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DIRECTION OF INJECTION OF LAMBDA GENOME: GENETIC ANALYSIS OF DONOR CONTRIBUTION FOLLOWING GAMMA IRRADIATION

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Marker rescue experiments following ^{60}Co irradiation of lambda bacteriophage have been carried out on three different hosts; *rec*$^+$, *rec* B and *rec*BC*sbc*A. Rescue of markers representative of the whole genome was carried out. No differences in radiosensitivities of individual markers were observed in the *rec*$^+$ host. The ability of any gene to be rescued was about one half as sensitive to ^{60}Co radiation as whole phage survival. Difference in the radiation sensitivity of markers was observed in both *rec*B and *rec*BC*sbc*A hosts. All markers were more sensitive to radiation when marker rescue was carried out on *rec*$^-$ hosts. Markers residing on the right arm of the lambda molecule showed a lower sensitivity to radiation than markers on the left. The results are consistent with the idea that the lambda molecule has a unique end for initiation of injection and a polar entry. It is possible that portions of the molecule on the origin side of a radiation induced break are transferred to the host bacterium and under special conditions recovered by recombination.

Key words: lambda, marker rescue, gamma irradiation.
Ključne reči: lambda, spasavanje markera, gama zračenje.

INTRODUCTION

Following X–irradiation, lambda bacteriophage show a dose dependent reduction in the amount of DNA injected into recipient bacteria (S h a r p and F r e i f e l d e r,

1971). This has been interpreted as a partial reduction in the amount of DNA injected by some or all of the damaged phage. Genetic tests have revealed such partial contributions of the SP82G genome take place following shearing during injection (M c A l l i s t e r, 1969), and following ^{32}P decay (M c A l l i s t e r and G r e e n, 1973, K r i s c h, 1974) and in T7 following X–irradiation and ^{32}P decay (P a o and S p e y e r, 1973, K r i s c h, 1974). Cited experiments suggest that those markers which are introduced first are the least radiosensitive and those that enter last are the most sensitive. A similar correlation of radiosensitivity with marker position on the lambda genome has not been observed, and attempts to correlate the contribution of functional complementation with marker location have been ambigous (S h a r p, D o n t a and F r e i f e l d e r, 1971).

Because the *rec*BC$^+$ function has been shown to be prejudicial to the survival od DNA fragments (O i s h i and C o s l o y, 1972, W a c k e r n a g e l, 1972, B e n z i n g e r, E n q u i s t and S k a l k a, 1975), an examination of the marker rescuability of irradiated lambda injecting into *rec*$^+$, *rec*B and *sbc*A supressed *rec*BC$^-$ cells was undertaken.

MATERIAL AND METHODS

A coded set of eight amber mutants representative of different regions of the lambda genome was kindly provided by F. W. S t a h l. The present study was carried out in a double blind manner. No attempts were made to map following mutants: Sus R5, Sus Q21, Sus L63, Sus V458, Sus A32, Sus D123 and Sus J6. One of the mutant, Sus D123 was consistently found to have high reversion titer (1–4 revertants/10^5 viable phages) and was not used.

All bacterial strains were derivatives of *E. coli.* Three different permissive host bacteria were C600 (*rec*$^+$), MMS1 (*rec*B21) and JC8679 (*rec*B21*rec*C22*sbc*A23*sup*E44). As non–permissive host the strain 594 was used.

High titer phage stocks were prepared as described by A d a m s (1959). Mutant Sus J6 (previously selected by Stahl as the „middle most marker") was prepared for irradiation according to the procedure of S h a r p and F r e i f e l d e r (1971).

Irradiation in a ^{60}Co source and dosimetry (H o h n e and B e r r y, 1970) were carried out by Dr Brana Radak, whose valuable contribution we gratefully acknowledge. Radiation was carried out at ambient temperature, 18^{o}C, under nitrogen. The dose rate was 3.66 kilorads/minute. Sampes were typically removed at eight minute intervals. The ability of lambda bacteriophage to contribute genetic markers following irradiation was determined by scoring recombinant infective centers formed when the irradiated Sus J6 mutant was mixedly infected at low multiplicity (0.05 phage/bacteria) with a „rescuing" *sus* mutant phage at high multiplicity (5–10 phage/bacteria). Following mixed infection on a permissive host and a 10 minutes adsorption at 37^{o}C, infective centers were diluted and plated on non–permissive host *E. coli* 594. Tipically, between 0.01 – 0.4 % of the infective centers dependent on the rescuing phage were recombinant at zero dose.

RESULTS AND DISCUSSION

The frequency of recombinant infective centers as a function of dose to the irradiated Sus J6 shows an exponential relationship for each marker on the host bacteria examined (see Fig. 1). The results for three hosts tested (C600, MMSl, JC8679) are

presented in the Table 1. As it can be seen the three different hosts show only slight differences in measuring whole phage survival (2.63 – 2.98 lethal hits/100 kilorads). On the contrary, there are differences in the rescue of genetic markers which are significant: C600 shows the least sensitivity for marker rescue to irradiation (1.07 marker rescue inactivating events/100 kilorads) and only slight differences between the six markers examinde; MMS1 shows greater sensitivity (1.48/100 kilorads) and JC8679 is the most sensitive (1.7/100 kilorads). Upon completion of the radiation analysis, these findings were exchanged for the marker identification, i.e. the marker position on the lambda genome (F.W. S t a h l, personal communication, see Tab. 1a)).

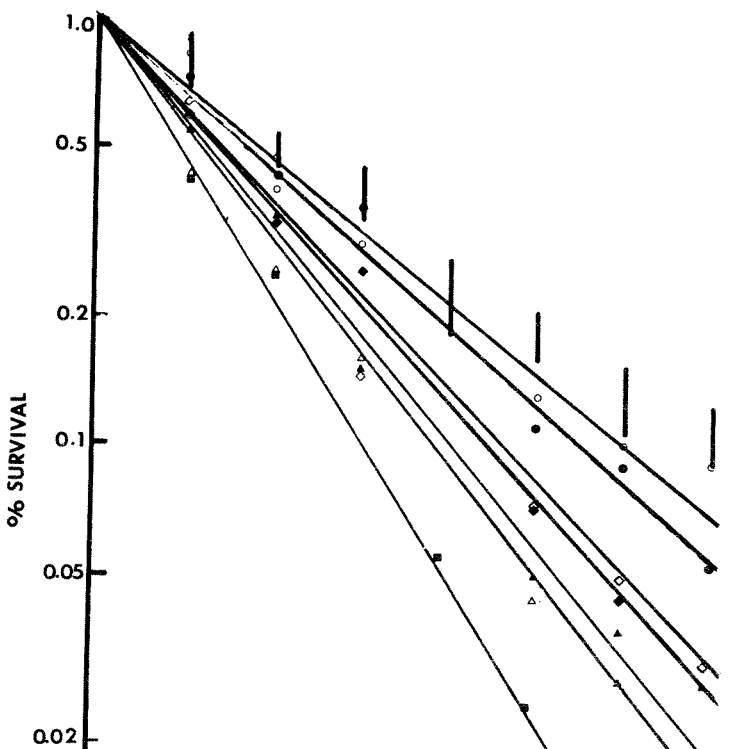

Fig. 1. – Frequency of recombinant infective centers as a function of dose to Sus J6. Genetic markers of rescuing phages: Sus P3 (o——o), Sus R4 (●——●), Sus V458 (◆——◆), Sus Q21 (◇——◇), Sus A32 (▲——▲), Sus L63 (△——△), whole phage (■——■).

Tab. 1. – Markers rescue

	Rescuing Phage							
	Sus R5 a) (5.5)	Sus Q21 (8)	Sus P3 (18)	Sus L63 (76)	Sus V458 (81)	Sus A32 (96.5)	Mean ± SEM	Whole Phage
	b) *Hits/100 kilorads*							
Host Bacteria								
C600 (*rec*$^+$)	1.09 1.17	1.15	0.89 0.98	1.24	1.24 0.94	0.97	1.07±0.04	2.63
MMS1 (*rec*B21)	1.38 1.34	1.40 1.65	1.20 1.17	1.60 1.65	1.58 1.43	1.72 1.62	1.48±0.05	2.88
JC8679 (*rec*B21*rec*C22*sbc*A23)	1.34 1.44	1.75 1.64	1.23 1.31 1.42	1.86 2.15	1.76 1.65	2.37 1.83 2.03	1.7 ±0.09	2.98

a) Marker position on the lambda genom (F. W. S t a h l) i.e. distance from right end.
b) Hits/100 kilorads are determined from the least squares analysis of slopes of phage survival or marker rescue. In each case the value reported is based on the average of two or more assays of six point curves in the range $0-2 \times 10^5$ kilorads.

Figure 2. represents the individual radiosensitivities of the markers on the three hosts ploted against their map position. The lines are the respective least square plots with the coefficient of correlation indicated for each host. Although the scatter is substantial there is a clear trend seen with MMS1 and JC8679 which indicates that the markers on the right end of the lambda molecule are less sensitive to gamma irradiation than those on the left. This finding is consistent with experiments which show that the right end of the lambda molecule can be attached chemically to the tail structure of lambda (T h o m a s, 1974, C h a t t o r a j and I n m a n, 1974), that the right end of the molecule is uniquely sensitive to micrococcal nuclease attack (P a d m a n a b h a n, W u and B o d e, 1972), and is released when partial injection is induced by formamide (T h o m a s, 1974). The physical association of the right end of the DNA with the tail and exterior of lambda is thus probably not trivial and reflects the introduction of the molecule in a unique polar fashion from this end into the host bacterium.

This study raises questions in respect to the levels of marker rescue observed. In general, the sensitivity of marker rescue events to radiation damage is above one half that of phage survival. A similar relationship was observed by S h a r p and F r e i f e l d e r

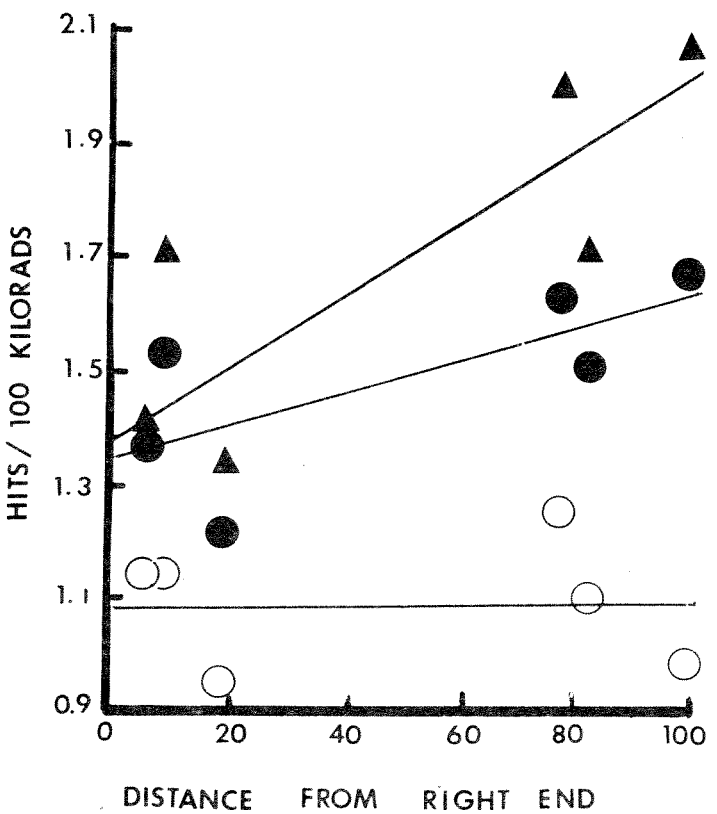

Fig. 2. – Individual radiosensitivities of the markers ploted against their map position (see Table I a). Hosts: C600 (o——o), MMS1 (●——●), JC8679 (▲——▲).

(1971) between the sensitivity of the lambda DNA to breakage and that of phage survival. It is thus probable that whole unbroken molecules normally comprise the primary contribution to recombinant clones. Our initial expectation was that, in the absence of a functional *rec*BC$^+$ enzyme, exonuclease V, fragment survival would be increased and the radiosensitivity decreased by contribution from these fragments. However, not only does this not occur, but the level of marker rescue is lower in the hosts with defective *rec*BC function (MMS1 and JC8679). These conflicting obsevations, the development of marker specific radiosensitivities related to molecule position and the increase in radiosensitivity of lambda marker rescue, might be resolved by hypothesizing a specific role in gamma irradiation damage repair for exonuclease V. If in addition to its capacity to remove broken fragment, the *rec*BC function acts in repair of transferred damages in whole molecules, these observations could be reconciled. In *rec*BC$^-$ cells the major source of recombinant molecules, i.e. repaired whole molecules, is lacking and probably less efficient recombinantion pathways utilize the fragments conserved in the absence of exonuclease V.

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Rezime

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PRAVAC UBRIZGAVANJA GENOMA LAMBDA FAGA: GENETIČKA ANALIZA KONTRIBUCIJE DONORA NAKON GAMA ZRAČENJA

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Ukoliko se bakterija istovremeno inficira ozračenim i neozračenim fagima u procesu rekombinacije dolazi do spasavanja („rescue") jednog ili više genetičkih markera ozračenog faga. Koristeći metodu „spasavanja markera" (Marker rescue) permisivni sojevi *E. coli (rec,*$^{+}$ *rec*B i *rec*BC*sbc*A) inficirani su gama–zračenim (niski multiplicitet infekcije) i *sus* mutantima (visoki multiplicitet infekcije) lambda faga. Posle određenog vremena inkubacije smeše u uslovima koji omogućavaju ubrizgavanje DNK faga u ćelije domaćina, odgovarajuća razblaženja su zasejavana na nepermisivnom soju *E. coli.* Samo ukoliko ubrizgani fragment iz ozračenog faga nosi gen koji je nefunkcionalan u odgovarajućem *sus* mutantu, fag raste na nepermisivnom bakterijskom soju, te prema tome jednom fragmentu odgovara jedan „spasen" (rescued) infektivni centar.

Dobijeni rezultati ukazuju da nema razlike u radioosetljivosti individualnih markera u *rec*$^{+}$ domaćinu. Međutim, značajne razlike u osetljivosti markera na gama–zračenje su otkriveni kod *rec*BC sojeva defektnih u rekombinaciji. Genetički markeri koji se nalaze na desnom kraju molekula DNK lambda faga pokazuju manju osetljivost na gama–zračenje u poređenju sa markerima na levom kraju molekule.

Ovi rezultati ukazuju na jedinstveni pravac ubrizgavanja genoma lambda faga u ćeliju domaćina, odnosno ulazak molekula DNK desnim krajem. Specifična uloga egzonukleaze V u ispravci lezija indukovanih gama–zračenjem je diskutovana.