. In any case, the assay measured a phenomenon that resulted from a disintegration of the integrity of the cell envelope and paralleled the absorbance decrease classically associated with cell lysis.

Cefsulodin lysis of synchronized cells. To answer the question of whether cefsulodin-induced lysis is a cell cycle-specific event, we synchronized cells by membrane filtration. We collected newly divided cells from the membrane filter and immediately placed them in a 37°C shaking water bath. These samples exhibited the characteristic pattern of cell counts expected during the Helmstetter cell synchronization procedure (Fig. 2). The number of cells eluted from the membrane cycled up and down with a period of 28 min, equal to the generation time under these growing conditions (Fig. 2A). Twenty-five populations of eluted cells, collected between 20 and 116 min in Fig. 2A, were sampled for cell counting (Fig. 2B). After 30 min of growth there was a sharp increase in cell numbers, which occurred twice more at 28-min intervals. Each population therefore represented cells at different times in the growth cycle. Thus, the cells at 34, 66, and 94 min in Fig. 2B were dividing, and those at 26, 54, and 82 min were at a late stage of elongation.

Seven minutes after the last (25th) cell sample had been collected, cefsulodin (100 μg/ml) was added to each population except for the control, and cell lysis was monitored in each population. Figure 3 shows the lysis curves of eight cell populations, each of which was at a different stage in the cell cycle when cefsulodin was added. The plots were similar, with the fluorometer counts rising rapidly 10 min after cefsulodin addition. The plots from the other 16 populations were essentially the same (data not shown). The cells to which no cefsulodin was added (sample 116) exhibited no increase in fluorometer counts. The experiment was re-

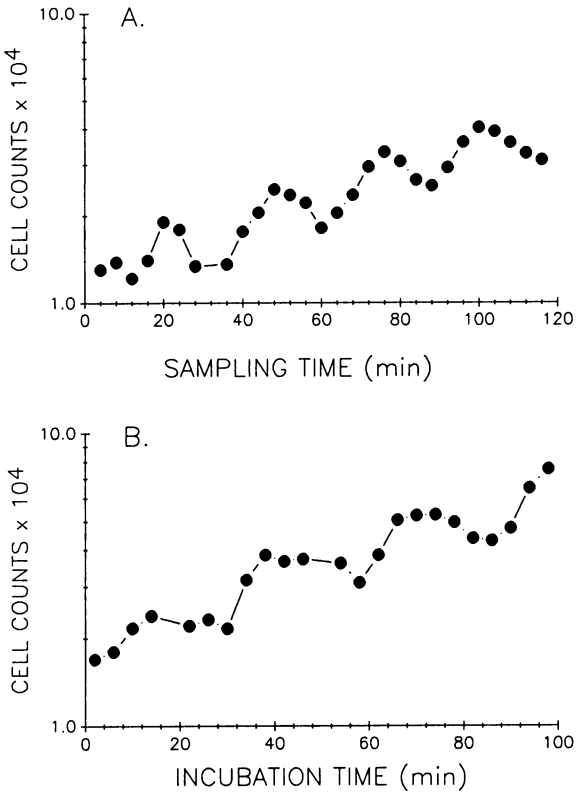

FIG. 2. Cell synchronization by the membrane filtration method. *E. coli* F26 cells were grown through at least two generations to an A_{550} of 0.2 at 37°C in M9 medium supplemented with Casamino Acids (0.1%), glucose (0.1%), and thymine (40 μg/ml). Cells (100 ml) were filtered onto a membrane filter, and newly divided cells were eluted as described in Materials and Methods. (A) Number of cells eluted during the synchronization procedure. Freshly collected cell samples (0.1 ml) were diluted into 10 ml of saline-Formalin. The cell count represents 0.1 ml of diluted cells. (B) Cell counts of bacteria collected as newly divided cells and incubated at 37°C with shaking for the indicated times (sampling times 20 to 116 min in panel A). The cells collected at 116 min in panel A correspond to the cells that were incubated for 2 min in panel B, etc. Two minutes after collection and incubation of the last sample (116-min sample of panel A), cells (0.1 ml) from each of the 25 flasks were diluted into 20 ml of saline-Formalin for counting. Each count represents 0.1 ml of diluted cells.

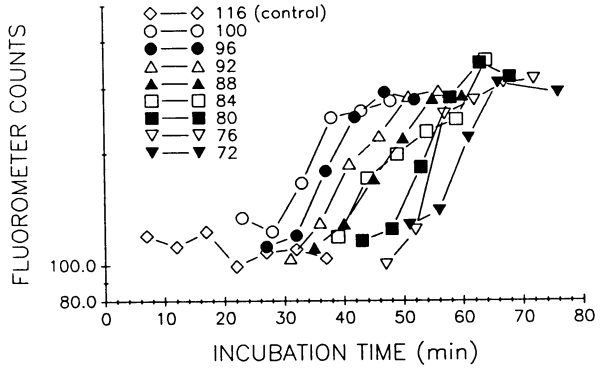

FIG. 3. Lysis of synchronized cells with cefsulodin. *E. coli* F26 cells were grown at 37°C in M9 medium supplemented with Casamino Acids (0.1%), glucose (0.1%), and thymine (40 μg/ml). Newly divided cells were collected and incubated as described in Materials and Methods. Each cell population, labeled with the starting time of collection, represents newly divided cells collected over a 4-min period. The incubation time refers to the time the cells had been growing after elution from the filter. Cefsulodin (100 μg/ml) was added to each flask except for the 116-min flask, which served as the control. A 0.5-ml sample was removed immediately and every 5 min thereafter and diluted into TNE-formaldehyde buffer for fluorometer analysis as described in Materials and Methods. Each curve represents the change in fluorometry readings of a population of cells at a different stage in the cell cycle (see Fig 2).

peated three more times with the same result: cefsulodin caused a rise in fluorometer counts (lysis) within 10 min.

To test cefsulodin-induced lysis in cells with a longer cell cycle, we used succinate as a carbon source, which lengthened the generation time to 60 min. As in the glucose experiments, all of the lysis curves were similar (Fig. 4).

If cefsulodin-induced lysis was related to septation, one would have expected plots approximating those in Fig. 5A. Cefsulodin added to cell populations at different stages in the cell cycle should have resulted in variable lysis delays. The length of each delay would have reflected the time each population had to incubate before division. Cells near septation should lyse more quickly than should cells further removed in time from division. Septation would be expected to occur at 60 and 120 min (for the succinate-grown cells), and lysis should have occurred predominantly at these times. On the other hand, if cefsulodin-induced lysis was independent of time of septation, one would have expected to see plots similar to those in Fig. 5B, a parallel set of lysis results indicating that cells at different stages of the growth cycle responded to cefsulodin in the same way. The data shown in Fig. 3 and 4 are similar to the idealized data shown in Fig. 5B.

DISCUSSION

Cells treated with cefsulodin presumably lyse because inhibition of PBPs 1a and 1b triggers the activity of murein hydrolases (19). A recent study used the methods of nutritional shift-up and chromosome replication alignment to achieve partial synchrony to determine whether lysis was related to the cell cycle (4). In the first division cycle after such synchronization, it was found that lysis began more rapidly when cefsulodin was added to cells nearer to the time of septation. Lysis appeared to begin just before or at the

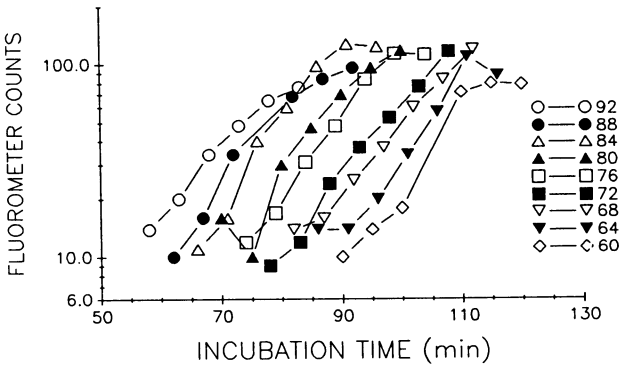

FIG. 4. Lysis of synchronized cells with cefsulodin. F26 cells were grown at 37°C as for Fig. 3 but with succinate (0.1%) rather than glucose. See legend to Figure 3 for procedure. The control is not included in this graph.

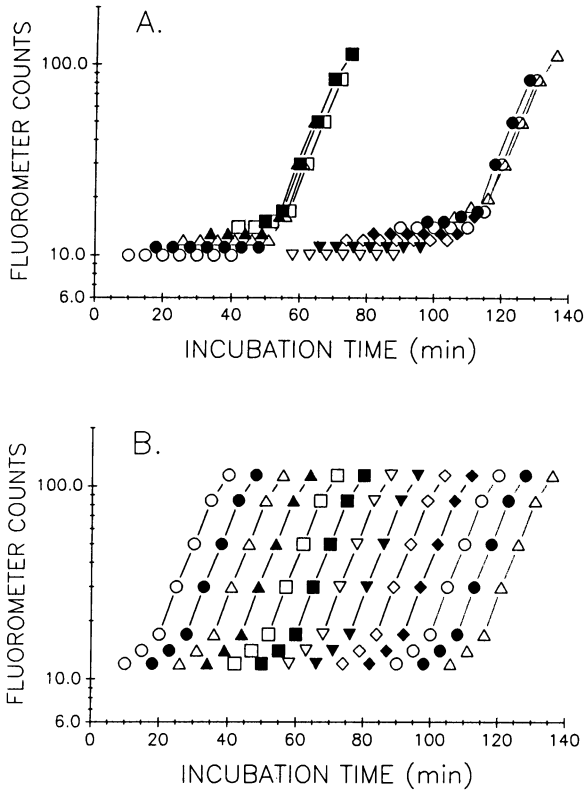

FIG. 5. Idealized lysis of synchronized cells with cefsulodin. Depicted are the expected fluorometry counts if cefsulodin (100 μg/ml) is added to populations of synchronized cells with a 60-min generation time. See legend to Figure 3 for procedure. (A) Pattern expected if lysis is related to septation; (B) pattern expected if there is no relationship between cefsulodin lysis and septation.

time of cell division, implying that cell lysis by cefsulodin occurred at the time of septation. Was the observed delay related to the actual septation event or to some other phenomenon occurring approximately at the time of septation? We have attempted to distinguish between these two alternatives.

The methods used in the previous study (4) may well alter peptidoglycan composition and hydrolase activities and, as a result, may change cell sensitivity to lysis by antibiotics. For example, during nutritional shift-up there is a change in growth rates (8), and such a change has been shown to result in modifications of peptidoglycan composition (21). Hydrolases may be more highly expressed during a shift-up (11). Changes in growth rate can also affect the relative concentrations of PBPs, with PBPs 1a and 1b increasing with higher growth rates (21).

In addition, as a result of the amino acid and thymine starvation involved in the chromosome replication alignment method, the stringent response (2) probably was activated, which also affects peptidoglycan composition (5, 9, 24). During amino acid deprivation, the composition of the peptidoglycan changes rapidly, becoming more resistant to antibiotic-induced autolysis (23). The effect is reversible (5).

As cells start to grow after a nutritional shift-up and after thymine addition, we may therefore assume that the composition of the peptidoglycan will begin changing to become increasingly less lysis resistant. With changing conditions, which may include increasing levels of PBPs 1a and 1b and more active autolysins, cefsulodin-induced lysis would become progressively easier. In fact, we have exposed cells to a similar inhibitor of PBPs 1a and 1b, cephaloridine, and have observed that as the cells exit the stationary phase the time required for lysis by penicillin decreases as they grow. The time required for such lysis onset reaches a minimum after the cells have divided five times (unpublished results). Thus, it is not surprising that cefsulodin added at staggered times after a shift-up would also exhibit staggered lysis times, and it may be only coincidental that lysis appears to occur at about the same time as does the first septation event.

It is interesting that when PBP 2 was inhibited by the antibiotic mecillinam, at the same time that PBPs 1a and 1b were inhibited by cefsulodin, cell lysis occurred at a constant time after addition of the antibiotics (4). At low concentrations, antibiotics that bind only PBP 2 produce spherical cells but do not induce lysis, but such antibiotics in combination with a β-lactam that binds PBPs 1a and 1b will accentuate the effect of the latter (1, 6). The synergistic effect of cefsulodin and mecillinam simply may have overcome the less sensitive peptidoglycan which we propose is present early in the changing growth conditions.

The membrane filtration method of Helmstetter (7) is a mechanical method in which newly divided *E. coli* B/r cells are collected and allowed to grow with no real interference with their cellular metabolism. With this method, the peptidoglycan composition should not change, nor should the relative concentrations of PBPs, which remain constant during the division cycle (25). We have shown (Fig. 3 and 4) that under these conditions there are no differences in the sensitivity of cells to cefsulodin during the cell cycle. If cefsulodin lysis were related to septation, we would expect the time of lysis onset to be variable and to depend on the stage of each population in the growth cycle (Fig. 5A). We saw no such delays in cell lysis. Instead, lysis occurred at a constant time after antibiotic addition. We therefore conclude that cefsulodin-induced lysis is not related to septation, so that the activities of PBPs 1a and 1b cannot be correlated directly with the timing of this event.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant GM40947 from the National Institutes of Health.

ADDENDUM IN PROOF

F. B. Wientjes and N. Nanninga (personal communication) synchronized *E. coli* cells by elutriation and have also found that lysis by cefsulodin is not dependent on the cell cycle (Res. Microbiol., in press).

REFERENCES

1. **Berenguer, J., M. A. de Pedro, and D. Vázquez.** 1982. Induction of cell lysis in *Escherichia coli*: cooperative effect of nocardicin A and mecillinam. Antimicrob. Agents Chemother. **21**:195–200.
2. **Cashel, M., and K. E. Rudd.** 1987. The stringent response, p. 1410–1438. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
3. **de la Rosa, E., M. A. de Pedro, and D. Vázquez.** 1985. Penicillin binding proteins: role in initiation of murein synthesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:5632–5635.
4. **García del Portillo, F., M. A. de Pedro, D. Joseleau-Petit, and R. D'Ari.** 1989. Lytic response of *Escherichia coli* cells to inhibi-

tors of penicillin-binding proteins 1a and 1b as a timed event related to cell division. J. Bacteriol. **171**:4217–4221.
5. **Goodell, W., and A. Tomasz.** 1980. Alteration of *Escherichia coli* murein during amino acid starvation. J. Bacteriol. **144**:1009–1016.
6. **Gutmann, L., S. Vincent, D. Billot-Klein, J. F. Acar, E. Mrèna, and R. Williamson.** 1986. Involvement of penicillin-binding protein 2 with other penicillin-binding proteins in lysis of *Escherichia coli* by some β-lactam antibiotics alone and in synergistic lytic effect of amdinocillin (mecillinam). Antimicrob. Agents Chemother. **30**:906–912.
7. **Helmstetter, C. E.** 1969. Methods for studying the microbial division cycle. Methods Microbiol. **1**:327–363.
8. **Ingraham, J. L., O. Maaloe, and F. C. Neidhardt.** 1983. Regulation at the whole cell level, p. 349–385. *In* Growth of the bacterial cell. Sinauer Associates Inc., Sunderland, Mass.
9. **Ishiguro, E. E., and W. Kusser.** 1988. Regulation of peptidoglycan biosynthesis and antibiotic-induced autolysis in nongrowing *Escherichia coli*: a preliminary model, p. 189–194. *In* P. Actor, L. Daneo-Moore, M. L. Higgins, M. R. J. Salton, and G. D. Stockman (ed.) Antibiotic inhibition of bacterial cell surface assembly and function. American Society for Microbiology, Washington, D.C.
10. **Kitano, K., and A. Tomasz.** 1979. Triggering of autolytic cell wall degradation in *Escherichia coli* by beta-lactam antibiotics. Antimicrob. Agents Chemother. **16**:838–848.
11. **Mengin-Lecreux, D., and J. van Heijenoort.** 1985. Effect of growth conditions on peptidoglycan content and cytoplasmic steps of its biosynthesis in *Escherichia coli*. J. Bacteriol. **163**: 208–212.
12. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Neumann, M.** 1981. Mechanisms of action of beta-lactam antibiotics: relation between PBP (penicillin-binding proteins) and autolysins. Drugs Exp. Clin. Res. **7**:363–367.
14. **Ogura, T., P. Bouloc, H. Niki, R. D'Ari, S. Hiraga, and A. Jaffé.** 1989. Penicillin-binding protein 2 is essential in wild-type *Escherichia coli* but not in *lov* or *cya* mutants. J. Bacteriol. **171**:3025–3030.
15. **Park, J. T.** 1987. The murein sacculus, p. 23–30. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
16. **Pisabarro, A. G., M. A. de Pedro, and D. Vázquez.** 1985. Structural modifications in the peptidoglycan of *Escherichia coli* associated with changes in the state of growth of the culture. J. Bacteriol. **161**:238–242.
17. **Spratt, B. G.** 1975. Distinct penicillin binding proteins involved in the division, elongation and shape of *Escherichia coli* K12. Proc. Nat. Acad. Sci. USA **72**:2999–3003.
18. **Suzuki, H., Y. Nishimura, and Y. Hirota.** 1978. On the process of cellular division in *Escherichia coli*: a series of mutants of *E. coli* altered in the penicillin-binding proteins. Proc. Natl. Acad. Sci. USA **75**:664–668.
19. **Tomasz, A.** 1979. The mechanism of the irreversible antimicrobial effects of penicillins: how the beta-lactam antibiotics kill and lyse bacteria. Annu. Rev. Microbiol. **33**:113–137.
20. **Tomasz, A.** 1986. Penicillin-binding proteins and the antibacterial effectiveness of β-lactam antibiotics. Rev. Infect. Diseases **8**(Suppl. 3):S260–S276.
21. **Tuomanen, E., and R. Cozens.** 1987. Changes in peptidoglycan composition and penicillin-binding proteins in slowly growing *Escherichia coli*. J. Bacteriol. **169**:5308–5310.
22. **Tuomanen, E., R. Cozens, W. Tosch, O. Zak, and A. Tomasz.** 1986. The rate of killing of *Escherichia coli* by β-lactam antibiotics is strictly proportional to the rate of bacterial growth. J Gen. Microbiol. **132**:1297–1304.
23. **Tuomanen, E., Z. Markiewicz, and A. Tomasz.** 1988. Autolysis-resistant peptidoglycan of anomalous composition in amino-acid starved *Escherichia coli*. J. Bacteriol. **170**:1373–1376.
24. **Waxman, D. J., and J. L. Strominger.** 1983. Penicillin-binding proteins and the mechanism of action of β-lactam antibiotics. Annu. Rev. Biochem. **52**:825–869.
25. **Wientjes, F. B., T. J. M. Olijhoek, U. Schwarz, and N. Nanninga.** 1983. Labeling pattern of major penicillin-binding proteins of *Escherichia coli* during the division cycle. J. Bacteriol. **153**:1287–1293.
26. **Yousif, S. Y., J. K. Broome-Smith, and B. G. Spratt.** 1985. Lysis of *Escherichia coli* by β-lactam antibiotics: deletion analysis of the role of penicillin-binding proteins 1A and 1B. J. Gen. Microbiol. **131**:2839–2845.