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Proposed Mechanism of Bacteriophage Lambda Induction: Acquisition of Binding Sites for Lambda Repressor by DNA of the Host

(ultraviolet light/mitomycin C/thymine starvation/damaged DNA/repressor of cell division)

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ABSTRACT **Interference with the *in vitro* binding of λ phage repressor to λ operator DNA was observed when *Escherichia coli* DNA containing the following lesions was present in the reaction mixture: (*a*) DNA with single-strand breaks from pancreatic DNase (nicked DNA); (*b*) DNA isolated from thymine-starved cells; (*c*) DNA from ultraviolet-treated cells; (*d*) DNA of mitomycin-treated cells; and (*e*) DNA from a temperature-sensitive ligase mutant after 1 hr at 42°. Normal *E. coli* DNA did not interfere. Binding of λ cI*ind*⁻ repressor to operator DNA was not affected by *E. coli* DNA with lesions. DNAs from cells treated with increasing doses of mitomycin were proportionately more effective in competition for repressor, suggesting increasing binding sites per unit of DNA. A general model of virus induction is proposed, based on binding affinity of ultraviolet-sensitive repressors for single-strand breaks in the host DNA. The model is extended also to the presumptive repressor of cell division.**

Temperate viruses have evolved two alternative and mutually exclusive developmental states: (*1*) a lysogenic condition in which the viral DNA is integrated into the host genome or (*2*) a lytic condition in which the viral DNA replicates autonomously, producing viral progeny and killing the cell. The choice is greatly influenced by the physiological condition of the host. Treatments that damage host DNA or that lead to the cessation of host DNA synthesis [ultraviolet (UV) or x-irradiation; exposure to mitomycin, nitrogen mustard, nalidixic acid, or fluoropyrimidines; thymine starvation; etc.] induce the lytic cycle (1–7).

The lysogenic condition is maintained by negative control (8) via a repressor protein, the product of a viral gene, which in the case of phage λ has been shown to bind to the viral DNA at two specific sites (9) and to prevent the transcription of the remaining viral genes (10–12). Despite the fact that the λ repressor has been purified and its binding to the operator sites on the λ DNA has been studied in detail (13), the mechanism of lytic induction, i.e., the molecular events that cause the dissociation of the repressor from viral DNA and thereby trigger transcription of the viral genes, is still unknown. The purpose of this communication is to propose a general model of viral induction that is consistent with previously known facts and to describe experimental results obtained with the λ phage-*Escherichia coli* system that provide empirical support for the model.

We assume that the viral repressors have affinity not only for their homologous operators on the viral genome, but also can bind nonspecifically to nicked host DNA. Blocking DNA synthesis leads to an accumulation of small fragments of DNA (14), an increase in nonconservative DNA replication (15), and appearance of single-strand breaks (16, 17). Under conditions where ligase activity is rate-limiting, the single-strand breaks in the host DNA would be available for binding with the viral repressor. Given the limited supply of repressor molecules per cell (18), this could lead to derepression of the viral genome and entrance into the lytic cycle. In general, no matter by what means the single-strand breaks are generated (nucleases, inhibitor of ligase, treatments that increase DNA lesions, or introduction of DNA from an irradiated episome), the end result would be the titrating out of the repressor followed by derepression of the viral operons.

Three specific predictions follow from this model: (*a*) *E. coli* DNA, nicked as a result of prior treatment of the cells with mitomycin C, UV irradiation, or by thymine starvation, should reduce the extent by which λ DNA binds to repressor in an *in vitro* assay. The degree of interference should increase with the severity of the treatment. (*b*) Normal *E. coli* DNA should not interfere in such an assay. (*c*) The damaged host DNA should not interfere in the assay if the repressor has been produced by the cI*ind*⁻ mutant of λ (which is not inducible by any of the treatments outlined above). The experimental results to be described are consistent with these predictions.

MATERIALS AND METHODS

Strains. E. coli B/r was obtained from J. Aronovitch. K12 strains W3350; W3350(λcI857S_7); W(λ$^+S_7$); Hfr 3000 (thymine-requiring); H(λi^{434}cI$_{ts}S_7$); and λ phage, wild type and λ cI*ind*⁻, were from the collection of Amos Oppenheim. Strain N2672 *lig*ts_7 was supplied by M. Gellert (19).

Media. Tryptone broth and synthetic medium TCL (20) containing 0.4% glucose were used. Solutions included KCl/EDTA/Tris (1 mM EDTA, 20 mM KCl, 10 mM Tris·HCl, pH 7.4) and binding solution: (10 mM Tris·HCl, pH 7.4, 10 mM Mg acetate, 20 mM KCl, 0.1 mM EDTA, 14 mM 2-mercaptoethanol).

Preparation of Phage DNA. Procedures for labeling with ^{32}P, heat-inducing, and isolating phages have been described in detail (20). The source of λ$^+$ DNA was W3350(λcI857S_7) and that of i^{434} DNA was H(λi^{434}cI$_{ts}S_7$). DNA was extracted with sodium dodecyl sulfate and phenol (21) and dialyzed against KCl/EDTA/Tris.

Bacterial DNA. E. coli DNA was extracted according to Marmur (22). UV or mitomycin-treated bacteria had to be

Abbreviation: UV, ultraviolet.

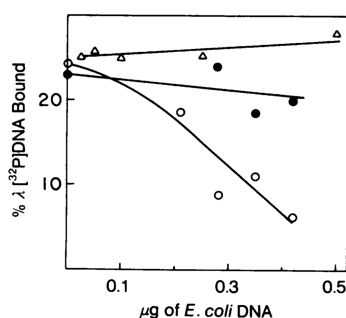

FIG. 1. Interference with the binding of λ DNA to *ind*$^+$ repressor by DNA from thymine-starved *E. coli*. In a reaction volume of 0.35 ml containing 0.25 ml of binding buffer and 50 µg of sonicated calf thymus DNA, various amounts of *E. coli* DNA (indicated in the abscissa) were mixed with a constant volume of repressor extract and then 0.5 µg of ^{32}P-labeled λ DNA was added. After 5 min at 0°, ^{32}P-labeled repressor–operator complex was assayed by membrane filtration of 0.1 ml in triplicate. The nonspecific label retained by an extract containing no repressor has been subtracted. Results are given as percent of the λ DNA input (11,400 cpm/0.1 ml). Δ, Normal *E. coli* DNA added to *ind*$^+$ repressor; O, DNA from thymine-starved *E. coli* added to *ind*$^+$ repressor; ●, DNA from thymine-starved *E. coli* added to *ind*$^-$ repressor.

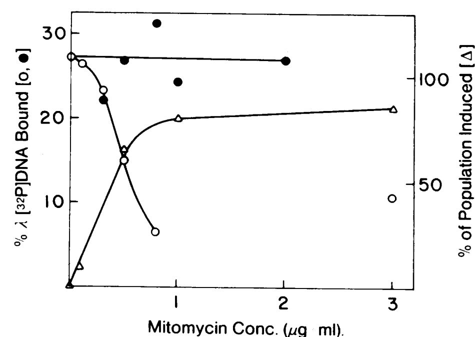

FIG. 2. Interference with the binding of λ DNA to *ind*$^+$ repressor by DNA from *E. coli* treated with different amounts of mitomycin C. A constant volume of repressor extract was present in the assay with 0.12 µg of *E. coli* DNA isolated from cells exposed to the indicated doses of mitomycin C. The residual binding activity of the repressor was determined after adding 0.5 µg of ^{32}P-labeled λ DNA. Conditions were as in Fig. 1 (input 13,500 cpm/0.1 ml). Δ, Infective centers obtained after treatment of Hfr 3000(λ) with the indicated dose of mitomycin, as described in *Methods and Materials;* O, for each point, DNA from Hfr 3000 treated with the indicated amount of mitomycin was added to *ind*$^+$ repressor extract; ●, same DNAs as above were added to *ind*$^-$ repressor extract.

incubated overnight with 100 µg/ml of Pronase in the presence of 1% sodium dodecyl sulfate at 37° before deproteinization, because of DNA cross-linking to protein (23). After purification, all DNAs were incubated with RNase (20 µg/ml, 37° for 1 hr) and Pronase (50 µg/ml, 37° for 1 hr) and then deproteinized with neutralized phenol. DNAs were dissolved in KCl/EDTA/Tris and stored over chloroform.

Repressor Extracts. Strain W3350 infected at a multiplicity of 5 to 10 phages (λ$^+$S$_7$ or λ*ind*$^-$) per cell was the source of repressor. Extracts were made following the procedure of Echols and Green (24).

Repressor–Operator-Binding Assay. To determine repressor activity, the membrane-binding technique was used (24). Details of the assays are presented in the legends to figures.

Incised DNA. Controlled enzymatic breakage of DNA was performed according to Weiss *et al.* (25).

Thymine Starvation. Strain Hfr 3000 was grown in TCL with 5 µg/ml of thymidine to a density of 2×10^8/ml. The cells were sedimented, washed, and resuspended in TCL without thymidine but supplemented with 50 µg/ml of uridine. After 30 min of agitation at 37°, the cells were collected and the DNA was extracted.

Induction by Mitomycin. A logarithmic culture (4×10^8/ml) of W3350(λ$^+$) in tryptone broth was distributed in 2-ml aliquots and increasing amounts of mitomycin C were added. Incubation was continued at 37° in the dark for 15 min. Appropriate dilutions were plated for determination of colony survival and infective centers. The same procedure was followed with 50-ml aliquots of mitomycin-treated nonlysogenic W3350, and the DNA was extracted.

DNA from UV-Treated E. coli. W3350 in the logarithmic phase was resuspended in 10 mM Tris·HCl (pH 7.4), 10 mM $MgSO_4$, and irradiated with a UV dose that induced 99% of the isogenic lysogens. Concentrated tryptone was added and incubation at 37° proceeded for 15 min in the dark before sedimentation and extraction of DNA.

RESULTS

The affinity of *lac* repressor protein for nonoperator DNA has been determined by Linn and Riggs (26) using competition experiments. The same technique was used in this study to determine if λ repressor had measurable affinity for nicked *E. coli* DNA. Thus, the binding of λ repressor to ^{32}P-labeled λ DNA was measured in mixtures containing *E. coli* DNA nicked by a controlled endonuclease reaction (25) with pancreatic DNase. Neutral gradients demonstrated the high molecular weight of the treated DNA, and alkaline gradients disclosed the presence of single-strand scissions. Since λ repressor binds to two operator sites per phage genome, the concentration of repressor was adjusted to sequester less than 40% of the input λ DNA. In the presence of nicked *E. coli* DNA, the binding of λ DNA to λ repressor was significantly reduced. Equivalent quantities of untreated *E. coli* DNA were ineffective. The specificity of the repressor-λ DNA binding was confirmed by the fact that when ^{32}P-labeled λ i^{434} DNA was used, only 1–3% of the input DNA was bound to the filters.

The proposed model predicts that DNA extracted from *E. coli* that has been exposed to agents or treatments known to induce the lytic cycle in lysogens should also interfere with the binding of λ repressor to λ DNA. This prediction was tested by using DNA from the thymine-requiring strain Hfr 3000 of *E. coli* extracted after 30 min of thymine starvation. Fig. 1 shows that this DNA did indeed compete significantly when extracts containing λ *ind*$^+$ repressor were used and failed to interfere significantly with the binding of λ cI*ind*$^-$ repressor. [*In vivo* experiments have shown that lysogens of λcI*ind*$^-$ are

considerably less sensitive to UV induction (27).] As control, equal amounts of normal *E. coli* DNA were used and, as can be seen in Fig. 1, the binding of λ repressor to operator DNA was either not changed or enhanced by the presence of the host DNA at these levels. This increase in binding activity was observed in some extracts of repressor more than others, and it is probably due to protection of the repressor oligomers when diluted into the assay mixture, or to elimination of nucleases present in the extracts, which are not completely inactivated by the calf thymus DNA used in the assay. The scattering of the points obtained in the competition experiments (as exemplified in Fig. 1) reflects the fact that we are measuring more than one parameter, competition being the predominant one.

Other non-operator DNAs tested were: (*a*) DNA isolated from nonlysogenic *E. coli* W3350 treated with inducing doses of UV radiation; (*b*) DNA from the same strain treated with inducing doses of mitomycin; and (*c*) DNA from a temperature-sensitive ligase mutant of *E. coli* N2672 after 1-hr incubation at 42°. All gave results similar to those obtained with the thymine-starved DNA.

The correlation between interference *in vitro* and induction *in vivo* was demonstrated by isolating DNA from nonlysogenic *E. coli* W3350 cultures that had been treated with various amounts of mitomycin. The mitomycin concentrations were chosen by trial experiments with a W3350(λ) culture, shown in Fig. 2. Assays were performed using the same concentration of competing DNA, and the results (Fig. 2) clearly show that, within this range of mitomycin concentrations, the higher the inducing ability, the better the competition obtained by equivalent amounts of DNA, implying more binding sites per unit of DNA. Again, binding activity of extract containing λ *ind*$^-$ repressor was not affected by these DNAs.

DISCUSSION

The concept that the inducer of λ phage repressor is damaged host DNA emerges from a consideration of the following experimental data: (*a*) Most treatments of the host that induce the lytic cycle are known to lead to the production of single-strand nicks in the host DNA. (*b*) Interference with the repair of the DNA increases the efficiency of induction in the treated host. This includes exposure to caffeine or acriflavine (28), *hcr*$^-$ mutations (29), *polA* mutation (30), and use of a mutant with a temperature-sensitive ligase (19). (*c*) Treatments that avoid single-strand nicks decrease the efficiency of induction. These include repair of pyrimidine dimers by photoreactivation (31) and suppression of induction in *recA* mutants of the "reckless type" (32). The enhanced exonuclease activity in the latter mutants prevents the accumulation of nicks by constant degradation. (*d*) Inhibiting protein synthesis or "liquid holding" suppresses the induction caused by UV radiation, thymineless death, etc. The probable mechanism of this type of suppression is that the cells have time to repair their DNA before the inhibitor, which is coded by the repressed operon(s), has been synthesized (37). (*e*) Induction occurs when an F^- (λ) lysogen is conjugated with a UV-treated nonlysogenic F^+ (33). DNA is the only macromolecule that is transferred in significant amounts during conjugation (34). Infection of a lysogen by γ-irradiated and ^{32}P-treated P_I phage also leads to induction (35).

However, it should be noted that the above evidence, as well as the experimental results described in this communication, is merely correlative. A more critical test of the model would be possible by determining directly the relative affinities of purified repressor from DNA possessing known numbers of different specific types of lesions, both before and after *in vitro* repairs.

Witkin (36) carried out a provocative comparison between the effects of radiomimetic agents on phage induction and inhibition of cell division in bacteria. She postulated the existence of a repressor that is inactivated by a process originating in replication-blocking lesions in the DNA, thereby causing the induction of an operon. The product of such an operon, directly or indirectly, inhibits septum formation and leads to the appearance of filaments.

The analysis of the *tif* mutation in *E. coli* (37) led her to postulate further that all UV-inducible activities are governed by repressors that respond to a common effector or effectors produced by a common pathway. Our model would predict that the common effector is nicked host DNA to which these repressors bind.

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