**U.V. induced enhancement of recombination among lambda bacteriophages:
relation with replication of irradiated DNA**

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ABSTRACT

Experimental results are reported showing the dependence of the U.V. induced enhancement of recombinants on the presence of the functional O gene product. This fact is tentatively interpreted as a replication dependence of the U.V. induced recombination.

INTRODUCTION

The frequency of genetic recombination among lambda bacteriophages is increased by U.V. irradiation as shown by Jacob and Wollman². These authors proved also that this increase can be reversed by photoreactivation during the early phase of phage infection. One can, therefore, infer that some relationship must exist between this enhancement in recombination and the existence of U.V. induced pyrimidine dimers in parental phage DNA³. Baker and Haynes⁴ studied this increase in recombination frequency using HCR⁺ and HCR⁻ bacteria, and showed that recombination events affect the neutralization of U.V. lesions both on HCR⁺ and HCR⁻ strains, and that recombination affecting this neutralization must occur before the completion of repair.

On the other hand, Rupp *et al.*⁵ proposed that, during recombination repair in *E.coli*, two main steps must occur:

- i) "Replication of DNA chain, leaving gaps opposite the original damage".
- ii) "Sister exchange involving a single strand of each duplex, that may insert the correct base sequence into the gap opposite the pyrimidine dimer, and thus promote the reconstruction of a genome with the complete base sequence needed for the survival of the colony-forming ability".

In the light of the above works the present paper has

been aimed at looking for the possible existence of a relationship between the DNA replication and the increase in genetic recombination in multiple infection of U.V. irradiated lambda phages.

We have performed genetic crosses of U.V. irradiated lambda phages in mutant E.coli strains known to be HCR^- and we compared the results with those obtained in HCR^+ host. Moreover, in order to ascertain whether DNA replication is needed for the expression of the U.V. induced enhancement of genetic recombination, we also performed crosses where DNA replication of lambda phage was blocked by using a thermosensitive lambda mutant whose DNA does not replicate at $40^\circ C$.

MATERIALS AND METHODS

Phage strains - The following lambda phage sus mutants have been used in the crosses related to this paper: λ sus N_{219} cII_{2002} ; λ sus $R_{221}c^+$ (Thomas et al.⁶). We also used the mutants λ sus $N_{219}O_{28ts}$ cII_{2002} and λ sus $R_{221}O_{28ts}c^+$ obtained through suitable crosses. Phage λ O_{28ts} have been isolated by A.W. Harris.

Bacterial strains - Genetic crosses have been performed on the following permissive bacterial strains: AB1157 uvr⁺ and AB1886 uvrA₆⁻, isolated and described by Howard-Flanders et al.⁷ and Howard-Flanders and Theriot⁸.

Sus⁺ recombinants were selected on the non permissive bacterial strain E.coli 594 (Campbell⁹). The titer of the lysates was scored on the permissive bacterial strain C600 (Appleyard¹⁰).

Media - TA_7 soft agar and TA_{12} agar media have been described by Kaiser¹¹. Other media are:

TM: 1% tryptone, 0.5% NaCl, $10^{-2}M$ $MgSO_4$

Mineral medium: $10^{-2}MgSO_4$, $2 \times 10^{-2}M$ phosphate buffer, pH 7.4

Tris-Mg-gelatine: $10^{-2}M$ -Tris, $10^{-2}MgSO_4$ pH7.4 and 0.01 gelatine added after autoclaving.

Ultraviolet irradiation - U.V. irradiation was performed using a Hanau type S500 quartz lamp. U.V. light was filtered by using an interferential filter (Infrared Industries Inc., Waltham, Mass.) centred at 2650 Å. Dose rates was measured with a Latarjet dosimeter¹². Phage suspended in Tris-Mg-gelatine at a con-

centration of 10^{10} /ml for each parent were irradiated in a layer 1-2 mm. deep in glass Petri dishes. All phases of the experiments which were susceptible to photoreactivation were performed under red light.

Phage titration - One of the two lambda phage mutants used in our crosses exhibit a clear and the other a turbid phenotype. Using phages resuspended in Tris-Mg-gelatine we prepared a large calibrated stock containing a mixture of both mutants which yielded an equal number of clear and turbid plaques. Phage stocks were titered on E.coli C600. The titer of revertants was determined by plating on E.coli 594.

Phage crosses - We shall call here $R(d)$ the titer of lambda sus⁺ progeny in the crosses at a given U.V. dose d and $R(o)$ the titer of lambda sus⁺ progeny in the unirradiated control.

In each experiment we performed up to ten crosses in tandem. An unirradiated control was performed for each experiment. Exponentially growing bacterial cultures were centrifuged, resuspended in equal volume of $10^{-2}M$ $MgSO_4$, pH 7.4 and then starved for about 90 minutes at $37^\circ C$. After starvation bacteria were again centrifuged and resuspended in mineral medium at a density of 10^9 /ml. The cells were then infected with a multiplicity of five phages of each parent. Twenty minutes adsorption at $37^\circ C$ were then allowed.

Following adsorption, two different pathways were followed in tandem for each cross:

i) Determination of the ratio $R(d)/R(o)$.

For the determination of the $R(d)/R(o)$ ratio, aliquots of the infected bacteria were directly diluted x 500 in nutrient medium and then incubated at a suitable temperature (37, 30, $40^\circ C$). After 120 minutes of incubation chloroform was added to the cultures.

ii) Determination of the recombination frequency.

For the determination of the recombination frequency, the infected bacteria were gently layered in a centrifuge tube at the top of 5 ml of mineral medium with 10% glycerol added, and then centrifuged at about $1.5 \times 10^3 g$ for 15 minutes. The pellet was then resuspended in 1 ml of mineral medium, diluted x 500 in TM and then incubated at a suit-

able temperature (37, 30, 40°C). After 120 minutes of incubation, chloroform was added to the cultures. By this procedure (Radman M., personal communication) a very satisfactory elimination of non-adsorbed phage particles was achieved.

We used separate tubes for the R(d)/R(o) measurements on one hand and the recombination frequency on the other in order to:

- i) Avoid alterations in the R(d)/R(o) ratio caused by differences in the recovery of infective centers after centrifugation.
- ii) Eliminate non-adsorbed phage in the determination of recombination frequency.

For determination of the R(d)/R(o) ratio, elimination of non-adsorbed phage was not necessary since we scored only on the non permissive bacterial strain E.coli 594.

The recombination frequency was measured by dividing the titer of each lysate scored on E.coli 594 by the titer on C600.

The R(d)/R(o) values were obtained from assays of the bursts on E.coli 594 as follows:

$$\frac{\text{titer of } \lambda \text{ } \underline{\text{sus}}^+ \text{ progeny phages in the cross at a dose d}}{\text{titer of } \lambda \text{ } \underline{\text{sus}}^+ \text{ progeny phages in the unirradiated control}}$$

This ratio was obtained in order to determine the dependence of the absolute number of recombinants on U.V. dose without regard for the total number of progeny which figures into the recombination frequency.

All crosses have been performed twice; some crosses have been further repeated in order to check reproducibility.

RESULTS AND DISCUSSION

Recombination among U.V. irradiated lambda phages - The curves in Fig.1,(a,b) show respectively the R(d)/R(o) ratio and the recombination frequency as a function of the U.V. dose in crosses: $\lambda \text{ } \underline{\text{sus}} \text{ N}_{219}^{\text{CII}} 2002 \cdot \lambda \text{ } \underline{\text{sus}} \text{ R}_{221}^{\text{c}^+}$ in uvr⁺ and uvrA⁻ bacteria. Curves for the recombination frequency are in agreement with those previously reported by Baker and Haynes (1967). The R(o) titer in the lysates for this and the following experiments are all reported in Table I.

Fig. 1 - Crosses of λ sus N₂₁₉cII₂₀₀₂: λ sus R₂₂₁c⁺ in *E. coli* AB1157 *uvr*⁺ ● and *E. coli* AB1886 *uvrA*⁻ ■ bacteria at 37°C.
 a) $R(d)/R(0)$; b) Recombination frequency %.
 The extremes of the segments reported in the figure represent the maximum and minimum experimental values obtained.

From the curves in Fig.1(a) one sees that the number of recombinants increases with U.V. dose and that this increase ends both in *uvr*⁺ and *uvrA*⁻ systems when a certain U.V. dose is reached. From Fig.1(a) it is also evident that, when this dose is

reached:

- i) In uvrA^- system (where no excision repair occurs), a sharp drop in the R(d)/R(o) ratio takes place.
- ii) In uvr^+ system within the dose range we explored, the above ratio maintains its high, almost constant, value.

On the other hand, from the results shown in Fig.1(b) one sees that for similar values of U.V. dose a sharp increase occurs in the recombination frequency in uvrA^- , while a saturation is reached for uvr^+ bacteria.

Table I
Average value of absolute number/ml. of
recombinants R(o) in non irradiated controls.*

Relevant genetic characteristics	30°C	37°C	40°C
$\text{uvr}^+ \text{Ots}^+$	$7,5 \times 10^8$	$7,5 \times 10^8$	$1,2 \times 10^9$
$\text{uvrA}^- \text{Ots}^+$	$7,5 \times 10^8$	$1,1 \times 10^9$	$1,3 \times 10^9$
$\text{uvr}^+ \text{Ots}^-$	$8,2 \times 10^8$		$3,7 \times 10^7$
$\text{uvrA}^- \text{Ots}^-$	$8,5 \times 10^8$		4×10^7

*The titers are indicated with reference to the original mixtures, prior to dilutions.

If replication is needed for U.V. induced enhancement of recombination, this behaviour could be related to the inhibition of λ DNA replication, after a certain critical U.V. dose as suggested by Radman *et al.*, Fornili *et al.*^{13,14}. We shall discuss Fig.1 also in connection with the results given in the following paragraph.

Recombination among U.V. irradiated thermosensitive lambda mutants during thermal inhibition of phage DNA replication - In order to ascertain the influence of DNA replication on the U.V. enhancement of genetic recombination, we performed the already described crosses by using phages in which a thermosensitive mutation $\text{O}_{28}(\text{ts})$ had been added to both parents.

As it is well known, the O-gene product is absolutely necessary for replication of λ phage DNA^{15,16,17}. DNA of phage

Ots mutants cannot then replicate at 40°C but it replicates normally at 30°C.

Fig. 2 - Crosses at 40°C of λ sus N₂₁₉CII₂₀₀₂ · λ sus R₂₂₁C⁺ in *E. coli* AB1157 uvr⁺ $\bullet$ and *E. coli* AB1886 uvrA⁻ $\blacksquare$ bacteria, and of λ sus N₂₁₉CII₂₀₀₂^{O₂₈ts} · λ sus R₂₂₁C⁺Ots in uvr⁺ $\circ$ and in uvrA⁻ $\square$ bacteria. a) $R(d)/R(o)$; b) Recombination frequency %. To avoid confusion in the figure, only the average values of the experimental results have been reported for Ots⁺ curves.

Curves in Fig.2(a) show the $R(d)/R(o)$ ratio in crosses performed at 40°C under replicating (Ots^+) and non replicating (Ots^-) conditions. From the analysis of these results one can deduce that a relationship must indeed exist between the U.V. induced enhancement of the absolute number of recombinants and the possibility for DNA to replicate. In fact, in both uvr^+ and $uvrA^-$ bacteria a decrease in the $R(d)/R(o)$ is present even for very low U.V. doses under O^- conditions. This decrease, however, is very slight in uvr^+ bacteria. One might therefore think that the lack of U.V. induced increase of the number of recombinants in λ phage is related to the absence of postreplicative DNA structures, or more generally to the fact that DNA replication is essential for the U.V. induced enhancement of the number of recombinants.

Curves in Fig.2(b) show that even under non replicative conditions an increase in the recombination frequency is found.

The recombination frequency increase in $uvrA^-$ bacteria when replication is blocked (in spite of the decrease in absolute number of recombinants) can be explained by considering that in this system, under these conditions, the "normal" i.e. non U.V. stimulated genetic recombination is the only way of increasing the viable progeny. This fact will cause the increase in the number of recombinants among the progeny.

At 40°C in uvr^+ bacteria, the recombination frequency for Ots mutants saturates at a higher value than for Ots^+ phage. This may be interpreted on the grounds that if DNA cannot replicate, postreplicative repair cannot take place and that again genetic recombination among parental phage DNA is a way of increasing the plaque forming progeny. Again this fact causes an increase of recombinants among the progeny.

In the light of the above results under $ts\ O^-$ conditions some suggestions can be advanced to explain the experimental curves obtained under replicative conditions.

The saturation of the recombination frequency (shown in both Figs.1(b) and 2(b)) in uvr^+ system, could be explained following the works of Radman *et al.*¹³, Fornili *et al.*¹⁴. In fact if λ DNA replication, above a certain critical U.V. dose, takes place only after the damages in the region controlling DNA replication have been repaired, it is likely that, on the average,

an almost constant number of U.V. damages will be left, when replication is allowed to start. This could bring about a constant number of genetic recombinants among the surviving progeny. It is interesting to note that, as shown in Fig.1(a) and 2(a), for similar U.V. dose also the $R(d)/R(o)$ ratio reaches its maximum value in uvr^+ system, and that in uvr^- bacteria, where no excision repair takes place, the $R(d)/R(o)$ ratio starts to decline about for the same U.V. dose.

Comparing the results in Fig.2(a) with those in Fig.2(b) one can deduce that, under O^- conditions, there is a decline in the number of parental types which parallels the decline in recombinants. This fact could lead one to think that the O_{ts} effect could be simply that these deficient infections were abortive because of the combination of thermal treatment and U.V. irradiation. In order to see whether this was the case or not we performed single infection survival experiments with our O_{ts} mutants, where single infected bacteria were incubated in TA_7 and kept at $40^\circ C$ in small tubes. After 120' incubation, plating bacteria were added and the cultures were directly plated and incubated overnight at $30^\circ C$.

The absence of lysis during the incubation time at $40^\circ C$ of single infected bacteria was carefully ascertained through control cultures maintained at $40^\circ C$ in TM. After 120' incubation $CHCl_3$ was added to these last cultures that were left a few more minutes at $40^\circ C$ in order to let the chloroform set while the cultures were at $40^\circ C$ ¹⁸.

The results of the above experiment are reported in Fig.3 together with: 1) the survival of O_{ts} phages at $30^\circ C$ without preincubation at high temperature; ii) the total progeny of our crosses performed under non replicative conditions.

As can be immediately seen, from single infection experiments no degradation is caused by the combination of U.V. irradiation and high temperature preincubation in uvr^+ while a very slight one is exhibited in $uvrA^-$ system¹⁹. Moreover, above a certain U.V. dose, the rate of decrease in the crosses progeny starts to be lower than the rate of decrease of survival in single infection. This behaviour is in agreement with the interpretation we have given for the high value of recombination fre-

Fig.3 - Survival of $N_{219}cII_{2002}Ots$ phages in single infection of:

Δ AB1157 *uvr*⁺ 30°C; ○ AB1157 *uvr*⁺ 40°C;

▽ AB1886 *uvrA*⁻ 30°C; □ AB1886 *uvrA*⁻ 40°C

The dashed lines represent the total surviving progeny for crosses under not replicative conditions respectively in *uvr*⁺ and *uvrA*⁻ bacteria.

Because of the procedure followed in our crosses (see Materials and Methods), the curve for the total surviving progeny could not be obtained by merely reporting the lysate titer on permissive bacteria. In fact fluctuations in the recovery after the centrifugations, should have brought about meaningless curves. The dashed curves have therefore been obtained from *Ots*⁻ curves reported in Fig.2(a,b) by using the following relation:

$$\frac{S(d)}{S(0)} = \frac{\text{Recombination frequency \% at dose zero}}{\text{Recombination frequency \% at dose } d} \times \frac{R(d)}{R(0)}$$

quency obtained under non replicative conditions. Indeed if the above increase in recombination frequency was to be ascribed to the fact that dimers behave as recombination promoting centers, the difference between crosses progeny and single infection survivors should have started from the lowest U.V. dose rather than when, according to our interpretation, phage rescue, due to recombination, starts to be dominant.

In the light of the experiments of Stahl *et al.*^{20,21} one could object to the interpretation of our *Ots* results that if

recombination is mandatory for successful maturation of lambda phage DNA molecules under non replicative conditions, it may happen that under O^- conditions, U.V. does not produce any effect since a very large fraction of DNA molecules undergoes recombination anyway, bringing about a saturation in the number of recombinants. In order to overcome this objection let us start from the following considerations: i) under non replicative conditions, no selective advantage of recombinants can take place and therefore the recombination frequency can at most reach the 25% value; ii) in our O^- crosses this value is almost reached, both in uvr^+ and $uvrA^-$ systems, in spite of the fact that the DNA region between the sus markers we used is only a small fraction of the total phage lambda DNA. Consistent with the whole set of our results we interpreted this fact as if in these deficient infections, phages were selected whose DNA through multiple events of "normal" (non U.V. stimulated) recombination have eliminated pyrimidine dimers. On the other hand the low value of the recombination frequency at zero U.V. dose indicates that among the total progeny the phages whose DNA has undergone multiple crossovers are a small fraction, the majority of the progeny having perhaps undergone a single recombination event, needed for maturation. One can therefore overcome the above objection by considering that the DNA of the majority of the phages in the lysates of unirradiated crosses could have undergone a higher number of crossovers.

In order to check whether under replicative conditions our sus mutants thermosensitive and non thermosensitive in O gene behave in the same way, control crosses were performed at 30°C . The results reported in Fig.4(a,b) confirm that O -thermosensitive and non-thermosensitive mutants exhibit the same kind of behaviour, although a difference is present in the $R(d)/R(o)$ ratio. This effect is in agreement with a plausible lower efficiency of the O gene product for O_{ts} mutants also at 30°C .

By comparing our experimental results obtained with non-thermosensitive mutants at different temperatures, the presence of thermal effects is evident. Although an explanation of these effects is beyond the aims of the present paper, they might tentatively be related to thermal effects on DNA replication.

Fig. 4 - Crosses at 30°C of λ sus $N_{219}cII_{2002}$ · λ sus $R_{221}c^+$ in *E.coli* AB1157 *uvr*⁺ ● and *E.coli* AB1886 *uvrA*⁻ ■ bacteria and λ sus $N_{219}cII_{2002}O_{28}ts$ · λ sus $R_{221}c^+O_{28}ts$ in *uvr*⁺ ○ and *uvrA*⁻ □ bacteria.
a) $R(d)/R(o)$; b) Recombination frequency %.
Again to avoid confusion in the figure, only the average values of the experimental results have been reported.

The interpretation we have given of our results is also consistent with the results of the very thorough work of Baker and Haynes²². We think in fact that the differences they obtain-

ed between uvrA⁻ viability index curve and their reference curve, could also be explained in terms of an increase of the viable λ -P progeny through recombination among replicated and/or non-replicated λ -P phage.

Moreover our results are in agreement with the results of Howard-Flanders and Lin²³ on the absence of pyrimidine dimers induced recombination in repressed phage λ .

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- 18 An alternative way of performing the above single infection experiments would have been to incubate plated infective centers for 120' at 40°C before shifting dishes at 30°C. We discarded this procedure for the following reasons: i) If Petri dishes prewarmed at 40°C, before plating, are used, the top agar does not harden during the incubation period at 40°C leading to rough and non countable dishes. ii) If Petri dishes taken at room temperature are used, the top agar does harden, when plating is carried out at 40°C, but non reproducible

- results are obtained. This fact is perhaps to be ascribed to the time interval elapsed before dishes reach the 40°C temperature.
- 19 The single infection survival experiments have been performed three times each. As can be seen from Fig.4 in the lower part of single infection curve experimental points of uvr⁺ at 40°C lie a little over those of uvr⁺ at 30°C. On the contrary, experimental points for uvr⁻ at 40°C lie a little below those of uvr⁻ at 30°C. We think it is worth mentioning here that experimental points were in each single experiment in the same way as for the mean values reported in the figure i.e. in agreement with results of Radman et al. (1970). However for the sake of clearness we thought better to draw single curves.
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