Correlation Between UV Dose Requirement for Lambda Bacteriophage Induction and Lambda Repressor Concentration

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Escherichia coli K-12 wild type and a *uvrA* mutant derivative were used to construct isogenic strains bearing one, two, three, or more phage λ cI genomes and containing increasing concentrations of λ repressor as measured by in vitro operator DNA-binding assays. The survival and phage induction in response to UV irradiation were determined. In both strains, dose-response relationships were obtained as a function of the cellular repressor concentration. The *uvrA* lysogens required one-tenth the UV fluence of the wild-type counterparts for induction. Lysogenic strains containing plasmids that overproduce the λ ind⁺ repressor and the same lysogens with plasmids overproducing the λ ind⁻ repressor displayed the same survival curves as the nonlysogenic parental strain; however, only the former produced infectious centers (at a frequency of 2×10^{-3} to 5×10^{-4}) in response to radiation.

The lysogenic state of coliphage lambda is maintained by the binding of a repressor protein specified by the virus to two adjacent operators, thereby shutting off transcription of most of the phage genome. In wild-type *Escherichia coli* lysogens, a small fraction of the cells lyse spontaneously, giving rise to active phage. This process of derepression followed by active virus production has been called induction. Since 1950 (24), many external and internal effectors have been shown to elicit induction in the majority of the cells, but the mechanism of induction itself remains a mystery.

All λ -inducing agents either interact directly with DNA (UV and gamma radiation, alkylating agents, cross-linking agents), interfere with its synthesis during chromosomal replication (thymine starvation, temperature increase in thermosensitive DNA elongation mutants), or promote the persistence of nicks and gaps in DNA [*polI*(ts), *lig*(ts), *dam*(ts) at nonpermissive temperature]. The only exception known is the *tif* mutation (11) found to map at the *recA* locus (5), which spontaneously induces λ phage at 41°C or in the presence of adenine, without detectable damage to the host DNA (22). Induction of λ requires a *recA*⁺ *lexA*⁺ genetic background (7, 19, 39). The characteristics just mentioned apply also to a few unrelated phenomena that seem to be coordinately regulated (46) and are termed "SOS functions" (33) because they are able to rescue the cell or virus from a DNA damaging event. These functions include (i) in-

hibition of septum formation, (ii) increased mutation rate, (iii) W-reactivation, (iv) inhibition of DNA degradation, and (v) synthesis of protein X. (For reviews, see references 33 and 47.)

Associated with the λ induction process are the disappearance of the operator-binding capacity of the repressor (31, 39, 44), brought about by host functions (43), and proteolytic cleavage of the repressor protein (36). UV and mitomycin C treatments require protein synthesis (39, 43) to exert their effect on repressor inactivation, whereas gamma radiation (44), *tif*-promoted induction (39, 44), and thermal induction of *dna*(ts) do not (31).

Studies by a number of investigators have led to the formulation of several putative mechanisms. Chief among these models are: that of Tomizawa and Ogawa (43), which postulates the induced synthesis of an effector protein capable either of binding to repressor molecules or competing with repressor for binding to operator sites; that of Sussman and Ben Zeev (41), which postulates the ability of repressor molecules to bind specifically to lesions that accumulate in nonoperator regions of the host DNA, thereby making repressor unavailable for binding to the λ operators; that of Roberts and Roberts (36), which postulates the induced synthesis or activation of a protease capable of cleaving repressor molecules; and other models (11, 14, 16, 19) based on the presumed activities of DNA precursors or degradation products.

Tomizawa and Ogawa (43) have shown a cor-

relation between the amount of repressor present and the UV dose necessary to abolish it, but only in segregating cells that contained significantly less repressor than do ordinary single lysogens. It is possible that the low UV fluences used were very close to the threshold amount needed to induce the effector, and therefore the correlation merely reflected the probability of inducing the effector. Because the confirmation of this relationship has important implications for possible models of viral induction, we were interested in extending their observation to cover a wide range of repressor concentrations. The results leave no doubt that, in the case of UV induction, at physiologically meaningful repressor concentrations, there is a correlation between the amount of repressor in the cell and the optimal dose necessary to inactivate it.

MATERIALS AND METHODS

Bacteria. *E. coli* strains were derivatives of *E. coli* K-12. AB1886 (*thr leu pro his arg thi rpsL lac gal ara mtl xyl tsx uvrA*) (38) and EK410 (*Δgal chl bio rpsE recA*)/F'*gal att λ* were obtained from K. Ihler. The repressor-overproducing strains RS294(pKB252), containing a plasmid bearing the repressor gene (*cI*) of λ phage, and strain RS294 (*pind*⁻), carrying the overproducer mutant repressor *cI ind*⁻ gene, were kindly provided by K. Backman (1). Strain RS294 (*endA thi r_k⁻ m_k⁺*) was obtained by loss of the plasmid pKB252, as a tetracycline-sensitive strain with no repressor activity. Single lysogens of these strains were prepared by conventional methods. The indicator bacterium was C600 Amp.

Phages. The coliphages used were λ^+ wild type, the noninducible mutant *λind*⁻ (20), and *λxis1* (13), provided by D. Freifelder.

Media. Supplemented M9 medium is M9 as described in Miller (29) containing 0.5% glucose and 0.1% Casamino Acids. Tryptone plates contain 10 g of tryptone (Difco), 8 g of NaCl, and 15 g of agar per liter.

Construction of multiple lysogens. Double lysogens were prepared by infecting bacteria suspended in 10 mM Tris-hydrochloride (pH 7.4)–10 mM MgCl₂ with a multiplicity of 20 phages per bacterium. When phage *λxis1* was used, the surviving lysogens were screened for the production of active phage by replica plating on a lawn of sensitive Su⁺ bacteria in the presence of 2 μ g of mitomycin C per plate. Induced bacteria that gave rise to a plaque were considered double lysogens (13).

The triple lysogen of strain AB1886 was obtained by conjugation of EK410 (λ^+) (this strain has the λ attachment site on the F'*gal* factor) with the double lysogen AB1886 (*λxis λxis*) and selection on EMB-galactose (29) plates containing streptomycin. The galactose-positive colonies chosen were able to transfer F'*gal* to F'*gal* (λ) strains and produce zygotic induction when conjugated with F' nonlysogenic strains.

Construction of strains bearing plasmids. Plasmid DNA from strains RS294(pKB252) and RS294 (*pind*⁻) was isolated by procedure II of Reuben et al. (35). Briefly, spheroplasts produced by lysozyme treat-

ment were lysed with sodium dodecyl sulfate at 65°C. After host DNA was precipitated with 1 M NaCl at 4°C, the plasmid DNA was recovered from the supernatant by ethanol precipitation at -20°C. The pellet was dissolved in 10 mM Tris-hydrochloride (pH 7.4)–10 mM NaCl–10 mM EDTA and used in transformation. Sensitive and lysogenic lines of AB1886 and RS294 were transformed by the procedure of Cohen et al. (6). Transformants were selected on tryptone plates containing 20 μ g of tetracycline per ml.

Growth of bacteria and UV irradiation. Cells were grown overnight in M9 medium. Plasmid-containing strains were grown in M9 with tetracycline (20 μ g/ml) to ensure that the plasmid had not been lost by segregation. Unless otherwise stated, all dilutions were made in M9 medium. The cells were diluted 1:50 and incubated with shaking at 37°C to an optical density at 550 nm of 0.2 (2×10^8 /ml). Optical density measurements were made on a Bausch and Lomb Spectronic 20 spectrophotometer. Bacteria were diluted to 2×10^6 /ml for the RS294 strains and to 2×10^7 /ml for the AB1886 strains. Samples of 20 ml were placed in glass petri plates (9-cm diameter) and irradiated. UV irradiation was performed with a General Electric germicidal lamp, 15 W, at a distance of 98 cm. The intensity, measured by a Blak-Ray UV meter, was 3.5×10^{-1} J/m² per s. The cells were immediately diluted and plated on tryptone plates to determine viable counts. The tryptone plates had been pretreated with catalase by spreading 0.1 ml of a 0.02% solution over the entire surface. This treatment produced a higher survival, as previously reported (27). To determine the production of infective centers after each UV dose, samples were removed, diluted, and incubated with aeration at 37°C for 30 min. After appropriate dilution, a portion was added to melted soft agar containing penicillin-resistant indicator bacteria and poured on tryptone plates containing catalase and 5,000 U of penicillin (32). All UV-treated bacteria were handled under dim light, and the plates were incubated at 37°C in the dark for at least 24 h.

Preparation of extracts for determination of repressor activity. The lysogenic strains were grown in 500 ml of M9 and the repressor-overproducer strains were grown in 10 ml of the same medium, both at 37°C with shaking. Conditions were maintained exactly as for the UV-irradiation experiments, and the cultures were harvested at the same cell density. After centrifugation at $2,500 \times g$ for 5 min, the bacteria were suspended in repressor buffer (10 mM Tris-hydrochloride [pH 8.0], 1 mM EDTA, 10 mM MgCl₂, 200 mM KCl, 0.1 mM dithiothreitol, and 5% glycerol) and disrupted by sonic treatment, and cell debris was removed by centrifugation at $29,000 \times g$ for 30 min. All manipulations were carried out at 0 to 4°C. The supernatants were then tested immediately for binding activity as described below. The protein concentrations of the extracts were determined by the method of Lowry et al. (23).

Binding assay for λ repressor. A membrane-binding technique (9) modified as follows was used to measure retention of operator-repressor complex. A volume of 0.1 ml of reaction mixture contained 10 mM Tris-hydrochloride (pH 7.4), 0.2 mM EDTA, 2.5 mM MgCl₂, 1 mM CaCl₂, 50 mM KCl, 0.2 mM dithiothreitol, 8.5 μ g of bovine serum albumin, 20 μ g of calf

thymus DNA, and 0.15 μ g of radioactive λ DNA. Dilutions of extracts in repressor buffer containing 50 mM KCl instead of 200 mM were made just before titration, and 1 to 10 μ l of these dilutions was added to each reaction mixture. After 10 min at room temperature, the content of each tube was passed through a nitrocellulose filter (Schleicher & Shuell B-6) that had been soaked in washing buffer (same as binding buffer but with dithiothreitol, bovine serum albumin, and calf thymus DNA omitted and 5% dimethyl sulfoxide added), and the filter was washed once by covering with washing buffer. The filters were either dried and covered with scintillation fluid or dissolved in scintillation fluid containing Triton X-100 and counted. Controls with radioactive λ imm⁴³⁴ DNA were run at the same time, and the percentage of nonspecific label retained was subtracted from the experiments. The amount of repressor was calculated from the dilution that gave 50% of maximum binding. One unit of activity is defined as the amount of repressor that binds 1 μ g of operator DNA.

Chemicals. Tetracycline was purchased from J. B. Roerig Division, Pfizer, Inc., penicillin G sodium was from Squibb, and beef liver catalase was from Sigma.

RESULTS

Single and multiple lysogens were constructed as described in Materials and Methods, and single lysogens were also transformed with λ repressor-overproducing plasmids. Two bacterial strains were employed. One (RS294) is wild type for all DNA repair enzymes. The second (AB1886) is a *uvrA* mutant, defective for correndonuclease II activity (4), and thus unable to incise DNA containing pyrimidine dimers (12). The lack of excision repair leads to single-strand gaps on the complementary strand opposite the dimers (38), which makes this strain 10 times more sensitive to UV (2).

The two isogenic series were examined with respect to concentration of λ repressor per cell, the incidence of spontaneous induction, and the UV dose response to killing and to λ induction.

Repressor concentration. The levels of λ repressor were measured in cells grown under the same conditions and to the same cell density as those used for measuring phage induction. Extracts were prepared and used in the λ DNA-binding assays as described above. The results (Table 1) indicate that, while multiple lysogens have more repressor than single lysogens, the levels are not strictly proportional to the number of *cI* gene copies per cell, perhaps due to a negative control exerted by the repressor protein upon its own promoter (8, 34, 43). The plasmid-bearing strains contained about 100-fold higher levels of repressor, as noted elsewhere (1).

Spontaneous induction. The lysogenic cell lines were checked for the proportions of spontaneously induced bacteria in cultures. These results, also presented in Table 1, show that double lysogens produced fewer infective centers spontaneously than did single lysogens, and the triple lysogen showed an even lower level. Both lysogens containing plasmids displayed an incidence of spontaneous induction 2 or more orders of magnitude lower than this. The AB1886 lysogenic strains yielded many more infective centers than their RS294 counterparts. For each bacterial strain the incidence of spontaneous induction was found to vary inversely with the repressor concentration.

UV-dose response of RS294 lysogens. Cells were irradiated with UV light to determine the kinetics of survival and the yield

TABLE 1. Concentrations of λ repressor and comparison of spontaneous and UV-induced infective centers

Strain	Repressor activity (U/mg of protein) ^a	Infective centers		Optimal UV dose ^b (J/m ²)
		Spontaneous ^c	Induced maximum ^d	
RS294 (λ)	4.0	8×10^{-3}	0.94	31.5
RS294 (λ xis λ xis)	6.0	7.4×10^{-4}	0.65	42
RS294 (λ) (pKB252)	590	$<1 \times 10^{-5}$	5×10^{-4}	63
AB1886 (λ)	4.0	4.3×10^{-2}	0.48	3.2
AB1886 (λ xis)	6.7			
AB1886 (λ xis λ xis)	8.0	3.3×10^{-3}	0.15	4.2
AB1886 (λ xis λ xis)/F' <i>gal</i> (λ)	9.0	9×10^{-4}	0.14	5.3
AB1886 (λ) (pKB252)	>500	$<1 \times 10^{-5}$	2×10^{-3}	5.3

^a Repressor activity of the cell extracts was determined as described in the text. Samples containing 2 to 20 μ g of extract protein were used in assays, except for plasmid strains, which had 100 times less. At 50% operator-binding activity, the nonspecific binding of λ imm⁴³⁴ was 10 to 12% for most extracts and less than 4% for plasmid strains.

^b This is the UV dose that gave maximal induction.

^c Infective centers per cell obtained at a cell density of 2×10^8 /ml, minus the free phage determined by plating the same cell suspension after treatment with chloroform. The figures are means of several experiments.

^d Ratios of the infective centers obtained at the optimal UV dose to the original number of bacteria treated. The figures are means of several experiments.

FIG. 1. UV survival curves of strain RS294 nonlysogenic and lysogenic sublines. ■, RS294; □, RS294 (λ); Δ , RS294 (λ axis λ); ●, RS294 (λ) (pKB252); ▼, RS294 (λ) (pind⁻). All curves in this and subsequent figures represent means of several experiments.

of induced bacteria (Fig. 1 and 2). It had been previously established that the greater sensitivity of lysogenic strains to UV light is due to induction of phage (26). The results of this study demonstrate that single lysogens are more sensitive to killing than double lysogens, whereas the single lysogens carrying plasmids that synthesize an excess of repressor were killed with kinetics similar to the nonlysogen. These differing sensitivities towards radiation were also seen in the production of infective centers (Fig. 2): the single lysogen was induced at lower doses than the double, and the plasmid-carrying lysogen needed still more UV fluence. The fraction of induced cells at the optimal dose was 1,000 times lower in the repressor-overproducer strain (Table 1). At higher doses yet, the cells lose the capacity to support growth of the phage (28).

UV-dose response of AB1886 lysogens. The survival of strain AB1886 lysogens at doses of 1 to 4 J/m² (Fig. 3) did not show a significant difference, because the sensitivity of this strain to killing is such that at maximum induction of the single lysogen only about 2% of the nonlysogenic cells were viable, whereas in

the wild type 20 to 30% of the nonlysogenic RS294 strains were still viable at the corresponding optimal induction dose. At optimal UV fluences for induction, the single lysogen reached a yield of 48% infective centers, while the double and triple lysogens reached a maximum of 15% phage-producing cells (Table 1). Nevertheless, a correlation of inducing UV dose with repressor concentration was apparent (Fig. 4).

UV-dose response of plasmid-bearing lysogens. When plasmids that overproduce λ ind⁺ or λ ind⁻ repressor were introduced into single lysogens, they imparted an increased UV resistance to the lysogens, to such an extent that the survival kinetics became similar to those of the respective nonlysogenic strains (Fig. 1 and 5). These results imply that the UV fluences used were not sufficient to inactivate the large amount of repressor synthesized by the plasmid-containing strains. The only difference between the lysogens with excess λ ind⁺ repressor and those with λ ind⁻ repressor is that the former

FIG. 2. UV induction of λ in lysogens of RS294 containing one or more active *cI* genomes of λ . Ordinate: number of PFU obtained at the UV dose indicated on abscissa, divided by the number of PFU obtained at the optimal UV dose.

FIG. 3. UV survival curves of nonlysogenic strain AB1886 and its sublines containing one or more active *cI* genomes of λ . ■, AB1886; □, AB1886 (λ); Δ , AB1886 ($\lambda_{xis} \lambda_{xis}$); $\times$, AB1886 ($\lambda_{xis} \lambda_{xis}$) / $F'gal(\lambda)$; ●, AB1886 (λ) (pKB252).

FIG. 4. UV induction of λ in lysogens of strain AB1886 containing one or more active *cI* genomes. Symbols as in Fig. 2.

produced infective centers in response to irradiation, while the latter never showed induction (Fig. 5). The optimal UV dose for the triple lysogen of AB1886 and for the *ind*⁺ plasmid-containing AB1886 (λ) was about the same, 5.3 J/m², but only 2 in 1,000 cells of the latter were inducible, compared to 14% of the former (Table 1).

DISCUSSION

The following conclusions stem from the data presented in this paper.

(i) Repressor is present in progressively increased concentrations in isogenic strains lysogenic for one, two, and three λ genomes (Table 1). The repressor levels in the single and double lysogens varied over a 1.5-fold range for strain RS294 and a 2.25-fold range between the single and triple lysogens of AB1886.

(ii) The occurrence of spontaneously induced cells is inversely related to the repressor concentration (Table 1).

(iii) Corresponding lysogens of a *uvrA* mutant, incapable of excising pyrimidine dimers, display a much higher incidence of spontaneous induction while maintaining the same general trend between repressor level and yield of infective centers (Table 1).

(iv) The same parameters, i.e., repressor level and capacity of the host for DNA repair, influ-

FIG. 5. UV-dose response of strain AB1886 and its λ lysogens containing repressor-overproducing plasmids. Survival (open symbols) or infective centers (closed symbols); ○, AB1886 nonlysogenic; □, AB1886 (λ) (pKB252); Δ , AB1886 (λ) (*pind*⁻). The fractions of infective centers of this last strain were much less than 10⁻⁵, but for convenience have been placed on the abscissa line.

ence the rate of induction elicited by UV radiation, much as they do the rate of spontaneous induction (Fig. 2, 4). This further supports the concept that both types of induction proceed via the same molecular mechanism, as concluded by Jacob and Wollman (21) based on the correlation between spontaneous induction of different strains and degree of inducibility.

(v) At extremely high repressor levels, produced by joining the *cI* region of λ to *lac* operators in the pKB252 plasmid strains, the UV fluences tried were incapable of inactivating all the repressor present in the cells, and the kinetics of cell death were equal to those obtained for the sensitive, isogenic strain (Fig. 5).

The purpose of performing these determinations was to gain insight into the events that follow the primary DNA lesion (produced by UV in this case) and the final inactivation of λ repressor. We know that the primary lesion has to be processed further, since blocking both pathways of DNA repair (excision and postreplication) prevents λ induction (3); therefore an intermediate in DNA repair is involved. A priori, we can reason that, if the role of this intermediate, or effector, is to activate a catalytic function necessary to inactivate repressor, then the optimal UV dose capable of inducing the catalytic function should be roughly the same in isogenic strains, regardless of a 1.5- to 2.5-fold increase in repressor concentration. On the other hand, if the effector interacts with the repressor, such as an allosteric inducer or the binding of repressor to a DNA lesion, we should expect a close correlation between the UV dose and the concentration of repressor. The results obtained support the latter possibility. Therefore, any model of λ induction should be able to explain this correlation. An unrestricted catalytic model such as that proposed by Roberts and Roberts (36) does not easily account for these facts. Roberts et al. (37) proposed an alternative model, postulating that the repressor may be altered by interaction with another molecule and thereby become sensitive to the protease. It would have to be further assumed that this interacting molecule is the effector produced by the repair of primary DNA lesions. A very recent model proposed by Gudas and Mount (15) states that a DNA damage product, possibly a nucleotide, is an activator of the *recA* protein which, once modified, is responsible for inactivation of repressors. These authors, as well as J. W. Roberts (personal communication), indicate that the *recA* protein is probably the ATP-dependent protease that cleaves λ repressor. If repressor inactivation is just a catalytic process, as the model of Gudas and Mount implies, it is difficult to explain the finding (40) that induction by

gamma rays in the presence of chloramphenicol abolishes operator-binding activity without cleaving all the repressor present in the cell. Shinagawa et al. (40) conclude from their results that there may be two steps leading to repressor cleavage. In the first step, the repressor could be modified, perhaps allosterically or by absorption to damaged DNA, so that it can no longer bind to operators. In the second step, an endopeptidase could cleave the modified repressor.

The induction model proposed by Sussman and Ben Zeev (41) is consistent with the results of this paper. Work performed in our laboratory (manuscript in preparation) shows that purified λ repressor has a very high affinity for double-stranded DNA containing single-strand gaps. In contrast, its affinity for intact or nicked non-operator DNA, as well as denatured DNA, is very low. Also, purified λ *ind*⁻ repressor has a considerably lower affinity for gapped DNA. Based on these results, we believe that the DNA repair intermediate is a double-strand DNA helix containing a persistent single-strand gap which would bind λ repressor. We explain the requirement for *recA*⁺ genotype (and also for the LexA⁺ phenotype, since *lexA*⁻ mutants do not synthesize *recA* protein after inducing treatments [14]) by assuming that the *recA* protein binds to single-strand DNA (17), thereby protecting its physical integrity (16, 42). In its absence, the single-strand gaps in the host DNA probably collapse and become substrate for the endonucleolytic activity of its *recBC*-coded exonuclease V (18, 25, 45), which destroys its inducing capacity. The thermal induction of the *tif*⁻ mutant (22) can be explained as follows. The *tif*⁻ product has been found to be a missense *recA* protein (10) which is constitutive at high temperature. We postulate that the mutation increases the affinity of this protein for single-strand DNA at 42°C, binding to temporarily denatured areas of the chromosome (recombination regions or others) and maintaining the single-strand bubbles for longer than usual. The DNA bubbles would not be detected by alkaline gradients, or be degraded by nucleases due to the presence of *tif-recA* protein, but would constitute repressor-binding sites.

In vivo, both repressor and *recA* protein might bind to the same single-strand structure, thus providing the continuity that would allow the latter to cleave the former. In this context, the results of Pollard and Fluke (E. C. Pollard and D. J. Fluke, *Biophys. J.* 17: 144a, 1977), showing lack of induced radioresistance in λ lysogens but not in λ *ind*⁻ lysogens, are highly interesting, suggesting that λ repressor may compete with *recA* protein for binding sites produced after UV treatment.

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LITERATURE CITED

- Backman, K., M. Ptashne, and W. Gilbert. 1976. Construction of plasmids carrying the *cl* gene of bacteriophage λ . Proc. Natl. Acad. Sci. U.S.A. **73**:4174-4178.
- Boyce, R. P., and P. Howard-Flanders. 1964. Release of UV light-induced thymine dimers from DNA in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **51**:293-300.
- Braun, A. G. 1976. Host DNA replication or excision repair requirement for ultraviolet induction of bacteriophage λ lysogens. Nature (London) **261**:164-166.
- Braun, A. G., and L. Grossman. 1974. An endonuclease from *Escherichia coli* that acts preferentially on UV-irradiated DNA and is absent from *uvrA* and *uvrB* mutants. Proc. Natl. Acad. Sci. U.S.A. **71**:1838-1842.
- Castellazzi, M., J. George, and G. Buttin. 1972. Prophage induction and cell division in *Escherichia coli*. I. Further characterization of the thermosensitive mutation *tif-1* whose expression mimics the effect of UV irradiation. Mol. Gen. Genet. **119**:139-152.
- Cohen, S. N., A. C. Y. Chang, and L. Hsu. 1972. Non-chromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110-2114.
- Donch, J., and J. Greenberg. 1974. The effect of *lex* on UV sensitivity, filament formation and λ induction in *lon* mutants of *Escherichia coli*. Mutat. Res. **25**:403-405.
- Dottin, R., L. Cutler, and M. Pearson. 1975. Repression and autogenous stimulation in vitro by bacteriophage lambda repressor. Proc. Natl. Acad. Sci. U.S.A. **72**:804-808.
- Echols, H., and L. Green. 1971. Establishment and maintenance of repression by bacteriophage lambda: the role of the *cl*, *cII*, and *cIII* proteins. Proc. Natl. Acad. Sci. U.S.A. **68**:2190-2194.
- 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.
- Goldthwait, D., and F. Jacob. 1964. Sur le mécanisme de l'induction du développement du prophage chez les bactéries lysogènes. C. R. Acad. Sci. Ser. D **259**:661-664.
- Grossman, L., A. Braun, R. Feldberg, and I. Mahler. 1975. Enzymatic repair of DNA. Annu. Rev. Biochem. **44**:19-43.
- Guarneros, G., and H. Echols. 1970. New mutants of bacteriophage λ with a specific defect in excision from the host chromosome. J. Mol. Biol. **47**:565-574.
- Gudas, L. S. 1976. The induction of protein X in DNA repair and cell division mutants of *Escherichia coli*. J. Mol. Biol. **104**:567-587.
- Gudas, L. S., 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.
- Gudas, L. S., and A. B. Pardee. 1975. Model for the regulation of *Escherichia coli* DNA repair functions. Proc. Natl. Acad. Sci. U.S.A. **72**:2330-2334.
- Gudas, L. S., and A. B. Pardee. 1976. DNA synthesis inhibition and the induction of protein X in *Escherichia coli*. J. Mol. Biol. **101**:459-477.
- Hertman, I. 1969. Survival, DNA-breakdown and induction of prophage Lambda in *Escherichia coli* K-12 *recA uvrB* double mutant. Genet. Res. **14**:291-307.
- Hertman, I., and S. E. Luria. 1967. Transduction studies on the role of a *rec*⁺ gene in UV induction of prophage λ . J. Mol. Biol. **23**:117-133.
- Jacob, F., and A. Campbell. 1959. Sur le système de repression assurant l'immunité chez les bactéries lysogènes. C. R. Acad. Sci. Ser. D **248**:3219-3221.
- Jacob, F., and E. L. Wollman. 1953. Induction of phage development in lysogenic bacteria. Cold Spring Harbor Symp. Quant. Biol. **18**:101-119.
- Kirby, E. P., F. Jacob, and D. A. Goldthwait. 1967. Prophage induction and filament formation in a mutant strain of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **58**:1903-1910.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
- Lwoff, A., L. Siminovitch, and N. Kjeldgaard. 1950. Induction de la production de bactériophages chez une bactérie lysogène. Ann. Inst. Pasteur (Paris) **79**:815-859.
- Mackay, V., and S. Linn. 1976. Selective inhibition of the DNase activity of the *recBC* enzyme by the DNA binding protein from *Escherichia coli*. J. Biol. Chem. **251**:3716-3719.
- Marcovich, H. 1956. Etude de l'action des rayons ultraviolets sur le système lysogène *Escherichia coli* K-12 (λ), K-12s, λ . Ann. Inst. Pasteur (Paris) **91**:511-522.
- Martin, S. E., R. S. Flowers, and Z. L. Ordal. 1976. Catalase: its effect on microbial enumeration. Appl. Environ. Microbiol. **32**:731-734.
- Mattern, I. E., M. P. van Winden, and A. Rorsch. 1965. The range of action of genes controlling radiation sensitivity in *Escherichia coli*. Mutat. Res. **2**:111-131.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Mount, D. W., and C. Kosel. 1975. Ultraviolet induced mutation in UV-resistant, thermosensitive derivatives of *lexA* strains of *Escherichia coli* K12. Mol. Gen. Genet. **136**:95-106.
- Noack, D., and S. Klaus. 1972. Inactivation kinetics of Lambda phage repressors in a mutant of *Escherichia coli* temperature sensitive in DNA replication. Mol. Gen. Genet. **115**:216-224.
- Ogawa, T., and J. I. Tomizawa. 1967. Abortive lysogenization of bacteriophage lambda b2 and residual immunity of non-lysogenic segregants. J. Mol. Biol. **23**:225-245.
- Radman, M. 1974. Phenomenology of an inducible mutagenic DNA repair pathway in *Escherichia coli*: SOS repair hypothesis, p. 128-142. In L. Prokash, F. Sherman, M. Miller, C. Lawrence, and H. W. Tabor (ed.), Molecular and environmental aspects of mutagenesis. Charles C Thomas, Publisher, Springfield, Ill.
- Reichardt, L. 1975. Control of bacteriophage lambda repressor synthesis: regulation of the maintenance pathway by the *cro* and *cl* products. J. Mol. Biol. **93**:281-309.
- Reuben, R., M. Geffer, L. Enquist, and A. Skalka. 1974. New method for the large-scale preparation of covalently closed λ DNA molecules. Virology **14**:1104-1107.
- 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.
- Roberts, J. W., C. W. Roberts, and D. W. Mount. 1977. Inactivation and proteolytic cleavage of phage λ repressor *in vitro* in an ATP-dependent reaction. Proc. Natl. Acad. Sci. U.S.A. **74**:2283-2287.
- Rupp, W. D., and P. Howard-Flanders. 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following UV irradiation. J. Mol. Biol. **31**:291-304.
- Shinagawa, K., and T. Itoh. 1973. Inactivation of DNA binding activity of repressor in extracts of lambda lysogens treated with mitomycin C. Mol. Gen. Genet. **126**:103-110.
- Shinagawa, H., K. Mizuuchi, and P. T. Emmerson.

1977. Induction of prophage lambda by γ -rays, mitomycin C and *tif*; repressor cleavage studied by immunoprecipitation. *Mol. Gen. Genet.* **155**:87-91.
41. **Sussman, R., and H. Ben Zeev.** 1975. Proposed mechanism of bacteriophage λ induction: acquisition of binding sites for λ repressor by DNA of the host. *Proc. Natl. Acad. Sci. U.S.A.* **72**:1973-1976.
42. **Tomizawa, J., and H. Ogawa.** 1968. Breakage of DNA in *rec*⁺ and *rec*⁻ bacteria by disintegration of radiophosphorus atoms in DNA and possible causes of pleiotropic effects of *recA* mutation. *Cold Spring Harbor Symp. Quant. Biol.* **33**:243-251.
43. **Tomizawa, J., and T. Ogawa.** 1967. Effects of UV irradiation on bacteriophage λ immunity. *J. Mol. Biol.* **23**:247-263.
44. **West, S. C., K. A. Powell, and P. T. Emmerson.** 1975. *recA*⁺-dependent inactivation of the λ repressor in *Escherichia coli* lysogens by γ -radiation and by *tif* expression. *Mol. Gen. Genet.* **141**:1-8.
45. **Willetts, N. A., and A. J. Clark.** 1969. Characteristics of some multiply recombination-deficient strains of *Escherichia coli*. *J. Bacteriol.* **100**:231-239.
46. **Witkin, E. M.** 1967. The radiation sensitivity of *Escherichia coli* B: a hypothesis relating filament formation and prophage induction. *Proc. Natl. Acad. Sci. U.S.A.* **57**:1275-1279.
47. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. *Bacteriol. Rev.* **40**:869-907.