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Model for Regulation of *Escherichia coli* DNA Repair Functions

(*recA*$^-$, *lex*$^-$ mutants/cell division/DNA binding proteins)

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ABSTRACT **A feedback loop for the regulation of the *rec/lex*-mediated DNA repair system is proposed. This model was formulated from experiments on the genetic and metabolic regulation of the rate of synthesis of protein X performed in this laboratory, and from genetic data obtained in other laboratories. Protein X is proposed to prevent DNA degradation by the *recBC*-coded exonuclease.**

The model states that: (*1*) The *lex* (or *exrA* in *E. coli* B) gene codes for a repressor. (*2*) This repressor binds to an operator region of DNA consisting of the *tif-zab* region at 51 minutes on the *E. coli* chromosome. (*3*) The operator region controls the production of several proteins involved in DNA repair, including protein X. (*4*) The *recA* gene product is required to remove the *lex*-coded repressor from the operator. The *recA* gene could code for an antirepressor (inducer protein or a protease) or a modifier of *recBC* nuclease action. (*5*) Low molecular weight products of DNA degradation are effectors that activate the system. (*6*) Protein X limits *recBC* nuclease action by binding to single-stranded DNA.

The *rec* genes code for components of a major system for DNA repair and recombination in *Escherichia coli*. The *recB* and *recC* genes carry structural information for exonuclease V, an ATP-dependent deoxyribonuclease (1). The *recA* gene limits the degree of this degradation by a hitherto unknown mechanism (2).

In addition to being recombination deficient, *recA*$^-$ mutants have many pleiotropic effects, such as high sensitivity to ultraviolet (UV) (3) and x-irradiation (4), inability to induce λ phage by UV (5) or thymine starvation (6), lack of mutability following UV-irradiation (7), and effects on cell division, such as the ability to divide in the absence of DNA synthesis (8).

Several other genes have effects that are related in their properties to the *rec* system. *lex*$^-$ mutants (*exrA*$^-$ in *E. coli* B) are similar to *recA*$^-$ mutants except that *lex*$^-$ mutants are not deficient in recombination (9). Moreover, the *lex*$^-$ mutation maps at 81 min on the *E. coli* chromosome, whereas the *recA* gene maps at 51 min (9, 10). Another mutation that maps within 0.04 min of *lex*$^-$ on the conjugation map is *tsl*$^-$. *tsl*$^-$ mutants were isolated as UV-resistant derivatives from *lex*$^-$ mutants, and they are temperature-sensitive for cell division, forming filaments at high temperature (11).

The *tif*$^-$ mutation, which maps very close to the *recA* and *zab* genes (12), mimics the effects of UV-irradiation at high temperature even though DNA synthesis is not inhibited. In the *tif*$^-$ mutant, induction of λ (13), increased mutability (14), and inhibition of cell division (13) take place at the nonpermissive temperature. Furthermore, the *tif*$^-$ mutant is suppressed by *recA*$^-$, *lex*$^-$ or *zab*$^-$ mutations (15). Finally, the addition of adenine or certain furfural derivatives to a *tif*$^-$ mutant lysogenic for λ causes λ induction even at the permissive temperature, 30° (16).

The pleiotropic effects of these mutations and the genetic mapping data, combined with the study of protein X, which is induced by the inhibition of DNA synthesis and binds to DNA, lead to a model for the regulation of DNA repair and related events.

Abbreviation: UV, ultraviolet.

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MATERIALS AND METHODS

Bacterial Strains. The bacterial strains used in this study are described in Table 1 (manuscript in preparation). Strains were grown in glucose M-9 medium at 37°, unless they were temperature-sensitive, in which case 30° was the permissive and 41° or 42° the restrictive temperature (8).

Labeling with [^{35}S]Methionine. Exponentially growing bacteria were labeled with [^{35}S]methionine (5 μCi/2.0 ml sample) for 5 min or 10 min at 37°, unless indicated otherwise in the figures. The bacteria were then poured onto frozen M-9 medium containing a 1000-fold excess of nonradioactive methionine to stop incorporation. The incorporation was linear over the labeling period.

Membrane Preparation. Membranes were prepared by sonication followed by differential ultracentrifugation (to be published). Soluble (cytoplasmic) proteins were obtained after cell sonication. These proteins remained in the supernatant after a 100,000 × *g* centrifugation for 40 min.

Gel Electrophoresis. Sodium dodecyl sulfate slab gel electrophoresis was performed as described in Laemmli and Favre (17).

Autoradiography and Microdensitometry. These techniques were performed as described (to be published).

DNA-Cellulose Chromatography. Essentially the method of Alberts and Herrick was used (18). A more detailed description of the method is given elsewhere (manuscript in preparation).

Chemicals. Bleomycin (Blenoxane) was a generous gift from Bristol Laboratories, Syracuse, N.Y. [^{35}S]Methionine (100–400 Ci/mmol) was purchased from New England Nuclear, Boston, Mass. Nalidixic acid was purchased from Sigma, St. Louis, Mo.

RESULTS

Protein X is induced during inhibition of DNA synthesis by several agents, including nalidixic acid, in wild-type *E. coli* K-12 or B/r (ref. 19; manuscript in preparation), and constitutes 3–4% of the total cell protein within 1 hr. It is found in the same relative proportion in both the cytoplasm and the

TABLE 1. *Production of protein X*

Bacterial strain		Source	30°	41°	Nalidixic acid*	Bleomycin†
E. coli B		This laboratory	−	−	++	++
E. coli B	*recA*	R. James	−	−	−	−
E. coli B	*exrA*	R. James	−	−	−	−
E. coli K-12	AB1157	D. Mount	−	−	++	++
E. coli DM49	*lex-3*	D. Mount	−	−	−	−
E. coli DM511	*lex-3 tsl-1*	D. Mount	+	++	+	NT
E. coli DM936	*lex-3 tsl-1 recA56*	D. Mount	+	++	+	NT
E. coli DM938	*recA56*	D. Mount	−	−	−	−
E. coli JC5489	*recC22*	A. J. Clark	−	−	−	+
E. coli JC5743	*recB21*	A. J. Clark	−	−	−	+
E. coli JC2926	*recA13*	A. J. Clark	−	−	−	−
E. coli JC5495	*recA13 recB21*	A. J. Clark	−	−	−	−
E. coli $WP2_s$	*tif*$^+$	E. Witkin	−	−	++	NT
E. coli $WP44_s$	*tif*$^-$	E. Witkin	+	++	++	NT

− Denotes absence of protein X synthesis; + denotes synthesis of protein X (+ = low amount of protein X; ++ = high amount of protein X). NT indicates not tested.

* Nalidixic acid was added at a final concentration of either 15 μg/ml or 40 μg/ml.

† Bleomycin was added at a final concentration of 5 μg/ml or 50 μg/ml.

membrane; the presence of protein X in the membrane was shown not to be due to contamination of the membrane with cytoplasmic protein X during membrane preparation (to be published).

Inouye first reported that a *recA*$^-$ mutant lacks protein X (8). That result led to this study, an examination of the ability of various temperature-sensitive cell division mutants and DNA repair mutants to produce protein X (20).

We have measured the rate of synthesis of protein X by the following methods. The various bacterial mutants were pulsed with [^{35}S]methionine for short periods at various times following the addition of nalidixic acid or the removal of thymine to stop DNA synthesis. Both cytoplasmic and membrane proteins were prepared and separated by sodium dodecyl sulfate discontinuous polyacrylamide slab gel electrophoresis. A quantitative measurement of the rate of synthesis of protein X was made by microdensitometry of autoradiograms of the slab gel (to be published).

A representative gel autoradiogram of membrane proteins from the three strains AB1157 (*lex*$^+$), DM49 (*lex-3*), and DM511 (*lex-3 tsl-1*) is shown in Fig. 1. Samples were pulsed with [^{35}S]methionine at various times after the addition of nalidixic acid. The detailed kinetics of induction of protein X in the various mutants is reported elsewhere (manuscript in preparation).

An example of a densitometry tracing of an autoradiogram of membrane proteins is shown in Fig. 2. In an *E. coli* B *rec*$^+$ strain, synthesis of protein X is turned on after the addition of nalidixic acid. However, in the *E. coli recA*$^-$ strain, no protein X is induced by the inhibition of DNA synthesis.

The results obtained from many such gels made from the various mutants are concisely summarized in Table 1. It can be seen that in the wild-type strains AB1157 and B protein X is synthesized at a rapid rate only after the addition of nalidixic acid. In either a *recA*$^-$, *recB*$^-$, *recC*$^-$, *recA*$^-$ *recB*$^-$, or *lex*$^-$(*exrA*$^-$) strain no protein X is synthesized after the addition of nalidixic acid, or at the high temperature (41°).

The *tsl*$^-$ mutant, the *tsl*$^-$ *recA*$^-$ mutant, and the *tif*$^-$ mutant synthesize protein X constitutively at 30°, and at an even faster rate at 41° even though DNA synthesis appears to be normal at 41° in these mutants. These three mutants also synthesize protein X after the addition of nalidixic acid (Table 1).

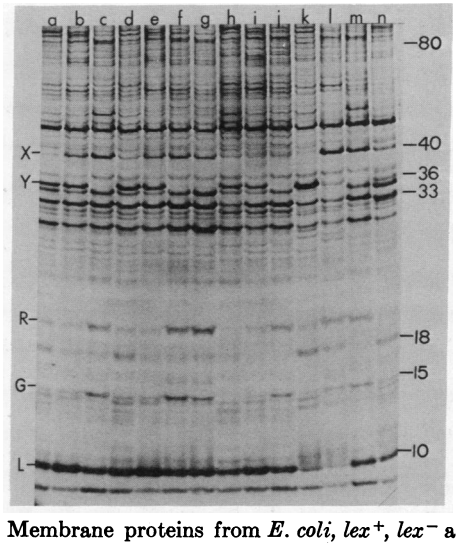

FIG. 1. Membrane proteins from *E. coli*, *lex*$^+$, *lex*$^-$ and *lex*$^-$-*tsl*$^-$ strains after nalidixic acid addition. The three strains *E. coli* AB1157 (*lex*$^+$*tsl*$^+$), DM49 (*lex-3*,) and DM511 (*tsl-1 lex-3*) were growing exponentially at 30° in glucose M-9 medium. Nalidixic acid was added at a final concentration of 40 μg/ml, and at various times thereafter samples were removed and pulsed with [^{35}S]methionine for 10 min at 30°. Isotope incorporation, membrane preparation, and gel electrophoresis were performed as described in *Materials and Methods*. The same amount of ^{35}S radioactivity was applied to each slot on the 12% slab gel. This figure is a photograph of the autoradiogram of the membrane protein gel. Molecular weights (in thousands) are indicated on the right of the figure. Proteins of interest are named on the left of the figure. The slots contained the following membrane preparations. The time of pulsing with [^{35}S]methionine after nalidixic acid is in parentheses. (a) AB1157, control; (b) AB1157 (10 min); (c) AB1157 (40 min). (d) *lex-3 tsl-1* (*tsl*$^-$), control; (e) *tsl*$^-$ (10 min); (f) *tsl*$^-$ (40 min); (g) *tsl*$^-$ (70 min). (h) *lex-3* (*lex*$^-$), control; (i) *lex*$^-$ (10 min); (j) *lex*$^-$ (40 min). (k) *E. coli* B/r, control; (l) B/r (40 min); (m) repeat of (c); (n) *tsl*$^-$ at 42° for 40 min.

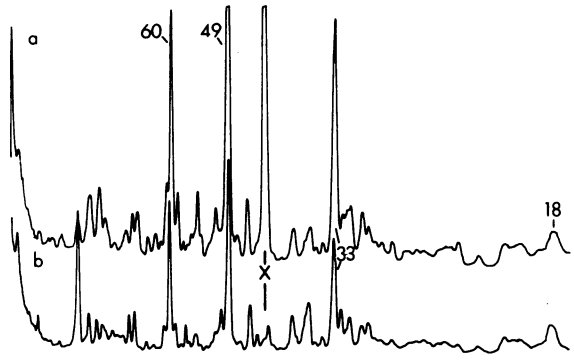

FIG. 2. Membrane proteins from *rec*$^+$ and *recA* strains. To cultures of *E. coli* B and B *recA*$^-$ growing exponentially in glucose M-9 medium at 35°, nalidixic acid was added at a final concentration of 40 μg/ml, and 40 min later 8 ml of cells from each culture were pulsed with 20 μCi [^{35}S]methionine for 10 min at 35°. Isotope incorporation, membrane preparation, gel electrophoresis, and autoradiography were performed as described in *Materials and Methods*. The slot traced in (a) and the slot in (b) contained the same amount of total ^{35}S radioactivity. (a) shows a microdensitometry tracing from the autoradiogram of a 12% acrylamide gel of *E. coli* B (*rec*$^+$). Protein X is indicated in the figure. (b) shows a microdensitometry tracing from an autoradiogram of *E. coli* B *recA*$^-$. Molecular weight markers (in thousands) and the position of protein X are indicated on the tracing.

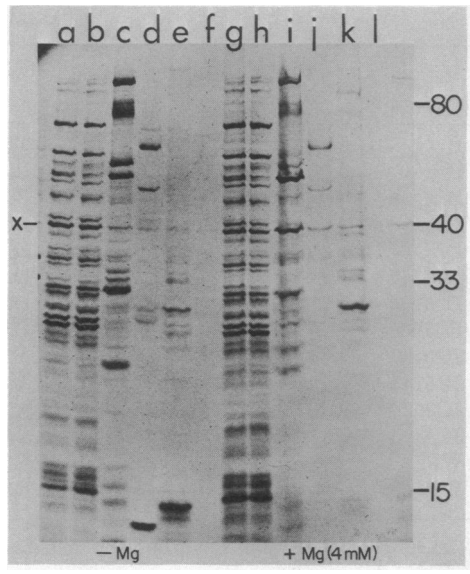

FIG. 3. Elution profiles from DNA-cellulose in the presence and absence of Mg^{++}. Membrane proteins prepared from exponentially growing *E. coli* B/r labeled with [^{35}S]methionine 40 min after the addition of nalidixic acid were loaded onto two single-stranded DNA cellulose columns as described in *Materials and Methods*. The column heights and flow rates of the two columns were identical. An equal mount of ^{35}S radioactivity (membrane proteins) was loaded onto each column. One column was loaded with membrane protein solubilized in the presence of 4 mM $MgCl_2$, and eluted with NaCl buffers containing 4 mM $MgCl_2$. Ten microliters from each fraction eluted was analyzed for ^{35}S, the peaks were pooled, and the membrane proteins eluted from the column in the peaks were prepared for gel electrophoresis as described in *Materials and Methods*. This figure is a photograph of the autoradiogram of the 12% gel. Molecular weights (in thousands) are indicated to the right of the figure. Protein X is indicated to the left of the figure. The slots are as follows: [(a) through (f) minus Mg^{++}; (g) through (l) plus Mg^{++}, as for (a) through (f).] (a) Total membrane applied to the column; (b) breakthrough in 50 mM NaCl; (c) proteins eluted by 0.15 M NaCl; (d) by 0.25 M NaCl; (e) by 0.6 M NaCl; (f) by 2.0 M NaCl.

Although the addition of nalidixic acid did not induce protein X in *recB*$^-$ or *recC*$^-$ strains, the antibiotic bleomycin, which has been shown to cause nonenzymatic degradation of DNA (21, 22), permitted a highly specific induction of protein X even in *recB*$^-$ or *recC*$^-$ strains. Only in the *recA*$^-$ mutant did bleomycin not cause the induction of protein X (Table 1). This result implicates a *recBC* exonuclease degradation product in the induction of protein X.

Protein X bound preferentially to single-stranded DNA; it bound less tightly to double-stranded DNA. Furthermore, Mg^{++} is extremely important for the binding of protein X to DNA. In the presence of 4 mM Mg^{++}, approximately 30–40% of the total amount of protein X loaded onto the single-stranded DNA-cellulose column remained bound to the DNA, whereas in the absence of Mg^{++}, only 2–3% of the total protein X applied to the column bound to the DNA (Fig. 3). This result suggests that protein X could protect the single-stranded DNA from the *recBC*-coded exonuclease.

DISCUSSION

In wild-type *E. coli*, the addition of nalidixic acid or thymine starvation of a strain auxotrophic for thymine inhibits DNA synthesis, and also leads to DNA degradation primarily by the *recBC*-coded exonuclease (23). This DNA degradation after DNA synthesis inhibition is more pronounced in *recA*$^-$ or *lex*$^-$ strains (23, 9), in which DNA repair is defective.

From our results concerning protein X, a model for the regulation of DNA repair functions can be formulated (Fig. 4). This model is a working hypothesis which attempts to explain the somewhat complex pleiotropic effects of the various DNA repair mutants.

First, we propose that the *lex* gene codes for a repressor for protein X, and possibly for the other inducible DNA repair functions described by Witkin (14). No protein X was induced in the *lex*$^-$ mutants; we suggest that the *lex*$^-$ mutation causes the repressor to have both a lower affinity for the inducer and(or) an increased affinity for the operator DNA. The *lex*$^-$ mutation is dominant (9), implying that the *lex* gene codes for a diffusable inhibitory element in the cell. There is a strong analogy between the noninducibility of protein X in the *lex*$^-$ mutant and the noninducibility of β-galactosidase in 11 *lac* super-repressor mutants (24). All of these *lac* super-repressors have reduced affinity for the inducer, isopropyl-β-D-thiogalactoside. Approximately 30% of the *lac* super-repressors also have increased operator affinity, and all the *lac* superrepressor mutants are dominant to wild type.

Second, we postulate that the *tsl*$^-$ mutation is a second mutation in the repressor for protein X. The *tsl*$^-$ derivative was selected for UV-resistance from the UV-sensitive *lex*$^-$ mutant. Synthesis of protein X in the *tsl*$^-$ derivative is constitutive (Table 1). This is analogous to revertants of super-repressor mutants in the *lac* system and the histidine utilization system; these revertants have predominantly been shown to be repressor-negative (constitutive) rather than wild type (inducible) (24, 25). Furthermore, after the addition of

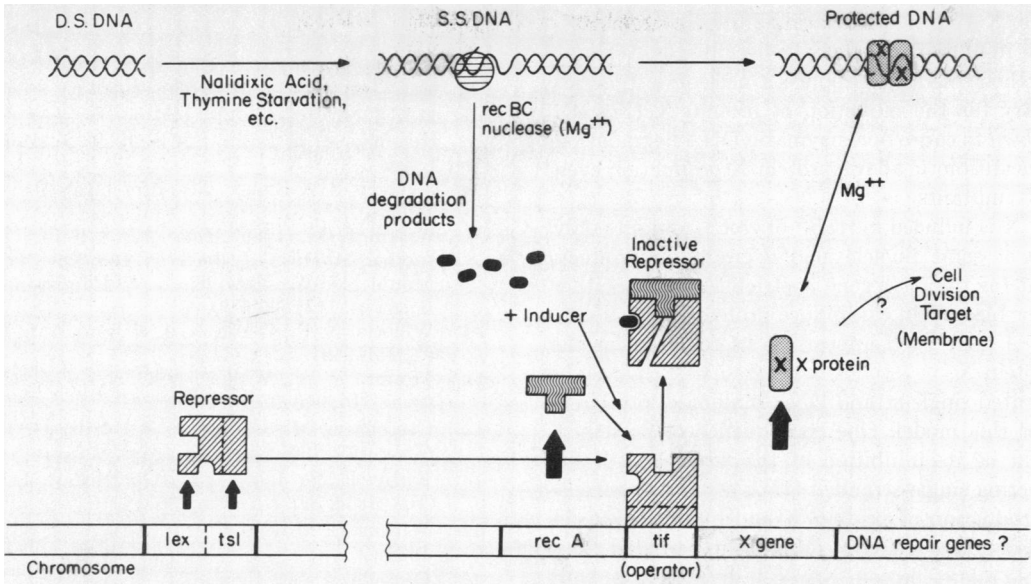

FIG. 4. A feedback loop for control of DNA degradation. D.S. and S.S., double- and single-stranded, respectively.

nalidixic acid, the *tsl*$^-$ mutant is only partially induced for protein X synthesis, suggesting that the repressor in the *tsl*$^-$ mutant still has a decreased affinity for the inducer molecule (Fig. 4, Table 1). Thus, the *tsl*$^-$ mutation could cause the repressor to have a decreased affinity for the operator region and a decreased affinity for the inducer, allowing protein X to be synthesized constitutively.

E. coli generally must be *recA*$^+$, *recB(C)*$^+$, and *lex*$^+$ in order to induce protein X. The only *recA*$^-$ strain which was able to produce protein X was the *tsl*$^-$ *recA*$^-$ double mutant (especially at 42°). Thus, the *recA* gene does not code for the X protein, but the *recA* gene product must be closely involved in the induction of protein X after inhibition of DNA synthesis.

We suggest that the *recA* gene product acts as an antirepressor which removes the *lex*-coded repressor from the operator, either by protease activity or by acting as an inducer protein. (Alternatively, the *recA* gene product could control functions related to the *recBC* nuclease to make an inducer.) Thus the *recA* gene product would not be needed in a *tsl*$^-$ mutant which has an altered, loosely binding repressor. This was found to be true; the *tsl*$^-$ *recA*$^-$ strains synthesized protein X constitutively at a low rate at 30° and at a faster rate at 42° (Table 1).

There is a precedent for a protein which functions as an antirepressor in bacteriophage P22 (26). Furthermore, Roberts and Roberts have recently found that the λ C_I repressor is not cleaved in a *recA*$^-$ strain after mitomycin C treatment (27). The *rate* of synthesis of protein X does not reach a maximum level for the first 20–30 minutes after the addition of nalidixic acid in wild-type *E. coli*, and these slow kinetics suggest that a protease may destroy the *lex*-coded repressor also (manuscript in preparation).

We propose that the *tif* region is the operator region to which the *lex*-coded repressor binds. The *tif*$^-$ mutant could be an operator constitutive mutant, since it synthesizes protein X at a low rate at 30°, and faster at 41°, even though DNA synthesis is normal. (The repressor is proposed to bind to the *tif*$^-$ operator region less strongly at 41°.) Although the *tif*$^-$ mutant synthesizes protein X at 30° and 41°, a greater induction of protein X in the *tif*$^-$ mutant can be achieved by DNA synthesis inhibition (unpublished result).

This model explains why a *lex*$^-$ mutation can suppress induction in the *tif*$^-$ mutation (15). If the *lex*$^-$-coded repressor binds more tightly to the DNA of the operator region than does the *lex*$^+$-coded repressor, then it may also bind better to the *tif*$^-$ DNA than the *lex*$^+$ repressor.

Most simply, the *tif* operator region could directly control transcription of genes involved in DNA repair lying adjacent to it. Alternatively, the *tif* operator region could control the transcription of an inducible protein which then could inactivate repressors of genes involved in DNA repair all over the chromosome.

That a small-molecular-weight compound such as an adenine derivative or a furfural derivative is involved in the induction of λ in the *tif*$^-$ mutant has been demonstrated by Kirby *et al.* (13). We have found that adenine at a final concentration of 100 μg/ml increases the production of protein X by the *tif*$^-$ mutant (manuscript in preparation). We propose that in addition to the *recA* gene product, small molecular weight compounds related to adenine are involved in the induction of protein X, and that the *tif*$^-$ mutant is more sensitive to these compounds because the repressor does not bind as well to the *tif*$^-$ operator as to the wild-type *tif*$^+$ operator. The addition of the adenine derivative to the *tif*$^-$ mutant could destabilize the repressor (in the presence of the *recA*$^+$ antirepressor) causing it to fall off the DNA more readily, and the gene for protein X would then be transcribed. Alternatively, a small molecular weight compound, possibly an adenine derivative, may destabilize or detach the *lex*-coded repressor from the DNA and thus render it susceptible to a *recA*-gene-controlled protease.

The *recA*$^+$ gene product (15) is needed in order for the *tif*$^-$ mutant to mimic the effects of UV-irradiation at 41°. Without the *recA* gene product to help remove (or degrade) the repressor, the *tif*$^-$ mutant presumably could not transcribe protein X at 41°.

We have further evidence that a small-molecular-weight compound, possibly an adenine derivative, is involved in the induction of protein X. The *recB*$^-$ and *recC*$^-$ strains do not

synthesize protein X after nalidixic acid addition even though DNA synthesis is inhibited. This may be because these strains cannot degrade their DNA to produce a necleotide product which is necessary for the induction of protein X (Fig. 4). Bleomycin is known to cause nonenzymatic DNA degradation (21, 22), and this antibiotic allows protein X production even in *recB*$^-$ or *recC*$^-$ mutants.

Thus, protein X is induced in *recB*$^-$ or *recC*$^-$ mutants only in the presence of some nucleic acid degradation product. The requirement for the *recA* gene is stricter in that neither nalidixic acid nor bleomycin can induce protein X in a *recA*$^-$ mutant. The *recA* gene itself appears to be required for the induction of protein X.

The demonstrated single-strand DNA binding of protein X is important for this model. One consequence of protein X production could be its inhibition of the *recBC*-coded exonuclease by covering single-stranded DNA regions. A correlation between production of protein X and inhibition of cell division was also noted (ref. 20; unpublished results), suggesting a possible role of protein X in cell division also. Only *tim-1* mutants make protein X at 41° and yet divide normally (manuscript in preparation). These mutants might be defective in a membrane receptor that is involved in cell division and is inhibited by protein X. Alternatively, another protein induced after nalidixic acid addition but not seen by sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis could inhibit cell division.

There are many predictions from this model that can be tested. First, the *tif*$^-$ mutation should be dominant in merodiploid strains. Second, the *zab* mutant (15) may actually be a pseudo-revertant of *tif*$^-$ which has an increased level of repressibility over the parent *tif*$^-$ strain and is not as inducible as the wild-type *tif*$^+$ strain (28). The *tif*$^-$ *zab*$^-$ mutant would be expected to synthesize almost no protein X after the addition of nalidixic acid or at 41°.

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1. Lieberman, R. P. & Oishi, M. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4816–4820.
2. Clark, A. J. (1973) *Annu. Rev. Biochem.* **7**, 67–86.
3. Clark, A. J. & Margulies, A. D. (1965) *Proc. Nat. Acad. Sci. USA* **53**, 451–459.
4. Howard-Flanders, P. & Theriot, L. (1966) *Genetics* **53**, 1137–1150.
5. Hertman, I. & Luria, S. E. (1967) *J. Mol. Biol.* **23**, 117–133.
6. Devoret, R. & Blanco, M. (1970) *Mol. Gen. Genet.* **107**, 272–280.
7. Witkin, E. M. (1969) *Mutat. Res.* **8**, 9–14.
8. Inouye, M. (1971) *J. Bacteriol.* **106**, 539–542.
9. Mount, D. W., Low, K. B. & Edmiston, S. J. (1972) *J. Bacteriol.* **112**, 886–893.
10. Willetts, N. S., Clark, A. J. & Low, B. (1969) *J. Bacteriol.* **97**, 244–249.
11. Mount, D. W., Walker, A. C. & Kossel, C. (1973) *J. Bacteriol.* **116**, 950–956.
12. Castellazzi, M., George, J. & Buttin, G. (1972) *Mol. Gen. Genet.* **119**, 139–152.
13. Kirby, E. P., Jacob, F. & Goldthwait, D. A. (1967) *Proc. Nat. Acad. Sci. USA* **58**, 1903–1910.
14. Witkin, E. M. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1930–1934.
15. Castellazzi, M., George, J. & Buttin, G. (1972) *Mol. Gen. Genet.* **119**, 153–174.
16. Kirby, E. P., Ruff, W. L. & Goldthwait, D. A. (1972) *J. Bacteriol.* **111**, 447–453.
17. Laemmli, U. K. & Favre, M. (1973) *J. Mol. Biol.* **80**, 575–599.
18. Alberts, B. & Herrick, G. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21, pp. 198–217.
19. Inouye, M. & Pardee, A. B. (1970) *J. Biol. Chem.* **245**, 5813–5819.
20. Pardee, A. B. (1974) *Eur. J. Biochem.* **43**, 209–213.
21. Haidle, C. W. (1971) *Mol. Pharmacol.* **7**, 645–652.
22. Müller, W. E., Yamazaki, Z., Breter, H. J. & Zahn, R. K. (1972) *Eur. J. Biochem.* **31**, 518–525.
23. Willetts, N. S. & Clark, A. J. (1969) *J. Bacteriol.* **100**, 231–239.
24. Jobe, A., Riggs, A. D. & Bourgeois, S. (1972) *J. Mol. Biol.* **64**, 181–199.
25. Hagen, D. C., Gerson, S. L. & Magasanik, B. (1975) *J. Bacteriol.* **121**, 583–593.
26. Botstein, D., Lew, K. K., Jarvik, V. & Swanson, C. A. (1975) *J. Mol. Biol.* **91**, 439–462.
27. Roberts, J. W. & Roberts, C. W. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 147–151.
28. Smith, T. F. & Sadler, J. R. (1971) *J. Mol. Biol.* **59**, 273–305.