Reports

Inactivation by Ultraviolet Light of an Acriflavine-Sensitive Gene Function in Phage T4D

Abstract. Mutants of phage T4D can be isolated which multiply in the presence of concentrations of acriflavine inhibitory to the growth of wild-type phage. Bacteria mixedly infected with resistant and sensitive phage are unproductive in the presence of acriflavine. Irradiation of the sensitive phage with ultraviolet light results in inactivation of the function of the gene for acriflavine sensitivity, permitting the mixedly infected complexes to yield phage. This function is inactivated in an exponential manner and has a cross section comparable to cross sections of other gene functions in T4D.

Mutants of phage T4D can be isolated which are capable of normal growth in low concentrations of acriflavine (0.25 per milliliter of "acriflavine neutral" [Nutritional Biochemical Corporation]). Under the same conditions, few (0.001 to 0.0001 percent) bacteria infected with wild-type phage are able to produce any infective particles. Crosses have shown that the sites of 12 out of 12 independently isolated acriflavine-resistant (*ac*) mutants are all about six map units from a terminal marker in the *r*IIB cistron, and the order *r*IIA, *r*IIB, *ac* has been established. Only 0.1 to 1 percent of cells infected with equal numbers of *ac* and *ac*$^+$ phage produce any phage in the presence of acriflavine. Thus acriflavine sensitivity (*ac*$^+$) is "dominant" over acriflavine resistance (*ac*) in the sense that acriflavine inhibits phage production in cells mixedly infected with *ac* and *ac*$^+$ phage.

Krieg (*1*) has measured the ultraviolet-light cross section of the *r*IIA$^+$ and *r*IIB$^+$ gene functions of phage T4D. In his experiments, *Escherichia coli*, strain K12(λ), bacteria were mixedly infected with unirradiated *r*II phage and irradiated wild-type phage. Krieg found that the fraction of mixedly infected bacteria which produced any phage decreased in an exponential manner as a function of ultraviolet dose to the *r*$^+$ phage, but at a rate much less than the rate of loss of infectivity of the phage particle. From this and other observations it was concluded that mixedly infected bacteria cannot produce phage unless the *r*II$^+$ gene of the infecting wild-type phage is free of ultraviolet damage affecting its function. The properties of the *ac*-*ac*$^+$ system described above make it possible to obtain a similar estimate of the ultraviolet target size of the *ac*$^+$ function. In contrast to the properties of the *r*II system, however, bacteria mixedly infected with *ac* and *ac*$^+$ phage are productive in the presence of acriflavine only if the *ac*$^+$ gene function in the infecting wild-type phage has been *damaged* by ultraviolet. Thus, in principle, one could mixedly infect bacteria with unirradiated *ac* and irradiated *ac*$^+$ phage and measure the increase in the fraction of mixedly infected cells yielding phage in the presence of acriflavine as a function of ultraviolet dose. From this one would obtain a measure of the *inactivation* of the *ac*$^+$ function, rather than of its survival.

In order to control effectively the condition of mixed infection, our experimental design was somewhat more complicated, combining both the *ac* and *r*II systems. In addition to the *ac* alleles, an *r*IIA(*r*61) and an *r*IIB(*r*73) marker were employed in these experiments. K12(λ) bacteria were infected at low multiplicity (0.2 of each phage) with unirradiated *r*IIA*r*IIB$^+$*ac* phage and with *r*IIA$^+$*r*IIB*ac*$^+$ phage which had been exposed to various doses of ultraviolet light (from a General Electric 15-watt germicidal lamp). Only mixedly infected bacteria will yield phage, because both the *r*IIA$^+$ and the *r*IIB$^+$ functions are required for productive infection of K12(λ). Since the multiplicity of infection is low, the majority of productive complexes will have received only one phage of each genotype.

Samples of the mixedly infected bacteria were plated on the indicator strain S/6. Any complex which yields phage forms a plaque on S/6. Thus we measure the number of mixedly infected cells which received a phage with an undamaged *r*IIA$^+$ gene function. This number decreases with increasing dose, because of damages of all kinds—that is, damages preventing injection as well as those specifically inhibiting the *r*IIA$^+$ function. Samples were also plated on plates supplemented with acriflavine. Here we measure the number of mixedly infected bacteria which received a phage with an undamaged *r*IIA$^+$ gene *and* a damaged *ac*$^+$ gene. The difference between the number on supplemented and the number on unsupplemented plates is the number of complexes which received both an undam-

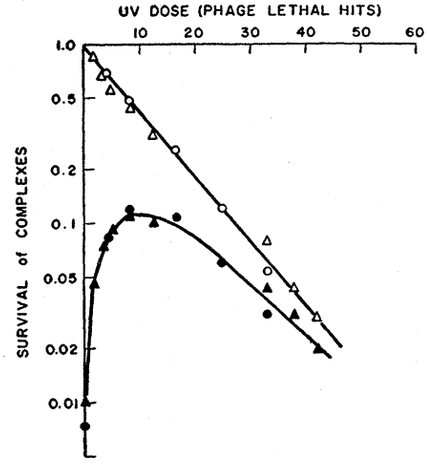

Fig. 1. Ultraviolet-light inactivation of infected bacteria plated with and without acriflavine. Strain K12(λ) bacteria infected with *r*IIA*r*IIB$^+$*ac* and ultraviolet-irradiated *r*IIA$^+$*r*IIB*ac*$^+$ phage are plated before lysis on plates seeded with S/6 (open symbols) and on plates seeded with S/6 and supplemented with acriflavine (solid symbols). Circles and triangles represent different experiments. The ordinate is the surviving fraction of plaque-forming infected bacteria relative to the plaque-formers at zero dose on unsupplemented plates. The abscissa is the dose expressed in phage lethal hits (*4*).

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

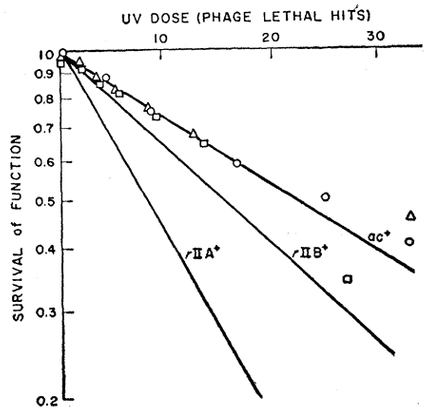

Fig. 2. Ultraviolet-light inactivation curve of the ac^+ function. Survival of ac^+ function is calculated as described in the text. Data from experiments illustrated in Fig. 1 (circles and triangles) and from an experiment with irradiated *r*IIA*r*IIB$^+$*ac*$^+$ and unirradiated *r*IIA$^+$*r*IIB*ac* (rectangles). Survival curves for the *r*IIA$^+$ and the *r*IIB$^+$ functions (obtained in these experiments) are shown for reference. For the ac^+ function, zero-dose values are less than 1, due to the small fraction of mixedly infected bacteria which form plaques in the presence of acriflavine (at zero dose).

aged *r*II$^+$ and an undamaged ac^+ gene. This number, divided by the number on unsupplemented plates, gives the fraction of complexes at each dose in which the ac^+ function survives *among* those in which the *r*IIA$^+$ function survives as well. Data from two experiments of this type are given in Fig. 1. The calculation of the data to give the survival of the ac^+ gene is presented in Fig. 2. Also given in Fig. 2 are comparable results from an experiment with unirradiated *r*IIA$^+$*r*IIB*ac* and irradiated *r*IIA*r*IIB$^+$*ac*$^+$. In this experiment the inactivation of the ac^+ function, together with the survival of the *r*IIB$^+$ rather than the *r*IIA$^+$ gene function, is measured.

Our results on the target sizes of the *r*IIA$^+$ and *r*IIB$^+$ functions are in good agreement with the previous measurements by Krieg (*1*). The survival of the ac^+ function, like that of the *r*IIA$^+$ and *r*IIB$^+$ functions, decreases in a simple exponential manner with increasing irradiation dose. The slope of the survival curve indicates that the ac^+ function is 0.03 of the total ultraviolet-sensitive target of the phage. No data are available at the present time which will permit a comparison between the ultraviolet cross section of the ac^+ function and an estimate of the length of the ac^+ gene obtained from mapping experiments.

In the experiments described here we measure only the damage to the ac^+ genes among those phage in which the *r*II$^+$ function survives. Any class of damages which simultaneously inactivate both the ac^+ and one of the *r*II$^+$ genes is not included in our estimate of the *ac* target size. One class of damage which might be expected to occur is that which renders the phage totally inoperative (for example, that which prevents injection). However, Barricelli (*2*) has suggested that irradiation damages, although discretely localized in the genome, may have a finite size. If this were true one would expect that some ultraviolet hits of this type could inactivate two neighboring genes at once. Indeed, Krieg (*1*) found a correlation between the inactivation of the *r*IIA$^+$ and the *r*IIB$^+$ genes which could be explained on this basis. If such extended damages contributed significantly to our data, the apparent target size of the ac^+ gene would be smaller in experiments requiring survival of the *r*IIB$^+$ gene than in experiments requiring survival of the *r*IIA$^+$ gene. As can be seen from the data presented in Fig. 2, no such effect is detectable. This only means that, if ultraviolet-induced lesions have a finite size, this size must be smaller than the distance between the *r*IIB$^+$ gene and the ac^+ gene (less than six map units).

Features of this system may make it useful in other studies on the genetic effects of ultraviolet irradiation. Since the cells which yield phage are those in which the irradiated parent has received at least one ultraviolet-induced lesion, there exists a selective system for examining the genetic consequences of very low doses of ultraviolet light. Since only that fraction of the irradiated phage which has a surviving *r*II$^+$ function yields phage, experiments are not influenced by damages which render the page totally inoperative (*3*).

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References and Notes

1. D. R. Krieg, *Virology* **8**, 80 (1959).
2. N. A. Barricelli, *ibid.* **11**, 99 (1960).
3. This work was aided by grants from the National Foundation and from the U.S. Public Health Service (RG-6965). One of us (R.H.E.) is a recipient of a postdoctoral fellowship from the U.S. Public Health Service.
4. F. W. Stahl, in *The Viruses*, F. M. Burnet and W. M. Stanley, Eds. (Academic Press, New York, 1959), vol. 2.
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Nonreinforced Trial Procedure for Probability Learning

Abstract. For rats in a T-maze, nonreinforced trials were interspersed with reinforced trials to insure that .67 of all reinforcements would be dispensed on the more frequently reinforced side. At asymptote, the proportion of responses to the more frequently reinforced side was .67.

Probability learning (as defined here) refers to experiments in which each trial ends with one of two responses. One response (called M in this report) is more frequently reinforced than the other response (called L). The independent variable is the ratio of reinforcements for M to all reinforcements; this ratio is called π. The dependent variable is the proportion of trials during which M is emitted; this proportion is called P. Functional relationships between π and P have been derived from theories about the effect of reinforcements on response probability, and much probability learning experimentation has been devoted to the development of these theories (*1*, *2*). Unfortunately, all the procedures used to control π in animal learning situations involve the introduction of factors not considered in these theories. In the light of generally applicable behavior principles, these factors should affect P as follows.

1) In the *forced trial procedure*, forced trials are interspersed with free trials in order to control π, and P is reckoned as the proportion of free trials during which M is emitted. An extraneous factor is a discrimination between forced trials and free trials which develops in the course of the experiment, so that reinforcement of forced trials affects P less than does reinforcement of free trials (*3*).

2) In the *correction procedure*, one response is selected for reinforcement on each trial, M being selected with probability π. If the initial response of the trial is not the response to be reinforced, the trial does not end until the reinforced response is emitted (that is, "correction" is permitted). P is reckoned as the proportion of trials during which M is the initial response of the trial. One possible extraneous factor in this procedure is that an initial response not scheduled for reinforcement may receive delayed reinforcement because it is followed by reinforcement of a correction. In addition, a correction is topographically different from an initial response, so that a discrimination between corrections and initial responses