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DNA Injection and Genetic Recombination of Alkylated Bacteriophage T7 in the Presence of Nalidixic Acid

BARBARA KARSKA-WYSOCKI,* MARGARET D. MAMET-BRATLEY, AND GRZEGORZ PRZEWLOCKI†

Département de Biochimie, Faculté de Médecine, Université de Montréal, C.P. 6128, Montreal H3C 3J7, Canada

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Marker rescue experiments with alkylated T7 bacteriophage carried out in the presence and in the absence of nalidixic acid suggest that the gradient in rescue is due to two alkylation-induced causes: a DNA injection defect and an interference with DNA synthesis.

T7 phage is reported to inject its DNA into the host bacterium in a unique direction (7, 11, 13). This conclusion is based on the results of marker rescue experiments carried out on phage particles containing physically or chemically induced lesions in their DNA. Treatment of T7 phage by X rays (13) or monofunctional alkylating agents (7), as well as ^{32}P decay in T7 phage (11), causes increasing marker sensitivity progressing from left to right on the genetic map.

Such evidence is not, however, unambiguous, since there are two possible causes for the observed genetic effect. Genes may show high or low marker rescue, depending on whether or not they are injected or expressed after injection. Levy (12), in discussing the effects of ^{32}P decay on marker rescue in the T4 system, has suggested two possible models to explain variations in genetic expression. High marker rescue could be due to preferential replication of certain segments of the damaged genome or could be characteristic of regions in which there is preferential repair of primary lesions. The first possibility may be visualized as follows. Lesions in phage DNA could block replication; segments of DNA located between the initiation sites for replication and those lesions would exist in multiple copies; and rescue of genetic markers localized in these segments would be favored. In contrast, regions beyond the lesions would not be copied, and their rescue would be much less probable. This model has been used by Doermann and co-workers (2, 3, 10) and Levy (12) to explain the effects of UV radiation, X rays, and ^{32}P decay on marker rescue in T4 phage; it finds experimental support in Rayssiguier's investigation of multiplicity reactivation of UV-treated T4 phage (2, 10).

† Present address: Institute of Microbiology, University of Warsaw, Warsaw, Poland.

Partial replicas of damaged DNA also appear to participate in repair by recombination in UV-treated *Escherichia coli* (6).

T7 phage has a unique origin for DNA replication, located about 17% from the left end of the phage genome; replication occurs bidirectionally from this site (4). All markers used in the previous studies lie to the right of this site (7, 11, 13). Should lesions block replication, markers on the left of the map (e.g., in gene 2) would be expected to be copied more often and, thus, give higher recombination frequencies than those on the right (e.g., in gene 19). Thus, one could argue that the gradient in recombination frequencies is due not at all to an injection defect, but to an interference with DNA replication.

There are at least two ways in which this objection might be answered. Use of genetic markers to the left of the origin of replication should permit a distinction between an injection defect and an interference with genetic expression. However, in the case of T7 phage, only one essential gene (gene 1) lies to the left of the origin; it is so close (8 to 15% of the DNA length; 14) to that origin that it would be experimentally very difficult to distinguish between the two possibilities. Another approach is to carry out marker rescue experiments under conditions in which DNA synthesis is inhibited. In this case, any contribution of replication or replication-dependent repair recombination (2) to the gradient should disappear.

We have thus carried out recombination experiments with alkylated T7 phage in the presence and in the absence of nalidixic acid, an inhibitor of DNA synthesis (1, 9) but not of recombination (9) in T7 phage. If the previously observed gradient in marker rescue is due entirely to an effect on replication, we would expect the gradient to disappear in the absence of

DNA replication. If replication effects made no contribution whatsoever to the gradient, we would expect to find similar gradients in the presence and absence of the inhibitor. A reduction in slope of the gradient in the presence of the inhibitor would indicate that replication effects contribute to but are not the sole cause of the gradient.

The bacterial strains and the T7 amber mutants were those described previously (7); they were multiplied at 30°C.

Alkylation with methyl methane sulfonate was carried out on purified phage as described previously (7).

The experimental protocol, slightly modified from that used previously (7), was as follows: *E. coli* O11′ (Su^+) cells from an overnight culture were transferred into fresh medium (broth [15] supplemented with 1 mM $MgSO_4$ [9] and 200 μg of thymine per ml) and grown with vigorous agitation to a density of 5×10^8 cells per ml at 30°C. The culture was infected at a multiplicity of one phage particle per cell with the alkylated or nonalkylated (control experiment) T7 amber mutant and at a multiplicity of three phage particles per cell with a different amber mutant (helper phage). In these experiments, the multiplicity of infection of the purified phage was based on the titer of the nonalkylated phage sample, since it is known that alkylation does not change adsorption of T7 phage to the host bacterium (8). Subsequent dilution and incubation of phage-infected cells were carried out in broth containing 40 μg of thymine per ml at 30°C. All crosses were carried out in duplicate.

Nalidixic acid (Schwarz/Mann, Orangeburg, N.Y.) was prepared as a stock solution for each experiment at a concentration of 1 mg/ml in 0.02 N NaOH (5). It was added, at a final concentration of 50 μg/ml, to the bacterial culture 4 min after infection. This concentration reduced the rate of DNA synthesis, measured by the method of Studier (15), by more than 95%.

Recombination experiments were carried out in the presence and absence of nalidixic acid. The complete results of one experiment with alkylated T7 phage *am*37 (gene 11) are shown in Table 1. Table 2 summarizes the effects of nalidixic acid on burst and recombination frequency for three different experiments with this phage. Nalidixic acid, at a concentration of 50 μg/ml, effectively inhibited phage production (ca. 0.3% of control) in crosses with alkylated phage as well as in crosses with control phage. In contrast, there was relatively little effect of the inhibitor on recombination frequencies, which is in agreement with results of Kerr and Sadowski (9).

The results for the three separate experiments, carried out with alkylated T7 *am*37, are presented graphically in Fig. 1 as the percentage of normal recombination. In the absence of nalidixic acid, we observed a gradient in percentage of normal recombination, decreasing from left to right on the genetic map. This confirms our previous experiments (7). In the presence of nalidixic acid, we continued to see a gradient; the percentage of normal recombination was larger for gene 2 mutant (*am*64) than for gene 19 mutant (*am*10). The gradient was, however, less steep in the presence of

TABLE 1. *Phage production and recombination frequency in the presence and absence of nalidixic acid*[a]

Cross	Phage titer on permissive host (PFU/ml)		Frequency of wild-type recombinants (%)	
	Control ($\times 10^{10}$)	+Nalidixic acid (50 μg/ml) ($\times 10^7$)	Control	+Nalidixic acid (50 μg/ml)
*am*37 × *am*64 (gene 11 × gene 2)	1.6	6.0	15	6.8
*am*37 (alkylated) × *am*64	1.2	3.0	13	6.4
*am*37 × *am*208 (gene 11 × gene 4)	2.0	12	12	8.4
*am*37 (alkylated) × *am*208	2.6	7.0	5.2	5.7
*am*37 × *am*9 (gene 11 × gene 16)	3.6	9.0	7.3	6.0
*am*37 (alkylated) × *am*9	3.0	5.0	3.2	4.0
*am*37 × *am*10 (gene 11 × gene 19)	3.4	11	7.3	9.9
*am*37 (alkylated) × *am*10	4.2	7.0	2.6	5.2

[a] Results represent the average values of data from duplicate samples belonging to the same experiment. Phage T7 *am*37 (gene 11) was treated for 2 h, 37°C, at a concentration of 0.002 M methyl methane sulfonate; phage survival after alkylation was 53%.

TABLE 2. *Effect of nalidixic acid on phage production and recombination frequency*[a]

Helper phage in cross	Expt	Phage production[b] (% of control)		Recombination frequency[b] (% of control)	
		am37	Alkylated *am37*	*am37*	Alkylated *am37*
am64 (gene 2)	1	0.30	0.30	110	150
	2	1.2	0.71	130	140
	3	0.38	0.25	44	51
am208 (gene 4)	1	0.27	0.11	220	230
	2				
	3	0.60	0.27	70	110
am9 (gene 16)	1				
	2				
	3	0.25	0.17	82	120
am10 (gene 19)	1	0.31	0.25	120	300
	2	0.50	0.42	120	180
	3	0.32	0.17	140	200

[a] Phage T7 *am37* (gene 11) was treated for 2 h, 37°C, at a concentration of 0.002 M methyl methane sulfonate; phage survival was 53, 45, and 53% in experiments 1, 2, and 3, respectively.

[b] Control refers to cross in the absence of nalidixic acid.

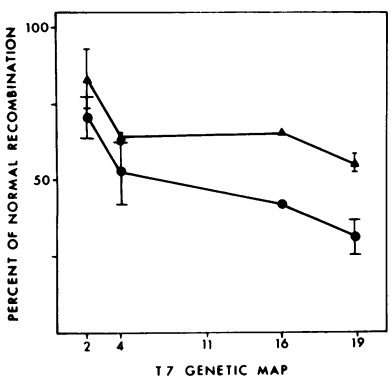

FIG. 1. *Frequency of recombination between alkylated T7 am37 (gene 11) and other T7 amber mutants (identified by gene number on genetic map) expressed as percentage of normal recombination (i.e., the ratio [×100] of the frequency of wild-type recombinants in the cross with alkylated phage to that in the control cross). Recombination was carried out in the presence of 50 μg of nalidixic acid per ml (▲) and in the absence of nalidixic acid (●). Results are presented as the mean values of percentage of normal recombination for the three experiments cited in Table 2; vertical bars represent the average error in this mean. All crosses in the presence of nalidixic acid were carried out on the first day after alkylation. All crosses in the absence of nalidixic acid were carried out on the second day after alkylation.*

nalidixic acid. The difference between the two curves increases from left to right on the genetic map (Fig. 1).

To show that these results are not unique to alkylated T7 *am37*, we repeated the same type of experiment with T7 *am208* (gene 4) as the alkylated phage. With this phage, the effect of nalidixic acid on phage production and recombination frequency was similar to that observed for T7 *am37*. Figure 2 presents the results (percentage of normal recombination) of two separate experiments carried out with alkylated T7 *am208*. Here also, the gradient was reduced but did not disappear in the presence of nalidixic acid.

We interpret these results as follows. If the gradient in the percentage of normal recombination, observed in the absence of nalidixic acid, is due to two types of alkylation-induced causes—an injection defect and an interference with genetic expression—then, in the presence of nalidixic acid (an inhibitor of both DNA synthesis and phage production), any interference with genetic expression that depends on replication must be significantly reduced. We may thus estimate its contribution, at the alkylation dose used, by comparing the gradients observed in the presence and absence of nalidixic acid. The values of percentage of normal recombination for the marker in gene 2 are identical (within the limits of experimental error) in the absence and presence of nalidixic acid (Fig. 1 and 2). In contrast, the values of percentage of normal recombination for the marker in gene 19 are higher in the presence of the inhibitor than in its absence. An interference with DNA synthesis thus contributes to the gradient under normal conditions (without nalidixic acid). This illustrates the potential danger of using marker rescue experiments alone as a proof or disproof of injection of T7 phage DNA.

Since a gradient persisted even in the ab-

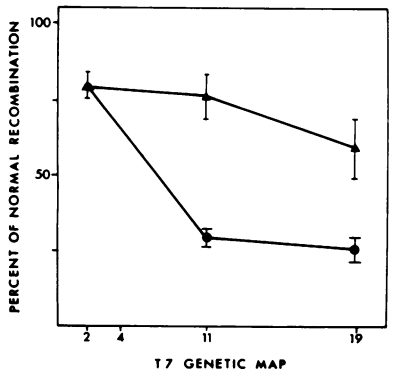

FIG. 2. *Frequency of recombination between alkylated T7 am208 (gene 4) and other T7 amber mutants (identified by gene numbers on genetic map) expressed as percentage of normal recombination. Other details are as explained in the legend to Fig. 1, except that results here come from two separate experiments in which the values of percent survival of purified T7 am208 after alkylation (0.002 M methyl methane sulfonate) were 32 and 44%, respectively.*

sence of DNA synthesis, we conclude that this gradient must be due to causes other than an interference with DNA synthesis. Alkylation prevents T7 phages from fully injecting their DNA into host cells (8). In addition, marker rescue experiments carried out on phages inactivated by X rays (13), ^{32}P decay (11), and alkylating agents (7) gave similar results. We thus feel that the most plausible explanation of the residual gradient is a partial injection of DNA by alkylated phages. It appears unlikely that region-specific excision repair of primary lesions contributes to this gradient. Our results suggest that the gradient in marker rescue is due to two alkylation-induced causes: a DNA injection defect and an interference with DNA synthesis. Thus, these results provide unambiguous support for Pao and Speyer's (13) proposal that T7 phage injects its DNA in a unique direction, starting from the end containing the genes coding for the early proteins.

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