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UV Sensitivity of a Nonrepressor Regulatory Protein of Bacteriophage P22

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The product of phage P22 gene *c*1 has two functions: it promotes synthesis of P22 repressor and it retards expression of some lytic genes. We present evidence that this product is inactivated in UV-irradiated hosts. The conditions for inactivation of *c*1 product include a functional DNA recombination system involving the host *recA* gene.

Patterns of regulation in the temperate phages λ of *Escherichia coli* and P22 of *Salmonella typhimurium* are quite analogous (1, 6). Specifically, maintenance of the prophage in a lysogenic cell requires a repressor molecule (the product of *c*I in λ and of *c*2 in P22; Fig. 1) that binds to a leftward operator O_L defined by *v*2 in λ and *V*x in P22 and a rightward operator O_R defined by *v*1*v*3 in λ and *K*5 in P22. This repressor-operator interaction prevents transcription of phage lytic genes. The interaction has been demonstrated in vitro for λ (15) and inferred for P22.

UV irradiation and the radiomimetric drug mitomycin C induce both prophage λ and P22 (20, 21), but the induction process has been much better studied in λ. Shinagawa and Ito (13) found that λ repressor activity, as assayed by its ability to bind λ DNA, decreased after exposure of a lysogen to mitomycin C. Roberts and Roberts (12) showed that UV irradiation or mitomycin C causes proteolytic cleavage of λ repressor. A λ mutant (*ind*$^-$) that is noninducible by UV produces a repressor that is not subject to cleavage, and both mitomycin C induction and cleavage are dependent on a functional *E. coli recA* gene (12).

Among the lambdoid phages that are inducible by UV is phage 21. The immunity regions of P22 and phage 21 are identical by heteroduplex mapping (1). It is reasonable to assume that if a UV-induced cleavage enzyme inactivates λ repressor, it also inactivates the repressor of all the lambdoid phages, including P22.

An alternate idea to that of proteolytic repressor cleavage has been advanced by Sussman and Ben Zeev (17). Their data suggest that UV irradiation produces new repressor binding sites in the host DNA. This results in λ repressor vacating the O_L and O_R operators and binding to the new sites on the host DNA. The open operators then allow transcription of λ genes and induction occurs. This model is applicable to all UV-inducible phages.

The establishment of lysogeny in λ and P22 is a complicated process (4, 6). In P22 the products of *c*1 and *c*3, which are made soon after infection, interact at a promotor site defined by *c*27 and (i) promote repressor synthesis and (ii) retard expression of lytic genes (e.g., *18* and *12*) to the right of *K*5.

Preirradiated *E. coli* are lysogenized at a lower frequency than nonirradiated cells (5, 10). This observation led us to look for effects of UV on the establishment of lysogeny by P22. In this paper we present data that show P22*c*1 product is sensitive to processes induced by UV and that this sensitivity depends on *recA*.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. *S. typhimurium* MG3 is a wild-type strain. MG109 is *ara*$^-$*leu*$^-$. It is the parent of Pox, which channels P22 to lysogenize with a frequency of 1.0 (18). A partial revertant of Pox, PR-Pox, causes a frequency of lysogenization intermediate between that seen with MG109 and Pox. Wild-type phage P22 plates on Pox at 5×10^{-4} and on PR-Pox at 0.12 the efficiency seen on MG3 or 109. Both Pox and PR-Pox synthesize DNA more slowly than does MG109 (16). Strain 330 is a *recA*$^-$ from the collection of M. Levine. P22 *c*$^+$ (wild type), *c*1^7, and *c*2^5 were originally obtained from M. Levine. The temperature-sensitive mutants *ts 18.1* and *ts 12.1* are unable to synthesize phage DNA at ≥37 C (9). P22 *ts 18.1* *c*1 and *c*2, and P22 *ts 12.1* *c*1 and *c*2, were isolated as spontaneous clear mutants arising in P22 *ts 18.1* and P22 *ts 12.1*; they were picked and assigned to *c*1 or *c*2 as a result of complementation against *c*1^7 and *c*2^5 (8). P22 *ts 18.1* *c*1^7 and P22 *ts 18.1* *c*2^5 were made by recombination.

Media. Nutrient broth was used for one-step growth curves and in experiments to determine burst size; it is 8 g of nutrient broth (Difco) and 5 g of NaCl in 1 liter of distilled water. Bacterial and

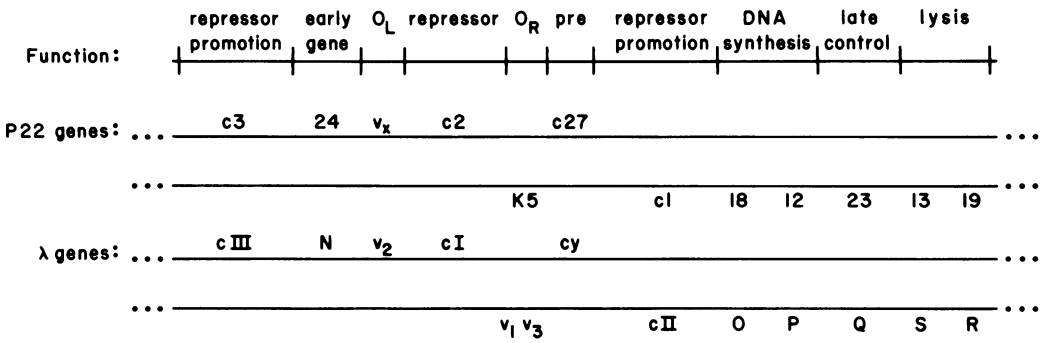

FIG. 1. *Partial maps of phages P22 and λ.*

phage platings were made on tryptone agar (10 g of tryptone [Difco], 5 g of NaCl, and 12 g of agar [Difco] in 1 liter of distilled water). Soft tryptone agar has 8 g of agar rather than 12. [^{3}H]thymidine incorporation was measured in M9-CAA medium (14).

Determination of burst size. Exponentially growing bacteria at 30 C were infected with the desired phage. After 10 min for adsorption and 5 min of treatment with P22 antiserum (K = 2) in nutrient broth, the infected cells were diluted into fresh nutrient broth and incubated for 3 h at 30 C. The number of phage was determined by plating on MG3 as indicator bacteria. Burst size is the number of progeny PFU divided by the number of infectious centers. For determination of burst size in UV-irradiated cells, exponentially growing cells were harvested and concentrated 2× by centrifugation, resuspended in 0.02 M $MgSO_4$, and exposed to an 8-W UV light at 28 cm for 6 s. Survival fractions of MG109 are indicated for each experiment. After irradiation, the culture was diluted 1:2 into 2× nutrient broth, and phage were added at 10 min.

One-step growth curves. Exponentially growing cells (MG109) were infected in nutrient broth at 30 C, as described above. At the desired times after infection, samples were removed from the culture and plated on MG3 to determine the number of phage.

Lysis curves. Turbidity changes in cultures of cells infected with P22 were followed on a Klett-Summerson colorimeter. When these experiments involved irradiated cells, the cells were collected and irradiated as described above except that irradiation was done in M9 salts rather than 0.02 M $MgSO_4$.

Measurement of [^{3}H]thymidine incorporation. A 25-ml culture of exponentially growing MG109 in M9-CAA was harvested by centrifugation and resuspended in 5 ml of M9 salts. The 5 ml of salts was irradiated as above and diluted to 20 ml with M9-CAA. At this time the culture was split and P22*c*^{5} at a multiplicity of infection (MOI) of 10 was added to 15 ml. The remaining 5 ml of the culture was used for the uninfected control. This experiment tested the effect of UV irradiation immediately before infection. In another experiment, the culture was incubated for 15 min after UV irradiation, and then phage were added and the experiment was carried out in the same way.

One-minute pulse labeling was done by the method of Smith and Levine (14), using [^{3}H]thymidine (New England Nuclear Corp.) at a final specific activity of 50 mCi/mmol and a concentration of 4 μCi/ml. Nonirradiated cells were treated as described above, but exposure to UV was eliminated.

RESULTS

Gene c1 retardation of lytic genes is exaggerated when DNA synthesis is altered. The interaction of the products of genes *c*1 and *c*3 at a site defined by *c*27 retards expression of lytic genes to the right of *K*5 (6, 19). This retardation is responsible for the "*c*1 repression" (14) of DNA synthesis.

We have found that the mutant hosts Pox and PR-Pox exaggerate the *c*1-imposed restriction on lytic gene expression. Phage P22*c*1 caused lysis of PR-Pox to begin at 60 min, whereas P22*c*2, which has a functional *c*1, did not cause lysis to start until 210 min (see Fig. 3). Increasing the number of *c*1 genes, and presumably the amount of *c*1 product, increased the severity of retardation of lytic gene expression. At MOIs of 2, 10, and 20, the burst sizes from P22*c*2 infections of PR-Pox were 140, 70, and 25, respectively. DNA synthesis rates were slower in Pox and PR-Pox than in MG109. The exaggerated *c*1 retardative effect in these hosts is thought to result from an altered DNA synthesis rate, allowing a high ratio of *c*1 product to phage DNA (19).

Two phage genes, *18* and *12*, are known to be involved in phage DNA synthesis (9). Because decreased host DNA synthesis capacity enhances *c*1 retardation, we expected that phage mutations affecting DNA synthesis would also exaggerate *c*1 retardation. The lesion in mutant *ts 18.1* did not prevent phage growth at 30 C, but we found that it affected DNA synthe-

sis sufficiently to enhance c1 retardation of lytic gene expression.

One-step growth curves of P22 *ts 18.1c*1 and *ts 18.1c*2 in MG109 at 30 C are shown in Fig. 2. Phages lacking a functional c1 gene had the same latent period at MOI = 5 or 15. However, increasing the MOI of P22 *ts 18.1c*2 from 5 to 15 prolonged the latent period. We interpret this prolongation as resulting from c1 retardation of lytic gene expression being enhanced by a greater dosage. We will refer to this phenomenon as "c1 multiple retardation." The experiments shown in Fig. 2 were repeated with phages P22 *ts 18.1c*1^7 and P22 *ts 18.1c*2^5. Identical results were obtained. These results show that c1 multiple retardation is a property not likely to be associated with particular c1 or c2 alleles.

None of phages P22*c*1^7, *c*2^5, *ts 12.1c*1, or *ts 12.1c*2 caused c1 multiple retardation at 30 C in MG109. Multiple retardation was seen in MG109 at 25 C after infection with P22 *ts 12.1c*2, although it was not seen at 30 C. We most clearly detected c1 multiple retardation

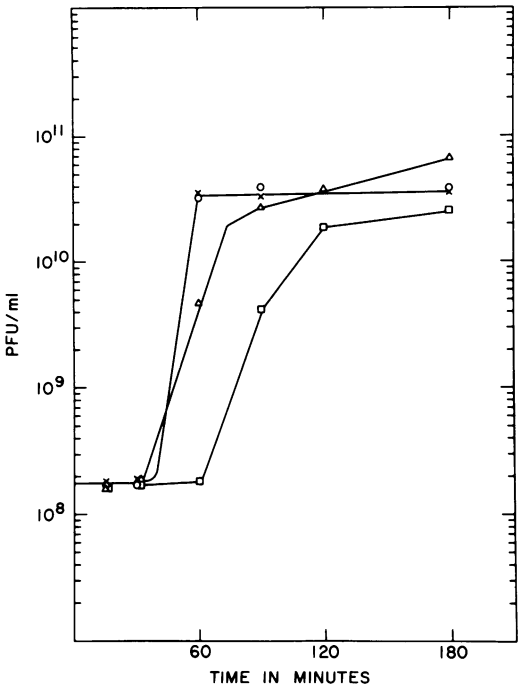

FIG. 2. *Effect of MOI upon growth of P22 ts 18.1c1 and c2 mutants in MG109. Exponentially growing bacteria were infected with the indicated multiplicity of phages. Phage adsorption and serum treatment are described in the text. Symbols: ○, ts 18.1c1, MOI = 5; ×, ts 18.1c1, MOI = 15; △, ts 18.1c2, MOI = 5; □, ts 18.1c2, MOI = 15.*

under conditions in which DNA synthesis was limited by mutations in the host (PR-Pox) or the phage (P22 *ts 18.1* or P22 *ts 12.1*), but it was exaggeration of an event present in wild-type conditions. We infer this because P22*c*1 in MG109 lyses 10 or 15 min earlier than does *c*2 (19). We ascribe this difference to retardation.

c1 multiple retardation was a transient effect (Fig. 2). Although the latent period was extended, there was little change in the rise period or in the final yield. The difference in burst size seen at 180 min from P22 *ts 18.1c*2 infections at low and high MOIs varied greatly from experiment to experiment. The difference seen in Fig. 2 is about threefold; in other experiments (MOI = 2 and 20) the difference was as great as 5- to 17-fold. We think that the larger differences are a result of greater prolongation of the latent period, and that if we sampled at times greater than 180 min the differences would become smaller. We do not understand the variation in conditions affecting the extent of c1 multiple retardation and thus yield at 180 min. To compensate for this variation, all comparisons of yield were made from cultures grown at the same time in the same medium.

UV irradiation interferes with c1 multiple retardation. The burst size of MG109 infected with P22*c*$^+$, P22 *ts 18.1c*$^+$, and P22 *ts 18.1c*2 decreased with increasing multiplicity (Table 1). The decreased yield seen in *c*$^+$ infections has been reported by Bronson and Levine (2), who ascribed it to increased dosage of repressor. Burst size was little affected by increasing the MOI in the case of P22 *ts 18.1c*1. Irradiation of the host before the phages were added greatly reduced c1 multiple retardation by the *ts 18.1c*2 mutant (Table 1). These data show that c1 retardation reduced burst size and that the retardation was sensitive to UV. The c1 multiple retardation by *ts 18.1c*2 and its elimination by UV was also observed in strain MG3.

As mentioned in reference to the results in Fig. 2, we again want to emphasize the transient nature of c1 multiple retardation. Although the burst size of cells infected at high MOI with P22 *ts 18.1c*2 was lower at 180 min, we expected that at later times the burst size would approach that found for a low MOI. We interpret the effect of UV to be a shortening of the length of c1 retardation.

UV sensitivity of c1 multiple retardation depends on recA. P22 *ts 18.1c*2 caused c1 multiple retardation when it infected the *recA*$^-$ host 330 (Table 2). P22 *ts 18.1c*1 behaved normally in this host; increasing the MOI had little effect on burst size. Irradiation of 330 before infection with P22 *ts 18.1c*2 did not appreciably lift c1

TABLE 1. *Burst sizes of P22 wild type, ts 18.1c⁺, c1, and c2 in MG109*

Phage MOI	Wild type		*ts 18.1c⁺*		*ts 18.1c1*		*ts 18.1c2*	
	No UV	UV[a]	No UV	UV	No UV	UV	No UV	UV
2	649	698	138	91	260	166	442	360
10	10	345	39	193	209	182	226	300
20	4	288	3	127	194	210	38	301

[a] Cell survival after UV varied between 0.08 and 0.03.

TABLE 2. *Burst sizes of P22 ts 18.1c1 and c2 in S. typhimurium recA⁻*

Phage MOI	*ts 18.1c1*		*ts 18.1c2*	
	No UV	UV[a]	No UV	UV
2	196	103	256	162
10	131	74	93	78
20	149	60	14	24

[a] Cell survival after UV was 8×10^{-6}. The low survival fraction results from the *recA* lesion.

multiple retardation. This result shows that the UV sensitivity of *c*1 multiple retardation depends upon a functional *recA* gene. This same dependence has been observed for UV induction of prophage P22 (21) and suggests that the same pathway that inactivates *c*2 repressor also inactivates *c*1 product.

P22*c*2[5] did not cause observable lysis of PR-Pox before 210 min; P22*c*1[7], on the other hand, caused lysis at about 60 min (Fig. 3). We expected the *c*1 retardation exhibited by P22*c*2 to be partially lifted by exposing PR-Pox to UV before infection. The results show that UV had this effect; the time of lysis was shifted to about 120 min. Irradiation had little effect on the lysis time of P22*c*1[7]-infected cultures. These results again illustrate that functional *c*1 genes retard expression of lytic phage functions and that the extent of this retardation can be lessened by exposure to UV.

Irradiation reduces c1 retardation of phage DNA synthesis. Phage P22*c*1⁺ infections cause a transient repression in DNA synthesis at about 6 min after infection (14). We expected that UV irradiation would eliminate or reduce the retardative effects of *c*1 product on DNA synthesis rates. UV caused this result (Fig. 4). Figure 4a shows the pattern of DNA synthesis when MG109 was infected immediately after UV irradiation with P22*c*2[5]; Fig. 4b presents results obtained when infection was delayed until 15 min after irradiation. In the latter case the cells were incubated at 37 C in M9-CAA between irradiation and infection. In both cases, irradiation decreased DNA synthesis rates in infected and noninfected cells. Infection of nonirradiated cells (Fig. 4a) resulted in the typical P22 DNA synthesis pattern (14). Immediately after infection, the synthesis rate dropped for about 4 min, but at that time the rate increased until about 6 min. Smith and Levine (14) have shown that the decrease in DNA synthesis rate at about 6 min is due to *c*1 activity. There was no detectable effect of *c*1 on DNA synthesis rate when cells were infected immediately after irradiation (Fig. 4a). We interpret these results to show that UV irradiation interferes with *c*1 product activity.

We reached the same conclusion from the results in Fig. 4b, which were obtained with

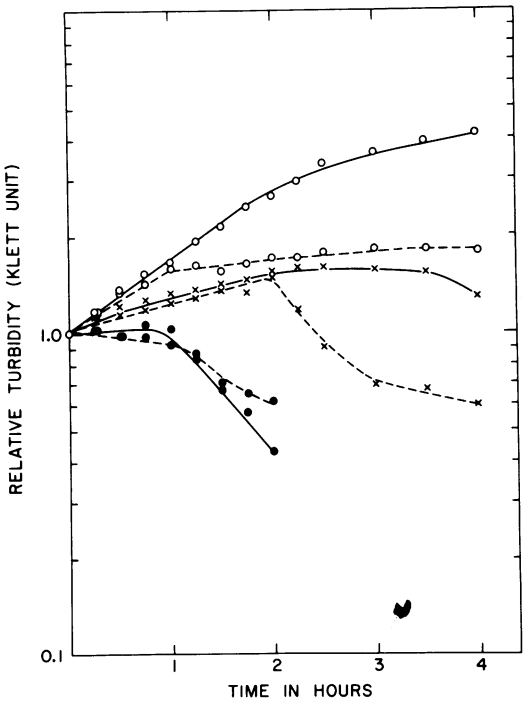

FIG. 3. *Lysis of PR-Pox after infection with P22c1[7] and c2[5]. Irradiated (dashed line) and nonirradiated bacterial cultures were infected with the indicated phages at MOI = 25. The fraction of cells that survived UV was 0.16. Uninfected control: —○—, no UV; - - -○- - -, irradiated. P22c1[7] infected: —●—, no UV; - - -●- - -, irradiated. P22c2[5] infected: —×—, no UV; - - -×- - -, irradiated.*

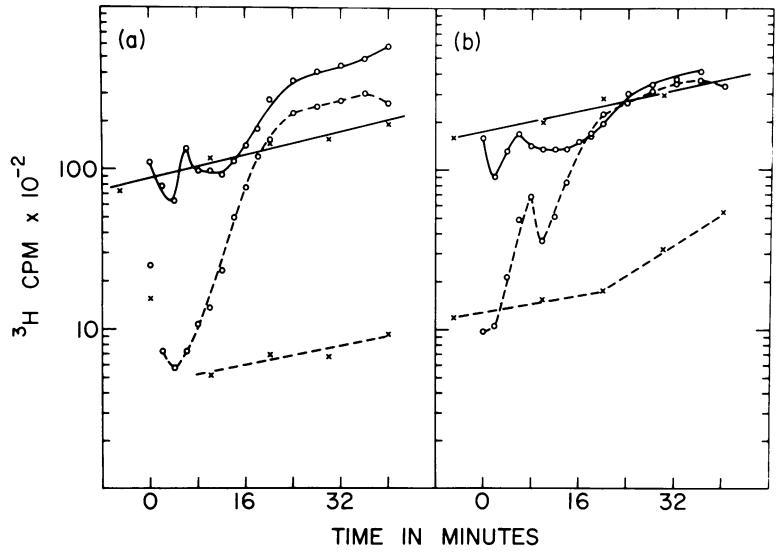

FIG. 4. *Effect of UV irradiation on rate of [^{3}H]thymidine incorporation in MG109. (a) The culture was infected immediately after UV irradiation. Cell density was 1.1 × 10^8 cells/ml, survival fraction was 0.11; MOI = 10. (b) The culture was incubated in M9-CAA for 15 min before infection. Cell density was 4.0 × 10^8 cells/ml; survival fraction was 0.26; MOI = 10. Nonirradiated uninfected control, —×—; irradiated uninfected, - - -×- - -; nonirradiated P22c2^5-infected, —O—; irradiated P22c2^5-infected, - - -O- - -.*

cells infected 15 min after irradiation. The infection on nonirradiated MG109 with P22*c*2^5 showed *c*1 retardation of DNA synthesis. The rate of synthesis was repressed beginning at 6 min and did not recover to the 6-min rate until 18 min. Irradiation 15 min before infection changed the duration of observed *c*1 retardation of DNA synthesis rates. The *c*1 retardation of DNA synthesis was seen at 8 min, but in another 5 min the rate had recovered to that seen at 8 min. In the nonirradiated infected culture, recovery to the same level required 12 min. These results are consistent with our hypothesis that UV affects *c*1 retardation.

UV irradiation diminished but did not eliminate *c*1 activity (Fig. 4b). Infection of MG109 immediately after irradiation with P22*c*1^7 (not shown) resulted in faster DNA synthesis between 4 and 20 min than was seen in the case of P22*c*2^5. This difference may indicate that there is residual *c*1 activity in the infection shown in Fig. 4a. The conditions that we used for irradiation were sufficient to diminish but not eliminate *c*1 retardation of DNA synthesis.

DISCUSSION

We have shown that the presence of a functional *c*1 gene in phage P22 retards expression of phage vegetative genes. This retardation can be lifted as a result of UV irradiation, and the effects of UV are mediated by *recA*. These results show that the *c*1 product, a nonrepressor regulatory function, is sensitive to UV.

The functions of P22*c*1 (6) and λ*c*II (3) are quite complex. They promote repressor synthesis and at the same time retard some lytic functions. UV inactivates all lambdoid phage repressors and many colicin repressors (11), suggesting that all of these repressors have a common sequence or shape that allows them to serve as substrate for a UV-induced inactivation enzyme(s). Perhaps the repressor-like function of P22*c*1 product requires that it share the sequence or shape of phage repressor. This would result in its being sensitive to *recA*-mediated UV inactivation. Alternatively, some entirely fortuitous similarity between the *c*1 protein and phage repressors may render it UV sensitive.

Smith and Levine (14) reported that preirradiation of the host did not change the pattern of phage DNA synthesis. Our results (Fig. 4) show that our conditions of irradiation altered DNA synthesis rates. There are at least three differences between their experiments and ours. After irradiation they aerated cultures under non-nutritive conditions; we did not. They used P22*c*$^+$ and we used P22*c*^{5}, but we do not think that these differences in genotypes would alter the activity of *c*1. However, their UV irradiation resulted in 0.01% bacterial survival, whereas in our experiments survival was 10 to 20%. The higher dose of UV may have resulted

in so much killing that no UV-induced enzymes could be formed.

Tomizawa and Ogawa (20) found that incubation in nutrient broth for 15 min after irradiation increased the efficiency with which λ immunity is lifted; incubation for 10 min under non-nutritive conditions did not result in lifting of immunity. These results showed that time is required for the production of the UV-induced product that inactivates λ repressor. We expected that there would also be a delay before UV inactivation of *c*1 activity could be detected. The results in Fig. 4a show that no period of incubation between irradiation and infection is necessary for the detection of UV inactivation of *c*1 activity. However, *c*1 activity is not detectable until 6 min after infection, and during the period from 0 to 6 min the UV-induced product would also be made. This product could then inactivate *c*1 product as it appears. We have no explanation for why *c*1 activity is more reduced in cells infected immediately after irradiation than in cells infected at 15 min after irradiation.

Hong et al. (7) isolated *cly* mutants of P22. These mutants lysogenize *S. typhimurium* with such frequency that P22 *cly*-2, for example, cannot form a plaque on wild-type hosts. We think that the *cly* mutation may be a "super *c*1 and that the super *c*1 increases repressor production and *c*1 retardation of lytic genes. If this is so, we expect *cly* activity to be UV sensitive. We have found that this is so. As expected, P22 *cly*-2 does not lyse nonirradiated MG109. However, MG109 preirradiated with UV are lysed by the mutant phage.

An immediate extension of our findings is to predict that λ *c*II is also UV sensitive.

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