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Deoxyribonucleic Acid Synthesis and Cell Division in a *lon*⁻ Mutant of *Escherichia coli*

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The *lon*⁻ mutants of *Escherichia coli* form long filamentous cells after temporary inhibition of deoxyribonucleic acid (DNA) synthesis by ultraviolet irradiation, treatment with nalidixic acid, or thymine starvation. The kinetics of DNA synthesis and cell division after a period of thymine starvation have been compared in *lon*⁺ and *lon*⁻ cells. After this treatment, both kinds of cells recover their normal DNA to mass ratio with the same kinetics. In contrast to previous reports, cell division is found to recommence in both *lon*⁺ and in *lon*⁻ cells after such a temporary period of inhibition of DNA synthesis. However, the delay separating the recommencement of DNA synthesis and of cell division is approximately three times as long in *lon*⁻ as in *lon*⁺ cells. Low concentrations of penicillin inhibit cell division in both *lon*⁺ and *lon*⁻ cells. In this case, cell division recommences with the same kinetics in both strains after the removal of penicillin. This suggests that different steps in the cell division process are blocked by inhibition of DNA synthesis and by penicillin treatment. The *lon*⁻ mutation appears to affect the former of these steps.

The *lon*⁻ mutants of *Escherichia coli* are a class of mutants which form filaments and fail to divide after ultraviolet (UV) irradiation (12). They are able to repair UV-induced lesions in their deoxyribonucleic acid (DNA; 12, 17), increase in mass, and synthesize DNA normally after UV (17). However, cell division is inhibited after irradiation, and the cells grow into long filaments (3). Filamentation is also induced by other agents which temporarily inhibit DNA synthesis (12, 18), and the effect of UV on cell division is, therefore, probably not a direct one but an indirect effect of temporary inhibition of DNA synthesis (18). In *lon*⁺ cells, inhibition of DNA synthesis also inhibits cell division (7), but in this case, cell division recommences in the treated cells as soon as the DNA to mass ratio in the cells is restored to its normal value (8, 9).

A possible explanation for the failure of *lon*⁻ populations to divide normally after inhibition of DNA synthesis, therefore, is that such populations do not recover a normal DNA to mass ratio. We therefore observed the course of DNA synthesis after a period of thymine starvation (or after UV irradiation) in *lon*⁺ and *lon*⁻ populations, to test whether the recovery of a normal DNA to mass ratio is delayed in the *lon*⁻ mutant. The kinetics of DNA synthesis after a period of inhibition appears to be the same in both mutant and wild-type, and both types of cells recover their DNA to mass ratio at the same time.

It has been reported that inhibition of cell division in *lon*⁻ cells can be prevented or reversed by a number of treatments, e.g., by "liquid holding" (13), by growth at 42 C (3, 5), or by a treatment with pantoyl lactone (2, 11, 16; *unpublished data*). A common result of these treatments is a decrease in the growth rate of the cells. It has also been reported (10) that cell division can be induced by a cell extract. This extract does not appear to inhibit growth (H. I. Adler, *personal communication*) and its action is so far not understood. Conversely, those treatments which increase the growth rate enhance the inhibition of cell division (3, 12, 18). In the present paper, we show that the inhibition of cell division in *lon*⁻ cells reverses spontaneously during post-treatment growth in the same medium. However, after restoration of the DNA to mass ratio, division is delayed longer in *lon*⁻ cells than in *lon*⁺ cells. The period of delay depends on the length of thymine starvation with a maximum delay of 120 to 140 min.

MATERIALS AND METHODS

Strains. *E. coli* AB1157 was obtained from H. I. Adler. The filamenting (*lon*⁻) derivative of this strain, *E. coli* AB1899NM (a nonmucoid mutant of the original *lon*⁻ isolate) was obtained from A. Hardigree, and

E. coli AB2497, a low-thymine (2 μg/ml)-requiring derivative of AB1157 was obtained from P. Howard-Flanders. The two isogenic strains used in these experiments were derived from AB1899NM. TG894, a low-thymine (2 μg/ml)-requiring derivative of the *lon*⁻ strain was obtained by treatment with trimethaprim (obtained through the courtesy of L. G. Petty, Burroughs Wellcome; reference 14). TG894LL, a *lon*⁺ derivative of TG894, was obtained by transduction with P1kc W3110 (from W. Brammar) and selection for *lac*⁺ colonies.

Media. Experiments were performed in minimal medium consisting of 300 ml of distilled water, 100 ml of M9 salts (4×; reference 1), $MgSO_4$ (10^{-3} M), glucose (0.2%), 8 mg of each of the required amino acids (arginine, histidine, threonine, leucine, and proline), and 8 mg of vitamin B_1. Where necessary, thymine (10 μg/ml) was added to the culture flasks. The L-agar used consisted of 1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5% agar, and 1 liter of water. The L-soft agar for pour-plating the bacteria contained only 1.2% agar.

Radioactive counting. DNA synthesis was followed by the uptake of ^{14}C-thymine (The Radiochemical Centre, Amersham). ^{14}C-thymine (10 μg/ml, 0.1 μc/ml) was added to the medium, and 0.5-ml culture samples were collected into 0.5 ml of 20% trichloroacetic acid in ice. These were then filtered on Oxoid membrane filters (2 cm, 0.45 μm pores). The filters were washed with 5% trichloroacetic acid and dried under an infrared heat lamp. The dry filters were placed in vials containing 10 ml of 0.4% Scintillator *BBOT* (Ciba) in toluene. These were counted in a liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

Cell number. Increase in the cell number of a culture was followed by using an electronic counter (Coulter Electronics, Dunstable). Samples were taken into 10 ml of azide saline (0.9% NaCl, 0.08% NaN_3), and a 0.05-ml volume was counted. The penicillin (Solupen; Dista Products Ltd., Liverpool) was used in the experiments at a concentration of 15 μg/ml. The penicillinase (obtained from R. Ambler) was derived from *Staphylococcus aureus* P.C.1. Optical density was measured at 540 nm with a spectrophotometer (Hilger & Watts Ltd., London).

RESULTS

UV sensitivity. Testing sensitivity to UV irradiation was used to show these two isogenic strains were in fact *lon*⁻ and *lon*⁺. Figure 1 shows UV survival curves for these two strains (TG894 and TG894LL) and for the known *lon*⁻ and *lon*⁺ strains AB1899NM and AB1157. The derived strains showed the same UV-sensitivities as the original strains.

DNA synthesis after thymine starvation. It has been previously reported that there is a correlation between the restoration of the DNA to mass ratio of *lon*⁺ cells and the recommencement of cell division (9). A slower rate of recovery of the DNA content of *lon*⁻ cells could, therefore, lead to the increased delay in restoration of the ability to divide. We have, accordingly, compared the kinetics of DNA synthesis in TG894LL (*lon*⁺) and TG894 (*lon*⁻) cells after 80 min of thymine starvation. The course of DNA synthesis is identical in the two strains (Fig. 2). DNA synthesis continues at a decreasing rate for about 20 min after the removal of thymine from the medium and then stops, suggesting that, in this K-12 strain as in others (6), there is a pool of thymine in growing cells. DNA synthesis recommences as soon as thymine is added, and continues at a higher rate than in the unstarved controls, until the DNA content of the treated cells is restored almost to the level in the control cultures. The kinetics of DNA synthesis in these two K-12 strains are, therefore, similar to that described and discussed earlier for strain 15 (8).

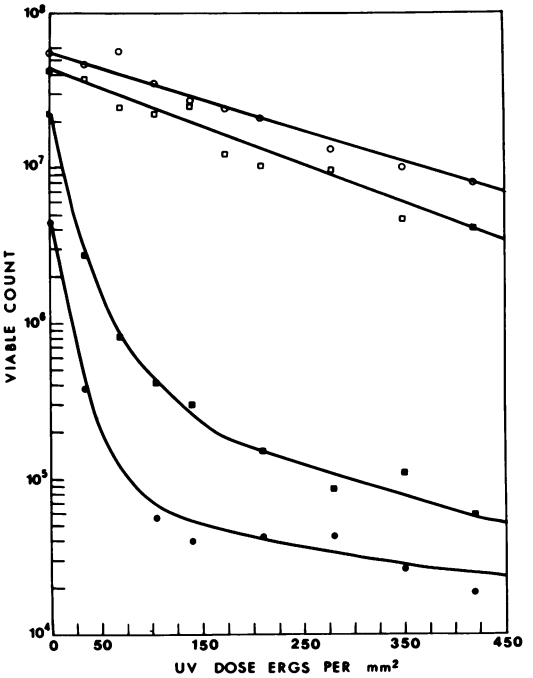

FIG. 1. *Viable count after UV irradiation. Cultures in the logarithmic phase of growth in minimal medium were irradiated. After irradiation, samples were pour-plated on L-agar containing added thymine (10 μg/ml) and incubated in the dark. AB1157 (lon⁺), □; AB1899 (lon⁻), ■; TG894LL (lon⁺), ○; and TG894 (lon⁻), ●.*

The kinetics of DNA synthesis after UV irradiation have also been compared in TG894LL (*lon*⁺) and TG894 (*lon*⁻). In both *lon*⁺ and *lon*⁻ cells, DNA synthesis stopped after irradiation and recommenced at an accelerated rate after a delay. The normal DNA to mass ratio was restored at about the same time in both strains.

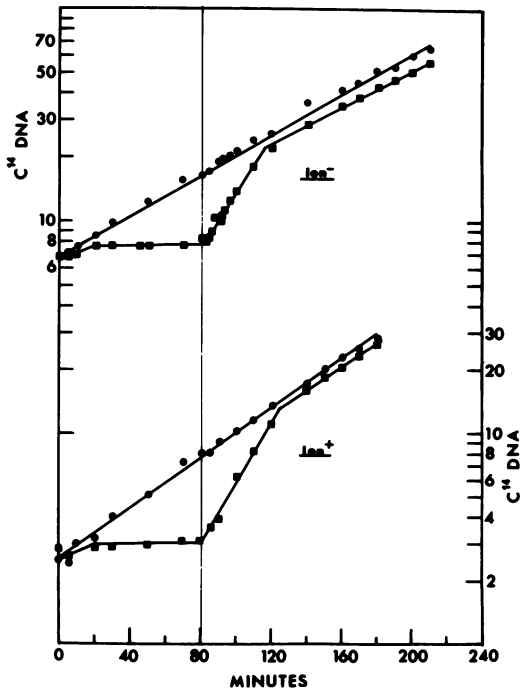

FIG. 2. *DNA synthesis in TG894LL (lon⁺) and TG894 (lon⁻) during and after thymine starvation. Cultures were grown for three mass doubling times in minimal medium containing ^{14}C-thymine. At 0 min, the culture was filtered, washed, and resuspended in fresh minimal medium containing ^{14}C-thymine (●), and in the absence of thymine (■). At 80 min, ^{14}C-thymine was added back to the starved cultures.*

Cell division after thymine starvation. Cultures of the thymine-requiring strains TG894LL (*lon⁺*) and TG894 (*lon⁻*) in the logarithmic phase of growth in minimal medium with thymine were grown in the absence of thymine by the method previously described (8). After 80 min, when the total cell mass (as measured by optical density) had increased about threefold, thymine was re-added to the thymine-starved cultures. Figure 3 shows the course of increase in total cell mass and in total cell number in such an experiment.

The cells continued to divide for some time after removal of thymine until the cell number had increased about 40%. After the readdition of thymine, the cell number remained constant for a period, and then began to increase again. The delay between the readdition of thymine (after 80 min starvation) and the recommencement of cell division was 40 to 50 min for *lon⁺* cells (8). However, the delay in the *lon⁻* cells was consistently found to be 120 to 140 min (Fig. 3). Apart from this difference in the length of the delay period, the kinetics of division on the *lon⁻* cell appears to be the same as previously reported for *lon⁺* strains (8). Similar results were obtained when the experiment was performed in an enriched medium.

As previously reported (8), the length of the delay between readdition of thymine and recommencement of division is a function of the length of the preceding period of growth without thymine. The results with the *lon⁺* strain AB2497 used in the present work (Fig. 4) are closely similar to those reported earlier. However, the results for the *lon⁻* strain, TG894, show a much more rapid increase in the length of delay of cell division after thymine starvation. Also, the maximal length of the delay is 120 to 140 min (as opposed to 45 min for the *lon⁺* strain). This means that the *lon⁻* cells show about three times the maximal delay before cell division shown by

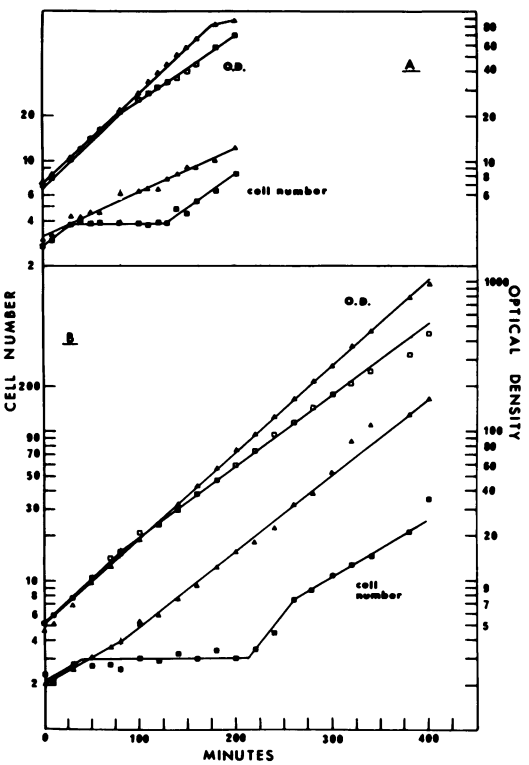

FIG. 3. *Cell number in TG894LL (lon⁺) and TG894 (lon⁻) during and after thymine starvation. At 0 min, log-phase cultures growing in minimal medium were filtered, washed, and resuspended in the presence (▲, △) and absence (■, □) of thymine (solid symbols, cell number; open symbols, optical density). At 80 min, thymine was added back to the starved cultures. B, To maintain the culture in logarithmic growth, the thymine-starved culture was diluted into fresh warm medium at 80 and 250 min, and the control unstarved culture was similarly diluted at 80, 250, and 300 min. The graph has been corrected for these dilutions. A, lon⁺; B, lon⁻.*

the *lon*⁺ cells, although both exhibit this maximal delay after starvation for the same length of time, 60 min. The length of the delay before cell division is dependent (up to a maximum) on the length of the period of inhibition of DNA synthesis for both *lon*⁺ and *lon*⁻ strains.

Cell division after penicillin treatment. Thymine starvation results in a cessation of DNA synthesis, and subsequent to this, an inhibition of cell division. The *lon*-induced filamentation could be due to inhibition of DNA synthesis or to the subsequent inhibition of the cell division process itself. If the latter is the case, inhibition of cell division without affecting DNA synthesis should also result in filamentation. To test this hypothesis, we inhibited cell division with low concentrations of penicillin, which inhibit cell division without altering the rate of growth or DNA synthesis (15). Therefore, in the presence of such a concentration of penicillin, cells grow into long filaments. The removal of penicillin from the medium (e.g., by the addition of penicillinase) leads to a resumption of cell division in wild-type cells (15).

The kinetics of cell division after penicillin filamentation was compared in *lon*⁺ and *lon*⁻ cells. Figure 5 shows that, in this case, the kinetics of cell division in the wild type and in the mutant are identical. Both cultures recommenced cell division at the same rate, about 45 min after the addition of penicillinase.

Figure 5 shows the combined effect of penicillin and thymine starvation. At zero minutes, thymine was removed and penicillin was added. At 80 min, thymine and penicillinase were added together. The effect of the combined treatment is the same as with thymine starvation alone.

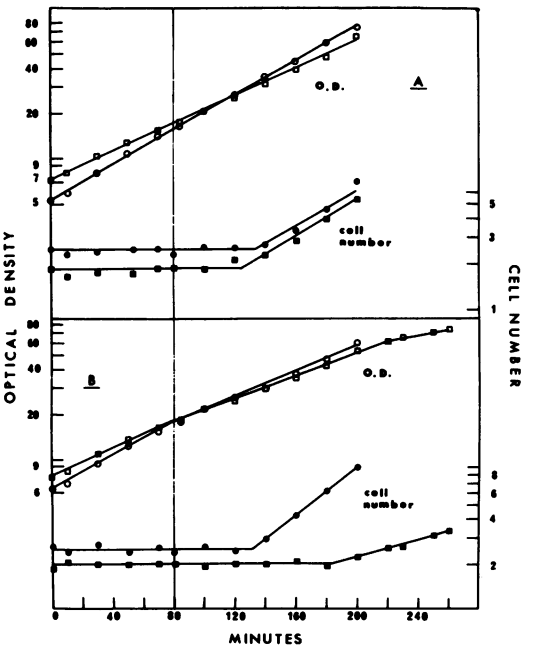

FIG. 5. *Effect of penicillin (A) and penicillin with thymine starvation (B) on cell division in TG894 (lon⁻, ■, □) and TG894LL (lon⁺; ●, ○); solid symbols = cell number, open symbols = optical density. At 0 min, penicillin (15 μg/ml) was added to the cultures (A), or the cultures were filtered, washed, and resuspended in minimal medium containing penicillin (15 μg/ml) in the absence of thymine (B). At 80 min, penicillinase (0.3 μg/ml; A), or penicillinase (0.3 μg/ml) with thymine (10 μg/ml; B) was added to the cultures.*

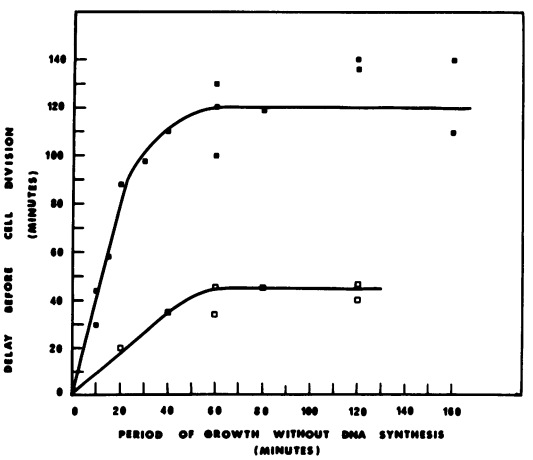

FIG. 4. *Relationship between pregrowth in the absence of DNA synthesis (thymine starvation) and the delay before cell division after DNA synthesis recurs (readdition of thymine). To allow for the thymine pool, the length of time of no DNA synthesis is taken as the time of thymine starvation less 20 min. TG894 (lon⁻), ■; AB2497 (lon⁺), □.*

DISCUSSION

The experiments described here were performed to test the possibility that the inhibition of cell division in *lon*⁻ cells, which follows thymine starvation, is due to an inability of the cells to restore their normal DNA to mass ratio after a temporary inhibition of DNA synthesis. Such a failure might have arisen from a defect in the normal regulation of DNA synthesis, such as, for example, the synthesis of an unstable initiator protein in the mutant. However, the regulation of DNA synthesis in this mutant appears to be normal, at least with respect to the kinetics of DNA synthesis after thymine starvation or UV irradiation. The mutant defect, however, clearly involves those steps which link DNA replication to cell division. Thus, treatments which temporarily inhibit cell division without interfering with

DNA synthesis have exactly the same effects in *lon*$^+$ and *lon*$^-$ cells, whereas any treatment which temporarily inhibits DNA replication inhibits cell division for a much longer period in *lon*$^-$ than in *lon*$^+$ cells.

Previous reports have suggested that filamentation in *lon*$^-$ cells, once induced, is irreversible except by special treatments which generally have the effect of slowing or preventing cell growth (3, 4). The experiments described here were carried out on continuously growing log-phase cells in liquid minimal medium. They have shown that, in fact, cell division resumed in *lon*$^-$ as in *lon*$^+$ cells after temporary inhibition of DNA synthesis. The difference between mutant and wild-type appears to lie in the length of the delay between restoration of the normal DNA to mass ratio and recommencement of cell division. Thus, in the experiments shown in Fig. 3, cell length (taken to be proportional to optical density) increased about 4-fold in the *lon*$^+$ strain, and by 15-fold in the *lon*$^-$ strain before cell division recommenced.

The data plotted in Figure 3 suggest that, as in other strains (8), cell division is restricted to an average of one per cell during the first period of rapid division. Such a restriction must lead to a perpetuation of the filaments in the culture. More detailed observations of the exact pattern of division in these cells are also in progress. Preliminary experiments also show that the filaments themselves do divide, and the increase in cell numbers is not due only to the division of cells of normal length in the culture.

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