JOURNAL OF BACTERIOLOGY, Jan. 1968, p. 123-131
Vol. 95, No. 1

Evidence for a Relationship Between Deoxyribonucleic Acid Metabolism and Septum Formation in *Escherichia coli*

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Received for publication 9 October 1967

Septum formation is a key step in bacterial division, but the mechanism which controls periodic septum formation is unknown. In an attempt to understand this mechanism, *lon*$^-$ mutants, in which septum formation is blocked by very low doses of ultraviolet light (UV), were investigated. UV must act on some part of the apparatus of cytokinesis; thus, identification of the UV target would identify part of this apparatus. As likely possibilities, UV might damage the septum-forming site or it might damage deoxyribonucleic acid (DNA), since DNA replication is normally coordinated with septum formation. To distinguish between these possibilities, DNA was specifically sensitized by incorporating bromodeoxyuridine into *lon*$^-$ bacteria. These bacteria were strongly sensitized to longer wavelength UV (2,900 to 3,100 A) so that they failed to form septa, grew into filaments which lysed, and did not form colonies. Various control experiments supported the conclusion that UV inhibits septum formation as a result of alterations in DNA metabolism. A relationship thus exists between DNA metabolism and septum formation.

Strains of *Escherichia coli* K-12 mutant at the *lon* locus do not form septa after very mild ionizing or ultraviolet (UV) radiation (17). They are conditional cell division mutants. Studies on the biochemical basis of the failure to divide may yield information on the normal regulation of cell division.

After radiation and plating on rich medium, the *lon*$^-$ mutant grows in the form of long, nonseptate filaments (2). They attain a maximal length of about 100 times that of unirradiated cells after 4 to 5 hr of incubation and then lyse (3). Formation and lysis of filaments results in greater radiation sensitivity, since the fraction of cells killed by a given dose equals the fraction which formed filaments (2).

Filaments appear to possess nuclear regions evenly spaced along their length, and deoxyribonucleic acid (DNA) synthesis in irradiated cultures proceeds for at least 2 hr (4).

Apparently, *lon*$^-$ possesses enzymes necessary for reparing pyrimidine dimers, since it supports the growth of UV-irradiated bacteriophage T1 to the same extent as does the wild type (*lon*$^+$), and hence is Hcr$^+$ (17, 37).

As a result of the *lon*$^-$ mutation, cells become mucoid; i.e., they produce copious amounts of a polysaccharide capsule when grown at 37 C on minimal medium (17). This polysaccharide, which is composed of glucose, glucuronic acid, galactose, and fucose, has the same composition as the polysaccharide produced by wild-type strains at low temperatures (24, 25).

E. coli K-12 *lon*$^-$ mutants are similar to *E. coli* B, which is UV-sensitive and forms filaments, except that *E. coli* B is nonmucoid (12, 28, 29).

A specific part of *lon*$^-$ involved in cytokinesis must be highly sensitive to UV (and to X-ray). Identification of this target would identify part of the mechanism for cell division. The septum-forming apparatus itself might be the target. On the other hand, DNA generally is considered the most critical UV target in living organisms (14). UV effects on DNA metabolism could lead to inhibition of septum formation if DNA metabolism is directly related to septum formation. Such a relationship was predicted by Kuempel and Pardee (22), Jacob, Brenner, and Cuzin (20), and Lark (23). However, no biochemical evidence for this relation has been found.

To distinguish between these targets, a means to sensitize specifically either DNA or the septum-forming apparatus is needed. It is possible specifically to sensitize DNA by incorporating

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5-bromodeoxyuridine (BUdR) into it (5, 7, 13, 21, 36). Evidence will be presented that this procedure strongly sensitizes *lon*$^-$ bacteria to light of 2,900 to 3,100 A. We interpret these data and those presented below as evidence that UV effects on the total chromosome inhibit septum formation in *lon*$^-$ bacteria. This suggests a relationship between DNA metabolism and septum formation.

MATERIALS AND METHODS

Strains. All strains used, their characteristics, and their source or derivation are listed in Table 1. AX14 (*lon*-8$^-$) is a spontaneous mutant isolated from 2e01c (37). (This mutant is designated *lon*-8$^-$ with the approval of P. Howard-Flanders.) AX53 and AX56 are spontaneous *thy*$^-$ mutants isolated by the trimethoprim technique (33) by Sergey Shestakov in this laboratory. AX69 and AX77 are *lon*$^+$ and *lon*-8$^-$, both isogenic *thy*$^-$. AX67 was prepared from P10 (31) by selection of spontaneous mutants.

Media. The YET agar and broth (0.5% yeast extract, 1% tryptone, 1% NaCl, with or without 2.2% agar) of Howard-Flanders, Simson, and Theriot (17) were used. Thymine (50 μg/ml) was added when desired. Minimal media had 1.5% agar in M63 base (26) and were supplemented with 2 mg/ml of separately autoclaved glucose, glycerol, and Casamino Acids as desired; 50 μg/ml of amino acids and thymine as required; and 5 μg/ml of thiamine. Streptomycin was used as a filter-sterilized solution at a concentration of 200 μg/ml and was added after autoclaving the medium. Nalidixic acid (a gift from Winthrop Laboratory) was dissolved in 0.1 N NaOH, diluted, and filtered.

BUdR incorporation. The conditions for BUdR incorporation and irradiation with light of 2,900 to 3,100 A were devised by Eisenberg and Pardee (*in preparation*). Thymineless strains, growing exponentially (10^8 cells/ml) in glycerol-Casamino Acid plus thymine (100 μg/ml) minimal medium were centrifuged, resuspended in M63 base, and diluted (1:2) with glycerol (4 mg/ml), Casamino Acids (4 mg/ml), adenosine (0.5 mg/ml), uridine (0.5 mg/ml), thiamine (10 μg/ml), and BUdR (100 μg/ml) or thymine (100 μg/ml) in M63 base. All operations involving BUdR were carried out with samples shielded from light and in a dark room illuminated by a yellow lamp. Under these conditions, wild-type (*lon*$^+$) cells grew in BUdR at normal rate for over two generations. Control cultures were grown on thymine in place of BUdR.

UV irradiation (2,900 to 3,100 A). Cells were harvested from BUdR-incorporation medium by centrifugation at 4 C and resuspension in M63 base. They were kept cold until irradiated and plated. Portions (5 ml) were placed in 50-ml beakers and stirred magnetically while being irradiated.

An atmospheric pressure mercury lamp provided a line spectrum (6). A Pyrex petri dish containing 100 μg of uracil per ml (0.65 cm in depth) was used to filter out all wavelengths below 2,893 A. The effective wavelengths (and relative intensities) of each were: 2,893 A (1); 2,925 A (0.4); 2,967 A (3); 3,021 A (6); and 3,131 A (11). With this combination, BUdR adsorbs about 17 times as much light as does thymidine; equimolar BUdR plus deoxycytidine adsorb about 4 times as much as do thymidine plus deoxycytidine.

TABLE 1. *Characteristics of strains*

Strain	Sex	Characteristics[a]	Source
2e01c	F$^-$	*lac*$^-$ *thi*$^-$ *thr*$^-$ *leu*$^-$ *str-r Su-11*$^+$ λ$^-$ *lon*$^+$	M. Malamy
AX14	F$^-$	*lon*$^-$ derivative of 2e01c	2e01c
AX53	F$^-$	*thy*$^-$ derivative of AX14	AX14
AX56	F$^-$	*thy*$^-$ derivative of 2e01c	2e01c
AX69	F$^-$	*thy*$^-$ derivative of 2e01c	AX67 × his$^-$ 2e01c
AX77	F$^-$	*thy*$^-$ derivative of AX14	AX67 × *his*$^-$ AX14
P10	Hfr	*lac*$^-$ *thi*$^-$ *thr*$^-$ *leu*$^-$ *malB*$^-$ *str-s* λ$^-$ λ^R *ton-r tfi-r*; injection order *met, mtl, str, ...*	A. Newton
AX67	Hfr	*thy*$^-$ *str-r* derivative of P10	P10

[a] Genetic symbols used: *lac*; lactose; *thi*, thiamine; *thr*, threonine; *leu*, leucine; *str*, streptomycin; *Su*, suppressor gene; *thy*, thymine; *his*, histidine; *mal*, maltose; *ton*, response to bacteriophage T1; *tfi*, response to bacteriophage T5; *met*, methionine; *mtl*, mannitol; Hcr, host cell reactivation; *uvr*, ultraviolet radiation damage repair; s, sensitive; r, resistant; −, inability to synthesize or utilize.

Effective intensity was 75 ergs per mm^2 per sec, as determined by comparison of data with those of Boyce and Setlow (7). A neutral filter of aluminum screen was sometimes used to reduce intensity to 7.5 ergs per mm^2 per sec.

Plating after irradiation. Samples were diluted in M63 and plated on appropriate media. The plates were incubated in the dark.

Radioactive counting. The amount of BUdR-*2-*^{14}*C*, thymine-*2-*^{14}*C*, and thymidine-*2-*^{14}*C* incorporated into DNA was determined by counting both total trichloroacetic acid-insoluble material and isolated DNA. For trichloroacetic acid precipitation, 1 ml of culture was mixed with 1 ml of bovine serum albumin (5 mg/ml) and 2 ml of 100% trichloroacetic acid containing 1.8 mg/ml of nonlabeled BUdR, thymine, or thymidine, as needed. After 30 min in the cold, the precipitates were centrifuged and washed twice with 5% trichloroacetic acid (containing 1.8 mg/ml of nonlabeled compounds). They were again centrifuged and dissolved in 0.5 ml of Hydroxide of Hyamine (Packard Instrument Co., La Grange, Ill.). The solutions were dissolved in 15 ml of toluene plus 0.6% 2,5-diphenyloxazole (PPO) plus 0.01% 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) and counted with 40% efficiency in a Packard Tri-Carb scintillation spectrometer.

For DNA separation, the modification of the Schmidt-Thannhauser procedure described by Roodyn and Mandel (27) was used. Carrier protein (5 mg of bovine serum albumin) and DNA (3 mg of sperm DNA) were added. Precipitates were collected by centrifugation. Hydrolysis with 0.5 N NaOH for

1.75 hr was used. The final aqueous solution (3 ml) was dissolved in 15 ml of Bray's solution (naphthalene, 60 g; PPO, 4 g; POPOP, 0.2 g; ethylene glycol, 20 ml; methanol, 100 ml; *p*-dioxane, to 1 liter) and counted with 45% efficiency.

Analytical ultracentrifugation. To prepare lysates, exponentially growing bacteria (10^8 cells/ml) were centrifuged in the cold and resuspended in one-tenth the original volume in M63 base plus 2% Sarkosyl NL97 (Geigy Industrial Chemicals, Ardsley, N. Y.). They were incubated at 37 C with gentle shaking for 15 min.

Lysate and water were added to a stock solution of 65% (w/w) CsCl (Harshaw Chemical Co., Cleveland, Ohio) to give a final CsCl concentration of 52%. Approximately 0.7 ml of solution was placed in a cell containing a 12-mm Kel-F centerpiece and was centrifuged in a Spinco model E analytical ultracentrifuge at 44,770 × *g* at 25 C for 20 hr. UV-absorption photographs were made only at the end of the run. Tracings were made with a Joyce-Loebl double-beam recording microdensitometer. Densities were calculated by using the position of ^{15}N-labeled *Pseudomonas aeruginosa* DNA [density, 1.742 g/cm^3 (30)] as a reference (34).

Chemicals. Thymine, thymidine, and BUdR were obtained from Calbiochem, Los Angeles, Calif. BUdR-2-^{14}C (30.8 mc/mmole) and thymine-2-^{14}C (21 mc/mmole) also were obtained from Calbiochem. BUdR-2-^{14}C (22.8 mc/mmole), thymine-2-^{14}C (30 mc/mmole), and thymidine-2-^{14}C (30 mc/mmole) were obtained from New England Nuclear Corp., Boston, Mass.

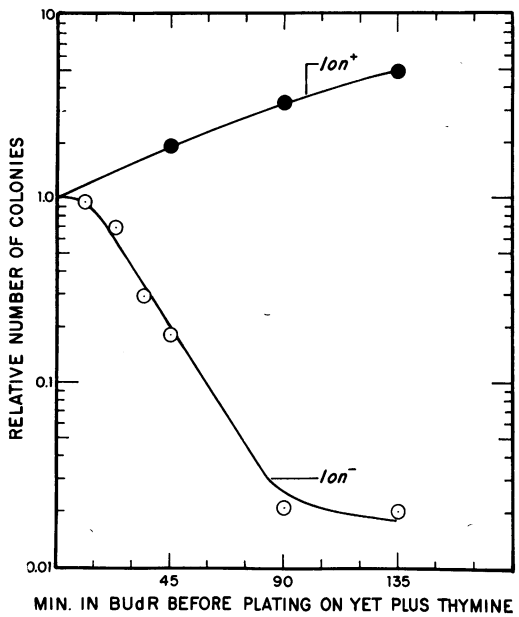

FIG. 1. *Effect of growing thymineless lon$^+$ and lon$^-$ in BUdR without irradiation. AX56 (lon$^+$) (●) and AX53 (lon$^-$) (○) were grown in BUdR for 45, 90, or 135 min, centrifuged, washed once in M63 base and plated on YET plus thymine.*

RESULTS

Production of filamentous growth of lon$^-$ by BUdR (without irradiation). Before determining the effect of BUdR on sensitivity to irradiation, it was necessary to determine the consequence of growing thymineless *lon*$^+$ and *lon*$^-$ in BUdR. BUdR was incorporated in the dark for periods up to three normal generation times (i.e., the period required for doubling in thymine medium, which was 45 min). Cells were harvested periodically and plated on YET plus thymine (Fig. 1). The *lon*$^+$ bacteria grew at almost the normal rate in BUdR for over two generations. On the other hand, 80% of the *lon*$^-$ cells were killed after one generation. This killing is not related to thymineless death, since the *lon*$^-$ used did not undergo thymineless death under conditions of thymine deprivation.

When *lon*$^+$ and *lon*$^-$ cells were transferred after one generation from BUdR into YET broth plus thymine, *lon*$^+$ grew normally, but *lon*$^-$ cells grew as long, nonseptate filaments, morphologically similar to those induced by UV. Thus, growth in BUdR alone (without irradiation) was sufficient to inhibit septum formation in a manner which seems analogous to the UV effect on these cells.

Amount of BUdR incorporated into DNA of lon$^+$ and lon$^-$. To compare BUdR sensitization of the wild type and *lon*$^-$, it was necessary to demonstrate that BUdR was incorporated in both strains to the same extent. Both *lon*$^+$ and *lon*$^-$ incorporated the same amount of BUdR into DNA during the 45-min period (Fig. 2) which was routinely used for cultures to be irradiated (e.g., those shown in Fig. 4). Thus, any difference in sensitization in the two strains was not due to different amounts of BUdR incorporated.

The BUdR incorporated during this relatively short interval was 2.8 times the amount of thymine incorporated, with the same ratio being observed in both *lon*$^+$ and *lon*$^-$. If thymidine was used, the amount incorporated was 1.3 times the amount of BUdR incorporated. The reason for higher degrees of incorporation of the deoxyribonucleosides BUdR and thymidine than of the free base thymine is unknown. A slight leakiness of the *thy*$^-$ marker is suggested. The conclusion that *lon*$^+$ and *lon*$^-$ incorporate BUdR to the same extent is not affected.

Ultracentrifuge analysis of unsubstituted and BUdR-containing DNA. It was possible, *a priori*, that DNA of *lon*$^-$ cells, growing normally, would have a buoyant density different from that of *lon*$^+$. Its density was, however, the same as that of wild-type DNA, as shown by CsCl density gradient centrifugation.

After 45 min in BUdR, the density of *lon*$^+$ and *lon*$^-$ DNA was determined to ascertain whether the patterns of DNA synthesis (in BUdR) were the same in both strains. Moreover, this served as an independent check on the amount of BUdR incorporated in the strains. Isogenic thymineless derivatives of *lon*$^+$ and *lon*-8$^-$ (which give results quantitatively similar to those of Fig. 5) were examined (Fig. 3A, B). Normal semiconservative replication occurred in both (during the 45-min period studied). In each strain, 85% of the DNA was converted to hybrid density (1.752).

An exception of possible significance must be pointed out. Another strain, also *lon*-8$^-$ but harboring a nonisogenic and independently obtained thymineless marker, did not replicate DNA normally in the presence of BUdR (Fig. 3C). Instead, material of density 1.742 and of greater heterogeneity was accumulated. The chemical nature of this DNA and its significance are being studied.

BUdR sensitization of lon$^+$ *and lon*$^-$ *to 2,900- to 3,100-A light.* Both *lon*$^+$ and *lon*$^-$ were grown in BUdR for 45 min, exposed to 2,900 to 3,100-A light, and plated on YET plus thymine. Control cultures were grown in thymine before irradiation. The *lon*$^+$ cells (Fig. 4A) were sensitized by BUdR, in agreement with data of others (5, 7, 13, 21). Aoki, Boyce, and Howard-Flanders (5) attributed this sensitization to incorporation into DNA and subsequent interference with repair enzymes.

When grown in thymine, *lon*$^-$ was more sensitive to irradiation than was *lon*$^+$. The 15% of the *lon*$^-$ which survived growth with BUdR in the dark were strongly sensitized (Fig. 4B).

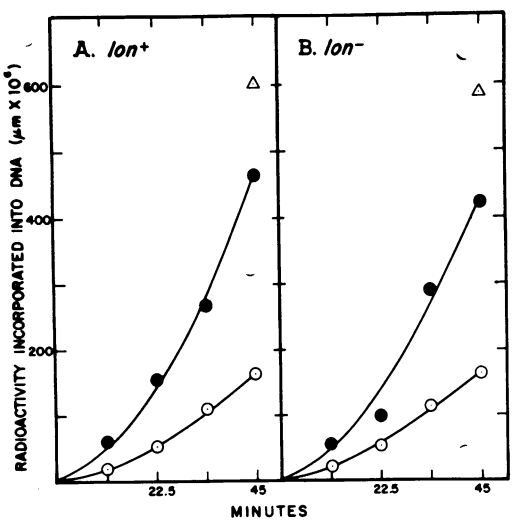

FIG. 2. *BUdR 2-*^{14}*C* (●), *thymine-2-*^{14}*C* (○), *and thymidine-2-*^{14}*C* (△) *incorporation into DNA of AX56 (lon*$^+$*) and AX53 (lon*$^-$*). Specific activity was 5.1, 3.18, and 2.68 mc/mmole, respectively. All cultures were adjusted to the same initial optical density.*

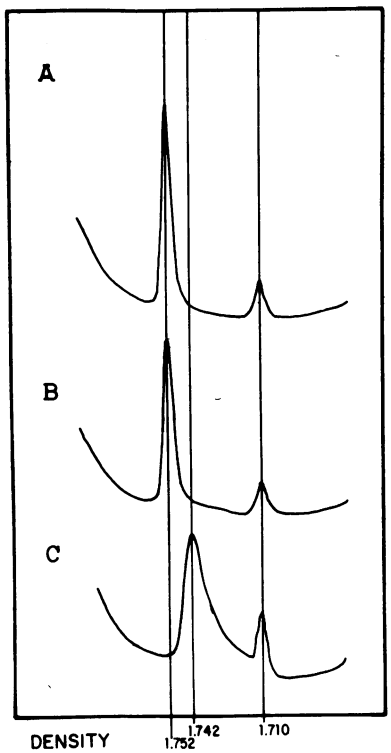

FIG. 3. *Buoyant density of BUdR-containing lon*$^+$ *and lon*$^-$ *DNA. Bacteria were grown in BUdR in the dark. (A) lon*$^+$ *(AX69) which had a thy*$^-$ *marker isogenic with (B) lon-8*$^-$ *thy*$^-$ *(AX77). (C) lon-8*$^-$ *(AX53) had an independent thy*$^-$ *mutation.*

BUdR-containing *lon*$^-$ cells, after 2,900- to 3,100-A irradiation, grew into nonseptate filaments, all of which lysed after 3 to 5 hr of incubation. Cells not converted to filaments grew and divided normally. The filaments were morphologically similar to those which result from 2,537-A irradiation. Identically treated *lon*$^+$ cells grew with normal morphology. Loss of colony-forming ability (Fig. 4B) thus reflects formation and lysis of filaments.

For quantitative treatment, the lines of Fig. 4 are replotted (Fig. 5) with viable cells at the time of irradiation normalized to 1. First, consider *lon*$^+$. Its sensitivity after growth in thymine is indicated by the final slope of the inactivation curve (k_1); the slope after growth in BUdR is designated k_2. The factor by which sensitivity was increased by BUdR ($\alpha = k_2/k_1$) was 6.

Consider now *lon*$^-$. Assuming a new, independent target, its sensitivity after growth in thymine

(k_4) is the sum of sensitivity of *lon*$^+$ cells (k_1) and sensitivity as a result of the *lon*$^-$ mutation (k_3). Sensitivity after BUdR incorporation (k_5) is the sum of BUdR sensitization of wild type (αk_1) plus any BUdR sensitization of the *lon*$^-$ gene (βk^3):

$$k_5 = \alpha k_1 + \beta k_3 \quad (1)$$

If BUdR had no effect on increasing *lon*$^-$ sensitivity, β would be equal to 1. The dotted line of Fig. 5 is calculated for survival of *lon*$^-$ after BUdR incorporation if β were 1. If BUdR increased *lon*$^-$ sensitivity by being incorporated into DNA, β should be equal to 6.

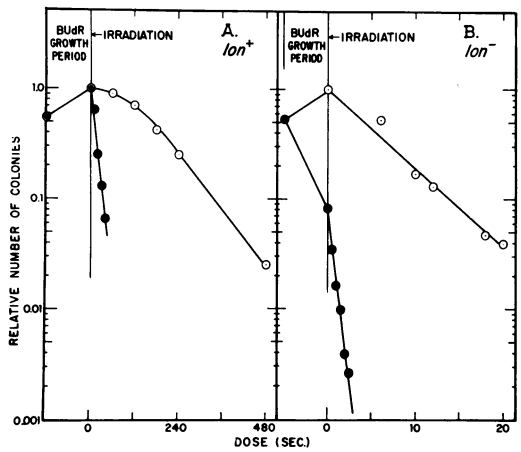

FIG. 4. *BUdR sensitization to 2,900- to 3,100-A irradiation of (A) AX56 (lon$^+$) and (B) AX53 (lon$^-$). The bacteria were grown in BUdR (●) or thymine (○) for 45 min, harvested, irradiated, and plated on YET plus thymine.*

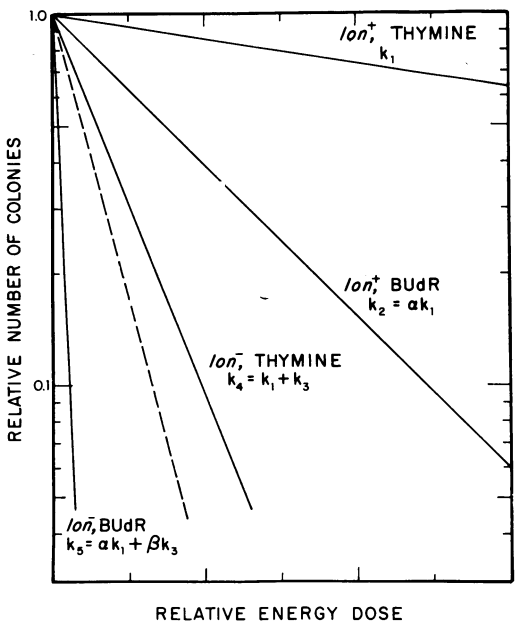

FIG. 5. *Relative sensitivity of lon$^+$ and lon$^-$ to 2,900- to 3,100-A light after growth in thymine or BUdR. For the lon$^+$, thymine curve, the shoulder on Fig. 4 has been eliminated. Dashed line would be the survival curve of lon$^-$ after growth in BUdR if BUdR had no effect on sensitivity resulting from the lon$^-$ allele.*

The extra UV sensitivity of *lon*$^-$ was strongly increased by BUdR incorporation (Fig. 5). The factor β was 8, within experimental error of α, a result which is consistent with the conclusion that DNA was the target of the radiation.

BUdR sensitization of lon$^-$ as a function of incorporation period. If BUdR sensitized *lon*$^-$ by being incorporated into macromolecules, sensitization should increase gradually over about 45 min (one normal generation time). Alternatively, if its effect were caused by incorporation into pools of low molecular weight compounds, sensitivity should be effected within a few minutes.

To distinguish between these possibilities, BUdR was incorporated into *lon*$^-$ for varying lengths of time and the cells were exposed to 2,900- to 3,100-A irradiation (Fig. 6). Sensitivity increased gradually over a 90-min period, a result consistent with the conclusion that DNA is the BUdR-containing target of irradiation.

Effect on radiation sensitivity of chasing BUdR with thymidine. If BUdR were incorporated into macromolecules, as suggested by the preceding experiment, sensitization caused by it should not be removed rapidly by incubation in thymidine. On the other hand, sensitization due to BUdR incorporation into low molecular weight compounds should disappear after a relatively short period of thymidine incorporation. A *lon*$^-$ strain was grown in BUdR for 45 min, during which time viable cells decreased 84%. One portion of the culture was irradiated, with typical results (Fig. 7); a second portion was shifted to the incorporation medium plus thymidine (without BUdR) for 45 min. During this chase, the loss of colony-forming ability due to BUdR (without irradiation) was gradually reversed. Afterwards, sensitivity to 2,900- to 3,100-A irradiation was unaltered. This result is consistent with the conclusion that sensitization to radiation is the result of BUdR incorporation into DNA.

Reversal of lon$^-$ sensitivity to BUdR and of BUdR-produced radiation sensitivity. Inhibition of cytokinesis in *lon*$^-$ by UV (2,537 A) is observed in rich medium and is readily and completely reversible by plating on minimal medium (17, 37). Division of X ray-induced filaments is promoted by growth on YET plus pantoyl lactone or incu-

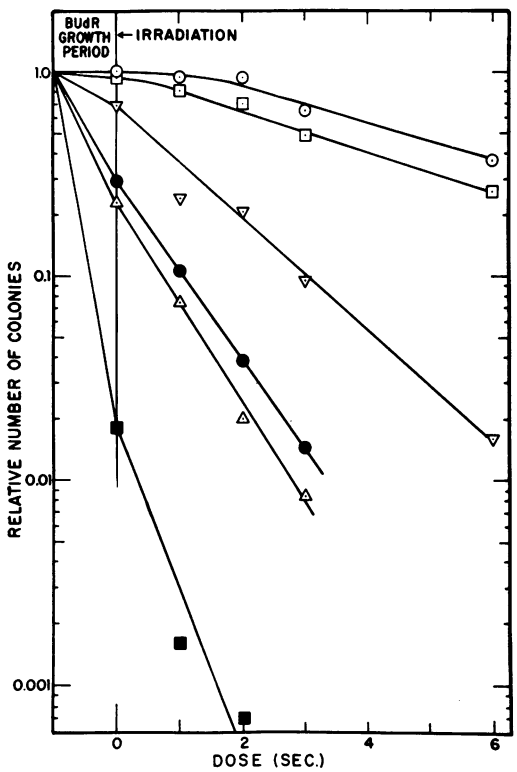

FIG. 6. *Sensitization of lon⁻ to 2,900- to 3,100-A irradiation by growth in BUdR as a function of incorporation period. AX53 was grown in the BUdR incorporation medium for the desired interval, harvested, irradiated, and plated on YET plus thymine. Intervals:* (○), *zero generations;* (□), *one-fourth generation;* (▽), *one-half generation;* (●) *three-fourths generation;* (△) *one generation;* (■), *two generations.*

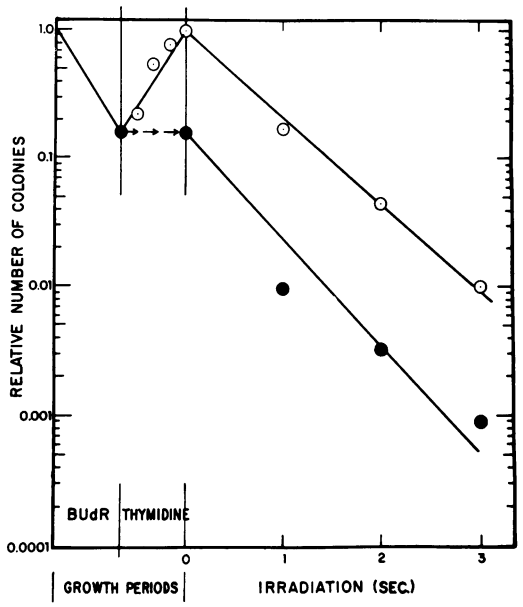

FIG. 7. *Effect of thymidine chase on BUdR sensitization to 2,900- to 3,100-A irradiation. AX53 (lon⁻) was grown with BUdR* (●) *for one generation, irradiated, and plated; or on BUdR for one generation and shifted to thymidine for one generation* (○) *and then irradiated. All plating was on YET plus thymine.*

bation at 42 C (4). Growth of *lon⁻* on minimal agar after incubation in BUdR for 45 min and after subsequent irradiation completely reversed sensitivity to these two treatments (Fig. 8). The reversal of BUdR-produced loss of colony-forming ability by growth in liquid thymidine-containing minimal medium (Fig. 7) was similar. Thus, both filament production by BUdR and by irradiation of BUdR-containing cells appear to be reversed by the same treatments as is filament induction by irradiation at 2,537 A.

Nalidixic acid production of filaments in lon⁻. The effect of a completely different, reversible inhibition of DNA synthesis on *lon⁻* is of interest, since the inhibition of septum formation by UV (2,537 A or 2,900 to 3,100 A) might be the result of transitory alterations in DNA metabolism. Nalidixic acid inhibits DNA synthesis, without interfering with protein or ribonucleic acid synthesis (10, 11). Its effect is readily reversible, for DNA synthesis resumes immediately upon its removal (11).

Both *lon⁺* and *lon⁻* cells were incubated in YET plus nalidixic acid under conditions which are know specifically to inhibit DNA synthesis, and then were shifted to YET medium without nalidixic. Optical density was used as an index of mass increase. The *lon⁺* cells quickly regained normal growth rate (Fig. 9). On the other hand, *lon⁻* cells regained normal growth rate more slowly, and total mass increase stopped after 2 hr. The *lon⁻* cells, after this temporary nalidixic acid treatment, grew as long, nonseptate filaments all of which lysed after 2 to 5 hr of incubation. The *lon⁺* cells were elongated somewhat, but were not converted to filaments under the conditions used. Thus, a temporary inhibition of DNA synthesis in *lon⁻* caused subsequent filamentous growth which continued until lysis.

DISCUSSION

UV appears to act on a macromolecule when it inhibits septum information in *lon⁻*. Loss of colony-forming ability (i.e., inhibition of septum formation) is extremely sensitive to UV, being inhibited to a greater extent than any other event, including DNA synthesis. The strain *lon-8⁻* is more sensitive than some mutants deficient in the

ability to repair pyrimidine dimers (16, 37). The dose required to cause an average of one hit per *lon*-8$^-$ cell (10 ergs/mm^2) causes about 60 to 80 chemical changes per chromosome (19, 32). At least 90% of these appear to be repaired by dark repair enzymes (18, 19) in *uvr*$^+$ *lon*$^-$ cells, by comparison with *uvr*$^-$ *lon*$^-$ mutants. Thus, less than half a dozen chemical changes per chromosome are adequate to inhibit septum formation. By comparison, cytokinesis is inhibited in 99% of the cells by a dose that had no noticeable effect on function of the single gene required for tryptophanase synthesis (37). The cytokinesis target must be nearly the size of the entire chromosome.

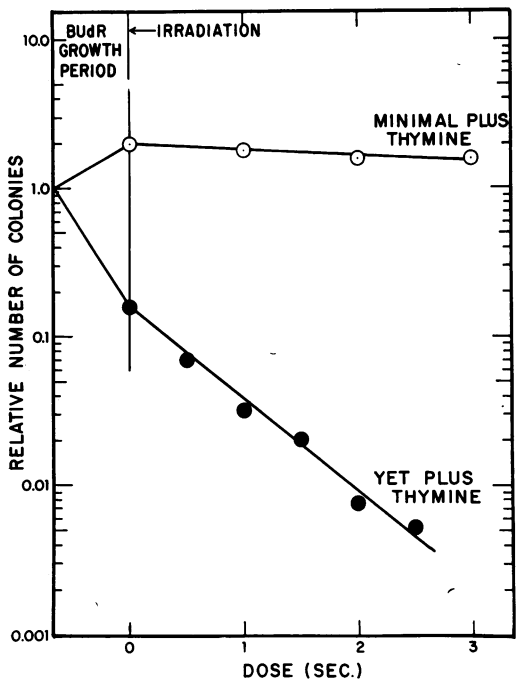

FIG. 8. *Reversal by minimal medium of lon$^-$ sensitivity to BUdR and of BUdR-produced radiation sensitivity. AX14 (lon$^-$) was grown in BUdR for 45 min. Unirradiated and irradiated (2,900 to 3,100 A) samples were placed on YET plus thymine (●) or on glucose minimal plus thymine (○).*

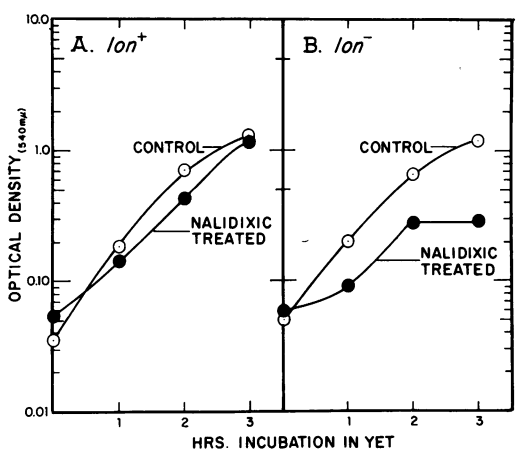

FIG. 9. *Effect of nalidixic acid on lon$^+$ and lon$^-$ growth. 2e01c (lon$^+$) and AX14 (lon$^-$) were grown in YET plus 50 μg/ml of nalidixic acid for 30 min, harvested, and shifted to YET.*

Other evidence indicating a macromolecular target is the gradual sensitization by BUdR and the persistence of this sensitivity after a thymidine chase. These results would not be expected if a low molecular weight metabolic intermediate were the target.

The UV-sensitive macromolecule appears to be DNA. BUdR increases *lon*$^-$ sensitivity, and in the same proportion as it increases sensitivity of wild-type strains. The latter effect is attributed to inhibition of repair by BUdR incorporation into DNA (5). Thus, the effect of BUdR on *lon*$^-$ probably similarly results from incorporation into DNA. [See also the similar sensitizations to X rays of bacteria and transforming DNA by BUdR (35).]

Ribonucleic acid (RNA) could not have been the target, since *E. coli* does not incorporate 5-bromouracil into RNA (8). The possibility that some thymine-containing macromolecule other than DNA is the target is not considered likely. Its size would have to be nearly that of a chromosome to account for the extreme sensitivity observed.

The manner in which BUdR kills *lon*$^-$ in the dark is unclear. Its effect is established gradually, but disappears after one generation of growth with thymidine.

Howard-Flanders, Simson, and Theriot (18) prepared *uvr*$^+$ *lon*$^+$, *uvr*$^-$ *lon*$^+$, *uvr*$^+$ *lon*$^-$, and *uvr*$^-$ *lon*$^-$ strains. The *uvr*$^+$ *lon*$^+$ to *uvr*$^-$ *lon*$^+$ mutation increased UV sensitivity by a factor of 10 to 60. The *uvr*$^+$ *lon*$^-$ to *uvr*$^-$ *lon*$^-$ mutation increased sensitivity over that caused by the *lon*$^-$ allele by a factor of 60. These results suggest that DNA repair mechanisms of *uvr*$^+$ *lon*$^-$ strains reverse most of the UV effect and that retention of DNA lesions is important for filament formation. It follows that septum formation is not inhibited by the excised photoproducts which are produced by *uvr*$^+$ bacteria.

Inhibition of DNA synthesis is adequate to account for these results, since nalidixic acid causes *lon*$^+$ bacteria to form filaments but has little effect on transcription of DNA under the conditions used (S. Barbour, J. Mol. Biol., *in press*). The accumulation of some DNA precursors might be responsible for the effect of UV, as has been proposed for UV induction of lysogenic bacteria (9, 15). Witkin (38) has recently proposed

a model for filament formation in *E. coli* B in which UV action on the chromosome was assumed to promote the formation of a division inhibitor.

The following observations obtained with *lon*⁻ mutants relate septum formation, cell envelope, and DNA metabolism. (i) Modified DNA metabolism blocks septum formation in *lon*⁻. (ii) The *lon*⁺ allele is dominant over *lon*⁻ and restores cytokinesis if introduced into an irradiated *lon*⁻ (37). (iii) Cytokinesis inhibition is readily reversible by a variety of chemical and physical conditions, including extracts of *E. coli* (1). (iv) The *lon*⁻ mutation also results in overproduction of capsular polysaccharide (17). Markovitz (24) suggested that *lon*⁻ bacteria lack a repressor which limits biosynthesis of the polysaccharide. (v) The capsular polysaccharide, per se, is not responsible for filament formation, since mucoid colonies are formed on minimal agar, on which irradiated *lon*⁻ cells survive to the same extent as do irradiated *lon*⁺ cells, but little polysaccharide is formed on enriched medium on which UV sensitivity is exhibited. Moreover, Adler et al. (1) isolated from a *lon*⁻ strain, a nonmucoid mutant which retained characteristic UV sensitivity.

These results are consistent with our earlier suggestion (37) that UV similarly damages the link between DNA replication and septum formation in both *lon*⁺ and *lon*⁻, but recovery occurs in *lon*⁺. This recovery is inhibited by cell envelope metabolites produced in rich medium by *lon*⁻ cells. This model is represented schematically in Fig. 10. Compound A is imagined to be overproduced in *lon*⁻ mutants. It is converted to mucoid capsule in minimal medium. This compound, the steady-state concentration of which is higher in the rich medium, is imagined to block restoration of the link between DNA replication and septum formation.

These events in *lon*⁻ might derive from modification of a control mechanism that coordinates septum formation, DNA replication, and cell envelope synthesis in normally growing bacteria.

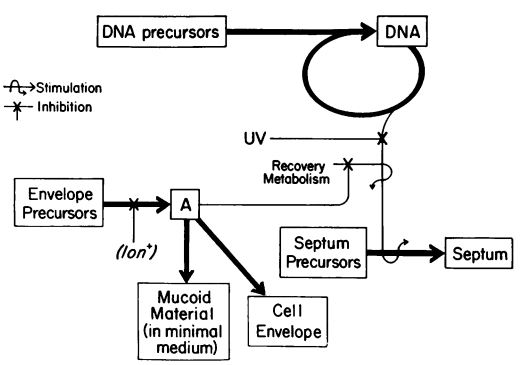

FIG. 10. *Model for UV-produced filamentous growth of lon⁻.*

Possibly, positive and negative cross inhibitions and activations (at the gene level or the enzyme level, as shown here for simplicity) could set up oscillations which periodically initiate septation. These could be switched by irradiation of *lon*⁻ to a permanent inability to initiate septation and subsequently to form colonies.

This model is highly tentative, as would be expected from the complexity of the problem and the shortage of data. For example, compound A might, with appropriate modifications, activate rather than inhibit.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-04409 from the National Institute of Allergy and Infectious Disease and by National Cancer Institute Fellowship 1-F2-CA-29, 413-01.

We are grateful to Noboru Sueoka and Robert Armstrong for discussions and a gift of *P. aeruginosa* ^{15}N-labeled DNA. We acknowledge the technical assistance of Etheria Robinson and we thank Barbara Bamman and L. R. Hyde for performing the ultracentrifugation.

ADDENDUM IN PROOF

While this manuscript was in press, E. P. Kirby, F. Jacob, and D. A. Goldthwait (Proc. Natl. Acad. Sci. U.S. **58**:1903, 1967) described a strain of *E. coli* K-12 (λ), which carries a mutation in the host cell which results in the induction of λ when grown at 40 C, although growth is normal at room temperature. If the strain is cured of its prophage, it forms filaments at elevated temperatures. They suggested that levels of purine and pyrimidine derivatives in the small molecule pool may be factors in prophage induction and in filament formation.

A. Markovitz and B. Baker (J. Bacteriol. **94**:388, 1967) presented genetic evidence that the product of the *lon* gene is a protein.

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