Molec. gen. Genet. 168, 69–80 (1979)

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Degradation of *Escherichia coli* DNA: Evidence for Limitation in vivo by Protein X, the *recA* Gene Product

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Summary. DNA is more extensively degraded after it is damaged in *recA* mutants of *E. coli* than in wild type cells. All data presented here are consistent with the *recA* gene product, protein X, being an inhibitor of nalidixic acid induced degradation of the bulk DNA (but not of newly replicated DNA). Production of protein X also is correlated with appearance of various "S.O.S." repair functions. Evidence was obtained by comparing the rates of protein X synthesis and solubilization of uniformly-labeled DNA in intact cells, incubated in the presence of nalidixic acid. A set of mutants at the *lexA* locus produced protein X at different rates and degraded their DNA at rates which were inversely correlated to their rates of protein X production. A low concentration of rifampicin quite specifically inhibited protein X production by wild type *E. coli*, and allowed more rapid DNA degradation. After the DNA was damaged by the incubation of cells in the presence of nalidixic acid, cells preloaded with protein X degraded their DNA more slowly. We propose that protein X could protect DNA against degradation by binding to single-stranded regions, thereby inhibiting nuclease action.

Introduction

The *recA* dpendent, inducible repair system of *Escherichia coli* is important in a variety of processes that follow DNA damage (Witkin, 1976). These processes, for convenience called "S.O.S." functions, include inhibition of DNA degradation, some types of DNA repair, phage induction, mutation, and inhibition of residual cell division. Genes involved include *recB* and *recC*, which code for exonuclease V (Marsden et al., 1974; Mackay and Linn, 1976) and *recA* and *lexA*, which limit DNA degradation (Witkin, 1976). *recA* also affects cell division after DNA damage (Inouye, 1971; Satta and Pardee, 1978). Biochemical events that may be related to S.O.S. functions include protease activity (Roberts and Roberts, 1975), creation of single-stranded DNA (Sussman and Ben Zeev, 1975), production of DNA degradation products (Gudas and Pardee, 1975; Smith and Oishi, 1978), and appearance of protein X of 40,000 M.W. (Inouye and Pardee, 1970). A similar inducible DNA repair system in *Bacillus subtilis* has been reported (Yasbin, 1977).

Protein X was originally described by Inouye and Pardee (1970) and was independently found by Siccardi et al. (1971), Sedgwick (1975), and McEntee et al. (1976). It is synthesized extremely rapidly, and it becomes at least 3% of the total protein, under the same conditions that generally induce S.O.S. functions (Gudas and Pardee, 1976). Its synthesis depends on *recA* and *lexA* genes, also required for expression of the S.O.S. functions (Gudas, 1976). A *recA* mutant was shown not to produce protein X (Inouye, 1971). The *recA* gene, originally proposed to be regulatory (Gudas and Pardee, 1975; Gudas, 1976), has now been shown to be the structural gene for protein X, both by transduction (McEntee, 1977; Little and Kleid, 1977) and because certain *recA* mutants, when combined with a specific *lexA* locus mutation to allow expression of protein X, produce protein X molecules with altered charges (Gudas and Mount, 1977).

A defective *recA* gene has long been associated with extensive "reckless" DNA degradation (How-

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ard-Flanders and Theriot, 1966; Marsden et al., 1974). We propose that the *recA*$^+$ gene product, protein X, limits DNA degradation. This hypothesis is tested by several new approaches. We show that rifampicin increases the rate of DNA degradation following damage to cellular DNA caused by incubation of cells in the presence of nalidixic acid. We propose that this effect is due to the specific inhibition of protein X induction by rifampicin, and that the lower concentration of protein X is less able to limit DNA degradation. By using temperature-sensitive mutants in combination with rifampicin, we have been able to modify the amount of protein X in a cell, either before or during DNA damage. In all cases, the rate of DNA degradation was inversely related to the amount of protein X present.

We studied DNA degradation induced by nalidixic acid within intact cells because these in vivo conditions avoid artifacts that could be created using purified protein X, isolated DNA and nucleases. Further in vitro studies should elucidate details of the mechanism.

Materials and Methods

Strains used were *E. coli* B/r; *E. coli* B/r (P1)-32, which is a lysogenic derivative obtained by infecting *E. coli* B/r with phage P1Ke, as described by Luria et al. (1960); *E. coli* NH5013 which is *recA44*, a temperature-conditional mutant (Hall and Howard-Flanders, 1975); AB1157 (*thr-1, leu-6, argE3, his-4, proA2, thi-1*); DM1187 (*tif-1, sfiA11, lexA3, spr-51*); DM1180 (*tif-1, sfiA11, lexA3*); DM1451 and DM1285 (derivatives of DM1187 with mutations at *lexA*) (Gudas and Mount, in preparation, 1978; Mount, 1977); and *rpoB225*, a mutant with modified RNA polymerase, and its parental strain (Fietta and Silvestri, 1975). The general methods used have been described (Gudas and Pardee, 1976).

DNA Labeling. Cell DNA was labeled uniformly by growing the cells overnight in M9 medium containing ^{3}H-thymidine, 5 μCi/ml, and 200 μg/ml of cold uridine. Cells grown overnight were always diluted with fresh medium not containing thymidine and incubated until they were growing exponentially. Cell DNA was pulse-labeled by adding 30 μCi/ml of ^{3}H-thymidine to growing cells for 1 min. Labeling was terminated and nalidixic acid treatment was initiated by washing and resuspending the cells with M9 medium containing an excess of cold thymidine plus nalidixic acid 30 μg/ml. At intervals TCA-precipitable radioactivity was determined, to measure DNA degradation (Marsden et al., 1974).

Determination of Radioactivity. Only TCA-precipitable radioactivity was determined. Samples of 0.1 ml each were transfered in triplicate to Whatman paper filter discs, 1.5 cm in diameter, and immediately dropped into cold 10% TCA. After 30 min the filters were washed twice with cold 5% TCA, twice with methanol and twice with acetone, dried and each one transfered into a vial containing 5 ml of a scintillation fluid of the following composition: PPO 4 g, POPOP 150 mg, and toluene 1 liter.

Gel Electrophoresis. Sodium dodecyl sulfate slab gel electrophoresis (Laemmli, 1970) was employed using gels containing either 12% or 15% acrylamide. The following proteins were used as molecular weight standards and run on the gels: β-galactosidase (130,000, Worthington); phosphorylase A (92,000 to 100,000, Worthington); bovine serum albumin (68,000, Sigma); pyruvate kinase (57,000, Sigma); Ovalbumin (43,000, Sigma); lactic dehydrogenase (36,000, Sigma); carbonic anhydrase (29,000, Sigma); chymotrypsinogen (25,000 to 26,000, Sigma); myoglobin (17,200, Sigma); lysozyme (14,300, Sigma); and cytochrome c (11,700, Sigma). A mixture of the proteins was made up 1:1 with final sample buffer, mercaptoethanol was added, and the mixture was incubated 5 min at 100° C.

Results

Inhibition of Protein X Production by Rifampicin. We have found that 4 μg/ml rifampicin selectively affects

Table 1. Interference with several S.O.S. functions by rifampicin and CAP

Function studied	Control	Rifampicin 4 μg/ml	CAP 1.5 μg/ml
Protein synthesis after 1 hr growth in the presence of the antibiotic[a]	100%	75%	80%
Induction of bacteriophage P1[b]	3.2×10^9/ml	0.13×10^9/ml	2.8×10^9/ml
Survival after UV-irradiation[c]	4%	0.54%	3%
Residual division in nalidixic acid-treated cells[a]	25%	80%	22%

[a] For methodology see Satta and Pardee, 1978. Protein synthesis was measured by rate of ^{3}H-leucine incorporation into TCA-insoluble material

[b] A culture of strain B/r (P1)-32 exponentially growing in M9 medium was subdivided into three parts: to one rifampicin was added; to another, chloramphenicol (CAP); the third was the control sample. After 2 min a 2 ml sample was taken from each culture to evaluate the P1 titer as described by Luria et al. (1960). Mitomycin, 1 μg/ml (final concentration), was added to remaining cultures. After 2, 4, and 6 hr incubations, samples were taken from all cultures to evaluate the phage P1 titer. For each culture the highest phage titer obtained at the three interval times has been recorded. The control, CAP, and rifampicin cultures before mitomycin treatment contained 2×10^3, 1.3×10^3, and 2.6×10^3 P1 phages/ml, respectively. Strain B/r was used as indicator strain for P1 phage plating

[c] A culture of *E. coli* B/r exponentially growing in M9 was resuspended in phosphate buffer (0.1 M, pH 7.2), plated to evaluate the CFU (colony forming units), and UV-irradiated by a 10 s exposure in the dark to a Desaga UV lamp (60 watts) at a distance of 10 cm. The culture was then subdivided into three parts. To one rifampicin was added, and to another, chloramphenicol (CAP); the third was the untreated control. The three samples were then incubated at 37° C for 2 hr and plated in the dark in triplicate on plates with rifampicin, CAP, and on control plates not containing drugs. The percentage of survivors in each sample was calculated by comparing the CFU values immediately before irradiation with those calculated from the abovementioned plates after 72 hr incubation

both DNA degradation and protein X synthesis, indicating that these phenomena could be related. In preliminary experiments low concentrations of rifampicin also interfered noticeably with several S.O.S. functions (Table 1). Rifampicin increased the number of cell divisions occurring after DNA synthesis was inhibited (Satta and Pardee, 1978), reduced cell survival after UV-irradiation, and reduced P1 phage induction. As a control, chloramphenicol (CAP), at a concentration that inhibited protein systhesis similarly, did not markedly influence most of these functions.

Protein X was produced by adding nalidixic acid, probably as a consequence of DNA degradation (Gudas and Pardee, 1976; Smith and Oishi, 1978; although also see Little and Hanawalt, 1977). Appear-

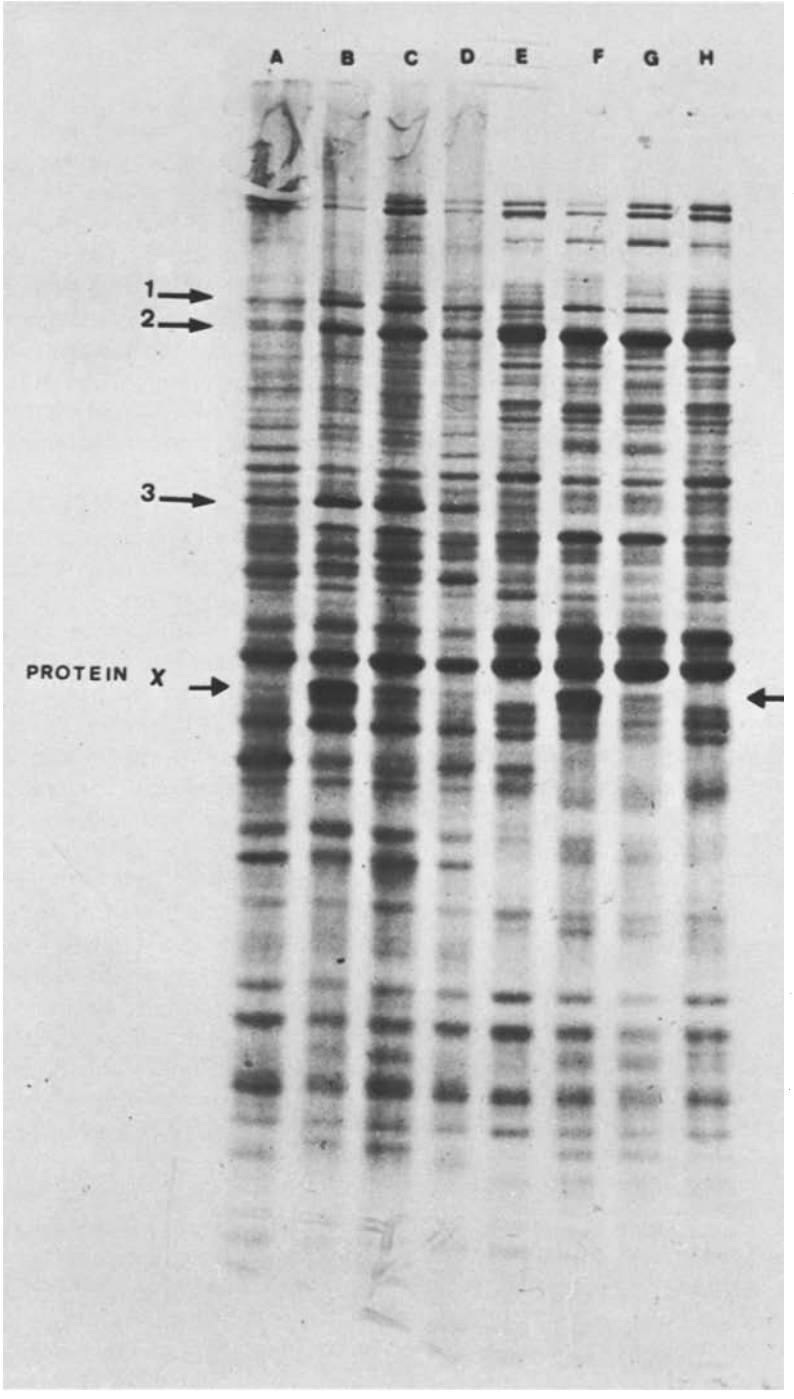

Fig. 1. Effect of rifampicin on the rate of synthesis of protein X. A culture of *E. coli* B/r exponentially growing in M9 medium was subdivided into 4 parts. To two of these samples rifampicin (4 μg/ml) was added. After 20 min incubation at 37° C, nalidixic acid (30 μg/ml) was added to one sample containing rifampicin and to one without rifampicin. All samples were then incubated at 37° C for an additional 40 min and pulsed 5 min with [^{35}S]-methionine. Radioactive protein preparation and gel electrophoresis were as described (Gudas and Pardee, 1976): (a) to (d), inner membrane proteins; (a) of the untreated control cell; (b) of the cells grown with nalidixic acid only; (c) of the cells grown with nalidixic acid and rifampicin; (d) of the cells grwon with rifampicin only. (e) to (h) cytoplasmic proteins, in the same order as (a) to (d). The location of protein X was determined in relation to the positions of reproducible major bands

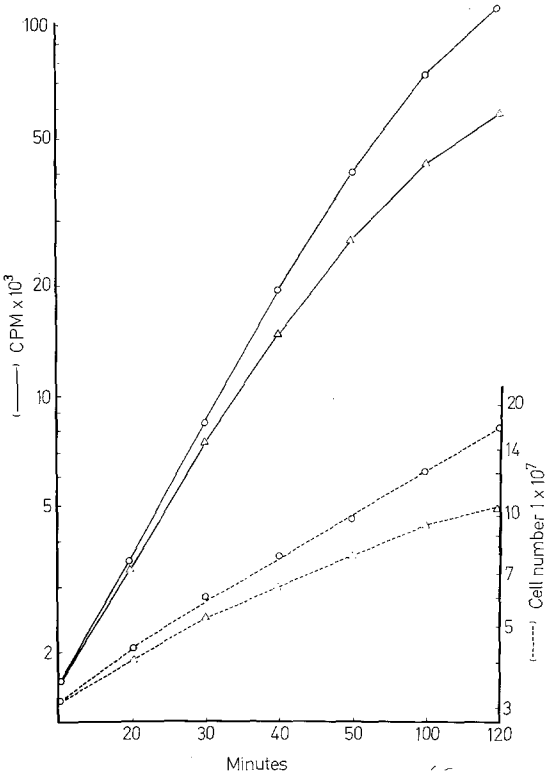

Fig. 2. Effect of 4 μg/ml rifampicin on both [^{3}H]-leucine incorporation and cell division of *E. coli* B/r. A culture growing exponentially in M9 medium was subdivided into two parts. One was incubated without further treatment (○—○). To the other was added rifampicin 4 μg/ml (△—△). At intervals samples were taken. Both the cell number (dashed lines) and the [^{3}H]-leucine incorporated into TCA-precipitable material (solid lines) were evaluated as described in the text

ance of protein X was markedly inhibited by a low concentration of rifampicin (Fig. 1). Rifampicin was at least 85% inhibitory (by gel densitometry) after 40 min' exposure to nalidixic acid, when the rate of protein X synthesis was highest. By contrast, total protein synthesis was inhibited by no more than 25% (Fig. 2). No striking differences were seen in the patterns of other membrane or cytoplasmic proteins. It is important to note the specificity of inhibition: nalidixic acid induced several proteins, such as inner membrane proteins 1, 2, and 3, indicated by arrows in Figure 1, but aside from protein X, none of these was selectively inhibited by rifampicin. Furthermore, this same dose of rifampicin slowed down the rate of cell division but did not reduce the colony-forming units of *E. coli* B/r cultures on Difco DST plates after 72 h incubation.

Other antibiotics (chloramphenicol, streptomycin, and tetracycline at 2 μg/ml, not shown), at concentrations which inhibited total protein synthesis by about 25%, did not specifically affect the rate of protein X synthesis, although they influenced synthesis of some other proteins relative to the majority (Fig. 3). Therefore, rifampicin cannot simply inhibit protein X synthesis as a consequence of inhibition of total protein synthesis or by a general effect of antibiotics.

Specific inhibition by rifampicin could occur if induction of protein X were a several-step process which requires the synthesis of several proteins or messenger RNAs in sequence, e.g., if sequential inhibitions were cumulatively expressed. This several-step mechanism is unlikely: when rifampicin was added 0, 20, 50, or 115 minutes after nalidixic acid, subsequent inhibition of protein X production was as shown in Fig. 1.

To determine whether binding of rifampicin to RNA polymerase was necessary to inhibit protein X induction, a mutant with altered RNA polymerase β-subunits unable to bind rifampicin and its parent strain were compared (Fietta and Silvestri, 1975), Rifampicin had no effect on the induction of protein X by nalidixic acid in this mutant strain. In addition, the mutants' rate of synthesis of protein X, measured after 1 h of induction, was 20% lower than the rate of synthesis of protein X by the parental *E. coli* strain K12 with wild type RNA polymerase, indicating that a structural change in the polymerase (β-subunits) affected induction of protein X.

To determine whether inhibition of RNA synthesis is responsible, actinomycin D, which inhibits RNA synthesis without binding RNA polymerase, was used with *E. coli* cells made permeable after EDTA treatment (Leive, 1965). Actinomycin D at 8 μg/ml reduced [^{3}H]-leucine incorporated into TCA-precipitable material similarly to rifampicin at 4 μg/ml. Fig. 4 shows that actinomycin D caused no apparent effects on the rate of protein X appearance in membrane (nor in cytoplasm, not shown). The induction of a membrane protein of roughly 80,000 daltons M.W. appears to depend on the EDTA treatment; it was not seen in the unpermeabilized cells, although it was present in all the permeabilized samples whether or not they were treated with nalidixic acid or actinomycin D. Other changes caused by EDTA are also evident. In this same experiment rifampicin inhibited the induction of protein X in non-permeabilized cells by 70%. Thus, specific inhibition of protein X induction is apparently a property of rifampicin that is separate from inhibition of RNA synthesis.

Effect of Rifampicin on the Rate of DNA Degradation. Nalidixic acid causes an increased rate of DNA degradation (Cook et al., 1966; Boyle et al., 1969; Hill and Fangman, 1973) (Fig. 5). The rate was higher if rifampicin was also present: 74% of the radioactivity became TCA-soluble in contrast to only 30% in cells treated with nalidixic acid alone. As a control, rifam-

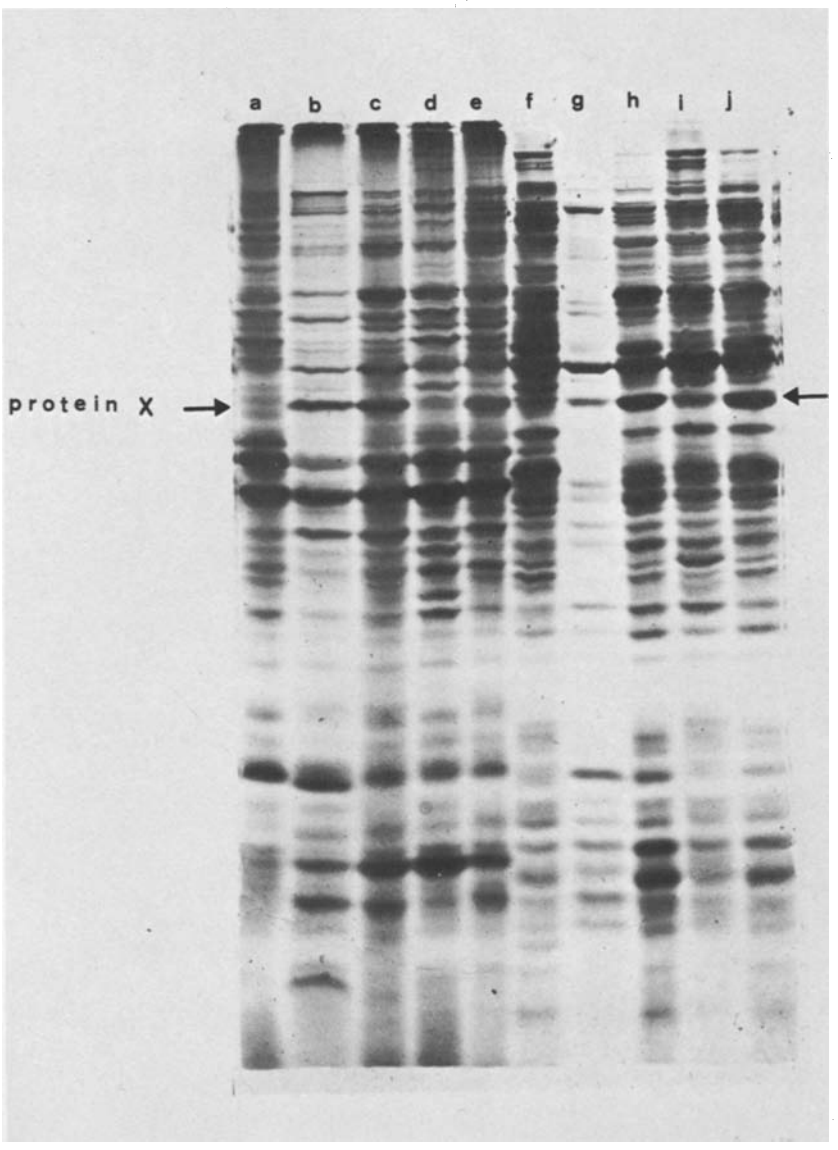

Fig. 3. Effect of chloramphenicol (1.5 μg/ml), rifampicin (4 μg/ml), and streptomycin (2 μg/ml) on the induction of protein X. A culture of *E. coli* B/r exponentially growing in M9 medium was subdivided into 5 parts. One of the above antibiotics was added to one part each and all were incubated 20 min at 37° C. Nalidixic acid (30 μg/ml) was added to these three samples plus one of the remaining two and all were incubated for an additional 55 min. After that, all the samples were pulsed with [^{35}S]-methionine and radioactie protein preparation and gel electrophoresis were done. (a) to (e), total membrane proteins of cells: (a) untreated control, (b) incubated with nalidixic acid only, (c) with nalidixic acid and chloramphenicol, (d) with nalidixic acid and rifampicin, (e) with nalidixic acid and streptomycin. (f) to (j) cytoplasmic proteins in same order as (a) to (e)

picin alone increased the degradation only moderately; not more than 18% of the label was solubilized within 8 h. The results are consistent with the hypothesis that rifampicin inhibits synthesis of protein X and thereby enhances the degradation of DNA in the presence or absence of nalidixic acid.

If there is a direct relation of protein X to DNA degradation, rifampicin's ability to block protein X synthesis should have relatively little effect on DNA degradation after the cells have produced protein X. Degradation of uniformly labeled DNA following nalidixic acid addition depended greatly on the subsequent time of rifampicin addition (Fig. 6). Rifampicin added during the first 30 min enhanced DNA degradation, but added later, it did not show a measurable effect on DNA degradation. It did not even cause the 15–20% increase in DNA degradation which it had produced in untreated cells (Fig. 5). Furthermore, when cells were allowed to accumulate protein X by 30 min treatment with nalidixic acid followed by resuspension in fresh medium, without nalidixic acid, rifampicin did not cause them to degrade DNA any more rapidly during the following 4 h (not shown).

Inverse Correlation of Protein X Production and DNA Degradation in lexA Mutants. A set of mutants which map at the *lexA* locus (Gudas and Mount, in preparation) produced protein X at different rates after nalidixic acid was added (Fig. 7). With the exception of DM1285, those that produced the most protein X degraded their DNA the least rapidly, whithin experimental error (Fig. 8). DM1285, although it produced

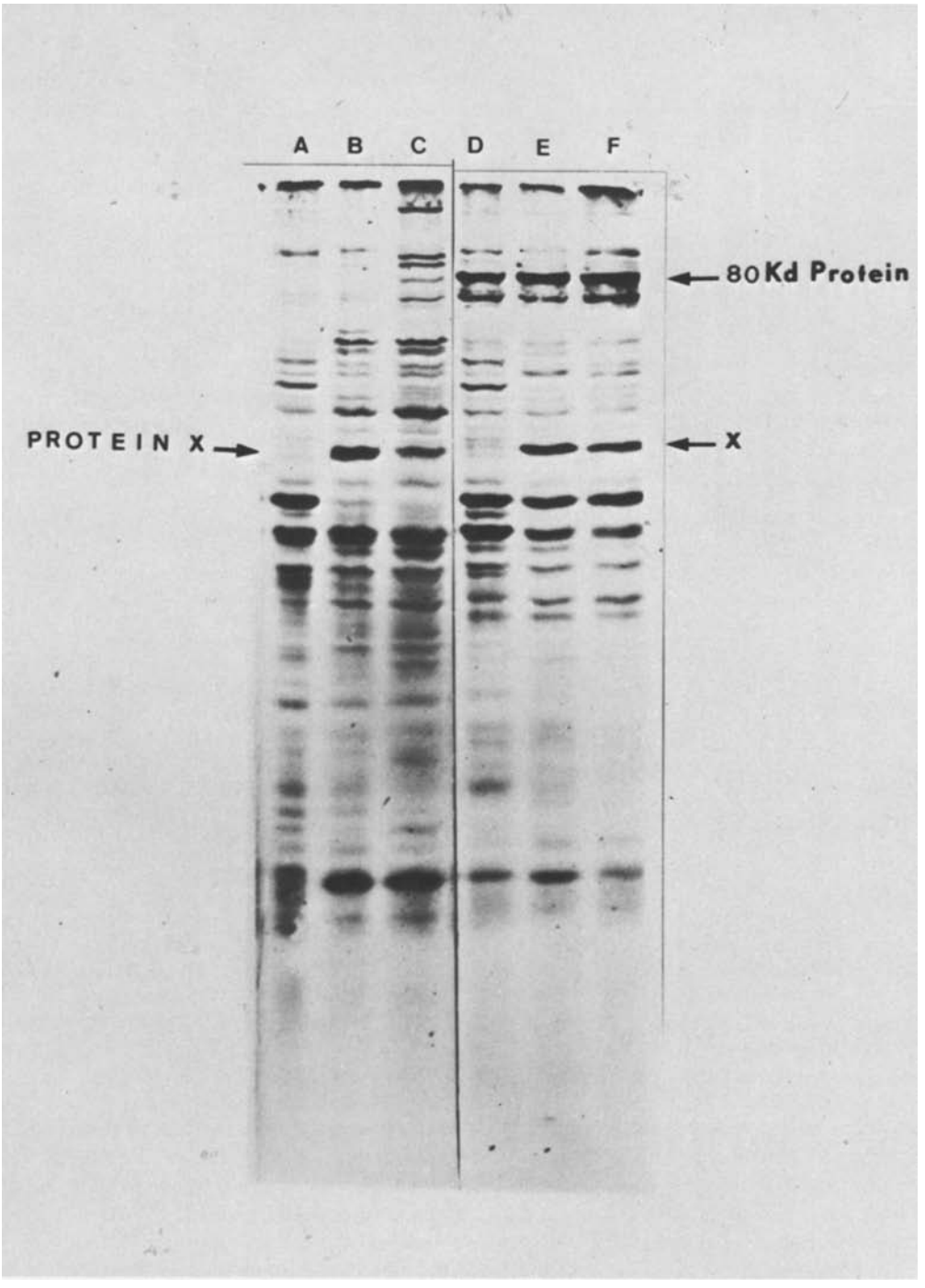

Fig. 4. Effect of actinomycin D on the induction of protein X. The experimental procedure was as described in Figure 3 except that cells to be treated with actinomycin D (8 μg/ml) but not those treated with rifampicin were first permeabilized with EDTA as described (Leive, 1965). (a) to (c), total membrane proteins of unpermeabilized cells: (a) ntreated control cells, (b) cells treated with nalidixic acid, (c) cells treated with nalidixic acid and rifampicin. (d) to (f) total membrane proteins of permeabilized cells: (d) cells not treated with antibiotics, (e) cells treated with nalidixic acid, (f) cells treated with nalidixic acid and actinomycin D

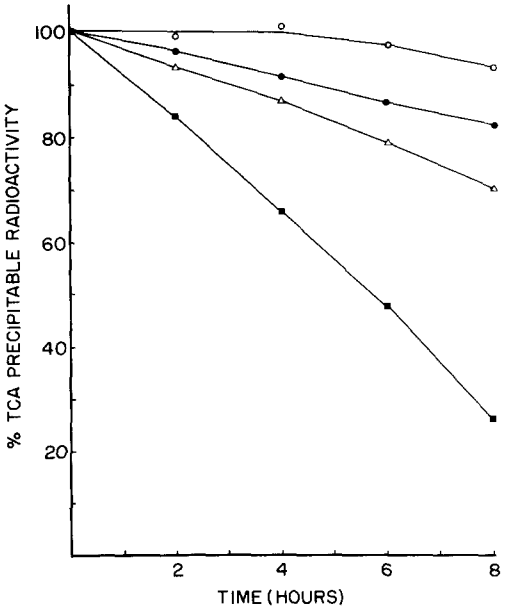

Fig. 5. Degradation of DNA in uniformly labeled cells treated with nalidixic acid, rifampicin, nad nalidixic acid plus rifampicin. A 40 ml culture of *E. coli* B/r growing exponentially in M9 medium not containing the radioactive label, at the optical density of 0.3 optical units and uniformly prelabeled with ^{3}H-thymidine as described, was subdivided into four parts. One was incubated without additional treatments (o—o), to one nalidixic acid 30 μg/ml was added (△—△), to one rifampicin 4 μg/ml (●—●), and to the last nalidixic acid 30 μg/ml plus rifampicin 4 μg/ml (■—■). At intervals samples were taken and the TCA-precipitable radioactivity was evaluated as described in "Materials and Methods"

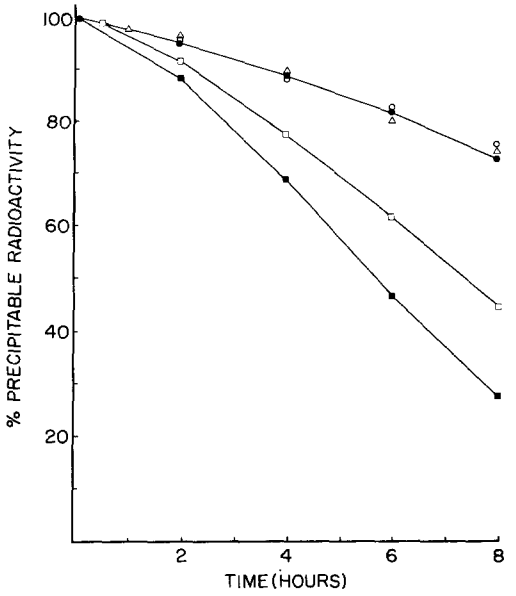

Fig. 6. Effect of time of addition of rifampicin on the rate of degradation of uniformly labeled DNA in cells treated with nalidixic acid. To a 50 ml culture of *E. coli* B/r growing exponentially in M9 medium not containing radioactive label, at an optical density of 0.3 optical units and uniformly prelabeled with ^{3}H-thymidine as described, nalidixic acid 30 μg/ml was added. One 10 ml sample was incubated at 37° C without further treatment (●—●). To the other 10 ml samples at 37 4 μg/ml of rifampicin was added at 0 min, (■—■), 30 (□—□), 60 (△—△), and 120 (o—o) min, and incubation continued at 37° C. At intervals samples were taken to evaluate TCA-precipitable radioactivity

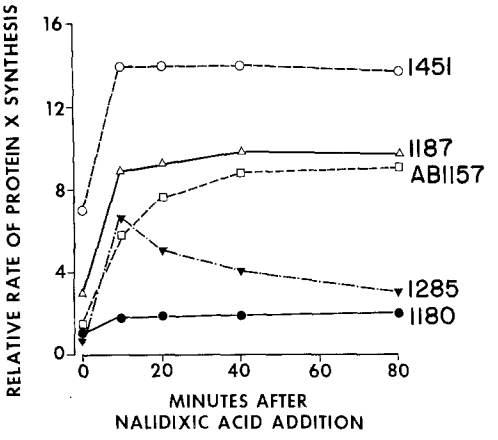

Fig. 7. Rate of protein X synthesis after nalidixic acid addition. The following *E. coli* strains, all of which have mutations at the *lexA* locus except AB1157, were grown exponentially at 30° C. At various times after addition of 40 μg/ml nalidixic acid, 1.0 ml samples of the mutants were pulsed with 5 μCi of [^{35}S]-methionine (about 400 Ci/mmol), SDS-gel elctrophoresis was performed, and densitometry tracings of the protein X peaks were measured (Gudas and Pardee, 1976). The numbers on this and the next figure refer to corresponding DM mutants

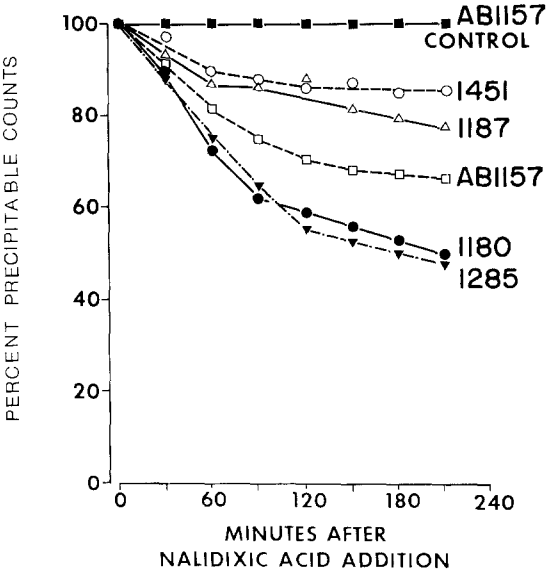

Fig. 8. Percent DNA degradation after nalidixic acid addition. Exponential *E. coli* as used in Figure 7 were grown in M9 medium in the presence of 20 μCi/ml, [^{3}H]-thymidine (20 Ci/mmol), and 1 mM uridine for the final 4 h. The bacteria were centrifuged to remove the [^{3}H]-thymidine, and resuspended in fresh medium to which 40 μg/ml nalidixic acid was added. At intervals samples were taken to evaluate TCA-precipitable radioactivity

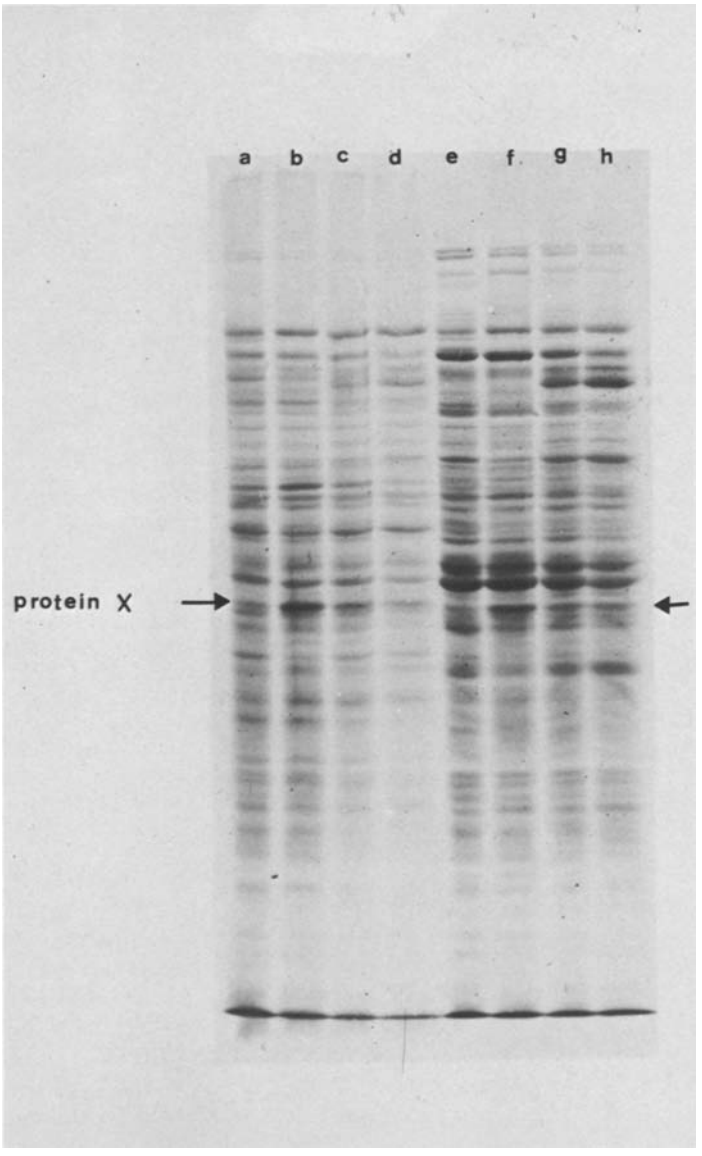

Fig. 9. Electrophoretic pattern of the protein from *recA444* grown at the permissive and nonpermissive temperature, both in the presence and the absence of nalidixic acid. A culture of *E. coli* NH5013 growing exponentially in M9 medium was subdivided into two samples which were incubated, one at 30° C and the other at 44° C. After two hours incubation each sample was subdivided into two parts. Nalidixic acid, 30 μg/ml, was added to one part; the other was the untreated control. After 40 min incubation all four samples were pulsed 5 min with [^{35}S]-methionine. Inner membrane and labeled protein preparation, electrophoresis and autoradiography were as described by Gudas and Pardee (1976). (a) to (d), inner membrane proteins of the (a) untreated control cells grown at 30° C, (b) of the cells treated with nalidixic acid at 30° C, (c) of the untreated control cells grown at 44° C, (d) of the cells treated with nalidixic acid at 44° C, (e) to (h), cytoplasmic proteins of the above samples

protein X early following addition of nalidixic acid, lost this ability, possibly explaining why it degraded its DNA similarly to DM1180 in which protein X was barely induced.

Experiments with a ts recA Mutant. *recA* mutants degrade DNA extensively, both during exponential growth and after treatment with agents which inhibit DNA synthesis. They do not produce protein X under any conditions, which initially suggested a relation between *recA* and protein X (Inouye, 1971). These facts suggested that protein X exerts a protective effect against DNA degradation (Gudas and Pardee, 1975).

A recently isolated mutant *recA444* (Hall and Howard-Flanders, 1975) produced protein X following nalidixic acid treatment at 30° but not at 44° (Fig. 9). We compared DNA degradation at these two temperatures. At 30° the rates of DNA degradation in *recA444* were the same under all conditions as for the wild type (see Fig. 5). At 44° *recA444* spontaneously degraded its DNA much faster (Fig. 10) than at 30°, as expected. The DNA degradation was even more rapid in the presence of nalidixic acid. An important observation is that rifampicin did not enhance DNA degradation in these cells at 44° either with or without nalidixic acid addition; at this temperature they did not induce more protein X.

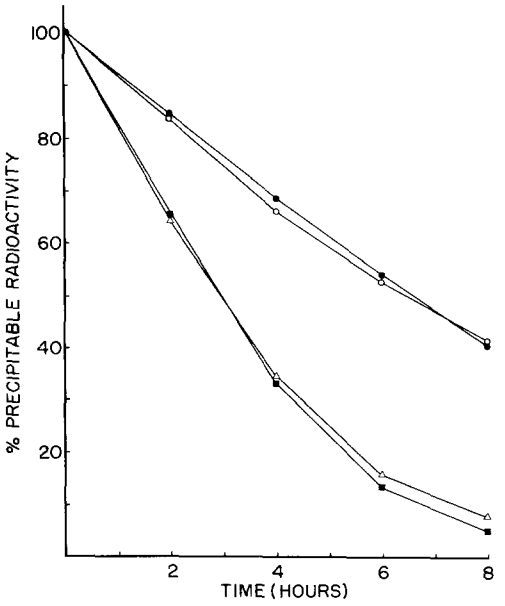

Fig. 10. Degradation of DNA in uniformly prelabeled cells of the *recA44* strain. A 40 ml culture of *E. coli* NH5013 uniformly prelabeled with ^{3}H-thymidine as described in the text was grown exponentially in M9 medium not containing radioactive label at an optical density of 0.3 optical units at 44° C. It was subdivided into four parts. One was incubated without treatment (○—○), to one was added nalidixic acid 30 μg/ml (△—△), to one rifampicin 4 μg/ml (●—●), and to one nalidixic acid 30 μg/ml plus rifampicin 4 μg/ml (■—■). Samples were taken at intervals for measurements of DNA degradation

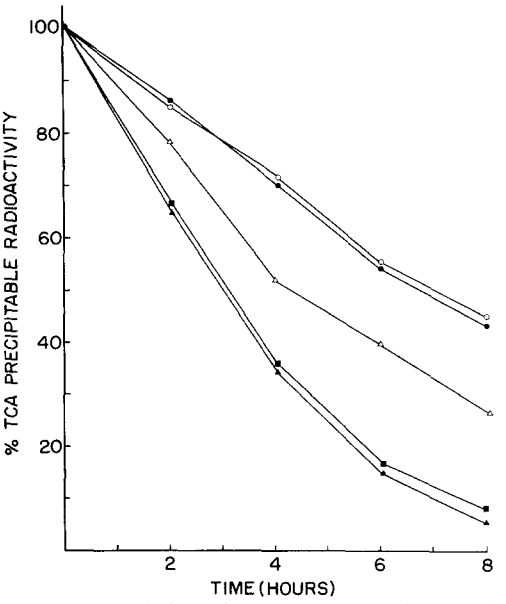

Fig. 11. Degradation of DNA in uniformly labeled cells of the *recA44* strain of *E. coli* shifted to the nonpermissive temperature. A 50 ml culture of *E. coli* NH5013 uniformly prelabeled with ^{3}H-thymidine growing exponentially in M9 medium not containing radioactive label, at an optical density of 0.3 optical units at 30° C was subdivided into five parts. One part was incubated without treatments (○—○), to one was added nalidixic acid 30 μg/ml (△—△), to one, rifampicin 4 μg/ml (●—●), and to one, nalidixic acid 30 μg/ml plus rifampicin 4 μg/ml (▲—▲). After one hour the four samples were shifted to 44° C and incubation was continued. Nalidixic acid was added also to the fifth sample, initially at 44° (■—■). TCA-precipitable radioactivity was evaluated at intervals

In the next experiment, the *recA44* cells were preincubated at 30° with nalidixic acid (to permit induction of protein X) or with nalidixic acid and rifampicin (to inhibit this protein X induction), and then were compared for DNA degradation at 44° (Fig. 11). Preincubating with nalidixic acid at 30° clearly slowed the DNA degradation at 44°. Preincubating with rifampicin alone at 30° did not influence the rate of DNA degradation at 44°. However, when both drugs were added during preincubation (and also were present at 44°), DNA degradation at 44° was faster than it was for cells pretreated with nalidixic acid only, and quite close to the rate for cells not pretreated but exposed to nalidixic acid only at 44°. Thus, if protein X was first produced at 30°, the rate of DNA degradation subsequently at 44° was decreased.

Degradation of Newly Labeled DNA. To determine whether protein X can protect the DNA near the replicating fork from degradation, we studied release of radioactivity from DNA labeled during a 1-min pulse (Fig. 12). Loss of label followed a different pattern from that observed with uniformly labeled cells, after nalidixic acid was added (Fig. 12). Radioactivity was very slowly released for the first hour; afterwards it was released much faster so that 75% of the radioactivity was solubilized within the following 140 min. Since protein X synthesis reaches its greatest rate after about an hour of exposure to nalidixic acid, a protecting effect of protein X does not seem likely from this experiment. Rifampicin at 4 μg/ml did not increase the label loss to any significant extent, in contrast to stimulating degradation of uniformly labeled DNA (Fig. 5).

Cells also were treated first for 30 min with nalidixic acid to accumulate protein X, washed and labeled for 1 min with ^{3}H-thymidine. Then nalidixic acid was added again. Release of the label by these cells was no slower than it was for cells in which protein X had not been previously accumulated (Fig. 12). These preliminary results indicate that protein X does not affect degradation of DNA near the growth point, in contrast to its affect on bulk DNA.

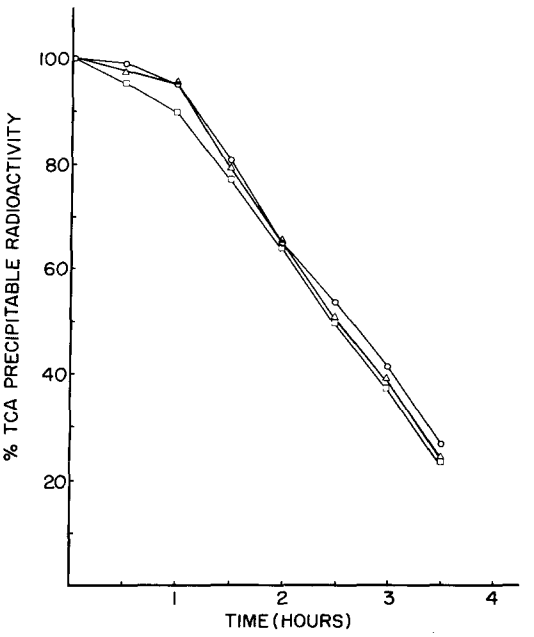

Fig. 12. Degradation of DNA in pulse-labeled cells treated with nalidixic acid or nalidixic acid plus rifampicin. Two-thirds of a 30 ml culture of *E. coli* B/r growing exponentially in M9 medium at an optical density of 0.3 optical units were pulse-labeled for 1 min with ^{3}H-thymidine. To this culture was immediately added nalidixic acid, 30 μg/ml, and it was subdivided into two parts. To one rifampicin, 4 μg/ml, was added (△—△); the second (○—○) received no further addition. To a third part (□—□) nalidixic acid, 30 μg/ml, was added first for 30 min at 37° C, and the drug was removed by filtration; then the cells were resuspended in prewarmed fresh medium and pulsed 1 min with ^{3}H-thymidine. Immediately, nalidixic acid, 30 μg/ml, was restored. Samples from all three cultures were taken at intervals during incubation at 37° C to determine the TCA-precipitable radioactivity

Discussion

We previously proposed that protein X maintains a balance between degradative activities and DNA repair enzymes, probably by limiting nuclease activity following damage to DNA (Gudas and Pardee, 1975). Protein X would first be induced by DNA degradation products, including the oligonucleotides containing thymine dimers that are formed after UV-irradiation, and then would automatically suppress its own induction as it blocked further degradation, and as repair occurred.

Further observations with bleomycin also suggest that protein X induction is linked to DNA degradation. Bleomycin causes DNA degradation by interacting directly with DNA (Suzuki et al., 1969; Haidle, 1971; Ross and Moses, 1976). It induces protein X, even under conditions where nalidixic acid is ineffective, as in synchronous *dnaA* at the non-permissive temperature after the end of DNA replication, and in *recB* and *recC* mutants (Gudas and Pardee, 1976) which are deficient in repair-nuclease activity (Oishi, 1969). Nalidixic acid presumably causes DNA degradation only if there is an active replication fork at the time the drug is added (Cook et al., 1966; Ramareddy and Reiter, 1969).

Our present data are consistent with protection by protein X of uniformly prelabeled DNA from extensive DNA degradation after nalidixic acid addition. We present four kinds of evidence to support this hypothesis: (1) A set of *lexA* mutants exhibited a general inverse relation between protein X synthesis and DNA degradation. The constitutive level of protein X present at the time of nalidixic acid addition in the various *lexA* derivatives is probably important in confering some resistance to DNA degradation. However, since the rate of synthesis of protein X increases dramatically after nalidixic acid addition in both the wild type and the mutants, the contribution of the protein X initially present in the mutants at the time of nalidixic acid addition to the final level of protein X induced is negligible. Thus, the rate of induction of protein X is more important for protection from DNA degradation after nalidixic acid addition than the constitutive level of protein X in the mutants. Also, it is not clear that the constitutive protein X is in an active state in the *lexA* derivative (Gudas and Mount, 1977). (2) Rifampicin added with nalidixic acid to cells increased the rate of DNA degradation. We propose that it does so by its specific ability to diminish the rate of induction of protein X, this smaller quantitiy of protein X being less able to inhibit DNA degradation. (3) Prior synthesis of protein X made the effects of rifampicin on DNA degradation negligible, as would be expected if the main role of rifampicin is to block the synthesis of this protein. (4) Rifampicin had no effect on DNA degradation in a *recA* mutant, under conditions where the cells could not synthesize protein X. Further support for the suggestion that protein X limits DNA degradation comes from pretreatment experiments which provide the cells with protein X prior to exposure to nalidixic acid. Thus, with *recA44*, after protein X was induced by pretreatment with nalidixic acid for 1 h at 30°, the rapid DNA degradation observed in the control culture at 44° was markedly decreased. However, this was not observed when preincubation at 30° was done in the presence of rifampicin, which inhibits protein X induction. We propose that nalidixic acid pretreatment induces protein X, rifampicin prevents its synthesis, and the level of preformed protein X determines the rate of DNA degradation at 44°. Since the protein is inactivated at 44° our interpretation rests on the assumption that the self-regulatory properties of the *recA*

protein are inactivated more effectively than those which prevent degradation.

The molecular mechanism by which rifampicin specifically inhibits protein X induction is not clear. The beta subunit of RNA polymerase is involved because rifampicin did not inhibit protein X induction in a mutant whose RNA polymerase does not bind rifampicin. However, a general inhibition of RNA synthesis cannot be the basis for selective inhibition of protein X synthesis, since actinomycin D did not have a similar effect. RNA polymerase could be directly involved in regulating expression of the *recA* gene, similar to the way that arginine biosynthesis is reported to be regulated by a complex between RNA polymerase and the gene *argR* (Wozny et al., 1975). Formation of a complex of a DNA fragment with RNA polymerase might similarly be necessary for induction of protein X. Another possibility is that rifampicin might inhibit induction of protein X by affecting DNA replication rather than transcription. RNA polymerase is known to be important in both initiating DNA replication and in extending the replication fork (Gefter, 1975; Dressler, 1975).

Preliminary experiments revealed a much faster degradation of the most recently replicated DNA than of the bulk DNA. There was no protection of this recently replicated DNA by conditions that increased the level of protein X. Other experiments show a general inhibition of S.O.S. repair functions by rifampicin. To the degree that rifampicin is specific for protein X synthesis in contrast to general events of cell synthesis and growth, our results support a role of protein X in the various S.O.S. functions, including that of limiting DNA degradation.

Protein X is made by wild type *E. coli* when DNA is damaged, and by several DNA synthesis conditional mutants at the non-permissive pemperature (Inouye and Pardee, 1970; Gudas, 1976). It has been suggested to inhibit cell division (see Satta and Pardee, 1978). Less protein X is produced in mutants with various alterations in the S.O.S. functions, it is not induced in *recA* and in *lexA* mutants, and it can bind to single-stranded DNA; these results suggested that this protein also may protect DNA from degradation, and in particular, against the *recB*, *recC* nuclease (Gudas and Pardee, 1975).

With the discovery that the *recA* gene codes for protein X (McEntee, 1977; Gudas and Mount, 1977; Little and Kleid, 1977), it is not surprising that this protein is important in determining the rate of DNA degradation. All our data show a correlation between the presence of the *recA* protein (protein X) and a lower rate of DNA degradation, by both genetic and inhibitor studies. However, other factors could be involved in protecting DNA against degradation. The system is complex and incompletely understood (Witkin, 1976), and there is a report that *rnm* mutants do not fit this pattern (Volkert et al., 1976; Witkin, personal communication). Thus, there might be other agents that protect DNA, either by themselves or in concert with protein X. In vitro studies of DNA degradation, as influenced by protein X and by other components purified from wild type and mutant cells, should clarify the situation.

Acknowledgments. This work was aided by grant CA-11595 from the U.S. Public Health Service, Department of Health, Education, and Welfare.

G.S. was supported by a NATO-CNR Scholarship. We are indebted for the following gifts: to Dr. B.M. Shapiro for the *dnaA* ts mutant, to Dr. P. Howard-Flanders for the *recA44* mutant, to Dr. L.G. Silvestri for the RNA polymerase mutant and its parent, and to Dr. D. Mount for the *lexA* mutants. We acknowledge the assistance of D. Schneider and J. Ragland preparing this manuscript.

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Communicated by A.J. Clark

Received December 9, 1977 / September 13, 1978