JOURNAL OF BACTERIOLOGY, Jan. 1979, p. 568-573
0021-9193/79/01-0568/06$02.00/0

Vol. 137, No. 1

Isolation and Characterization of Amber Mutations in the *lexA* Gene of *Escherichia coli* K-12

LAURA Z. PACELLI, SUSAN H. EDMISTON, AND DAVID W. MOUNT

Department of Microbiology, College of Medicine, University of Arizona, Tucson, Arizona 85724

Received for publication 30 October 1978

We describe the isolation and characterization of amber mutations in the *lexA* gene of *Escherichia coli* K-12. These mutations, designated *spr*(Am), were isolated and characterized in a *lexA tif sfi* genetic background. They abolished the sensitivity of the strain to UV light and resulted in high rates of synthesis of *recA* protein. Phage λ^+ failed to lysogenize the strains as observed with similar strains carrying non-amber *spr* mutations described previously, thereby indicating a constitutive expression of the phage induction pathway. Introduction of an amber suppressor mutation into a strain bearing the *spr*(Am) mutation restored expression of the LexA mutant phenotype. We conclude that *spr* mutations either inactivate or prevent synthesis of the *lexA* gene product and that loss of this product results in constitutive expression of the *E. coli* induction system in the *tif sfi* genetic background.

When *Escherichia coli* is treated with agents which block DNA replication, such as UV light and mitomycin C, a coordinated set of events occur which are thought to aid cellular survival (25). These phenomena have been termed "SOS functions." They include prophage induction, inhibition of cell division, induced mutagenesis, and increased synthesis of the *recA* protein (6, 9, 15, 17), formerly also known as protein X (10, 11). The SOS hypothesis proposes that all these events are part of a general response signal to a block in DNA replication (25). *lexA* mutations which map at 90 min (3, 21) on the *E. coli* linkage map (2) block SOS functions and render the cell UV sensitive (21). These mutations are also dominant to *lexA*$^+$, suggesting that they act through the synthesis of a diffusible product (3, 21). Several classes of resistant derivatives have been isolated; these carry mutations that map at or near the *lexA* locus. In *tsl* derivatives, thought to make a *lexA* product that is thermosensitive and gives rise to thermosensitive growth, certain SOS functions are induced constitutively at 40-42°C (8, 22); in the *spr* derivatives, thought to make very little or a nonfunctional *lexA* protein, there is a high rate of constitutive expression of all known SOS functions in a *tif sfi* background at 30 to 42°C (19).

In this paper, we report the isolation and characterization of *spr*(Am) mutations in a *lexA tif sfi* genetic background of *Escherichia coli* K-12. These mutants are UV resistant and show an increased rate of synthesis of *recA* protein and constitutive expression of all the known SOS functions. This finding further supports a model for the regulation of the *recA* gene by the *lexA* protein.

MATERIALS AND METHODS

Media. Oxoid broth was used to grow cultures before and during matings (18); Davis minimal media (5) was used for selection of recombinants and transductants; L broth (16), supplemented with 2 mg of maltose per ml and 10 mM $MgSO_4$, was used to grow cultures for measuring UV survival; and N agar (21) containing 0.5 μg of mitomycin C (Sigma Chemical Co.) per ml was used for selection of mitomycin C-resistant derivatives. Davis minimal salts is Davis minimal medium lacking glucose, thiamine, or amino acid additions.

Bacterial strains. Table 1 lists the bacterial strains and relevant genetic markers. Wild-type λ phage was obtained from R. Devoret; λc*IInd*$^-$ came from D. Kaiser; λ*imm*434c*I*$^-O_{29}$ was obtained from B. Knoll.

Mating and transduction crosses. The mating procedure was as described by Moody and Hayes (18). P1 transductions were performed as previously described (4, 21).

For matings to introduce an amber suppressor mutation, donor strain JG75 (Hfr *his*$^+$ *supD*) was mated with recipient DM2001 (F$^-$*spr-55 lexA3 his-4 sup*$^+$ *strA*). Cultures (9 Hfr:1F) were mated for 60 min at 37°C without agitation, the mating was interrupted by blending in a Vortex mixer for 1 min, and the cells were spread on selective medium and incubated overnight at 37°C. Recombinants were replica plated onto plates containing 10^9 λ^+ or λ*imm*434c*I*$^-O_{29}$ phage. The loss of the STS (constitutive SOS) phenotype was indicated by growth on the λ^+ phage-containing plate, whereas the presence of the suppressor was indicated by the lack of growth on the λ*imm*434c*I*$^-O_{29}$ phage-containing plate. Sensitivity to UV light was measured as previously described (21).

TABLE 1. *Bacterial strains*

Strain no.	Relevant genetic markers[a]	Origin (reference)
DM1187	F⁻ *thr-1 leu-6 proA2 sfiA11 tif-1 strA1 sup-37 galK2*	(19)
AB259	HfrH *sup*⁺	A. J. Clark
DM1590[b]	as DM1187, also *gal*⁺ *sup*⁺	DM1187 × AB259
DM960	Hfr-Ra-2 *metA28*	(19)
DM972	Hfr Ra-2 *lexA3*	(19)
JG75	Hfr *supD*	J. Gross
JG70	Hfr *supE*	J. Gross
DM1700	as DM1590, also *thr*⁺ *leu*⁺ *lexA3*	DM972 × DM1590 Thr⁺ Leu⁺ [Str^r] recombinant
DM1600	as DM1590, also *thr*⁺ *leu*⁺ metA28	DM960 × DM1590 Thr⁺ Leu⁺ [Str^r] recombinant
DM2000	as DM1590, also *thr*⁺ *leu*⁺ *lexA3 spr-54* (amber)	Mitomycin C-resistant derivative of DM1590
DM2001	as DM1590, also *thr*⁺ *leu*⁺ *lexA3 spr-55* (amber)	Mitomycin C-resistant derivative of DM1590
DM2002	as DM1590, also *thr*⁺ *leu*⁺ *lexA3 spr-56* (amber)	Mitomycin C-resistant derivative of DM1590
AB2480	F⁻ *recA13 uvrA6*	P. Howard Flanders
DM1180	*lexA3 tif-1 sfiA11 strA31 sup-37*	(19)
JM1	*tif*⁺ *sfi*⁺ *lexA*⁺ *spr*⁺	J. George

[a] The nomenclature is that followed by Bachmann et al. (2). To avoid confusing nomenclature changes, *spr* has been retained to designate *lexA* mutations that inactivate or block synthesis of the *lexA* protein.

[b] This strain was provided by B. Knoll. Loss of the uncharacterized amber suppressor mutation, which is probably *sup-37* because the strain is derived from the AB1157 series, was detected by failure to grow λimm^{434} $O_{29}cI^-$ phage.

For mapping of *spr-55* by P1 transduction, phage previously grown on donor strain DM2001 (*metA*⁺ *spr-55 lexA3*) were used to infect recipient strain DM1600 (*metA28 spr*⁺ *lexA*⁺ *tif sfiB*), and Met⁺ transductants were selected at 37°C. The STS phenotype was scored by the absence of growth after replica plating transductants to plates spread with λ⁺ phage.

UV irradiation. The procedure for UV irradiation was as previously described (21).

NTG and EMS mutagenesis. The procedure for NTG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) mutagenesis was that described by Adelberg et al. (1). Late exponential-phase cells, suspended in 100 mM sodium citrate (pH 5.0) and 100 μg of NTG per ml (Calbiochem), were incubated for 15 min at 37°C, diluted 100-fold into L broth, and grown overnight at 37°C. Cells were spread on plates containing 0.5 μg of mitomycin C per ml (Sigma) and incubated overnight at 37°C to select resistant derivatives.

For EMS (ethyl methane sulfonate) mutagenesis, stationary-phase cells were centrifuged, washed in Davis minimal salts, centrifuged again, and suspended in Davis minimal salts at their original volume. To 5 ml of cells was added 25 μl of EMS (Eastman Organic Chemical Div., Eastman Kodak Co.), the tube was blended in a Vortex mixer for 30 s, incubated for 15 min at 37°C, and diluted 100-fold into L broth, and the cells were incubated overnight at 37°C. The resulting culture was spread on mitomycin C-containing plates to select resistant derivatives.

Gel electrophoresis. Sodium dodecyl sulfate (SDS) slab gel electrophoresis was performed as described previously (13, 14).

Labeling with [^{35}S]methionine. Exponentially growing bacteria were labeled with [^{35}S]methionine (2.5 μCi/1-ml sample) for 5 min at 37°C. A 1,000- to 10,000-fold excess of unlabeled methionine was added, and the cells were chilled. They were collected by centrifugation, and the pellets were suspended in 0.1 ml of 10% glycerol–10 mM tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride (pH 7.4) and stored in 24-μl portions at −20°C (14).

Rationale for mutant isolation. *spr lexA tif sfi* indicator bacteria yield clear plaques of λ⁺ phage but turbid plaques of the noninducible phage mutant λ*ind*⁻ (19). This plating behavior is observed presumably because the wild-type phage repressor is inactivated by the constitutive phage induction pathway, thus preventing lysogenization, whereas the mutant λ*ind*⁻ phage can establish stable repressor levels and make lysogens.

This behavior of λ⁺ phage provides a simple method for the detection of *spr* mutations. When colonies bearing a *spr* mutation in a *lexA tif sfi* genetic background are replica plated to plates spread with λ⁺ phage, the colonies are eaten because no lysogenization can occur (19). To obtain *spr* mutants, a *lexA tif sfi* strain is grown on plates containing mitomycin C at a level which kills *lexA* mutants but allows survival of *lexA*⁺ strains and derivatives of *lexA* mutants, some of which produce little *lexA* product or a faulty product. These are detected by their being eaten by λ⁺ phage and are referred to as *spr* mutants. Their phenotype is referred to as the STS phenotype (19).

If *spr* mutations do inactivate the *lexA* gene product (see Introduction), then it should be possible to isolate amber *spr* mutations in the *lexA* gene. Like *spr* mutations already described, strains bearing these should

not be lysogenized by λ phage in a *tif sfi* genetic background. Introduction of an amber suppressor mutation into a *spr*(Am) *tif sfi* strain should restore lysogenization ability and lead to synthesis of the *lexA* mutant product which would likely render the suppressed strain again sensitive to UV and mitomycin C.

Detection of amber *spr* mutations was not readily achievable previously due to the presence of an amber suppressor mutation in the *lexA tif sfi* strain (DM1180) previously used for selection of *spr* mutations. The amber suppressor mutation was removed (Table 1), and the resulting strain (DM1700) was subjected to EMS and NTG mutagenesis. Mitomycin C-resistant mutants were selected and screened for the STS phenotype.

RESULTS

Ten of 44 EMS- and 1 of 10 NTG-induced mutants showed the STS phenotype. These were mated with Hfr JG75 *supD* and His^+ [Str^r] recombinants selected, most of which had inherited the *his*-linked *supD*(Am) suppressor mutation carried by JG75. In the case of one mutant, strain DM2001, 54 of 140 His^+ [Str^r] recombinants tested had acquired *supD*, and all of these had lost the STS phenotype and become UV sensitive. This UV sensitivity must have resulted from suppression of the *spr* mutation and restoration of synthesis of the diffusible product that renders *lexA* mutant cells UV sensitive. We conclude that this *spr* mutation, designated *spr-55*, is an amber mutation in the *lexA* gene.

In similar crosses, two other mutants, DM2000 and 2002, also appeared to carry amber mutations as judged by the simultaneous loss of their STS phenotype with the acquisition of *supD*. However, these suppressed strains did not become UV sensitive. This may be accounted for by the reduced quality or quantity of the *lexA* product upon suppression of their amber mutations.

An additional cross was performed between Hfr JG70 *supE* and DM2001, and Pro^+ [Str^r] recombinants were selected. All recombinants that had inherited the linked *supE* amber suppressor mutation had lost the STS phenotype. Upon subsequent testing (see below), one of these recombinants was shown to have an intermediate UV sensitivity.

Additional support for the conclusion that *spr-55* is in the *lexA* gene was provided by showing that *spr-55* is contransducible with $metA^+$ at a frequency of 16%. This is similar to the frequency of cotransduction of *lexA3* with $metA^+$ (21), which means that *spr-55* maps near the *lexA* gene.

Characteristics of amber *spr* mutants. UV survival curves for strain DM2001 *lexA3 spr-55*, its parent DM1700 *lexA3* spr^+, and derivatives of DM2001 carrying the amber suppressors *supD* and *supE* are shown in Fig. 1. It is quite apparent that the presence of *spr-55* abolishes the extreme UV sensitivity due to *lexA3*, and that the introduction of *supD* again restores UV sensitivity to almost the same level as that seen in DM1700. *supE*, which is generally a less efficient suppressor than *supD* (12), does not restore as great a UV sensitivity, presumably because less *lexA* mutant product is made, although it is also possible that the amino acid substitution by *supE* yields a product that is only partially functional.

λ^+ makes clear plaques and λind^- makes turbid plaques on DM2001, as described previously for another non-amber *spr* mutation in strain DM1187. This property is not observed in the amber suppressor-bearing derivatives of DM2001. Both the *tif* and *sfiA* mutations must also be present if this phenotype is to be observed (19), which correlates with a very high rate of synthesis of the *recA* protein (formerly also known as protein X) (6, 9, 15, 17). Additional characterization of DM2001 (Fig. 2) indicated that high survival levels of UV-irradiated λ phage, approximately equivalent to those observed on an appropriately UV-irradiated wild-type host strain (data not shown), were observed in unirradiated DM2001, but not in its suppressed derivatives. This result can be explained by spontaneous UV (Weigle) reactivation in

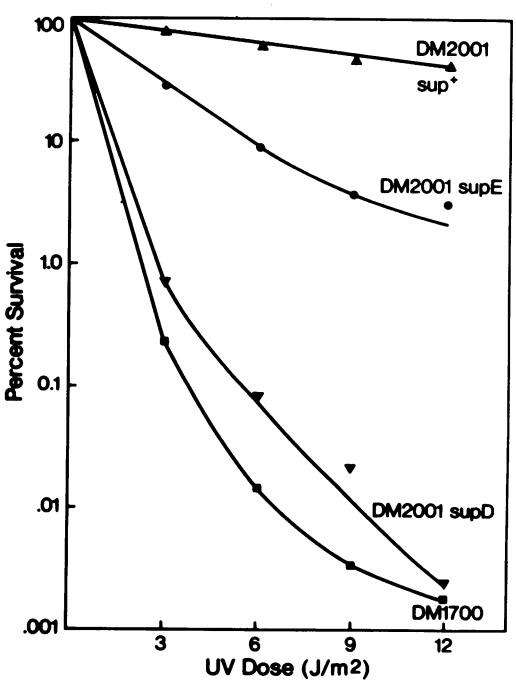

FIG. 1. *UV survival curves for mutant strains.*

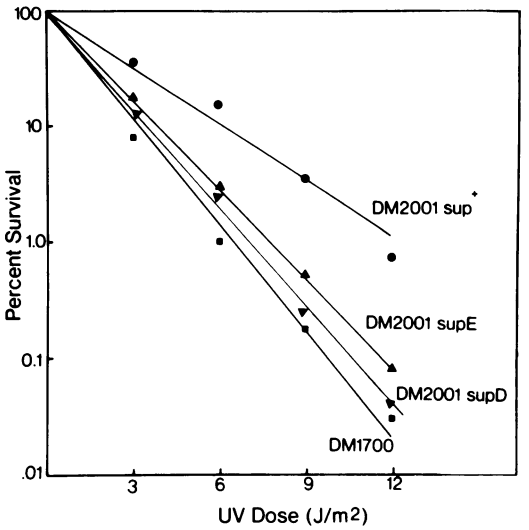

FIG. 2. *Reactivation of UV-irradiated phage λ⁺. Cultures were grown to exponential phase, centrifuged, suspended in one-fifth volume 10 mM $MgSO_4$, and infected at a multiplicity of infection of <0.001 with UV-irradiated phage λ⁺. The infected cells were incubated for 15 min at 37°C, diluted and assayed for plaque-forming infective centers on indicator strain AB2480. This strain was used because it is deficient in both host cell reactivation and UV reactivation and thus does not repair unabsorbed phage particles efficiently. The high level of reactivation observed with DM2001 is approximately equivalent to the level of UV reactivation in induced wild-type bacteria and is thought to be due to constitutive expression of an inducible repair mechanism.*

DM2001. Our interpretation (see below) is that the *spr*(Am) mutation derepresses UV reactivation in the *tif sfiA* genetic background.

In Fig. 3 are shown one-dimensional SDS polyacrylamide gel patterns of labeled soluble proteins from uninduced and nalidixic acid-induced strain DM2001. These show that protein X (the *recA* protein) was produced at a high rate in DM2001 without an inducing treatment being given, and that some further stimulation of synthesis occurred with nalidixic acid treatment. This same result has also been observed with a non-amber *spr* mutant (9). The high rate of synthesis of X was not observed in the DM2001 derivatives carrying amber suppressor mutations, even when an inducing treatment was given them (Fig. 3). These data demonstrated clearly that the presence of the *spr*(Am) mutation allows high levels of *recA* protein synthesis, but when this mutation was suppressed, *recA* protein was no longer inducible. Presumably, suppression restored synthesis of the *lexA* mutant product and this product blocked induction of *recA* protein.

DISCUSSION

We and others have previously shown that the presence near the *lexA* gene of suppressor mutations, such as *tsl* and *spr*, restores inducibility for some, and sometimes all, of the so-called SOS pathways of the cell. Under certain conditions, these suppressors even allow constitutive expression of apparently all of these pathways. Until the experiments described above were performed, it was not possible to determine whether such suppressor mutations altered the activity of the *lexA* product or some other nearby gene product, and, if they did alter *lexA* product activity, whether they actually inactivated the product or just altered its interaction with some other cellular component. The isola-

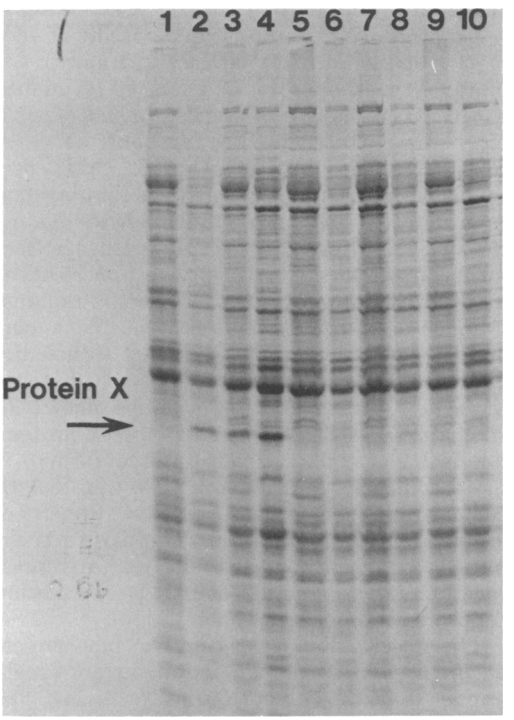

FIG. 3. *Induction of recA protein (protein X) by nalidixic acid. Cultures were grown exponentially at 37°C in glucose M-9 medium. Nalidixic acid was added at a final concentration of 40 μg/ml. After 30 min they were pulse labeled with [^{35}S]methionine for 5 min at 37°C and analyzed by SDS-polyacrylamide gel electrophoresis on an 11% gel. Untreated JM1 (lane 1), JM1 treated with nalidixic acid (lane 2), untreated DM2001 (lane 3), DM2001 treated with nalidixic acid (lane 4), DM2001 supD untreated (lane 5), DM2001 supD treated with nalidixic acid (lane 6), DM2001 supE untreated (lane 7), DM2001 supE treated with nalidixic acid (lane 8), DM1700 untreated (lane 9), DM1700 treated with nalidixic acid (lane 10). The position of protein X is indicated.*

tion of amber *spr* mutants and the properties of derivatives with amber suppressor mutations leave little doubt that *spr* mutations inactivate the *lexA* gene product and show, furthermore, that it is a protein. Moreover, inactivation of this product in the *tif sfiA* genetic background leads to high spontaneous synthesis of *recA* protein and an apparent constitutive expression of all the cellular SOS functions. A similar result was described recently with another strain carrying a non-amber *spr* mutation (19).

We have shown elsewhere that the *tif* mutation is essential for this constitutive expression (19). In *tif*$^+$ strains, either a non-amber or amber *spr* mutation restores inducibility of SOS functions, but most are not expressed in the absence of an inducing treatment (19; L. J. Gudas and D. W. Mount, unpublished data). An exception is *recA* (protein X) synthesis which occurs at very high rates in both non-amber and amber *spr* mutants, whether or not *tif* is present (9; unpublished data). There now appears to be a feasible biochemical explanation for this behavior. J. W. Roberts, C. W. Roberts, and N. L. Craig (personal communication) have shown that purified *recA*$^+$ protein has a proteolytic activity against the λ repressor in vitro, yielding a cleavage fragment similar to that seen in vivo in λ lysogens induced by mitomycin C (24). *spr tif*$^+$ mutants produce high levels of this protein, but apparently the proteolytic activity is not sufficiently great to cleave all the phage repressor because λ lysogens of *spr tif*$^+$ mutants can be made (19). *spr tif* mutants also produce much *recA* protein, but in an electrophoretically different form (6, 9, 17) that is several-fold more active proteolytically against the λ repressor in vitro (Roberts et al., personal communication). This extra activity must be sufficiently great to destroy the repressor of an infecting phage so that lysogenization cannot be established (19).

The observations above on the biochemical mechanism of phage induction have strongly implied that the *recA* protein is directly involved biochemically in the expression of all the induction pathways of the cell, and that the role of the *lexA* protein is to regulate the synthesis of *recA* protein (6, 9, 17; Roberts et al., personal communication). It has been suggested that the *lexA* product is a repressor of the *recA* gene which, like the λ repressor, is inactivated in the induced cell. The detection of an increased rate of synthesis of *recA* message in induced cells lends considerable credence to this model (A. McPartland, L. Green, and H. Echols, personal communication). The isolation of amber mutants in the *lexA* gene and their constitutive properties lends support to the argument that the *lexA* gene product is inactivated rather than modified (25), in the induced cell.

The simplest model (6, 9, 17) for induction of *recA* protein is that the available *recA* protein in the uninduced cell undergoes some biochemical modification, attacks the *lexA* repressor, and thereby derepresses its own synthesis. With the amber *lexA* mutations in hand, we hope to identify the *lexA* protein and demonstrate its presumed role in induction as a repressor of the *recA* protein synthesis.

ACKNOWLEDGMENTS

This research was supported by grant PCM77-06638 from the National Science Foundation and by Public Health Service grant GM24496 from the National Institute of General Medical Sciences.

We thank J. Little and J. Harper for helpful discussion, and B. Knoll for constructing and providing many of the phage and bacterial strains used in this study.

LITERATURE CITED

1. **Adelberg, E. A., M. Mandell, and G. Chein Chin Hem.** 1965. Optimal conditions for mutagenesis by N-methyl-N-nitro-N-nitrosoguanidine in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
3. **Castellazzi, M., J. George, and G. Buttin.** 1972. Prophage induction and cell division in *E. coli*. II. Linked (*recA, zab*) and unlinked (*lex*) suppressors of *tif*-1 mediated induction and filamentation. Mol. Gen. Genet. **119**:153–174.
4. **Clark, A. J., and A. D. Margulies.** 1965. Isolation and characterization of recombinant-deficient mutants of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **53**: 451–459.
5. **Davis, B. D., and S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17–28.
6. **Emmerson, P. T. and S. C. West.** 1977. Identification of protein X of *Escherichia coli* as the *recA*$^+$/*tif*$^+$ gene product. Mol. Gen. Genet. **155**:77–85.
7. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
8. **Gudas, L. J.** 1976. The induction of protein X in DNA repair and cell division mutants of *Escherichia coli*. J. Mol. Biol. **104**:567–587.
9. **Gudas, L. J., and D. W. Mount.** 1977. Identification of the *recA* (*tif*) gene product of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5280–5284.
10. **Gudas, L. J., and A. B. Pardee.** 1976. DNA synthesis inhibition and the induction of protein X in *Escherichia coli*. J. Mol. Biol. **101**:459–477.
11. **Inouye, M., and A. B. Pardee.** 1970. Changes of membrane proteins and their relation to DNA synthesis and cell division of *Escherichia coli*. J. Biol. Chem. **245**: 5813–5819.
12. **Kaplan, S., A. O. W. Stretton, and S. Brenner.** 1965. Amber suppressors: efficiency of chain propagation and suppressor specific amino acids. J. Mol. Biol. **14**: 528–533.
13. **Laemmli, U. K., and M. Favre.** 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Mol. Biol. **80**:575–599.

14. **Little, J. W., and P. C. Hanawalt.** 1977. Induction of protein X in *Escherichia coli*. Mol. Gen. Genet. **150:** 237-248.
15. **Little, J. W., and D. G. Kleid.** 1977. *Escherichia coli* protein X is the *recA* gene product. J. Biol. Chem. **252:** 6251-6252.
16. **Luria, S. T., and J. W. Burrous.** 1957. Hybridization between *E. coli* and *Shigella*. J. Bacteriol. **74:**461-476.
17. **McEntee, K.** 1977. Protein X is the product of the *recA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74:**5275-5279.
18. **Moody, E. E. M., and W. Hayes.** 1972. Chromosome transfer by autonomous transmissible plasmids: the role of the bacterial recombination (*rec*) system. J. Bacteriol. **111:**80-85.
19. **Mount, D. W.** 1977. A mutant of *Escherichia coli* showing constitutive expression of the lysogenic induction and error-prone DNA repair pathways. Proc. Natl. Acad. Sci. U.S.A. **74:**300-304.
20. **Mount, D. W., and C. Kosel.** 1975. Ultraviolet light-induced mutation in UV resistant, thermosensitive derivatives of *lexA*$^-$ strains of *Escherichia coli* K-12. Mol. Gen. Genet. **136:**95-106.
21. **Mount, D. W., K. B. Low, and S. J. Edmiston.** 1972. Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112:** 886-893.
22. **Mount, D. W., A. C. Walker, and C. Kosel.** 1973. Suppression of *lex* mutations affecting deoxyribonucleic acid repair in *Escherichia coli* K-12 by closely linked thermosensitive mutations. J. Bacteriol. **116:**950-956.
23. **Roberts, J. W. and C. W. Roberts.** 1975. Proteolytic cleavage of bacteriophage lambda repressor in induction. Proc. Natl. Acad. Sci. U.S.A. **72:**147-151.
24. **West, S. C. and P. T. Emmerson.** 1977. Induction of protein synthesis in *Escherichia coli* following UV or γ-irradiation, Mitomycin C treatment or *tif* expression. Mol. Gen. Genet. **151:**57-67.
25. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40:** 869-907.