LIMITED GENETIC AND FUNCTIONAL PARTICIPATION
IN MULTIPLE INFECTIONS BY UV-IRRADIATED
WILD-TYPE T4 PHAGES IN COMPETITION
WITH ONE UNIRRADIATED HELPER PHAGE*

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Received January 28, 1969

KRIEG (1957) observed that if a K12(λ) bacterium was infected by one wild-type T4⁺ phage and T4rII phages at a variable multiplicity, the complex often failed to yield any output. The phenomenon was more pronounced the greater the multiplicity of the T4r phage and could be interpreted by assuming that only a limited number, P, of phages was allowed to express its function in K12(λ) bacteria (limited functional participation). For two rII markers used in our experiments this limit was found to be around P = 3 or P = 4. KRIEG also suggested that some observations by LURIA and DELBRÜCK (1942) and by DULBECCO (1949) could be interpreted as a result of limited functional participation. The observation was that bacteria which adsorb one active phage and more than one irradiated phage may fail to produce progeny phages.

Evidence for limited functional participation also in S/6 and CR-63 bacteria was reported by SNUSTAD (1966 a, b). Experiments we have done with other markers (*am N82*, *am N122* and *am E355*) agree with his results as far as functional participation is concerned. All three markers show functional participation limited to 5 or 6 or 8 phages, the highest (8 phages) in *am N122*. These participation limits are not corrected for lysis from without, which is difficult to do in a nonpermissive host, and may therefore be higher. We also have found another amber mutant (*am E355* in cistron 24, a macrotarget function according to WOMACK and BARRICELLI 1965; for a description of the types of function quoted in this paper see BARRICELLI and WOMACK 1965) which, judging from its expression, is often considered a late function, but nevertheless shows the same limited participation (about 6 phages in B bacteria) as other amber functions. A similar case, described by SNUSTAD (1966b), is presented by *am N58* in cistron 34, also a macrotarget function (according to WOMACK and BARRICELLI 1965) which has been considered a late function because of its observed phenotypic expression. In both cases a possible explanation first given by SNUSTAD in connection with cistron 34, can be found in the function type assignments presented by WOMACK and BARRICELLI (1965). Both cistron 24 and cistron 34 represent macrotarget functions, and not just late functions. They are therefore needed

* This investigation was supported by Public Health Service Research Grants No. GM-06980 and GM-12518 from the Division of General Medical Sciences, U. S. Public Health Service.

both early and late, and can be expected to show the same limited participation as early functions. For readers unfamiliar with this concept we should point out that according to the Simplex D model (BARRICELLI and WOMACK 1965) only one of the two DNA strands injected by a phage (the so-called genetic strand or master strand) transmits hereditary information to the progeny. The other one (associated strand) is needed for certain classes of functions and is apparently used for early RNA production, but it does not transmit hereditary information to the progeny. Four different types of function are theoretically possible on this model, but only the following two have been detected so far (WOMACK and BARRICELLI 1965): *High GL functions*, requiring as a condition for the functions' expression that the segment (cistron) carrying the hereditary information of the function must be intact in the *genetic strand*, but need not be intact in the associated strand; *Macrotarget functions*, requiring that the cistron must be intact *both in the genetic and the associated strand*. *The macrotarget functions are therefore needed both early (when the associated strand produces the RNA) and later, when copies of the genetic strand are available, which can substitute for the associated strand in the RNA production.* (BARRICELLI and WOMACK 1965.)

The purpose of this paper is to present a different type of limited participation experiment in which wild-type phages irradiated with ultraviolet (UV) light were used together with unirradiated mutant (carrier) phages in order to find answers to the following questions.

(1) Is functional and genetic participation the same for UV-irradiated as for unirradiated phages?

(2) Is participation different in macrotarget functions and high-GL functions?

(3) Is there any correlation between participation in different functions? For instance, if a phage participates in one function, will it also participate in all the other functions?

An answer to these questions should throw light on the mechanism by which the DNA strands or segments (partial replicas) of such strands are selected to carry out the various functions.

MATERIALS AND METHODS

Bacteria: The following strains of *Escherichia coli* were used: B (CHASE and DOERMANN 1958)—used as a nonpermissive host for functional survival determinations for amber mutants. CR63 (EPSTEIN *et al.* 1963)—used as a permissive host for marker-rescue experiments, and as a plating bacterium for functional survival experiments. S/6 (CHASE and DOERMANN 1958)—used as a plating bacterium for wild-type survival in marker-rescue experiments.

Phage: Bacteriophage T4D and the following mutant derivatives of T4D were used: *am E355* (gene 24), *am B251* (gene 6), *am N82* (gene 44), *am B256* (gene 5), *am N122* (gene 42). In addition, one other phage was used which contained the following mutations: *r2-19* (*rIIA*), *r64* (*rIIA*), *r71* (*rIIA*), *r70* (*rIIA*), *r2-20* (*rIIA*), *ac41r67* (*rIII*), *S6* (gene 48), *S60* (gene 27), *am N131* (gene 26), *am B16* (gene 7), *am B251* (gene 6), *am B256* (gene 5), *am N135* (gene 5), *am N130* (gene 46), *am N81* (gene 41), *am N82* (gene 44). Sources of the *rII* mutants and the acriflavine-resistance mutant (*AC 41*) are given in WOMACK (1963). The amber mutants designated *S* were isolated by A. H. DOERMANN. All other amber mutants were isolated by R. EPSTEIN and R. EDGAR and supplied by R. EDGAR.

Phage stocks were made according to the method of CHASE and DOERMANN (1958). UV

irradiation was carried out by means of a low-pressure mercury arc (General Electric 15 watt germicidal lamp) powered through a Sola constant-voltage regulator.

Experimental techniques for marker rescue in a permissive host, and for survival of phenotype in a nonpermissive host are described in detail by WOMACK and BARRICELLI (1965). The marker-rescue experiment involves mixedly infecting CR63 with mutant (*amber*) carrier phage and irradiated wild type. Infective centers are plated prior to lysis on S/6 which is restrictive for the *amber* mutant. Survival-of-phenotype measurements, on the other hand are achieved by making similar mixed infections on the *amber*-restrictive host, B, and plating before lysis on the permissive strain CR63. The former procedure thus measures the success with which irradiated wild type contributes at least one *am*⁺ gene to the progeny phage emerging from a single cell. The latter scores the number of infected bacteria in which the *am*⁺ function has been carried out by the irradiated wild-type parent. Multiplicities of the unirradiated carrier phage were below 0.11 except for the multiply-marked stock ($m = 2.16$). Multiplicities of irradiated wild-type phage were varied as stated in RESULTS.

RESULTS AND DISCUSSION

Participation Experiments With Irradiated Phages: Functional or genetic participation has been tested by allowing a variable number of UV irradiated, wild-type phages to infect a nonpermissive or a permissive host together with a carrier phage. The survival of the complex in the nonpermissive host or of the wild genotype in the permissive host is expected to increase (see APPENDIX problem 1) with the average multiplicity, x , of the irradiated wild-type phage. But the increase will be different depending on the number, i , of irradiated phages which are able to participate together with the carrier phage (Figure 1).

A fundamental question of theoretical interest is presented by the following possibility. For the following reasons, the chance that a macrotarget function survives might increase much more rapidly than the chance that a high GL function survives when x increases without changing UV dose. In fact at low multiplicity x , macrotarget functions are at a disadvantage in irradiated phages because of the requirement that their cistron must be intact on both strands for survival. But whatever advantage an increase in multiplicity x may present for a high GL function, the same advantage may show up two times (one time for each strand) in a macrotarget function. For this reason, the survival probability for macrotarget functions may increase much more rapidly than for high GL functions with increasing multiplicity x . This is true provided the following *basic assumption* is correct: *In a macrotarget function, the associated strand providing the function at the initial stage of infection, and the genetic strand whose copies will provide the function later, do not have to be supplied by the same infecting phage.*

The results of participation experiments with irradiated phages are presented in Figure 1. In the same figure the theoretical survival diagrams for $i = 1, 2, 3$ (i = participation limit for the UV irradiated phages) and for unlimited participation of UV irradiated phages ($i = \infty$) are given for the following cases: (1) for macrotarget functions (dashed lines calculated by APPENDIX formula B on the above assumption concerning participation in macrotarget functions), and (2) for all other cases, including genetic participation and participation in a high GL function (solid lines calculated by APPENDIX formula A). In Table 1 the relative

FIGURE 1.—Survival of wild type in permissive host (CR) and survival of complex in nonpermissive host (SP) after infection with different mutant phages (listed below) and a variable number of irradiated (dose indicated below) wild-type phages at an average multiplicity, x (abscissa). Both the abscissa (multiplicity x) and the ordinate (survival s) are given on a logarithmic scale in order to obtain convenient spacing between plots. The survival values for each marker are multiplied by a constant normalization factor designed to obtain a series of values which extrapolates to 1 for $x \rightarrow 0$ since the relative increase for any given marker is the only result predicted by the theory (APPENDIX, formulae A and B).

Permissive host	Nonpermissive host	Mutants used	Cistron	Function type	UV dose (hits)
▲ Cr63	△ B	<i>am E355</i>	24	Macrotarget	35.8
▼ Cr63	▽ B	<i>am B251</i>	6	Macrotarget	35.8
● Cr63	✱ B	<i>am N82</i>	44	High GL	35.8
■ Cr63	✱ B	<i>am B256</i>	5	High GL	34.3
	◆ B	<i>am N122</i>	42	High GL	34.3
	* B	16 Mutants	In 9 cistrons	Both	17.1

Multiplicity of carrier was ≤ 0.11 in all single-mutant experiments, and equal to 2.16 in the

TABLE 1

Survival ratios giving the relative increase of survival from lowest x_{low} to highest x_{high} multiplicity of irradiated wild-type phage represented in the data of Figure 1 for each type of function and experimental condition

Function type	Host	x_{low}	x_{high}	Observed survival ratio* $S(x_{high})/S(x_{low})$	Theoretical $S(x_{high})/S(x_{low})$		
					$i = 2$	$i = 3$	$i = \infty$
Macrotarget	Nonpermissive	0.23	3.18	3.26	2.80	4.95	8.66
Macrotarget	Permissive	0.285	3.12	2.40	1.60	2.15	2.80
High GL	Nonpermissive	0.33	3.70	1.93	1.63	2.23	3.43
High GL	Permissive	0.34	3.60	2.04	1.61	2.19	3.09

* Each observed survival ratio is the ratio of the geometric averages of survival values determined from the data of Figure 1 at the x_{low} and x_{high} points. Diagrams were constructed for each marker (for permissive as well as nonpermissive host conditions) by joining with line segments the successive points referring to that marker.

increase of survival is given for macrotarget and high GL functions in permissive and nonpermissive hosts for the x intervals in which data are available. In the same table the theoretically expected increases are given for the same intervals according to APPENDIX formula (A) and (B).

16-mutant experiment. Theoretical curves for limited participation of irradiated phage $i = 1, 2, 3$, and for unlimited participation $i = \infty$ are calculated by APPENDIX formula B, for macrotarget functions (dashed lines) and by formula A for all other cases (solid lines).

In order to explain how the data plotted in this figure are calculated, we shall indicate how the SP values for cistron 5 (indicated by the symbol ■ in the figure) are determined. Each SP value for a given average multiplicity x (abscissa) of the irradiated wild type phages is calculated by the formula

$$SP = \frac{S}{C(1-e^{-x})}$$

where:

S = count of infective centers after plating on permissive host the bacteria mixedly infected with irradiated wild-type phages (average multiplicity x) and carrier phages (at low multiplicity).

C = Estimated titer of bacteria infected with carrier phages (carrier multiplicity always ≤ 0.11). $1-e^{-x}$ = fraction of bacteria receiving at least one irradiated wild-type phage (x being their average multiplicity).

For example, for the lowest multiplicity ($x = 0.325$ and $1-e^{-x} = 0.278$) used in the figure, we found $S = 2.2 \times 10^5$ and (for all multiplicities) $C = 8.8 \times 10^6$, yielding the SP value

$$SP = 0.09$$

In a similar way we obtained the other SP values given in the table below for various multiplicities:

x	0.325	0.65	1.3	2.6	5.2
SP	0.09	0.102	0.146	0.190	0.216

These SP values were multiplied by 1.27 in order to obtain values extrapolating to 1 when x vanishes and the results are represented in the figure by the symbols ■.

The CR values (represented by squares ■) are obtained in a similar way, with the only difference that S was determined by absorbing the phages on permissive host and plating the bacteria on nonpermissive host.

The following results and tentative conclusions are indicated by Figure 1 and Table 1:

(1) Genetic participation is generally limited to approximately two or three UV-irradiated phages (possibly with one exception presented by *am E355* in gene 24). This indicates that UV irradiation imposes a severe restriction to genetic participation by the irradiated phage (possibly by delaying their DNA replication) competing with an unirradiated carrier.

(2) Participation in high GL functions is also limited to two or three phages, like genetic participation, which is no surprise since the genetic strand is the one which must be intact in the cistron of a high GL function if the function is to be expressed. Furthermore in all those regions in which the genetic strand is not copied into messenger RNA—which is the case at least in the *rII* region (WOMACK and BARRICELLI 1965) and in the lysozyme mutants (BARRICELLI and DEL ZOPPO 1968) while no counter-examples have been found yet—the genetic strand's cistron must be duplicated and almost certainly participate genetically, in order to participate functionally.

(3) In agreement with the assumption presented above, survival of the two macrotarget functions (open triangles in Figure 1) shows a much greater increase with the multiplicity of the UV-irradiated phage than survival of high GL functions. However, when we compare with the theoretical prediction for this type of function (dashed lines in Figure 1), we find that participation in macrotarget functions is also limited to the equivalent of two or three phage's hereditary material (or more precisely, limited to two or three genetic DNA strands and two or three associated DNA strands).

(4) When participation in nine different functions was tested in the same experiment, using a carrier with mutations in nine different genes, the survival increased with growing multiplicity x much faster than in single-function experiments. This indicates that a phage which does not participate in one function is not necessarily excluded from other functions, and different phages may therefore either participate or be excluded from different functions, in agreement with KRIEG (1957 and 1959).

The different multiplicity response which, in agreement with theoretical expectation, has been found for participation in macrotarget functions, compared with high GL functions or with genetic participation, is considered a particularly significant new piece of evidence that the functional type assignments made by WOMACK and BARRICELLI (1965) can be used as a means to interpret and predict other properties of the respective functions. For example, the following markers *amB23*, *amB16*, *amN102*, *amB270*, *amN85*, *amS12*, *amS6*, *amN58*, *amB25*, *amA455*, classified as belonging to macrotarget functions according to WOMACK and BARRICELLI (1965), are expected to behave as the two macrotarget function markers in the kind of experiment described in Figure 1. Similarly, the following markers *amN135*, *amS52*, *amS29*, *amA452*, *amN55*, are expected to behave as the three high GL functions markers in Figure 1.

As the reader will certainly be aware of, no such predictions are possible at present on the basis of the Duplex theory, since the very notion of high GL and

macrotarget functions would have no meaning and these types of function should not exist at all if the Duplex theory were correct.

Thanks are due to Dr. FRANCES WOMACK for her valuable contribution in the experimental work on which this paper is based, and to Professor A. H. DOERMANN for his helpful advice in this work.

SUMMARY

The basic results of this paper, with respect to irradiated phages in competition with an unirradiated helper, are: (1) That there is a limited genetic participation for most irradiated phages lower than for unirradiated phages; (2) That in the high GL functions investigated, there is a limited functional participation for the irradiated phages lower than the limited functional participation found by SNUSTAD (1966, a, b); (3) The same low limit of functional participation by UV-irradiated phages is found in macrotarget functions as well, when the cause of their stronger response to irradiated phage multiplicity is accounted for; (4) An irradiated phage participating in (or excluded from) one function is not necessarily participating in (or excluded from) all the other functions. (5) The associated strand providing the early mRNA required for the expression of a macrotarget function, and the genetic strand whose copies provide the later mRNA for the same function do not have to be supplied by the same phage.

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APPENDIX

Problem 1: Assume that only i irradiated (h hits) wild-type phages co-infecting a bacterium together with one unirradiated mutant phage are able to participate by contributing a marker (genetic participation) in a permissive host or a function (functional participation) in a non-permissive host. Let $S(h)$ be the survival probability (assumed to be small relative to 1) of the wild-type (function or marker) in one of the irradiated phages infecting the bacterium. Assume that each phage can either participate or be entirely excluded. That would, for example, be the case if only one strand from each phage could participate, as in genetic participation or in a high GL function.

Calculate the probability $S_i(h, x)$ that one wild-type phage will participate if x is the average multiplicity of infection for the irradiated wild-type phages and i is their participation limit.

Problem 2: Same as problem 1 but applied to functional participation in a macrotarget function. Assume that the genetic strand and the associated strand providing the function at different stages of the infectious process do not have to be supplied by the same phage, and that the function in each strand has the same probability $S(h)$ (small relative to 1) to be unharmed by UV irradiation.

Answer to Problem 1:

$$A \quad S_i(h, x) = S(h) \frac{i - e^{-x} \sum_{r=0}^{i-1} (i-r) \frac{x^r}{r!}}{1 - e^{-x}}$$

In fact with a Poisson distribution of the multiplicities of infection the probability of survival with less than i irradiated phages infecting the bacterium (in which case each phage will participate) will be $S(h)e^{-x} \sum_{r=0}^{i-1} r \frac{x^r}{r!}$. The survival probability with no less than i phages infecting the bacterium will be

$$S(h)e^{-x} \sum_{r=i}^{+\infty} i \frac{x^r}{r!} = S(h)ie^{-x} \left(e^x - \sum_{r=0}^{i-1} \frac{x^r}{r!} \right).$$

The sum of these two probabilities shall be divided by the probability $1 - e^{-x}$ that at least one irradiated phage will infect the bacterium in order to obtain the survival $S_i(h, x)$ among infected cells.

Answer to Problem 2:

$$B \quad S_{iM}(h, x) = S_i^2(h, x)$$

where:

$S_{iM}(h, x)$ designates the survival probability of a macrotarget function. $S_i(h, x)$ is the expression determined by formula A in which $S(h)$ is used in this case to designate the probability that the function will survive in one particular strand.

In fact for the associated strands (or for the genetic strands) the probability that the necessary condition for survival of a macrotarget function will be fulfilled as far as these strands are concerned, will be $S_i(h, x)$, the same as above, when the changed meaning of $S(h)$ is taken into account. The probability that this condition will be fulfilled both for the genetic and the associated strands (which is necessary and sufficient for the survival of a macrotarget function) will therefore be $S_i^2(h, x)$.