Production of Cells Without Deoxyribonucleic Acid During Thymidine Starvation of \textit{lexA}\textsuperscript{−} Cultures of \textit{Escherichia coli} K-12

WILLIAM E. HOWE AND DAVID W. MOUNT\textsuperscript{*}
Department of Microbiology, College of Medicine, University of Arizona, Tucson, Arizona 85724
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When thymidine-requiring \textit{lexA}\textsuperscript{−} strains were starved for thymidine, the kinetics of survival were similar to those of a nearly isogenic \textit{lexA}\textsuperscript{+} strain. The size distribution of cells in the \textit{lexA}\textsuperscript{−} and \textit{lexA}\textsuperscript{+} cultures were, however, quite different. Whereas most of the cells in the starved \textit{lexA}\textsuperscript{+} cultures grew into long filamentous forms (longer than 4.0 \textmu m), many of the \textit{lexA}\textsuperscript{−} cells were found to have a normal rod shape (4.0 \textmu m or shorter). It was shown that \textit{lexA}\textsuperscript{−} cells undergo more divisions during thymidine starvation than \textit{lexA}\textsuperscript{+} cells. Furthermore, using an autoradiographic method to analyze deoxyribonucleic acid (DNA) distribution in the starved cells, we demonstrated that cells without DNA are produced in both normal and starved \textit{lexA}\textsuperscript{−} cultures at a much higher frequency than in \textit{lexA}\textsuperscript{+} cultures. Some of these cells may be produced by breakdown of DNA, but we favor the hypothesis that they result from an abnormal cell division process. Since \textit{lexA} mutations are dominant, we conclude that a diffusible product decreases the synthesis or activity of an inhibitor of cell division in \textit{lexA}\textsuperscript{−} strains when DNA synthesis is blocked by thymidine starvation.

\textit{lexA}\textsuperscript{−} cells are sensitive to ultraviolet (UV) light and a variety of agents that damage deoxyribonucleic acid (DNA), but are recombination proficient. The dominance of \textit{lexA}\textsuperscript{−} over \textit{lexA}\textsuperscript{+} indicates that the \textit{LexA}\textsuperscript{−} phenotype is due to the synthesis of a diffusible product (18). When UV-resistant derivatives of \textit{lexA}\textsuperscript{−} strains are selected, about 50\% appear to be defective in cell division and form filaments at 42 C. These derivatives are thought to carry a mutation that affects the same product as the original \textit{lexA} mutation. This product appears to be necessary for DNA repair and cell division (19).

Inouye has reported that cultures of thymidine (TdR)-requiring \textit{recA}\textsuperscript{−} strains divide and produce cells without DNA (N\textsuperscript{−} cells) during TdR starvation (15). The production of N\textsuperscript{−} cells by \textit{recA}\textsuperscript{−} strains has also been reported by Capaldo and Barbour in normal growing cultures (3, 4). Many of the mutant properties observed in \textit{lexA}\textsuperscript{−} strains are also observed in \textit{recA}\textsuperscript{−} mutants of \textit{Escherichia coli} K-12. However, \textit{recA}\textsuperscript{−} cells are recombination deficient and are more sensitive to UV than \textit{lexA}\textsuperscript{−} cells (17). These studies have indicated that \textit{recA}, like the \textit{lexA} gene, influences cell division in \textit{E. coli} K-12.

We have looked for further evidence of the involvement of the \textit{lexA} gene product in the control of cell division by examining cell division in cultures of TdR-requiring \textit{lexA}\textsuperscript{+} and \textit{lexA}\textsuperscript{−} cells with or without TdR. These studies have suggested a regulatory control of cell division by the \textit{lexA} gene.

MATERIALS AND METHODS

\textbf{Medium.} The media employed were described previously (10, 17, 18). TdR was added to the medium at a concentration of 2 \textmu g/ml unless otherwise noted. Complete Davis minimal medium (CDM) consists of Davis minimal salts (10), 0.1 \textmu g of thiamine per ml, 0.2\% glucose, and 2 \textmu g of TdR per ml.

\textbf{Bacterial strains.} The properties of the strains and their sources are given in Table 1. The \textit{lex} locus at 80.9 min on the \textit{E. coli} K-12 linkage map (23) is designated here \textit{lexA}.

\textbf{Transduction procedures.} The transduction procedures described by Willetts et al. (24) were used.

\textbf{UV survivals.} The UV survival technique described by Willetts and Mount (25) and Mount et al. (18) was used.

\textbf{Measuring survival during thymidine starvation.} Cells to be starved for TdR were grown in CDM broth to log phase (2 \times 10\textsuperscript{8} cells/ml). The cells were centrifuged and the pellet was washed once at 3 C and resuspended to the original volume in Davis minimal salts. The culture was then diluted 1,000-fold into CDM broth lacking TdR. Samples (0.1 to 1.0 ml)
were taken, inoculated to CDM plates (using Davis minimal overlays plus 3 μg of TdR per ml), and incubated at 37°C for about 24 h.

**Optical density measurements.** Cells were grown to log phase as stated before and then centrifuged, and the pellet was resuspended in CDM broth without TdR. The centrifugation and resuspension process was repeated twice and then the cells were starved for TdR. Optical density (OD) measurements were taken on a Klett-Summerson photometer.

**Cell number counts.** Cultures were treated in the same manner as in the OD experiments. Samples (0.9 ml) were taken and 0.1 ml of 20% Formalin was added to each. When necessary, the samples were diluted into Davis minimal salts containing 2% Formalin. Portions were placed on a Petroff-Hauser counter and photographed. Four sets of pictures were taken of each sample; each set was taken at two focal planes in the sample in order to score all cells. Kodak Tri-X film was used for the photography. The film was developed and printed on paper (8 by 10 inch; about 20 by 24 cm) (#3) for cell counting.

**Autoradiographs.** The cells were grown and starved in the same way as in the OD experiments except that the DNA was labeled by adding [3H]TdR (specific activity 20 μCi/μg, New England Nuclear Corp.) to cultures prior to starvation (minimum of 10 generations). Samples for the autoradiographs (5, 6) were taken before and after 4 and 8 h of TdR starvation. These were diluted into an equal volume of 4% Formalin. Samples were filtered (45 μm pore size, Millipore Corp., 13 mm) with a syringe, washed with distilled water, resuspended in distilled water, spread on subbed slides, and dried. Subbed slides were prepared by cleaning microscope slides in 95% ethanol, drying them, dipping them in a solution of 0.5% gelatine and 0.01% chromium potassium sulfate, and drying them in a vertical position. Slides with the dried cultures were dipped in cold 5% trichloroacetic acid for 10 min and distilled water twice for 2 min. These slides were then dipped in emulsion (Ilford L-4 at 40 to 45°C) in the dark. Developing of the slides was done after 1 to 8 days in the dark by dipping them into D19 developer (5 min), distilled water (1 min), F24 fixer (240 g of sodium thioulate, 25 g of sodium bisulfite, and 10 g of sodium sulfite in 1,000 ml of distilled water, 5 min), and then five consecutive baths of distilled water (2 min each). The autoradiographs were analyzed on a phase contrast microscope. The cells were classified as N+ if they had grains and N− if they did not (14). A normal cell was defined as a cell with a length of 4.0 μm or less and if a filament was defined as a cell longer than 4.0 μm. This definition was based on our observation that 90% of the cells in lexA+ cultures growing in CDM medium were found to have lengths in the range of 0.7 to 4.0 μm, the average length being 2.0 μm. A length of 4.0 μm for the normal cell was selected as the longest cell which, after completing the process of cell division, would yield progeny of lengths near the average.

**Measuring DNA degradation.** Preparation of the cultures was similar to the autoradiographs except [3H]TdR specific activity was 10 μCi/μg, and the cell concentration was 2 × 10^6/ml. Samples (10 ml) were taken, and then 5 ml of 15% cold trichloroacetic acid was added to each sample. Samples were kept cold for 105 min, filtered (0.45 μm pore size, Millipore Corp., white 25 mm), dried at 60°C for 30 min, placed in scintillation fluid (7.56 g of 2,5-diphenyloxazole and 0.189 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene per 1,900 ml of toluene), and counted on a Nuclear-Chicago Mark I or Beckman LS-250 liquid scintillation counter. Sample counts were in the range of 10^4 to 10^6 counts/min with background at 200 counts/min or less. To obtain the data points for Fig. 8B, the points in Fig. 3 were used in combination with those in Fig. 8A. Hour points 1 through 8 (lexA+, Fig. 3) were averaged and used to normalize the lexA+ curve in Fig. 8A to obtain the lexA+ curve in Fig. 8B. The 2-h point (lexA−, Fig. 3) was used to normalize the 2-h point in Fig. 8A for Fig. 8B; the hour points 3 through 8 (lexA+, Fig. 3) were averaged to normalize the same lexA+ points in Fig. 8A for construction of Fig. 8B.

**RESULTS**

**Survival and the change in OD during thymidine starvation.** Survival curves of lexA+ and lexA− cultures are shown in Fig. 1. The strains (Table 1) carry the same thyA and drm mutations (20) and are isogenic except for the malB-lexA region of the E. coli K-12 genetic map (23). The results shown are the average of three experiments each of which gave similar results. (All experiments to be described have been repeated at least two times and show the same general trends, except when noted.) The kinetics of survival are similar for the two
strains, indicating that the lexA gene product does not appear to affect the repair of DNA lesions arising from TdR starvation. Similar observations were made by Anderson and Barbour (1) with recA cells, but not by Inouye (15) who found a different recA- strain to be more resistant to starvation of TdR. Similar kinetics of survival (9) were also observed in exrA mutants (thought to be identical to lexA mutants of E. coli K-12 [13] of E. coli B and a related strain E. coli B/r). However, since these strains differ by at least one mutation other than exrA (sul[16]) that affects cell division, the interpretation of this result is difficult.

The OD changes seen in Fig. 2 are slightly greater for lexA- cells than lexA+ cells during TdR starvation. This infers that the increase in total mass during TdR deprivation is approximately the same and may be slightly greater in lexA- cultures.

**Petroff-Hausser cell count.** The total number of cells in TdR-starved lexA- cultures (Fig. 3) increases over an 8-h interval whereas the increase in lexA+ cells is very slight. This indicates that there is much more cell division during TdR starvation in lexA- cultures than in lexA+ cultures. In lexA+ cultures the number of normal-sized cells (4.0 μm or less) decreases (Fig. 4), while there is an increase in filaments (cells that are longer than 4.0 μm) (Fig. 5). This result suggests that most of the short rods in lexA+ cultures grow into filaments during TdR deprivation. The increase in the number of normal-sized cells (Fig. 4) and the total number of cells in lexA- cultures (Fig. 3) are very
similar. This suggests that the short rods are produced in lexA\(^{-}\) cultures during TdR starvation and that these probably do not elongate into filaments. Similar results were obtained with recA\(^{-}\) cells during TdR starvation by Inouye (15).

**Autoradiographs.** Typical examples of autoradiograms of lexA\(^{+}\) and lexA\(^{-}\) cultures that have been starved for TdR for 4 h are shown in Fig. 6 and 7, respectively. The following tables have been constructed from four different autoradiographic experiments. The distribution of cell sizes of all cells counted (sum of N\(^{+}\) and N\(^{-}\)) is shown in Table 2. lexA\(^{-}\) cultures have many more normal-sized cells (75\%) than filaments (25\%) during TdR starvation when compared to lexA\(^{+}\) cultures (75\% filaments). This infers that the average cell in lexA\(^{-}\) cultures is much smaller than those of lexA\(^{+}\) cultures. Since the increase in OD in these experiments is slightly larger in lexA\(^{-}\) versus lexA\(^{+}\) cultures, then the lexA\(^{-}\) cultures must contain more cells than lexA\(^{+}\) cultures, in agreement with the data in Fig. 3.

The classification of cells in the above experiment into N\(^{+}\) and N\(^{-}\) types is shown in Tables 3 and 4, respectively. There are substantially more N\(^{+}\) normal-sized rods in lexA\(^{-}\) (50\%) than in lexA\(^{+}\) cultures (25\%) (Table 3). The question as to why the lexA\(^{-}\) cultures have many more normal-sized N\(^{+}\) cells is under further investigation.

The number of N\(^{-}\) cells in lexA\(^{-}\) cultures is found to be very small (0.1\%) (Table 4). This low frequency could represent the lower limit of resolution of the autoradiographic technique for the detection of N\(^{-}\) cells. This percentage increases to 1.4\% at 4 h and 1.9\% at 8 h of TdR deprivation. This indicates that a small number of N\(^{-}\) cells are produced in lexA\(^{-}\) cultures. Unstarved lexA\(^{-}\) cultures have 2.1\% N\(^{-}\) cells. This is considerably more than lexA\(^{+}\) cultures (0.1\%), indicating that lethal sectoring is present in lexA\(^{-}\) cells as it is in recA\(^{-}\) cells (3, 4). At 4 and 8 h of TdR starvation, the N\(^{-}\) cells increase to 15.8\% and 18.4\% in lexA\(^{-}\) cultures, respectively. This result shows that there is a substantial number of N\(^{-}\) cells produced in lexA\(^{-}\) cultures during TdR deprivation. This amount (20\%) is 10-fold higher than that of lexA\(^{+}\) at the same time of starvation (2\%) and that of lexA\(^{-}\) cultures during normal growth (2\%). In summary, approximately 10 times more N\(^{-}\) cells are found in both unstarved and starved lexA\(^{-}\) cultures than are present in similarly treated lexA\(^{+}\) cultures.

**DNA degradation.** N\(^{-}\) cells could be formed by an abnormal cell division process (defined here as the sum total of the steps needed for the division of the cell and not just the step of septum formation) or by DNA degradation during TdR starvation. It has been reported that there is 15\% DNA breakdown during thymidine starvation (2) and that this breakdown is probably at the replicative fork(s) (21). The problem with measuring DNA degradation during TdR starvation is to stop the reutilization of labeled DNA precursors. To reduce such reutilization, DNA degradation experiments were performed at a low cell concentration (10\(^{6}\) cells/ml). The rate of loss of trichloroacetic acid-insoluble counts in lexA\(^{+}\) and lexA\(^{-}\) cultures (Fig. 8A) is approximately the same within a reasonable margin of experimental error. These DNA breakdown data assume that the cell number in each sample remains constant. This is probably not true because we have shown (Fig. 3) that lexA\(^{-}\) cells divide during TdR deprivation whereas lexA\(^{+}\) cells do not. If the DNA breakdown data is normalized to the total cell number (Fig. 3), the DNA degradation curves (Fig. 8B) are obtained. Approximately 10\% more breakdown per cell is calculated to occur in lexA\(^{-}\) cultures by this analysis. The implications of these results are discussed below.

**DISCUSSION**

lexA\(^{-}\) cultures are sensitive to UV (18), but not to TdR starvation. As they are starved for TdR, the lexA\(^{-}\) cells divide, and many of the normal-sized cells do not appear to elongate into filaments. This is in contrast to the lexA\(^{+}\) cultures where there are fewer cell divisions and most of the cells grow into filaments. Unstarved lexA\(^{+}\) cultures have very few N\(^{-}\) cells (0.1\%), whereas similar lexA\(^{-}\) cultures have a considerable number of N\(^{-}\) cells (2\%). There is an increase in N\(^{-}\) cells in both lexA\(^{+}\) (2\%) and
Fig. 6. Autoradiograph of TdR-starved lexA+ culture. Culture contains mostly filaments with a few normal-sized cells of which one is N-. The length of one of the small normal-sized cells is approximately 2 μm.
Fig. 7.Autoradiograph of TdR-starved lexA- culture. Culture contains some filaments and some normal-sized rods of which 5 are N-. The length of one of the smaller normal-sized cells is approximately 2 μm.
lexA (20%) cultures during starvation, indicating that N - cells are produced in both strains during TdR deprivation, but the production of N - cells in lexA - cultures is 10-fold more frequent than in lexA + cultures.

The major question that arises from these observations is: How are the N - cells which are present in lexA - cultures during TdR starvation produced? N - cells could be produced by the cell division process being abnormal or by DNA degradation in N + cells. Cell division and DNA breakdown do occur in lexA - cultures during TdR starvation and so both explanations are tenable. In the autoradiograph experiments,
cells could be seen that have the septum positioned such that there are grains on one side but not on the other. This observation supports the first hypothesis of cell division. Furthermore, 20% more DNA breakdown in the lexA− cultures would be necessary to account for the high yield of N− cells, assuming that the pattern of degradation in individual cells is the same in both lexA+ and lexA− cultures, and this is not observed (Fig. 8A and B). We conclude that some of the N− cells could arise by DNA breakdown, but that the most reasonable interpretation of our data is that cell division is perpetuated in lexA− cultures under conditions that block division in wild-type E. coli K-12 to produce N− cells.

reca− cells may react to TdR starvation in the same manner as lexA− cells, showing increased residual divisions and the production of N− cells. Inouye has also shown that reca− strains have higher levels of survival than reca+ cultures during TdR deprivation. He did not find N− cells in unstarved recA− or control recA+ cultures (15). On the other hand, Anderson and Barbour (1), working with a different reca− strain, reported no difference in survivals of starved reca− and reca+ cultures. Furthermore, Capaldo and Barbour (3, 4) have demonstrated that untreated reca− cultures contain 10% N− cells. Since there is DNA breakdown during normal growth in reca− cells (8), these N− cells could arise through DNA degradation. Clearly these discrepancies indicate that more work should be done with reca− strains. Inouye’s reca+ lexA− DNA degradation results are not in agreement with ours, because he found no DNA breakdown (15) and we show 50% DNA degradation. His procedure probably did not inhibit the reutilization of DNA precursors. His conclusion that N− cells are produced by cell division in reca− cultures during TdR starvation, based on his result of no breakdown, now appears to be open to question.

That the presence of a lexA mutation leads to the production of N− cells may or may not be related to the increase in cell number and normal-sized cells during TdR starvation. These effects show that the lexA gene product influences the process of cell division. tsl−, a UV-resistant derivative of a lexA mutant, forms filaments at 42°C (19). lexA mutations (or exrA) suppress the filamentation of tif− (λ−) cells at 42°C or of lon− cells after UV irradiation on complete medium (12, 13).

These reports provide further evidence that the lexA gene product affects the cell division process. Within (26) previously proposed that UV irradiation induces the synthesis of an inhibitor of cell division. Possibly TdR starvation has the same effect. lexA mutations are dominant and so produce a diffusible product (18). We propose that this diffusible product acts in some manner to decrease the synthesis or activity of an inhibitor of cell division during TdR starvation. This product may also influence cell division during normal growth because lexA− cultures have a significant number of N− cells and there are fewer long filamentous cells than in lexA+ cultures (Table 2).

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PRODUCTION OF CELLS WITHOUT DNA


