RECOMBINATION AND TRANSFECTION MAPPING OF CISTRON 5 OF BACTERIOPHAGE SP82G

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ABSTRACT

Recombination between transfecting SP82G DNA molecules has been studied in *Bacillus subtilis*. Recombinant progeny issuing from transfected cells show many of the features that characterize progeny production in multiplicity reactivated bacteriophage, such as: majority recombinant clones, non-reciprocity of recombinant clones and the frequent absence of input alleles. While transfection substantially lowers the linkage observed between markers in normal phage crosses, linkage is observed at small map distances in transfection either by plating transfected bacteria or the progeny phage. Maps constructed from transfection crosses are identical to those of normal phage crosses, except in magnitude.—Examination of the concentration response of two marker biparental crosses, and three marker triparental crosses using transfecting DNA leads to the conclusion that at all concentrations, transfective centers are saturated with respect to the number of molecules that can be taken up. Thus, the frequency of recombinant infective centers, or recombinant progeny is independent of concentration effects.

TRANSFECTION of *Bacillus subtilis* with two genetically marked DNAs results in a substantial increase in recombination between genetic markers when compared to the values observed in standard phage crosses (Okubo, Strauss and Stodolsky 1964; Green 1964, 1968; Spatz and Trautner 1970). Genetic analysis of all the progeny genotypes from such transfection crosses (Green 1968) shows random assortment of markers which, by phage crosses, are separated by only 1.3% recombination. Further studies, presented in this paper, lead to the view that transfecting DNAs are much like multiplicity reactivated (MR) bacteriophage in the clonal production of progeny. While phage recombination and transfection recombination have marked differences, it seemed that at some level of recombination, markers must be linked in transfection. Obviously, transfection crosses might serve as a useful tool in the development of fine-structure mapping of phage genes.

The linkage of a number of independently isolated markers in gene 5 of SP82G was examined by phage and transfection crosses to determine the relationship between these two processes and the validity of using the transfection cross for the determination of marker order and distance. The frequencies of wild-type infective center formation or wild-type progeny yield are proportional to map distances between the genetic markers on the transfecting DNAs for very small regions of the genetic map. It is possible to construct linear genetic maps from

the observed frequencies of wild-type progeny in transfection crosses where markers show 4% or less recombination in standard phage crosses, and from the frequency of formation of infective centers where markers show 2% or less recombination in standard phage crosses. Maps constructed from the wild-type frequencies of transfection crosses of two genetically different DNAs bearing temperature-sensitive markers are essentially identical to those constructed by standard phage crosses.

MATERIALS AND METHODS

The bacterial strain used in these studies was *Bacillus subtilis* SB-1 (NESTER and LEDERBERG 1961). Twelve temperature-sensitive markers of gene 5 were used in this study; three of which (*H50*, *H63*, and *H180*) were described by KAHAN (1966). The remaining mutants (e) were isolated following ethyl methanesulfonate-induced mutation of wild-type SP82G phage stocks as described by KRIEG (1963), or (the NG class) induced by N-methyl-N′-nitro-N-nitrosoguanidine (KAHAN 1966). Mutagenized stocks were grown for a single growth cycle and plated at the permissive temperature (33°C). These 33°C plates were replicated to plates which were then incubated at 47°C. The temperature-sensitive plaques revealed by comparison of the two sets of plates were tested against known representatives of cistrons of SP82G by a spot testing procedure. The unknown mutant was placed in a bacteria-seeded lawn at a concentration of approximately 10^7 phage particles/ml. An innoculating grid consisting of a 5×5 arrangement of sewing needles was stamped onto a 5×5 depression plate into each well of which a different testing phage was placed in soft agar at a titre of about 10^7 particles/ml. The grid was then stamped onto the seeded lawn and the plate incubated at the restrictive temperature, 47°C. The mutants used in these studies were those that showed no substantial increase in plaques over control plating with the markers assigned to gene 5 by KAHAN (1966).

Quantitative complementation studies were also carried out with markers from various regions of gene 5 in order to confirm the spot testing procedure. This complementation procedure consisted of adsorbing each of two *ts* mutants to 47°C-grown SB-1 at a multiplicity of 10 in the presence of 0.001M NaCN. After a ten minute adsorption period at 33°C, anti-SP82G rabbit serum was added for five minutes. The cells were appropriately diluted and plated for surviving

TABLE 1

Complementation of temperature-sensitive mutants assigned to gene 5

	H50	*H180*	*E75*	*E97*	*E107*	*E91*	*NG11*	*E9*	*E60*	*E59*	*NG8*
H50	.237	.90	.846	. .	1.11	3.61	3.12	. .	1.00	1.39	1.52
H180		.84	.830	. .	13.55	2.58	. .	. .	.83	.75	. .
E75			.492	1.13	1.88	1.30	2.65	1.25	1.12	. .	.814
E97				.98	.960	. .	. .	1.83	1.71	. .	. .
E107					.82	18.8	1.37	. .	1.33	.91	. .
E91						.78	1.26	1.66	. .	8.31	. .
NG11							1.36	1.23	. .	1.58	1.20
E9								.422	2.12	2.62	. .
E60									1.53	2.36	1.06
E59										.94	. .
NG8											1.37

Experiments were performed as described in the text. The values presented are the mean burst sizes of the individual mutants and the tested pairs. Typical burst sizes of wild-type phage and complementing mutant pairs grown at the restrictive temperature are in the range of 80–100 phage particles.

bacteria and infective centers within the next five minutes. A growth tube was incubated at 47°C and, after lysis, the viable progeny phage were assayed. The mean burst sizes of the various markers used are recorded in Table 1. In most cases the burst size is substantially below that of wild type or complementing pairs of mutant phage grown under similar conditions. There are clearly a number of cases in which a significant amount of intragenic complementation occurs. The levels achieved are, in every case, below 20% of expected control value.

Procedures for growth of phage stocks and genetic crosses with SP82G have been previously described (GREEN 1964, 1966).

The procedure for transfection crosses is similar to that previously reported (GREEN 1968), with the exception that the concentrations of DNA used in the present study were lower (5 μg/ml of each DNA, compared to 10 μg/ml previously used). This concentration was chosen following concentration response experiments with each DNA to insure that this was a saturating concentration of transfecting DNA for all DNA preparations. While most of the transfection experiments were performed using mixtures of independently isolated DNA preparations, in a small number of cases (Table 2 and Table 4) DNA preparations were isolated from a mixture of equal parts of concentrated purified phage. This procedure effectively controls for variations in biological activity introduced in the isolation of the DNA and is, while somewhat more time-consuming, the method of choice in performing quantitative genetic studies with transfection.

Following an incubation period of 50 min the DNA-infected bacteria were diluted, pour-plated with a bacterial lawn, and incubated at the restrictive or non-restrictive temperatures to measure infective centers. The fraction of total infective centers able to plate at the restrictive temperature was determined. Alternatively infected cells were incubated a further 70 min and assayed through chloroform or lysed with lysozyme in 0.001 M NaCN, to determine wild-type and total progeny phage. The wild-type frequencies were calculated by:

$$\left(\frac{\text{titer at } 47^\circ\text{C}}{\text{titer at } 33^\circ\text{C}}\right) (\text{e.o.p.}) - \text{mean reversion frequency} \times 100$$

where "e.o.p." is the titre of wild type plating at 33°C divided by that plating at 47°C.

RESULTS

Analysis of the phage yield of transfection crosses: In typical bacteriophage infection under conditions identical to those used for transfection, SP82G yields bursts in the range of 50–200 particles/bacterium. In contrast, the mean burst size of progeny from transfecting SP82G DNA ranged, in various experiments, from 4.1 to 30 particles/infected bacterium. This rather wide variation in burst size reflects a delay in phage production which characterizes SP82G-transfected cells (GREEN 1964). A clearer picture of the progeny yield and distribution of burst sizes was sought by singly distributing transfected bacteria and permitting them to release progeny under conditions where re-infection was negligible. Competent *B. subtilis* was transfected with a mixture of two DNAs, diluted and distributed to small tubes (0.17 transfected bacterium/tube). The tubes were incubated at 33°C for three hours, refrigerated, and the entire contents subsequently plated. Two experiments were done; in one, seventeen clones derived from transfected cells were examined, in the other thirty-seven such clones were observed. While the first experiment was, in salient features, like the second, the results were clouded by the occurrence of three exceptionally large clones of size 500–1000. We attribute this to occasional chains of bacteria, one member of which was competent (these do occur, see JAVOR and TOMASZ (1968)). The prog-

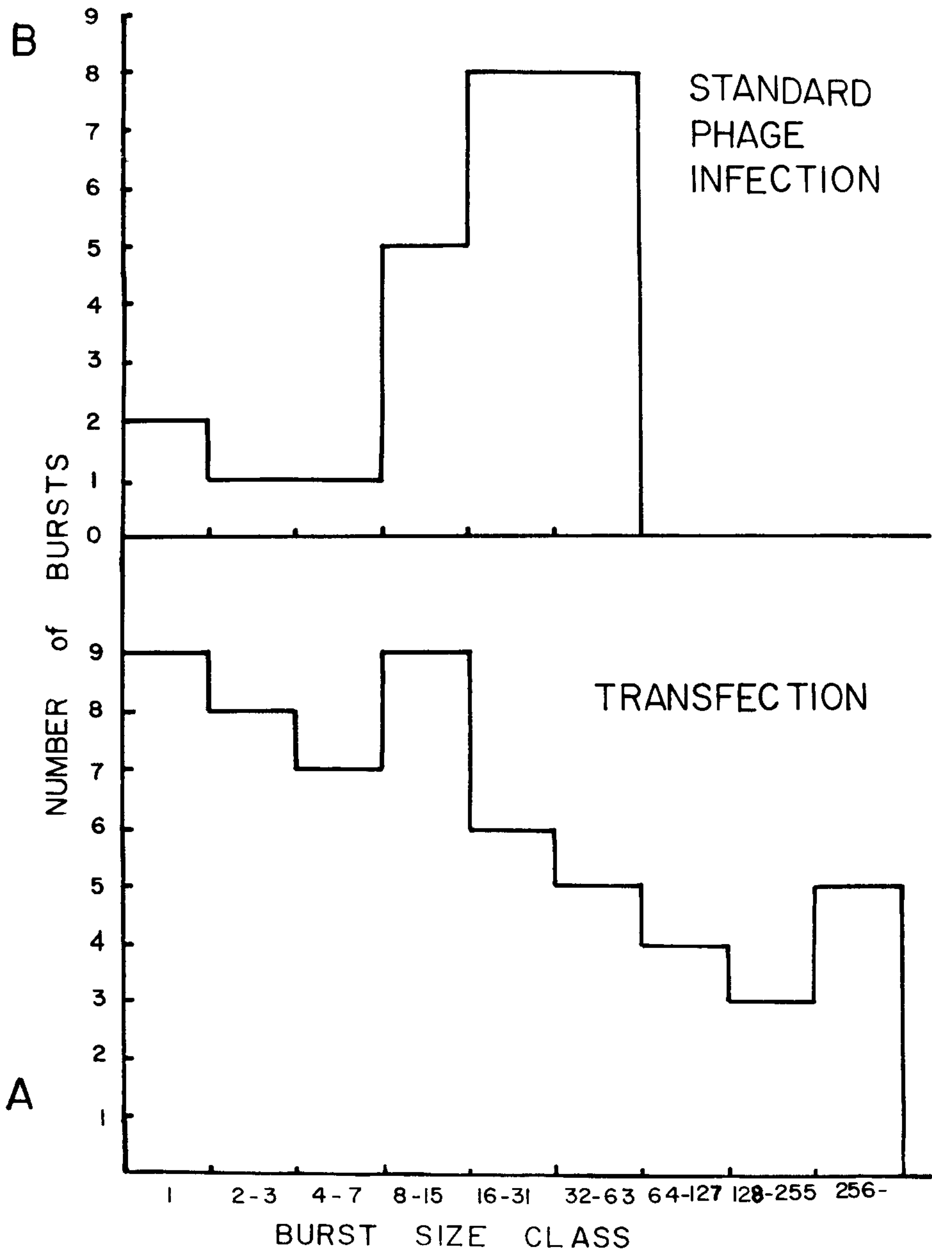


FIGURE 1.—The number of clones occurring in a given size class is plotted, for transfection (1A) and for a normal phage infection (1B). In the transfection distribution small clones up to size class 32–64 appear to be equally probable. Above the 32–64 clone size the probability of occurrence decreases. In the normal phage infection, clone size is distributed about a well defined mean.

eny of such a transfected cell are released in a locally high concentration of uninfected cells which favors adsorption and growth during the 3 hr period allowed for release of transfection progeny. A graph of the number of clones in a given size class (Figure 1A) indicates no evidence for a preferred size class in the distribution. A control distribution of burst sizes produced from infection with bacteriophages *e75* and *e91* in a typical phage cross (performed in competent cells) is presented in Figure 1B. In contrast to transfection the burst-size distribution in the phage cross has a well-defined mode. About half the total plaques in the transfection distribution derive from the three unusually large clones occurring in the first experiment. If we eliminate these clones and divide the total plaques by the Poisson calculated number of bursts, the mean burst size is 30/bacterium. The skew of the clone size distribution toward small bursts resembles that seen in multiplicity reactivation of ultraviolet-treated bacteriophage T4 (EPSTEIN 1958).

Analysis of genetic contribution to the progeny of transfection crosses: The transfecting DNAs used in the second of the single burst experiments were genetically marked so that contribution to the progeny of three different markers could be examined. The DNAs used were *e75* + *tu*, + *e91* tu^+. Two of the markers reside in gene 5, *e75* and *e91* (Figure 5), while the third marker, *tu* is not sensibly linked to this marker pair in standard phage crosses. It was thus possible to enquire (1) whether there is a majority genotype in the progeny of a given transfected cell, (2) whether recombinants occur reciprocally or otherwise in a given burst and (3) whether all markers are contributed to the progeny. The tu/tu^+ alleles were scored by plaque morphology. The temperature-sensitive alleles were scored by transferring plaques with a toothpick from a single-burst plate to a plate seeded with a lawn containing either the *e75* phage or the *e91* phage. These plates were then incubated at 47°C and the genotype characterized by the presence or absence of recombinants in the stab region. In an ideal experiment, all the progeny should be tested in each burst examined (particularly to rule on point 3). However, to avoid contamination from plaques in close proximity to one another, total scoring was possible in only the rather small bursts. All thirty-seven of the productive plates were analyzed and are presented in Table 2. To reduce sampling errors, we limit our discussion to the seventeen bursts in which more than fourteen plaques occurred or were scored. In thirteen of the seventeen bursts, one genotype comprises more than 50% of the progeny analyzed. This is the case in large bursts (up to size 178) as well as in the smaller bursts. Among the thirteen bursts with a majority genotype, six majority genotypes are nonrecombinant for one parent (*e75* + *tu*) and three genotypes for the other parent (+ *e91* tu^+); the remaining four are recombinant.

Of four bursts in which *all* the progeny phage were analyzed, three lack at least one of the input markers. It is also clear from a plot of a given recombinant class against its reciprocal class in each burst (Figure 2) that there is little marked correlation between the occurrence of reciprocal classes. In 20 out of 48 recombinant class pairs examined, one of the reciprocal recombinant classes is completely missing.

TABLE 2

Analysis of single burst distribution of transfection-produced phage

Plaques scored	Total plaques	Genotype of transfection clones: Parental DNA $e75 + tu$	$+ e91\ tu^+$	$e75\ e91\ tu$	Recombinant $- - tu^+$	$+ + tu$	$+ + +$	$- + tu^+$	$+ - tu$	Alleles missing
43	328	12	0	10	0	20	1	0	0	—
85	198	41	0	1	7	0	0	36	0	$e75^+$
14	178	12	0	0	0	0	0	0	2	tu^+
122	136	6	68	1	1	3	3	1	39	—
46	80	0	26	0	5	0	12	0	3	—
44	48	3	7	7	1	2	1	0	23	—
33	47	0	7	2	12	0	2	8	2	—
25	41	25	0	0	0	0	0	0	0	$e75^+\ e91^-\ tu^+$ (P)
34	40	24	1	0	1	0	0	8	0	—
28	33	1	5	8	1	0	5	2	6	—
28	32	16	1	0	0	6	1	4	0	—
30	30	0	17	0	1	0	0	0	12	$e91^+$
21	29	14	2	3	1	0	0	1	0	—
15	18	0	0	0	6	0	1	8	0	tu
14	14	8	0	6	0	0	0	0	0	$e75^+\ tu^+$
14	14	0	1	0	3	0	0	10	0	tu
14	14	1	0	7	1	1	4	0	0	—
13	19	13	0	0	0	0	0	0	0	$e75^+\ e91^-\ tu^+$ (P)
12	12	11	0	1	0	0	0	0	0	$e75^+\ tu^+$
12	13	0	5	1	0	2	1	0	3	—
10	10	3	0	1	0	0	5	1	0	—
9	9	9	0	0	0	0	0	0	0	$e75^+\ e91^-\ tu^+$ (P)
9	9	6	0	2	0	0	0	0	1	tu^+
7	7	5	0	0	0	0	0	0	2	tu^+
6	6	0	3	0	1	0	1	0	1	—
5	5	1	1	1	0	0	0	0	2	—
5	5	1	0	1	0	0	0	0	3	tu^+
4	4	1	1	0	0	1	0	0	1	—
4	4	0	0	0	0	0	0	0	4	$e75^-\ e91^+\ tu^+$
3	3	2	0	0	0	1	0	0	0	$e91^-\ tu^+$
2	2	2	0	0	0	0	0	0	0	$e75^+\ e91^-\ tu^+$ (P)
2	2	0	0	1	0	1	0	0	0	tu^+
2	2	0	0	0	1	0	0	1	0	$e75^+\ tu^+$
2	2	0	2	0	0	0	0	0	0	$e75^-\ e91^+\ tu$ (P)
1	1	0	0	0	1	0	0	0	0	$e75^+\ e91^+\ tu$
1	1	0	0	1	0	0	0	0	0	$e75^+\ e91^+\ tu^+$
1	1	0	0	1	0	0	0	0	0	$e75^+\ e91^+\ tu^+$

Competent *B. subtilis* cells were exposed to 10 μg/ml of an equal mixture of two DNAs bearing the markers $e75 + tu$ and $+ e91\ tu^+$. Following incubation, distribution, and plating as described in the text the plaques were picked and scored for the indicated genotypes. The symbol (P) in the column headed "alleles missing" indicates all alleles of one parent are missing.

An analysis of single-burst distribution of bacteriophage crosses was undertaken to compare the features observed in transfection crosses to those of the standard phage cross. Cells were infected with an equal multiplicity of the two parental phage, $e75 + tu$ and $+ e91\ tu^+$, at a total infected multiplicity of 8

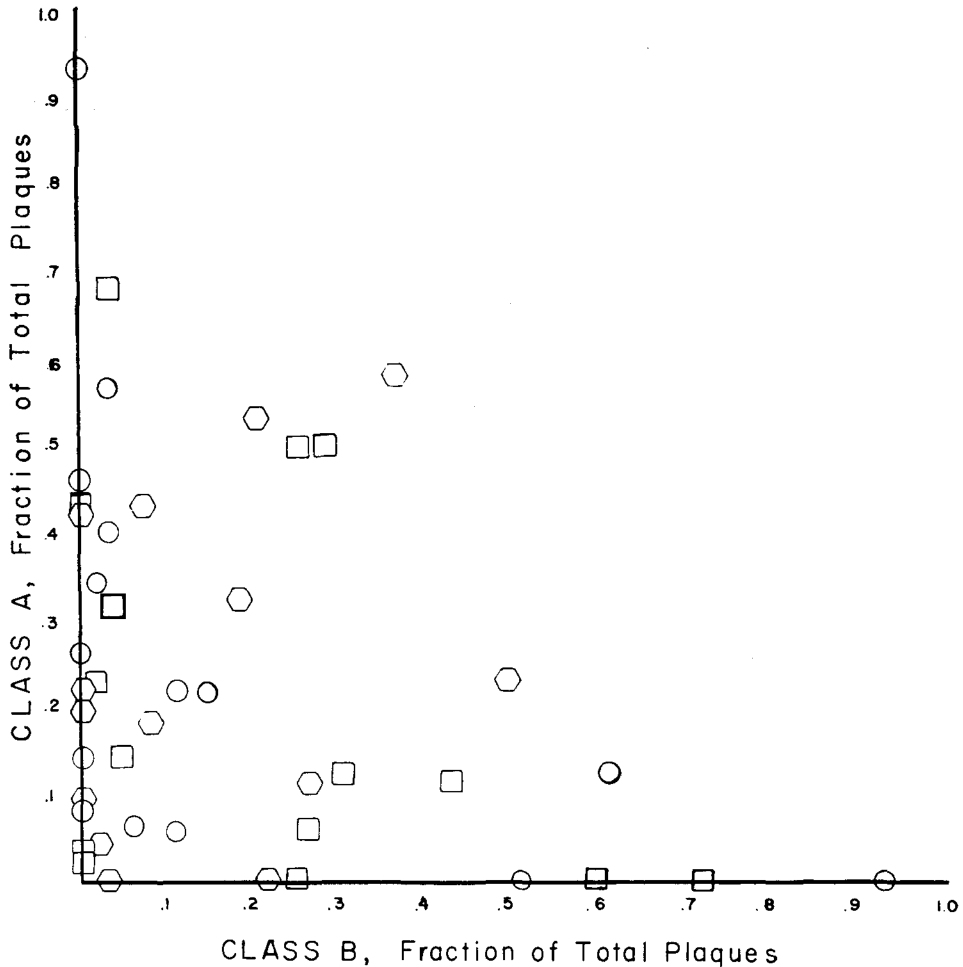


FIGURE 2.—A plot of the occurrence of reciprocal recombinant classes in the data recorded in Table 2. In this plot the percent that a given recombinant class (A) makes up the total clone is plotted against the percent that its reciprocal class (B) comprises. Symbols: ⬡ is *e75*+ *e91*+/ *e75*- *e91*-; ○ is *e75*+ *tu*/*e75*- *tu*+; □ is *e91*- *tu*/*e91*+ *tu*+

phage/bacterium, as determined by surviving bacteria. The infected cells were distributed at a mean number of infected bacteria/tube of 0.094, the tubes incubated for ninety minutes, and plated at 33°C. In contrast to the transfection cross where one or the other *tu* allele was missing in five of the seventeen bursts of size greater than 14, in the standard phage cross only one burst lacked one of the input *tu* alleles among the fifty-five examined. This one clone is expected from random infection.

Scoring for all genotypes in the *e75* + *tu* × + *e91* tu^+ phage cross was not feasible because of the low recombination frequency between the *e75* and *e91* alleles. In this experiment the recombination frequency was observed to be 2.02%

and the burst size 148 phage/bacterium. Wild-type recombinants can be scored selectively and thus infected cells were distributed to yield 0.135 wild type-yielding infected bacteria per tube. Following incubation at 33°C, these tubes were plated and the plates incubated at 47°C. Twenty-nine wild-type recombinant clones were observed; of these, nineteen were single plaques, and the largest recombinant clone was five. These results contrast with those of the transfection crosses (Table 2) where, even though much smaller burst sizes predominate, the wild-type recombinants occur as single plaques in only five of the fifteen bursts which showed wild-type recombinants. It thus appears that the major features of marker loss and the occurrence of majority recombinant clones seen in transfection crosses are not a normal characteristic of the SP82G bacteriophage infective process.

Some of these observations could result from our present inability to precisely control the infection conditions of transfection when using mixtures of separately prepared DNAs. This is evident from the gene output of the single-burst experiment which favors, by 2:1, those genes carried by the *e75* + *tu* parent over those of the + *e91* tu^+ parental DNA in spite of the fact that equal concentrations of the two input DNAs were used. However, the loss of markers in recombinant bursts suggests that unequal DNA input multiplicity cannot completely describe the observations. In general, the clonal nature of progeny in transfection parallels that seen for multiplicity reactivation in T4 bacteriophage (EPSTEIN 1958).

DNA multiplicity in transfection: The use of transfection for mapping purposes requires some insight into the relationship between the number of molecules infecting a competent cell and the frequency of infective centers and progeny phage which are recombinant for markers carried by the transfecting DNA. For limiting concentrations of DNA the response of total transfected centers and recombinant transfected centers follow a relationship in which the plaque-forming units are proportional to some power (between 2 and 4) of the concentration of the DNA. The variability of the proportionality relationship with different preparations of cells suggests that this is a physiological property of the competent cells. We have in the past interpreted (GREEN 1964, 1966) this concentration response to reflect the number of molecules which are necessary and sufficient to establish a viable infective center from the infecting molecules following (and during) their inactivation by an intracellular inactivation mechanism. The existance of a cooperative molecular requirement for transfection raises the issue of whether the number of molecules cooperating is concentration-dependent, e.g., whether at high concentrations more molecules interact in the establishment of an infective center than at limiting DNA concentrations. If this is so the use of transfection for determination of a relative recombination frequency may be limited to a narrow range of DNA concentration. Initial studies (GREEN 1964) indicated that the frequency of recombinants in mixtures of transfecting DNA was not concentration-dependent. We have undertaken a more detailed analysis of the situation with two-marker two-molecule transfection crosses and three-marker three-molecule transfection crosses in order to determine whether the recombinant frequency is affected by the concentration of DNA used.

A series of experiments were undertaken using DNA isolated from an equal mixture of purified phage preparations of two unlinked mutants *e112* (gene *2*) and *e119* (a new gene to the right, 0.2 map units, of gene *15*). Concentration response curves are presented for the mixture in Figure 3A. In order to estimate the efficiency of plating of infective centers under these conditions a control experiment using transfection with wild-type DNA was performed and plated at the selective and non-selective temperatures. The infective centers data presented in Figures 3A and B are corrected by the efficiency of plating factor (0.62) observed in the experiment. The frequency of wild-type recombinant infective centers remains unchanged throughout the concentration response curve and even through a range of DNA six fold in excess of that required to saturate the competent cells. The value for the corrected frequency of wild-type infective centers is 55%.

The frequency of wild-type transfection progeny observed in the above experiment, 25%, is that expected from a condition in which virtually every cell is infected with both genomes in nearly equal proportion. An examination of the frequency of wild-type progeny of nine other DNA crosses involving distant markers (Table 3, Figure 4) indicates that the frequency corrected for e.o.p., 0.9, is 20.4%.

A similar type of experiment was undertaken using a three-marker three-molecule (triparental) transfection cross. The triple DNA mixture was composed of equal amounts of DNA isolated from the doubly marked mutants, *H20 H167* +, *H20* + *H24*, and + *H167 H24*. Formation of a wild-type infective center of phage particles requires a cooperative infection involving each of the three molecules. The results of two experiments performed with this DNA mixture are presented in Table 4. The frequency of wild-type infective centers corrected for efficiency of plating was 20.5% and 23.3% in the two trials, and for wild-type progeny was 3.65% and 3.19%. Within experimental error the frequency of recombinants (infective centers or progeny phage) is independent of DNA concentration as it was in the biparental cross. The high frequency of wild-type infective centers suggests that the cooperation of three or more molecules in a transfection event occurs often.

The expectations for the frequencies of recombinant infective centers and progeny in both the two-molecule and three-molecule crosses are obscured by a number of factors such as the possibility of infection of cells by DNA molecules which are fragmented, the partial inactivation of infective molecules by the intracellular inactivation process and the possibility of variable adsorption properties of the infectable cells.

While the values observed for the slopes of the concentration response curves are consistent with the participation of approximately three independently infecting units for the formation of the transfection center, it is probable that the number of interacting molecules may exceed three. First, there is uncertainty in the determination of a final slope in transfection concentration response curves in phages like SP82G and ϕ25 (REILLY and SPIZIZEN 1965) because of a tendency for some experiments to show a continuously increasing slope to very low DNA

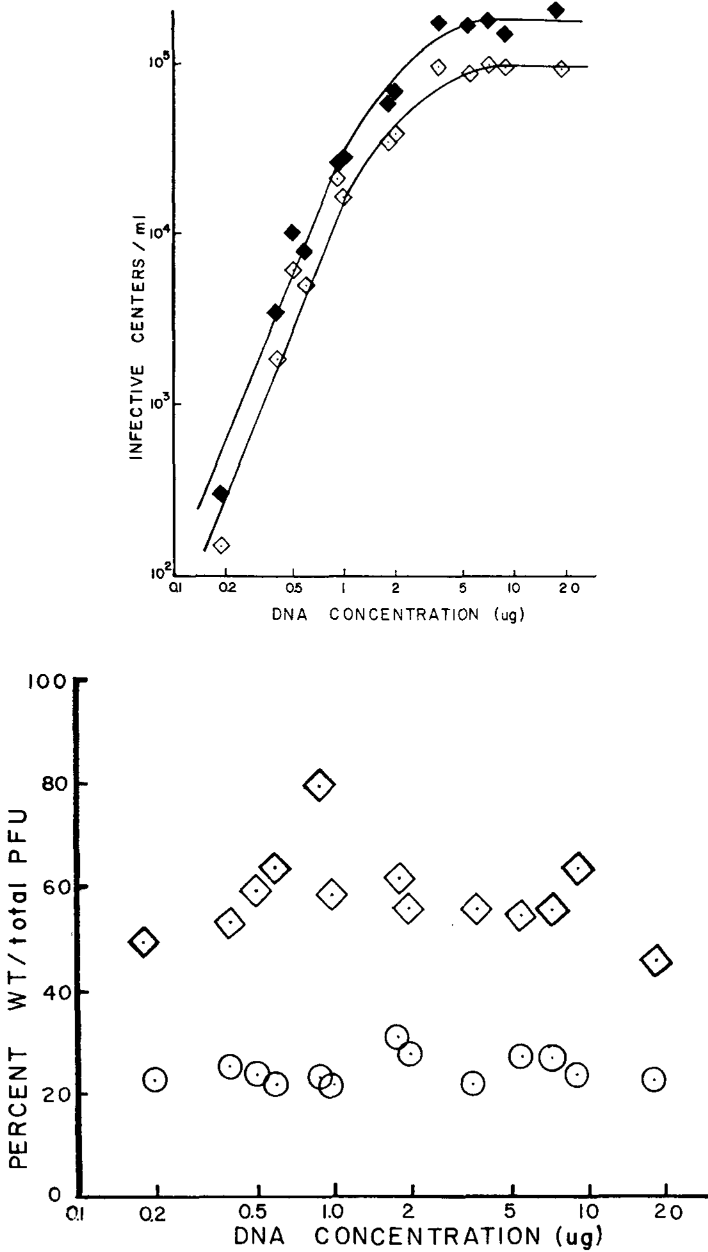


FIGURE 3.—A. The response of infective centers capable of plating at 33° (closed squares) and 47° (open squares) to a DNA preparation isolated from an equal mixture of purified phage lysates of *ts e119* and *e112*. B. The frequency of wild-type infective centers and wild-type progeny to total plaque-forming units in the preceding experiment; infective centers (squares), progeny phage (circles). The infective center data has been corrected for efficiency of plating.

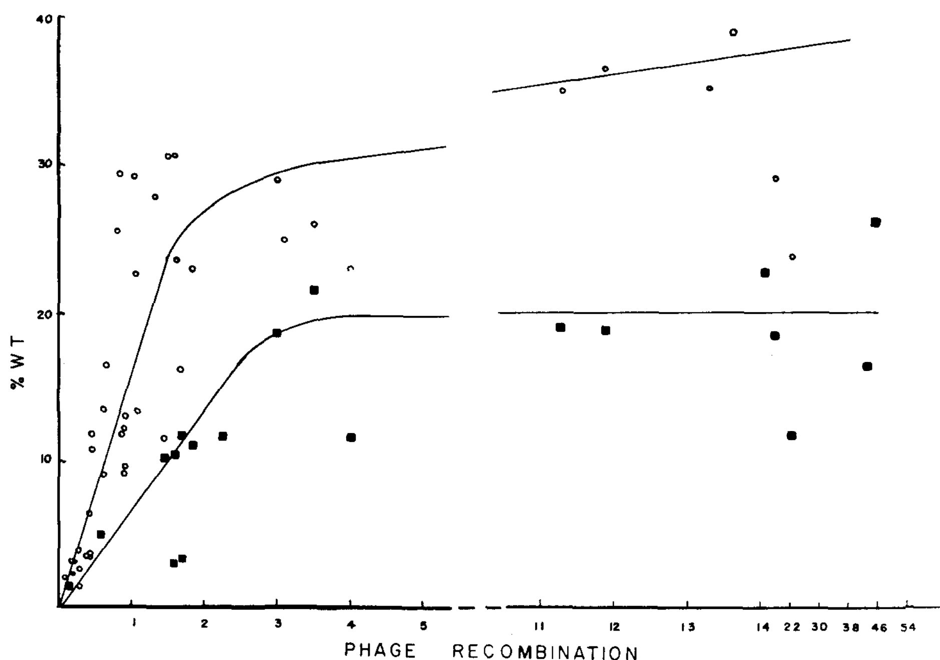


FIGURE 4.—A plot of the data in Table 3 relating the frequency of transfection produced wild-type infective centers, ○, and progeny, ■, to the recombination frequency observed in standard phage crosses.

concentrations. Secondly, it is probable that limitations imposed on the rescue of transfecting molecules by the intracellular inactivation process, the concomitant marker loss, and the strong clonality of transfectant bursts would reduce the fraction of potential recombinant infective centers which do form recombinant infective centers. Thus estimates based on the observed frequency of recombinant infective centers may be underestimated.

In a practical sense, the results of the biparental and triparental transfection crosses demonstrate a unique advantage to the transfection system in the determination of the relative recombination frequency; specifically, the independence of DNA concentration and recombination frequency. In order to take advantage of this independence in transfection crosses, two conditions should be met; equal concentrations of the two interacting DNAs, preferably isolated from mixed phage preparations, and the use of cells at the same level of competency in each experiment. Freezing cells at the four-hour growth stage as previously described (GREEN 1966) and careful replication of conditions using one batch of frozen cells in the subsequent 90 min growth phase is sufficient to insure repeatable competency. Under these conditions the measurement of the relative recombination frequency in transfection crosses using either infective centers or progeny phage is a reliable and useful tool for genetic mapping.

TABLE 3

A comparison of the frequencies of wild-type infective centers and wild-type progeny produced by transfection crosses with the recombination frequency observed in standard phage crosses

Marker pair crosses	Phage recombination (frequency recombinants per total progeny)	Transfection crosses Infective centers (wt/total)	Progeny (wt/total)
Markers in gene 5			
e107 × *e97*	1.46	11.6	10.25
H50	1.50		
e75	1.69	16.2	11.8
H63			
e109	1.5	30.6	
e101	2.24		11.67
H180	3.1	25.1	
e108	3.05		
e91	6.24	26.0	21.6
e97 × *H50*	0.10	2.19	
e75	0.28	3.20	2.05
H63	0.45	6.45	
e109	0.89	12.14	
e101	0.61	13.47	5.0
H180	0.92	13.05	
e108	1.34	27.9	
e91	1.61	30.7	10.5
H50 × *e75*	0.29	2.77	
H63	0.20	3.33	
e109	0.47	3.52	
e101	0.93	9.69	
H180	0.91	9.15	
e108	1.03	29.4	
e91	2.16		
e75 × *H63*	0.22	2.48	
e109	0.46	11.9	
e101	0.40	3.57	
H180	0.62	9.09	
e108	1.10	13.45	
*e91**	1.83	23.0	11.1
H63 × *e109*	0.47	3.52	
e101	0.29	4.04	
H180	0.46	10.93	
e108	1.03	29.3	
e91	2.16		
e109 × *e101*	0.05		
H180	0.30	1.51	
e108	0.57		
e91	0.68	16.51	
e101 × *H180*	0.16	2.37	1.56
e108	0.80	25.5	
e91	1.70		3.43
H180 × *e108*	0.86	11.8	
e91	1.04	22.7	
e108 × *e91*	0.89		

Markers outside gene 5			
H362 × *H385**	4.0	23.0	11.6
*H42**	11.3	34.9	18.9
*e7**	11.9	36.4	18.7
*e69**	14.3	38.7	22.7
*H20**	18.4	29.0	18.3
e69 × *e119**	1.6	23.6	3.03
*H42**	3.0	29.0	18.7
e91	13.3	35.1	19.9
e112	42.7	43.1	16.4
e112 × *e119**	44.4	43.0	26.0
H20 × *H385**	22.7	23.6	10.7

Most of the values presented here represent two or more independent determinations. Those values marked with an asterisk (*) were determined using DNA preparations isolated from equal mixtures of bacteriophage.

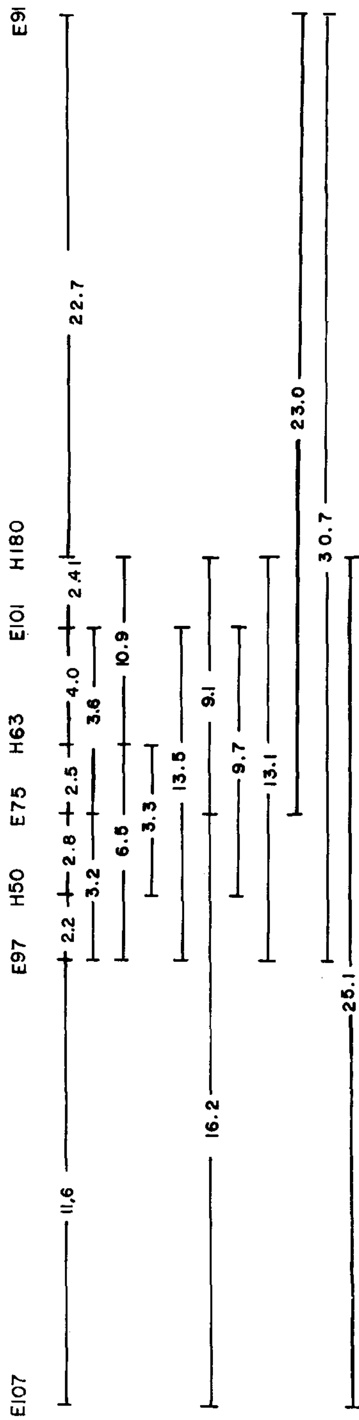
Mapping by transfection crosses: Twelve genetic markers in cistron 5 and various unlinked markers were crossed with each other by the transfection method (Table 3). The transfection values for total plaques and wild-type plaques were compared to the same markers using recombination frequencies for the standard phage crosses (for the more distant markers the phage recombination value is based on a summation of the smallest mappable intervals between

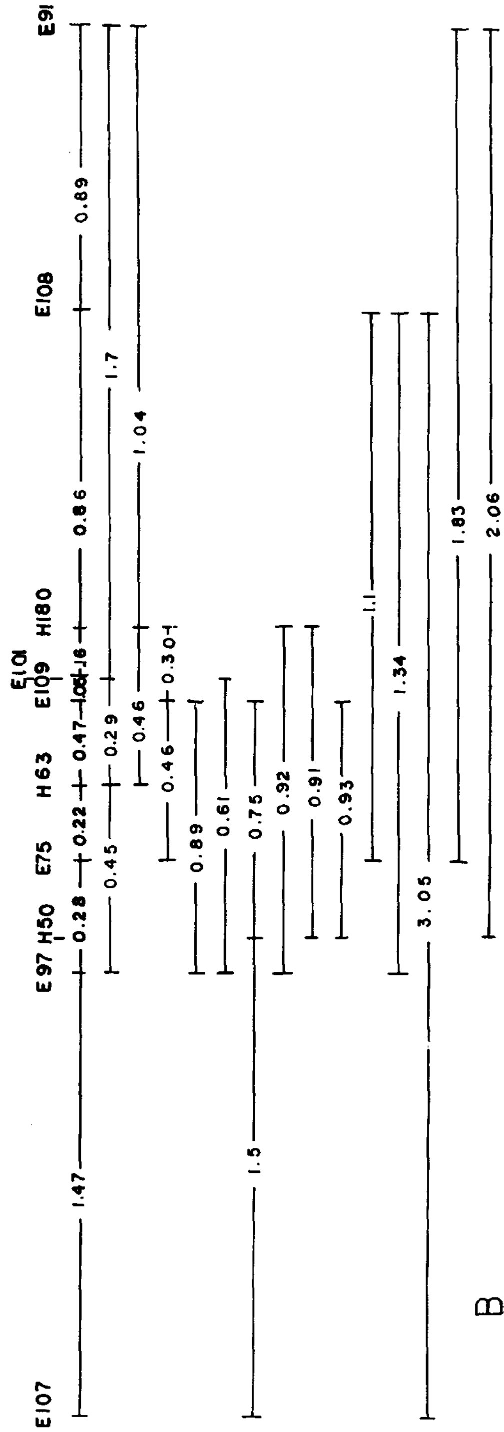
TABLE 4

Transfection with tri-parental DNA

DNA concentration μg/ml	Infective centers per ml 47/33	Freq.	Free phage per ml 47/33	Freq.	Infective centers per ml 47/33	Freq.	Free phage per ml 47/33	Freq.
6.6	$\frac{8.05\times10^4}{3.28\times10^5}$	0.246	$\frac{2.63\times10^5}{5.70\times10^6}$	0.0461	—	—	—	—
3.3	$\frac{3.8\times10^4}{2.35\times10^5}$	0.162	$\frac{5.25\times10^4}{2.88\times10^6}$	0.0182	$\frac{2.46\times10^4}{1.4\times10^5}$	0.176	$\frac{4.09\times10^4}{1.23\times10^6}$	0.034
1.65	$\frac{1.04\times10^4}{7.5\times10^4}$	0.139	$\frac{2.10\times10^4}{8.83\times10^5}$	0.0238	$\frac{2.51\times10^4}{1.0\times10^5}$	0.251	$\frac{2.38\times10^4}{1.01\times10^6}$	0.0241
0.825	$\frac{2.56\times10^3}{1.76\times10^4}$	0.145	$\frac{4.72\times10^3}{2.06\times10^5}$	0.0230	$\frac{8.17\times10^3}{4.72\times10^4}$	0.173	$\frac{6.30\times10^3}{3.13\times10^5}$	0.0202
0.4125	$\frac{9.05\times10^2}{7.09\times10^3}$	0.127	$\frac{2.0\times10^3}{4.52\times10^4}$	0.0441	$\frac{3.09\times10^3}{1.74\times10^4}$	0.178	$\frac{2.69\times10^3}{1.05\times10^5}$	0.0256
0.20625	—	—	—	—	$\frac{5.0\times10^2}{3.27\times10^3}$	0.153	$\frac{6.3\times10^2}{1.95\times10^4}$	0.0325
Mean freq.		0.164		0.031		0.186		0.0271
Corrected for e.o.p.		0.205		0.0365		0.233		0.0319

A mixture of equal parts of bacteriophage *H20 H167* +, + *H167 H24*, and *H20* + *H24*, was made and the DNA isolated. This DNA was used to infect cells and the frequency of infective centers and progeny phage determined as a function of the concentration of DNA used. Under these conditions wild-type infective centers and progeny can be produced only by those cells receiving at least one of each of the transfecting molecules.


E91
H180
E101
H63
E75
H50
E97
E107
22.7
2.41
4.0
2.5
2.8
2.2
11.6
3.6
3.2
10.9
6.5
3.3
13.5
9.1
16.2
9.7
13.1
23.0
30.7
25.1
A


E91
E108
H180
E101
E109
H63
E75
H50
E97
E107
0.89
0.86
0.47
0.22
0.28
1.47
1.7
0.29
0.45
1.04
0.46
0.46
0.30
0.89
0.61
0.75
1.5
0.92
0.91
0.93
1.1
1.34
3.05
1.83
2.06
B

markers). In transfection both the frequencies for the formation of wild-type infective centers and the production of wild-type progeny are linearly related to the phage cross recombination at low map values (Figure 4). Infective center formation is linear below 2% phage recombination and maximizes at a frequency of 38% wild-type infective center. Wild-type progeny are linearly related to phage recombination up to 4% and maximally constitutes 18% of the phage progeny. Efficiency of plating of wild-type infective centers and free phage were 0.65 and 0.9 respectively. Correction of the infective center data by this value gives a maximum frequency of wild-type infective centers of 58.5%. The e.o.p. corrected frequency of wild-type progeny for unlinked markers, 20.4, has been noted in the previous section.

The data for the frequency of wild-type infective centers produced through transfection can be used to construct a genetic map (Figure 5A) in the gene 5 region. This mapping procedure yields a map which is consistent with the map derived from standard phage crosses (Figure 5B). The progeny data can also be used to construct a similar map (not shown). While quite reasonable additivity is observed in most transfection crosses, exceptions do occur. Some of these may be attributed to variations in the ability of various DNA preparations to contribute markers. It is noteworthy that the wild-type frequencies observed in four of the five smallest summed intervals examined are lower than the expected summed values.

The use of transfection permits genetic mapping in very small regions of the genetic map. Based on a total genetic map for SP82G of 55 map units in a molecular structure of 108×10^6 daltons, the transfection crosses give linear mapping in the range of 4 to 0.1 map units or in the range of 1.7×10^4 to 3.5×10^2 base pairs. The lower limit reflects our failure to isolate more closely linked markers in gene 5.

DISCUSSION

Marker unlinking in transfection: Transfection in SP82G requires the interaction of several infecting DNA molecules. The failure of a single transfecting DNA molecule to yield productively is the result of an intracellular inactivating event which introduces partial damages on the infecting DNA molecule. The damages are partial in that superinfecting phage can rescue undamaged portions of the infecting DNA molecule. In an earlier study (GREEN 1968) it was observed that when a cell was transfected by a mixture of DNA molecules bearing two temperature-sensitive markers separated by only 1.3 recombination units, the progeny genotypes were randomly assorted. In the present study, complete random assortment of input alleles to the progeny of infecting DNA molecules occurs at a greater map distance, 4.0 recombination units. The differences between the two experimental values are probably attributable to the fact that in the for-

FIGURE 5.—Maps constructed by the frequency of wild-type infective centers observed in transfection (A) and standard phage recombination (B) of markers in gene 5. In all respects except the magnitudes of the values the two maps are consistent.

mer experiments transfectant progeny were permitted to grow until lysis (3–4 hr) whereas, in the present experiments, phage progeny were examined at two hours. Transfectants have a delayed bursting period (GREEN 1964) and selection of early bursts may favor, to some degree, phage clones that have less damage and require less recombination in the genetic region being analyzed.

The isolation of genetic markers on independently interacting units that comprise 1/14 or less of the whole genome is consistent with a random introduction of approximately 40 unlinking hits per genome.

The occurrence of recombination in transfection: The implication that genetic interaction occurs between transfecting DNA molecules has further support from the analysis of single-burst progeny carried out by OKUBO *et al.* (1964) who observed about 10% of the bursts produced by transfection with a mixture of two genetically marked DNAs of phage SP01 were composed of a recombinant majority class. Subsequent studies with SP82G (GREEN 1968) supported the idea that input alleles from such mixtures of transfecting DNAs were randomly assorted to the progeny even when by phage crosses, the marker pairs were closely linked. Such random assortment of input alleles leads to an apparent increase in recombination for small regions of the genetic map. However, at sufficiently small map distances it is possible to correlate the relative recombination of transfection with that of phage recombination.

It is clear that progeny produced by transfectants show about a twelve-fold increase in recombination.

While OKUBO *et al.* (1964) had undertaken single burst experiments with SP01 transfectants, a detailed genotype analysis was reported only for the "exceptional" majority recombinant clones. In order to gain a more complete picture of the marker contribution similar experiments were undertaken. The results of these experiments share a number of features in common with the progeny production by multiplicity reactivated T4 bacteriophage (EPSTEIN 1958). Central among these is the marked clonality of the progeny within a given single burst as evidenced by the frequent absence or marked rarity of one or more of the input alleles in the progeny of a transfectant, the existence of a substantial fraction of clones in which a single genotype predominates. In accord with EPSTEIN's (1958) conclusions on MR we feel that these results implicate a recovery process from the intracellular inactivation involving a recombination mechanism prior to (much, if any) multiplication of the transfecting DNA.

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