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Damage in DNA of an infecting phage T4 shifts reproduction from asexual to sexual allowing rescue of its genes

CAROL BERNSTEIN

Department of Microbiology and Immunology, College of Medicine, University of Arizona, Tucson AZ 85724

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Summary

The selective advantage of sexual reproduction is widely regarded as a major unsolved problem in biology. Recently, it has been proposed that the fundamental selective advantage of sex is its promotion of recombinational repair and hence survival of DNA in the germ line of organisms. A bacteriophage T4 system was set up to test this theory. After a phage T4 injects its DNA into an *Escherichia coli* cell it quickly establishes a barrier, through its immunity function, to infection by a second phage T4, arriving at a later time. This barrier causes phage T4 to reproduce asexually. The immunity barrier has a selective advantage in preserving the host cell as a sole resource for the first phage. If the first phage's DNA is damaged by UV irradiation, however, it has a reduced probability of being able to survive in the host cell when it is there alone. It was found that UV irradiation, in addition to reducing the first phage's viability, also prevents the first phage from raising an effective immunity barrier. This UV-induced reduction in immunity now allows sexual interaction with later-arriving secondary phage or sexual reproduction. It was found that these secondary phage enhanced survival of genes of the UV-damaged first phage. This supports the theory that under DNA-damaging conditions, which should be prevalent in nature, sex would have a selective advantage.

1. Introduction

The adaptive function of sexual reproduction is a major unsolved problem in evolutionary biology (Williams, 1975; Maynard-Smith, 1978; Bell, 1982). In other words, it is not known why sex exists. In the last few years it has been proposed that a major selective advantage of sex lies in the promotion of efficient recombinational repair of DNA damage in the germ line of organisms (e.g. Bernstein 1979; Bernstein, Byers & Michod, 1981; Bernstein *et al.* 1985). This increases survival by removing lethal lesions, thus increasing fitness in the Darwinian sense.

In phage T4 the level of sexual interaction has thus far been found to be regulated by the timing of infections and by genetic controls. If a primary-infecting phage injects its DNA into a host cell, it is able to largely prevent infection by a second phage T4 if the second one adsorbs at least a minute after the first (Dulbecco, 1952*b*). Thus, sexual interaction between two phage genomes is greatly reduced if the infections are separated in time. This time-dependent reduction of sexual interaction largely reflects expression of the immunity gene (Vallee & Cornett, 1972; Mufti, 1972), and, to a lesser extent, expression of the spackle gene (Cornett, 1974).

In the laboratory, recombination is known to be increased by DNA damage but in these cases mating is forced by reversible KCN poisoning or starvation. (See table 3 in Schneider, Bernstein & Bernstein, 1978, for a list of eight cases in which this was demonstrated in phage T4.) Under natural conditions, however, without starvation or simultaneous double infections, immunity strongly promotes asexual reproduction in phage T4. On the hypothesis that the major function of sex is to promote survival by allowing repair of DNA damage, it might be expected that expression of the immunity functions would be decreased if the primary genome is damaged, so that sexual interaction, including recombinational repair of DNA, can occur.

I show here that DNA damage shifts reproduction from an asexual mode to a sexual mode. To my knowledge, this has never been demonstrated before in phage T4 or in any other organism.

2. Materials and methods

(i) *Bacterial and phage strains*

Two phage T4 *amber* mutants were used, *am*N52 (gene 37), a tail fibre mutant, and *am*S1 (gene 51), a base plate mutant. The restrictive host for the *am* mutants was *E. coli* S/6/5(su$^-$) and the permissive host was *E. coli* CR63(su$^+$).

(ii) *Media and growth conditions*

Phage titres were determined after dilution by plaque formation on agar plates by the agar overlay method (Adams, 1959) using Hershey agar (Steinberg & Edgar, 1962). High-titre phage stocks were prepared by growing phage lysates in Hershey broth (Steinberg & Edgar, 1962) followed by low-speed centrifugation at about 2000 **g** in relative centrifugal force (RCF) to remove debris, high-speed centrifugation at about 26000 **g** RCF to pellet the phage, resuspension of the phage overnight in adsorption salts solution (see below), and low-speed centrifugation at about 2000 **g** RCF to remove debris that had aggregated during the high-speed centrifugation. Bacteria were grown in Hershey broth. *E. coli* to be used as host cells in phage infections were first grown overnight to stationary phase, about 2×10^9/ml, and stored in the refrigerator for up to one month. On the day of an experiment an aliquot was diluted to 2×10^7/ml in Hershey broth and incubated at 37 °C with aeration. The concentration of cells was determined at frequent intervals using a Petroff–Hauser counting chamber. The cells were grown to 1–2 $\times 10^8$/ml and then centrifuged at about 2000 **g** RCF. The centrifuge was kept at 25–32 °C to avoid chilling the cells since chilling affects the time course of induction of immunity (Dulbecco, 1952*b*; and our unpublished observations). The cells were resuspended at 4–8 $\times 10^8$/ml in pre-warmed 37 °C adsorption salts solution consisting of (in g/l of distilled water) NH_4Cl 1·0 g, KH_2PO_4 3·0 g, Na_2HPO_4 6·0 g, at pH 6·8–7·0. The cell number after resuspension was estimated in a Petroff–Hauser counting chamber and adjusted to 4×10^8/ml with adsorption salts solution prewarned to 37 °C. The actual number of cells/ml was determined by dilution and plating in duplicate. Cells were allowed to starve in this adsorption salts solution at 37 °C for periods of 0–2·5 h until they were required in an experiment.

(iii) *Treatment of phage with UV light*

Phage, suspended in 20 ml of adsorption salts solution, were placed in a Petri dish 9 cm in diameter. For irradiation the dish was slid into position 21 cm below a UV lamp with a GE bulb, G8T5, for timed intervals. The bulb was masked except for a rectangular opening 6·5 × 7·0 cm. At all times during and after UV irradiation to the phage, work was carried out with general illumination by yellow 40 W GE Bug Lamps, to prevent photoreactivation.

(iv) *Interpretation of plaque formation*

If an *E. coli* S/6/5(su$^-$) cell is simultaneously infected by *am*N52(37) and *am*S1(51) and then plated on *E. coli* S/6/5(su$^-$), a plaque can form because the wild-type gene 51 protein is made by *am*N52(37) and the wild-type gene 37 protein is made by *am*S1(51). That is, complementation occurs. Also frequent 37$^+$51$^+$ wild-type recombinant progeny will be formed (gene 37 and 51 are ~ 40 kb apart on the chromosome) and these will contribute to formation of a plaque on the su$^-$ indicator bacteria. If the *am*N52(37) phage are UV-irradiated, their wild-type gene 51 may be damaged. When a mixed infection of UV-irradiated *am*N52(37) and unirradiated *am*S1(51) in an *E. coli* S/6/5(su$^-$) cell forms a plaque on *E. coli* S/6/5(su$^-$) plating culture, this indicates that the gene 51$^+$ marker in *am*N52(37) was phenotypically active in the mixed infection and that the 51$^+$ marker also participated in genetic recombination and duplication (Luria & Dulbecco 1949; Krieg 1959). This is usually called survival-of-phenotype (SP) plus cross-reactivation (CR) or marker rescue (*ibid.*). I call this SP plus CR of gene 51$^+$ the 'survival of gene 51$^+$', and the converse as the 'inactivation of gene 51$^+$'.

3. Results

(i) *Induction of immunity*

After infection of *E. coli* S/6/5(su$^-$) by undamaged primary phage, *am*N52(37), the time course of induction of immunity to superinfection by secondary phage *am*S1(51), was measured. The primary phage were allowed 2 min to adsorb to the host bacteria. The end of this 2 min was designated time zero. Then, after adding broth (round symbols, Fig. 1) or not adding broth (square symbols), secondary-infecting phage

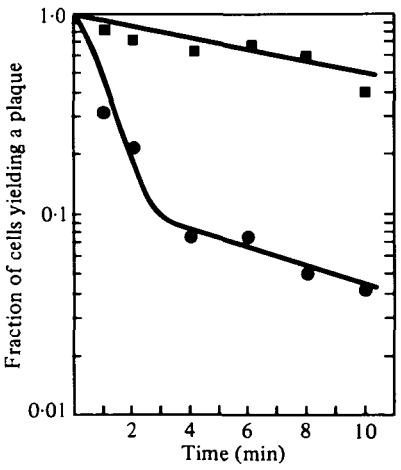

Fig. 1. Time course of induction of immunity. *E. coli* S/6/5(su$^-$) were infected by primary phage, *am*N52(37), and broth was added in order to allow full expression of immunity (●), or broth was not added (■). Secondary phage, *am*S1(51), were added at the times indicated. Each point represents plaques formed as a fraction of the number formed when secondary phage were added at time zero without addition of broth.

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were added at the times indicated. Addition of broth allows metabolism and thus expression of immunity. Each point represents infective centres formed as a fraction of the number formed when secondary phage were added at time zero without the addition of broth. Infective centres were measured by plaque formation on *E. coli* S/6/5(su$^-$) plating bacteria.

The results show that if broth is added there is a rapid decline in infective centre formation during the first 3 mins. After this, a slower decline in infective centre formation is observed. A second experiment, carried out in a similar manner, gave similar results (data not shown). We interpret the initial rapid decline as an expression of the immunity function of the primary phage. When no broth was added (square symbols) there is no initial rapid decline but rather a slow decline presumably reflecting a slow development of immunity.

(ii) *UV dosage measurements and side effects of UV dose*

Subsequent experiments require treatment of the primary-infecting phage with UV light in order to test the effect of DNA damage on expression of immunity. To standardize conditions of UV treatment, the UV lamp to be used was calibrated by irradiating a suspension of *am*N52(37) phage for a series of time intervals and plating on *E. coli* CR63(su$^+$). As discussed in the authoritative works of Harm (1980) and Adams (1959), the most accurate way of determining the UV dose delivered, in an experiment, is to compare the sensitivity of phage T4 under the conditions used to the sensitivity of phage T4 under standard conditions, such as those used by Harm (1980) in his fig. 4.5. Comparing the dose yielding 1% survival in our study to the dose yielding 1% survival by Harm's well calibrated methods, 1·0 second of irradiation in our study corresponds to 1·28 J m^{-2}. Thus, under our conditions, 1 lethal hit = 4 s irradiation = 5·12 J m^{-2}.

In the experiments described below, large doses of UV were administered to some aliquots of the primary infecting phage. Such doses might inactivate the ability of the phage to adsorb to host cells in addition to inactivating the phage's ability to carry out a successful infection once adsorbed (Dulbecco 1952*b*). Thus the doses of UV used here were evaluated for their effects on ability to adsorb. This was done by determining the phage's ability to kill the host cells. The data showed that at UV doses up to 100 phage lethal hits, the phages' ability to kill *E. coli* S/6/5(su$^-$) was reduced only by about 7% and the rate of this inactivation of killing ability is about 0·07% of the rate of inactivation of plaque-forming ability. Thus, observed effects of UV on expression of immunity should not be significantly affected by loss of capacity to adsorb. The relative rate of inactivation of killing ability to plaque forming ability that we obtained, 0·07%, is somewhat smaller than the relative rate of 1% obtained by comparing data of Dulbecco in two publications (1952*a*,*b*).

(iii) *Survival of gene* 51$^+$ *when sexual interaction is artificially promoted.*

As described in the Materials and methods, the survival of a gene during a phage T4 infection means that the gene is able to function phenotypically and to participate in genetic recombination and duplication so that it appears in progeny phage. If an *am*N52(37) phage is UV-irradiated and is within an *E. coli* S/6 cell along with an undamaged *am*S1(51) phage, wild-type progeny phage can only be produced if the gene 51$^+$ in the UV-irradiated parent can express its function, recombine and replicate (that is, if gene 51$^+$ survives). The open symbols in Fig. 2 show the survival of gene 51$^+$ of *am*N52(37) phage which received UV doses of 0–100 phage lethal hits (plh), and which were mixed with unirradiated *am*S1(51) phage and then allowed to undergo simultaneous heterologous infections of starved *E. coli* S/6/5(su$^-$) cells. The starvation of the host cells and the simultaneous presentation of the *am*S1(51) and *am*N52(37) phage to the cells artificially promotes the sexual interaction of the two phage.

For comparison, the survival of UV-treated *am*N52(37) phage which had been plated alone on the permissive host *E. coli* CR63(su$^+$) are shown by the filled symbols in Fig. 2. In this latter case survival depends on expression of all essential genes. The entire set of essential genes is the target and inactivation of any one inactivates the phage.

The experiments to measure survival of gene 51$^+$

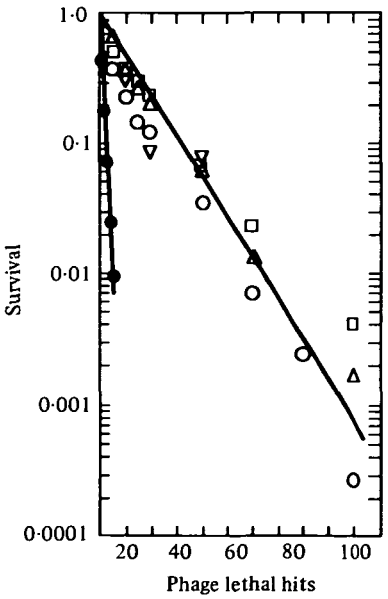

Fig. 2. Survival of gene 51$^+$ (open symbols) at different doses of UV light to the primary phage, *am*N52(37). The different open symbols represent independent experiments. For comparison the survival of the entire genome of *am*N52(37) is indicated by the filled symbols.

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were performed as follows. *E. coli* S/6/5(su⁻) was used as the host. A mixture of UV-irradiated *am*N52(37) and undamaged *am*S1(51) phage was brought to 37 °C. The titres of the phage had been adjusted so that when the mixture was added to the cells the damaged *am*N52(37) phage were at about 4×10^6/ml, the undamaged *am*S1(51) phage were at about 4×10^9/ml, and the *E. coli* host cells were at about 2×10^8/ml. Five minutes were allowed for adsorption. There were about ten *am*S1(51) phage and usually only one *am*N52(37) phage within each heterologously infected cell (only about 1% of the cells are heterologously infected). Then the infected cells were diluted appropriately and plated out on *E. coli* S/6/5(su⁻) indicator bacteria. The frequency of cells forming a plaque after 5–100 plh to the primary phage *am*N52(37) is plotted as a fraction of the number of heterologously infected cells forming plaques at zero UV exposure. The cells forming plaques have a surviving gene 51^+ from the UV-irradiated parent phage *am*N52(37) under these conditions of artificially promoted sexual interaction.

It should be noted that the frequency of plaques expected at 0 plh to *am*N52(37) (taken as 100% of survival) is less than the frequency of heterologously infected cells under these particular conditions. This is because of the limited number of genomes that can express per cell, an effect described by Snustad (1966*a*, *b*). Since about five genomes can express per cell, and there are about ten *am*S1(51) phage and one *am*N52(37) phage in a heterologously infected cell, the *am*N52(37) phage should be able to express its gene 51^+ only about half the time. The actual 0 plh survival of gene 51^+ was found to be 18, 29, 42 and 75% of the number of heterologously infected cells in four experiments. The fractional survival of gene 51^+ after irradiation however, should not be affected by this limited expression effect.

As shown in Fig. 2 the inactivation rate of gene 51^+ is about 13-fold less than the inactivation rate of the genome, as is to be expected of a smaller target. This rate of inactivation of gene 51^+ is similar to the inactivation of phenotype measured for other genes. Thus the phenotypes of genes $rIIA^+$, $rIIB^+$, and 42^+, and 43^+ are inactivated at rates that are respectively, 10-, 20-, 17- and 12-fold less rapid than inactivation of the genome (Krieg, 1959; Maynard-Smith & Symonds, 1973).

(iv) *Survival of gene 51^+ after UV treatment when addition of the second complementing phage is delayed to allow immunity function expression*

As implied in the Introduction, two important questions are whether damage to primary phage DNA increases sexual interaction with a delayed secondary phage, and whether this results in enhanced survival of genes in the damaged primary phage genome. The experiments in this section are set up to test these ideas.

E. coli S/6/5(su⁻) at about 4×10^8/ml were infected by primary phage, *am*N52(37), which were either untreated or treated with various doses of UV. The multiplicity of infection (moi) was 0·01 and 5 min were allowed for adsorption. After this primary infection an equal volume of pre-warmed 2 × -Hershey broth was added and metabolism was allowed to proceed for 3·4 min. The 2 × -Hershey broth consisted of twice the concentration of each ingredient except the NaCl. The NaCl was added at the standard concentration to keep the Na^+ concentration the same to promote optimal adsorption (Puck, Garen & Cline, 1951). Then, unirradiated secondary phage, *am*S1(51), also at an moi of 0·01 were added. Only cells receiving at least one primary and one secondary phage can form an infective centre on the *E. coli* S/6/5(su⁻) indicator bacteria used. As previously shown (Fig. 1), at zero UV dose an approximate three minute delay between primary and secondary infection, during which metabolism is allowed, causes about a 10-fold reduction in infective centers compared to simultaneous infection. This reduction is due to expression of immunity by the primary phage during the three minute delay which causes exclusion of the secondary phage.

Figure 3A–D shows the results of four separate experiments measuring the change in the survival of gene 51^+ in the circumstances described above, with increasing UV dose to the primary phage (round symbols). The conditions used here should mimic natural conditions since infection by the second phage

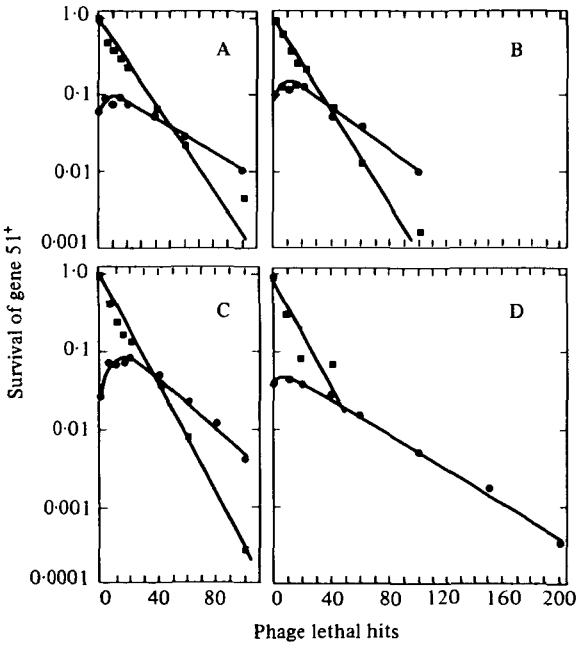

Fig. 3. Survival of gene 51^+ after UV irradiation of *am*N52(37) when primary infection is followed by metabolism and then secondary infection by unirradiated *am*S1(51) (round symbols). For comparison, survival of gene 51^+ is shown after a parallel simultaneous infection by the two phages (square symbols). The four panels show the results of four separate experiments.

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was not simultaneous with that of the first phage, and infection by the second phage took place in complete growth media. The number of plaques observed at zero UV dose is given as a fraction of the number of doubly infected cells as estimated from the cell concentration and the moi of each phage (viable plus inactivated). The square symbols in each panel, for comparison, show the survival of gene 51^+ in simultaneous infections (with artificial promotion of sexual interaction) determined in parallel with the more natural delayed-secondary infections. The data from the simultaneous-infections were obtained as described in the previous section of the Results and were also shown in composite form in Fig. 2. It was consistently observed that, at UV doses to the primary phage in the range of about 5–20 plh, infective centre formation and thus survival of gene 51^+ in the delayed secondary infections does not decline and is actually *higher* than at zero UV dose. This implies that UV-induced damage inhibits expression of immunity more rapidly than it inactivates gene 51^+. Sexual reproduction is promoted and asexual reproduction is decreased more rapidly than the measured gene is inactivated.

It can also be seen in Fig. 3 that the rate of decline of survival of gene 51^+ is slower in the delayed infections than in the simultaneous ones over the UV dose range from 40–200 phage lethal hits.

(v) *Comparing gene 51^+ survival in delayed and simultaneous infections*

It is of interest to make a quantitative comparison of gene 51^+ survival in delayed versus simultaneous infection. This can be done by calculating the ratio of [the fractional survival of gene 51^+ in the delayed infections] to [the fractional survival of gene 51^+ in the simultaneous infections]. The second bracketed parameter is the 'expected' fractional survival of gene 51^+ if there were no immunity and if survival of 51^+ is the same in either a simultaneous or delayed heterologous infection.

When these ratios of observed to 'expected' are less than one, it indicates expression of immunity and consequent asexual reproduction. The ratios from the four experiments depicted in Fig. 3 are plotted in Fig. 4. At zero dose of UV the ratio of observed to 'expected' was less than 0·1, indicating effective immunity to secondary infection. With 10 phage lethal hits of UV to the primary phage the ratio increases to about 0·25. This increase from $< 0{\cdot}1$ to 0·25 implies that immunity is inhibited by the UV treatment allowing more successful secondary infections. With about 45 plh to the primary phage, the ratio of observed to 'expected' is 1·0 suggesting that immunity is no longer a barrier to successful secondary infection. At 60–100 plh of UV to the primary phage the observed number of successful double infections rises above the 'expected' number.

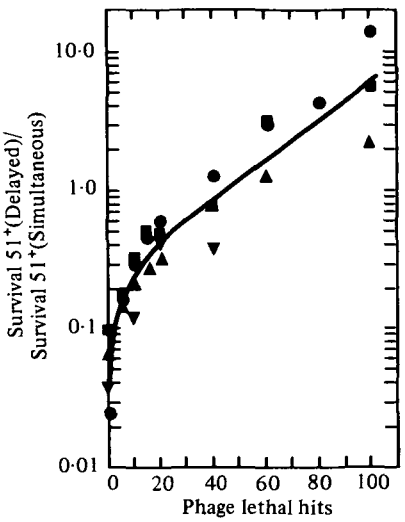

Fig. 4. Gene 51^+ survival after delayed secondary infection divided by gene 51^+ survival after simultaneous secondary infection, versus dose of UV to primary phage.

(vi) *Capacity of E. coli S/6/5 to support T4 functions is not affected by a period of starvation*

In each of the four experiments detailed above in Figs. 3 and 4, a set of eight delayed infections and a set of eight simultaneous infections were tested, using starved *E. coli* S/6/5(su^-) cells. After each set of infections, dilution and plating were immediately carried out. This meant that the *E. coli* S/6/5(su^-) cells often were starved for periods up to 2·5 h before use. To determine whether the capacity of the bacteria to support T4 function during the period of starvation was affected, aliquots of starved cells were tested during periods up to 2·5 h for their capacities to sustain phage infection and to allow the immunity function to be expressed. For this, aliquots of cells at $5{\cdot}5 \times 10^8$/ml were added to a mixture of phage, with *am*N52(37) at a final concentration of $7{\cdot}7 \times 10^6$/ml and *am*S1(51) at a final concentration of $1{\cdot}35 \times 10^5$/ml. Five minutes were allowed for adsorption and the infected cells were plated out with *E. coli* S/6/5(su^-) as the indicator bacteria. The data obtained indicated that virtually all simultaneously infected cells formed infective centres over the entire 2·5 h period.

To test the capacity of host cells to support the development of T4 immunity, delayed infections were carried out. In these experiments aliquots of cells at $5{\cdot}5 \times 10^8$/ml were infected at various times after initiation of starvation by primary phage, *am*N52(37), at $7{\cdot}7 \times 10^6$/ml and five minutes was allowed for adsorption at 37 °C. Then an equal volume of pre-warmed 2 × -Hershey broth was added to initiate metabolism, and, hence, expression of immunity. Three minutes after adding 2 × -Hershey broth, the secondary phage, *am*S1(51), were added at $1{\cdot}35 \times 10^5$/ml. Five minutes were allowed for adsorption of the secondary phage and the infected cells were plated out with *E. coli* S/6/5(su^-1) indicator bacteria.

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If immunity is induced as expected, a small fraction of the *am*N52(37) and *am*S1(51) heterologously infected cells should yield plaques. If the capacity of the host cells to support this immunity development remains constant during starvation the same small number of plaques should be produced at each of the times at which the cells are taken. It was observed that at each interval up to 2·5 h a fraction of about 0·1 of the heterologously infected cells were able to form infective centres.

A third kind of capacity experiment was also performed to test the ability of the primary-infecting phage to remain in a functional state during starvation. This involved primary infection in adsorption salts solution and, after an extended period of starvation, a short period of metabolism followed by secondary infection. Cells at $5{\cdot}5 \times 10^8$/ml were infected by primary phage *am*N52(37) at $7{\cdot}7 \times 10^6$/ml immediately after beginning starvation. At various times, from 5 min to 2·5 h, an equal volume of pre-warmed 2 × -Hershey broth was added, 3 min were allowed for immunity to develop and secondary phage, *am*S1(51) at $1{\cdot}35 \times 10^5$/ml, were added. Five minutes were allowed for adsorption of the secondary phage and the infected cells were plated with *E. coli* S/6/5(su$^-$) indicator bacteria. The data showed that during the first hour of starvation the ability to generate immunity against a second infecting phage increases by about 30%. From 1·0 to 2·5 h of starvation there was no further increase in ability to generate immunity. An ability to generate immunity under starvation conditions was also observed over a ten minute interval in the experiment shown in the top curve of Fig. 1. This immunity is developed even though very little metabolism should occur in the absence of an outside carbon source. Perhaps there is recycling of internal resources to allow some production of the immunity function.

4. Discussion

These experiments show that UV damage to a first-infecting phage shifts reproduction from a primarily asexual mode towards a sexual mode. In the sexual mode a second-infecting phage can enter and undergo recombination with the first-infecting phage. Genes from the damaged first-infecting phage have enhanced survival from the shift towards the sexual mode and their participation in the sexual reproduction process.

To my knowledge there have been no previous reports that a shift to sexual reproduction occurs in response to damage. The only somewhat similar study is one in which inhibition of immunity to superinfecting phage occured, caused by damage to primary infecting phage (Karska-Wysocki, Racine & Mamet-Brathy, 1982). In that study, when primary infecting phage T7 were treated with methyl-methanesulfonate to a survival of 0·5%, there was increased entry of second-infecting phage presented 5 min after entry of the first-infecting phage. This entry was measured by production of an enzyme by the second phage. Survival of genes of the damaged first phage, or its participation in reproduction after it was damaged, however, was not examined.

Other work relevant to the experiments reported here is that by Sinden & Pettijohn (1982). When they added inhibitors of the *E. coli* gyrase to phage T4 infected cells soon after infection, they observed a reduction in the synthesis of five proteins including two which they noted had molecular weights close to those of the immunity (*imm*) proteins. This suggests that reduction in the superhelicity of the phage T4 DNA by gyrase inhibition might block expression of *imm*. They also found that single strand breaks induced in the T4 DNA by incorporation of 5-bromodeoxyuridine plus irradiation wth 313 nm light blocked synthesis of similar proteins to those blocked by the gyrase inhibitors. Since single-strand breaks would also relax superhelicity this observation suggests that immunity functions might be inhibited by a reduction in DNA superhelicity, brought about by DNA damage.

In the work of Karska-Wysocki *et al.* (1982) discussed above with T7, alkylation damage to the first infecting phage also caused a delay in synthesis of proteins as well as permitting increased entry of secondary infecting phage. There, too, damage might have interfered with expression of immunity.

It was shown by Krieg (1957), in his fig. 2, that when both a UV-irradiated phage and a non-irradiated phage T4 infect simultaneously during a KCN metabolic-poison-forced mating, there is a strong tendency for genetic information from the undamaged phage to appear in progeny to the exclusion of information from the damaged phage. He showed that even when a gene on a damaged chromosome survived phenotypically there was only about a 15% chance it would appear in progeny phage if damage to the UV-irradiated genome exceeded 14 plh. The data in Fig. 4, however, show that if a damaged phage chromosome enters the cell first and is given 3·4 min to direct metabolism, then at high levels of UV damage a gene can be rescued up to 6-fold more effectively than expected from the gene survival under conditions of simultaneous infection during a starvation-induced mating. The rescue is beyond that expected from complete removal of immunity. This may indicate that, during the initial metabolic period after infection by a UV-damaged T4 genome, the damaged DNA enters a more rescue-prone form. Then, upon subsequent superinfection by a secondary phage the genes that escaped damage in the primary phage can be rescued with higher probability than could occur with simultaneous infection of UV-damaged phage plus non-damaged phage.

The immunity function in phage T4 apparently provides a gate to sexual interaction (sex) that may be

either closed or open. When there is no damage the gate is closed, presumably to preserve the host cell as a sole resource for the first-infecting phage. Damage to DNA, however, inhibits expression of the immunity function, allowing the gate to be open. This promotes sexual interaction yielding enhanced survival of genes from the damaged DNA.

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