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Short Communication

Gene 49 Endonuclease VII is not Essential for Multiplicity Reactivation of Bacteriophage T4

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Summary. Endonuclease VII, the product of phage T4 gene 49, has been shown previously to resolve Holliday structures in vitro. Two different processes, genetic recombination and multiplicity reactivation are presumed to have Holliday structure intermediates. Other workers have shown that genetic recombination is reduced in a gene 49 mutant infection. However, in the present study, multiplicity reactivation of UV-irradiated *ts* or *amber* mutant phage defective in gene 49 was nearly identical to that of UV-irradiated wild-type phage T4. Thus endonuclease VII is not thought to be essential for multiplicity reactivation of phage T4.

Endonuclease VII, the product of bacteriophage T4 gene 49, has been shown to play a critical role in maturation of phage DNA (Kemper and Brown 1976; Kemper and Garabett 1981). Late during infection, phage T4 DNA is in the form of concatemers which are packaged in "headfull" length pieces in phage heads. In gene 49 mutant infections, head filling can begin but cannot be completed, and very-fast sedimenting (>1000S) DNA accumulates (Kemper and Brown 1976). This DNA is compact and branched suggesting a steric hindrence to headfilling. Recently a second activity has been shown for endonuclease VII, that of Holliday structure cleavage and resolution (Mizuuchi et al. 1982). In a gene 49 mutant infection, Y structures, thought to be recombinational intermediates and probably related to Holliday structures, accumulate (Minagawa et al. 1983). Miyazaki et al. (1983) have shown that gene 49 mutants had reduced recombination between genetic markers, suggesting that the Y structures have to be resolved by endonuclease VII in order for most recombination events to be completed.

Multiplicity reactivation (MR) in phage T4 is considered to be a form of recombinational repair. This conclusion is based on the need for interaction of at least two phage chromosomes, and a requirement for several gene functions necessary for recombination (see Bernstein 1981, for review). It is thus reasonable to assume that multiplicity reactivation involves Holliday structure intermediates. To test whether activity of endonuclease VII was required for MR, the effect of mutations in gene 49 on MR was studied. MR experiments were performed as follows: Phage, suspended at a concentration of 2×10^{10} ml in adsorption salts (Nonn and Bernstein 1977) were irradiated by a broad band UV lamp (GE G8T5). At various times during irradiation, samples were withdrawn and diluted 10-fold in adsorption salts. *E. coli* S/6 was used as host for experiments involving *ts* mutants and *E. coli* CA167 (*supC*$^+$) was used for experiments involving the gene 49 amber mutant *amE727*(49). The bacteria were grown to a concentration of approximately 1.5×10^8 ml in Hershey's broth (Steinberg and Edgar 1962), centrifuged in the cold and resuspended in an equal volume of adsorption salts at a concentration of about 2×10^8 ml. The bacteria were then starved at 37° C with aeration for about one hour. For single infections, the phage suspension was diluted 10^4-fold in adsorption salts and then equal volumes of phage and bacterial suspensions were mixed to give an average multiplicity of infection (m.o.i.) of 0.001. For multiple infections, equal volumes of the phage suspension at 2×10^9 ml and bacteria at 2×10^8 ml were mixed to give an average multiplicity of infection of 10. Adsorption was allowed to occur for 15 min at room temperature and was stopped by dilution. A gene 46 mutant *tsC17*(46), used in control experiments, adsorbed poorly, and anti-T4 rabbit serum was added in this case to inactivate unadsorbed phage. Samples were further diluted as appropriate and then plated by the soft agar overlay method (Adams 1959). In all experiments involving *ts* mutants, the plates were prewarmed for at least an hour at the appropriate incubation temperature prior to plating the phage and bacteria. The plates were then incubated for 24 h at this temperature (see legend to Fig. 1).

Figure 1a shows the survival of the ability of singly infected or multiply infected *E. coli* to form plaques when infected by wild-type T4D (T4D$^+$) or *tsC9*(49) phage previously irradiated by the indicated doses of UV light. After infection, the phage-host complexes (infectious centers) were incubated on plates at 34° C, a partially restrictive condition for the mutant phage. Plaques formed by *tsC9*(49) at this temperature were small, indicating partial restriction, but were easily countable. The burst size produced by *tsC9*(49)-infected cells in liquid culture at this temperature has been measured and found to be less than 5% of the wild-type burst size (H. Bernstein, personal communication). Survival of wild-type infectious centers was found to be quite similar to that of mutant infectious centers, both under conditions of single and multiple infections. Figure 1b shows the survival of T4D$^+$ and *tsC9*(49) in singly-infected or multiply-infected *E. coli* when growth was at 25° C, a permissive condition for *tsC9*(49). The results are similar to those in Fig. 1a.

Figure 1c shows the survival of plaque-forming ability

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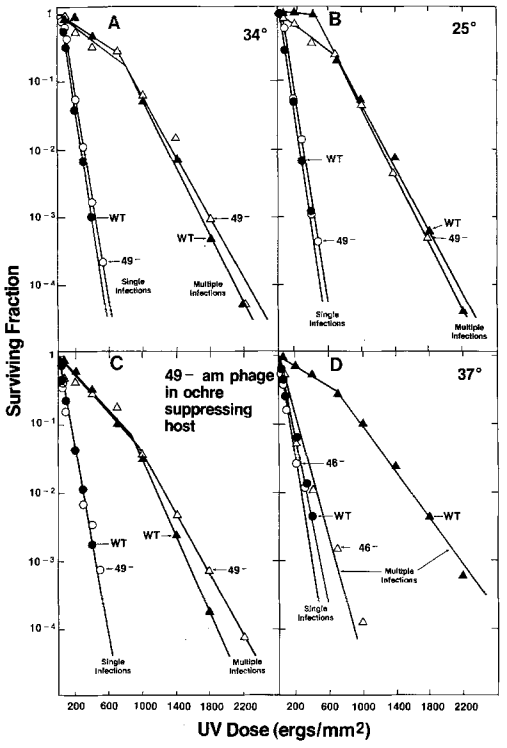

Fig. 1. Survival of wild-type or mutant phage T4 in singly or multiply-infected cells after various doses of UV light to the infecting phage. **a**. Wild-type T4D single infections (●) and multiple infections (▲); *tsC9*(49) single infections (○) and multiple infections (△). Growth was at 34° C. Results of a single experiment are shown. This experiment was performed four times and very similar results were obtained in all four experiments. **b**. Wild-type T4D single infections (●) and multiple infections (▲); *tsC9*(49) single infections (○) and multiple infections (△). Growth was at 25° C. Results of a single experiment are shown. This experiment was performed once. **c**. Wild-type T4D single infections (●) and multiple infections (▲); *amE727*(49) single infections (○) and multiple infection (△). Growth was at 37° C in *E. coli* CA167 which contains an ochre suppressor (*sup* C^+). Results of a single experiment are shown. This experiment was performed four times, and very similar results were obtained in all four experiments. **d**. Wild-type T4D single infections (●) and multiple infections (▲); *tsC17*(46) single infection (○) and multiple infections (△). Growth was at 37° C. Results of a single experiment are shown. This experiment was performed three times and very similar results were obtained in all three experiments

by bacteria singly or multiply infected by $T4D^+$ or *amE727*(49) previously treated with the indicated doses of UV light. Here the bacteria were *E. coli* CA167(*sup* C^+). The ochre suppressor in this host is only able to weakly suppress amber mutations (Brenner and Beckwith 1965). Again, the wild-type and mutant survival curves are quite similar.

As a control for the lack of effect shown by mutations in gene 49 in the MR experiments, a mutant, *tsC17*(46), defective in gene 46, was tested using the same procedures. Gene 46 codes for a nuclease (Prashad and Hosoda 1972) involved in a number of processes in phage T4 growth including DNA replication (Epstein et al. 1963) and recombination (Bernstein 1968). Previous work, using a partial revertant of a gene 46 *amber* mutant (Davis and Symonds 1974) and a gene 46 *ts* mutant (Nonn and Bernstein 1977) has shown that the gene 46 product is required for MR after induction of damage by UV or HNO_2 respectively. As can be seen in Fig. 1d, at 37° C, a partially restrictive condition, there is a dramatic decrease in MR of UV-damaged *tsC17*(46) mutant phage compared to $T4D^+$ phage.

Overall, the results obtained suggest that the gene 49 product is not essential for MR even though it is required for a normal level of genetic recombination (Miyazaki et al. 1983) and is the only phage T4 enzyme known to be able to cleave Holliday structures. Assuming that Holliday structures are intermediates in phage T4 recombination and MR, the following explanation is suggested for the results obtained.

Figure 2 shows a model for MR which is a modified version of one presented by Bernstein and Wallace (1983). It is consistent with available evidence on the genes required for MR and the likely structural intermediates in recombination. The model illustrates accurate bypass of a lesion by a recombinational exchange between two homologous chromosomes, rather than removal of the lesion. Thus, the model is analogous to the more established mechanism of post-replication recombinational repair in *E. coli* (e.g. Livneh and Lehman 1982). The intermediate formed at step *e* in Fig. 2 is a partial Holliday structure (Y branch structure) of a type that presumably could be resolved by endonuclease VII (Minagawa et al. 1983) as shown in step *f*.

It has been proposed, however, that MR of damaged parental phage takes place early in infection to allow replication past damaged regions in parental DNA (Bernstein 1981; Bernstein and Wallace 1983). In addition, Dannenberg and Mosig (1983) have concluded that in multiple infections, recombination intermediates are formed soon after the onset of replication, before the majority of parental chromosomes have completed their first round of replication. Thus gene 49, which is a late expressing gene (Frankel et al. 1971), would not be expected to be involved in MR. Any Y branched intermediates formed early are probably resolved by a different enzyme, a host or early phage Holliday structure resolving enzyme. Such an activity has been demonstrated in uninfected *E. coli* (West and Howard-Flanders 1983a) and this activity is apparently not under *recA* control (West and Howard-Flanders 1983b). Later in infection, after turn-off of host and early phage functions, the remaining branched structures are probably cleaved by the gene 49 product (Minagawa et al. 1983).

If a different enzyme acted at early times, MR would not be reduced in a gene 49 mutant infection. On the other hand, genetic recombination, which probably occurs both at early and late times, should be reduced in a gene 49 mutant infection as reported by Miyazaki et al. (1983). In gene 49 mutant infections, carried out with phage not treated by DNA damaging agents, recombination is reduced to 1/4 to 1/3 of wild-type levels. Presumably, in untreated phage, 2/3 to 3/4 of recombinants are resolved by endonuclease VII while the remaining 1/4 to 1/3 may be resolved by the other enzyme, whose presence is proposed here.

Other observations are also consistent with this explanation. In contrast to the gene 49 product, the gene 46 product (acting at step b in Fig. 2) appears to be required for all recombination events, those that occur early involving parental DNA, and those that occur late. This explains the drastic loss of genetic recombination (Bernstein 1968) and MR (Fig. 1d) in gene 46 mutant infections. The branched structure shown after step *e* (Fig. 2) would be expected to

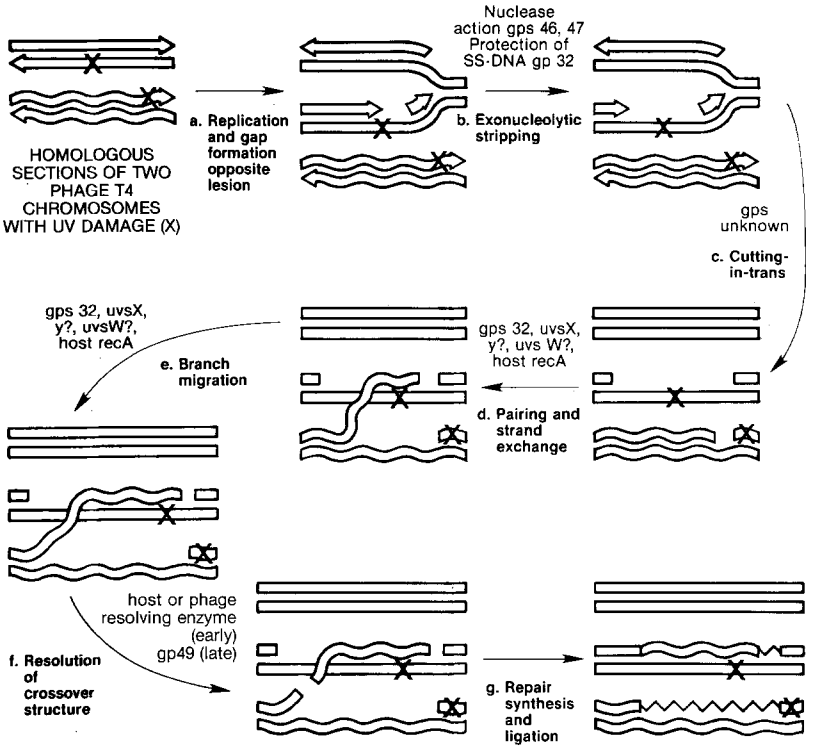

Fig. 2. A model of multiplicity reactivation in phage T4 infected cells adapted from Bernstein and Wallace (1983)

contain extended single stranded regions. This fits with the observation of Minagawa et al. (1983) that the very-fast sedimenting DNA found in gene 49 mutant infections contains branches, each branch containing a single-standed interruption about 60 nucleotides long at its proximal end.

In conclusion, the results reported here indicate that endonuclease VII does not have a significant role in multiplicity reactivation.

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