INHIBITION OF RECOMBINATION AND HETEROZYGOSIS IN PHENYL ETHYL ALCOHOL TREATED PHAGE T4-*E. coli* B COMPLEXES

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Phenyl ethyl alcohol (PEA) reversibly blocks bacterial DNA synthesis, but not protein or RNA synthesis (1). Phage infected PEA-treated bacteria demonstrate no detectable DNA synthesis; however lysis occurs at 20-30 min. and small numbers (one to eight per bacterium) of viable progeny phage are produced (9). Such a sequence of events suggests that in PEA-treated complexes parental phage DNA can function, but cannot replicate. The small numbers of viable progeny phage most probably contain entirely parental DNA which bears newly synthesized protein shells.

This article presents experiments on phage T4 which concern (a) the complete inhibition of recombinant formation, and (b) the complete inhibition of heterozygote formation due to PEA blockage of DNA synthesis.

Media and phage mutants employed for these studies have been described in a previous article (2). Bacteria *E. coli* B grown to a titre of 2×10^8 per ml at 37°C were treated with 0.34% (v/v) PEA and incubated for a further 90 min. During this interval no bacterial growth (as measured by cell counting) nor DNA synthesis (determined by the diphenylamine reaction) were detectable. Input phages at multiplicities of infection of four of each type were added to PEA-treated

bacteria in M/500 KCN, and the adsorption mixtures were aerated for 15 min. Aliquots of the adsorption mixtures were withdrawn for the estimation of unadsorbed phage, and for dilution into the growth tubes. For each cross, one growth tube contained broth, and a second contained broth plus 0.34% PEA. Growth tubes were incubated 90 min, chloroformed, and suitable volumes were plated on *E. coli* B to permit estimation of burst size and heterozygote frequency. For crosses of two rII mutants, r+ progeny were assayed on *E. coli* K.

Typical results of these experiments are presented in Table I below.

TABLE I: PEA EFFECTS ON HETEROZYGOTE FREQUENCIES AND RECOMBINATION

cross	no. plaques scanned	hets no.	hets %	map units	avg. burst size
control	10,498	36	0.34±.06	-	87
UF-115 x r+ PEA	5,249	1	0.02 (a)	-	0.69
control	11,774	32	0.25±.05	-	79
UF-9 x r+ PEA	6,158	0	0	-	0.41
control	9,683	51	0.53±.07	-	116
SF-312 x r+ PEA	15,722	0	0	-	0.75
control	-	-	-	4.1	51
UF-9 x UF-115 PEA	-	-	-	no r+ plaques found	0.13

notes:
(a) r type phage recovered from this plaque recombined with UF-115.

UF-115 (cistron B) and UF-9 (cistronA) are non-reverting mutants: SF-312 is a reverting mutant of spontaneous origin which is 2-aminopurine revertable: (ref. 3).

Heterozygous plaques appear in three modes on plates which contain 200-300 plaques. One observes mottled, sectored, and speckled plaques. Speckled plaques are infrequent and have been observed to yield newly arisen r mutant phages. Sectored plaques appear with a frequency dependant upon the total number of r and r+ plaques per plate. The frequency of sectored plaques declines to a negligible fraction when the total number of r and r+ plaques per plate is about 50: in the experiments reported above all determinations of heterozygote frequencies were based upon plates containing about 50 plaques. Presumably sectored plaques result from overlapping of r and r+ plaques since mixtures of r and r+ phages plated at 200 per plate yielded sectored plaques (0.21%). Mottled plaques represent true heterozygotes (6) and appear with a frequency independant of the number of plaques per plate in the range of 10-250 plaques. For PEA- treated crosses of rII x r+ phage no mottled plaques have been observed and few speckled plaques; when plaque overlapping is minimized by plating no more than 50 phage per plate, sectored plaques are not present. We conclude from these observations and the data of Table I that PEA completely blocks the formation of phage heterozygotes.

When UF-9 and UF-115 mutants are crossed, r+ recombinants are formed only in the control. PEA-treated complexes yield no detectable r+ recombinants. Since aliquots of the entire growth tube containing 10^7 infected bacteria and hence 1.3×10^6 progeny phage were plated on K, recombination is reduced at least four orders of magnitude, if not completely.

Since PEA permits progeny phage to be formed in the absence of detectable DNA synthesis, recombination, and

heterozygote formation, we conclude that DNA replication is required for genetic recombination.

DNA function occurs prior to replication: if DNA were to function during replication as well, the synthesis of different species of phage specific RNA would be random, which is not the case according to Kano-Sueoka and Spiegelman (7). Hence we surmize that phage DNA function is restricted to the early latent period and is manifested solely by the intact phage genome.

If fragmentation of input phage DNA were to occur, one might expect to recover some heterozygous phage from PEA-treated complexes. Either fragmentation occurs but the fragments cannot be reunited, or fragmentation does not occur when DNA synthesis is PEA inhibited. Since there is ample evidence for fragmentation under conditions permitting normal DNA synthesis (8, 10, 11, 13) one might infer that DNA synthesis is necessary to join newly formed and parental fragments (partial replicas) in order to form a complete phage genome. Various recent models for phage recombination have considered this point (2, 4).

Transformation and recombination have been shown to procede in D. pneumoniae in the absence of DNA synthesis via an intermediate single stranded state of the transforming DNA (5, 12). Thus, recombination in phage is different, or, more probably, PEA might act to prevent DNA from attaining a single stranded state. Recent in vitro experiments do suggest that PEA prevents DNA from becoming single stranded and will be reported in a further communication.

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