COORDINATE VARIATION IN DENSITY AND RECOMBINATION POTENTIAL IN T4 PHAGE PARTICLES PRODUCED AT DIFFERENT TIMES AFTER INFECTION¹

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FREQUENCIES of recombinants in phage crosses depend not only on the genetic markers under observation but also on the time of lysis (DOERMANN 1953), conditions of phage growth (HERSHEY 1958; SYMONDS 1962), multiplicity of infection (TRAUTNER 1960; MOSIG 1962), and, apparently, unknown variables (HERSHEY 1958). Information about the sources of these variations may provide additional clues to the mechanism of recombination. Do individual differences in the phage particles crossed affect recombination frequency? The results reported here show that recombination frequencies depend on the time of maturation, during the previous growth cycle, of the phage particles entering into the cross. The relevant variable seems to be the DNA content of the particles.

EXPERIMENTS

Crosses were made between pairs of either "early" or "late" phage particles (obtained by early-induced lysis or spontaneous lysis) of genotypes ++ and $r_{61}tu_{41}$ of *Escherichia coli* bacteriophage T4D. The different parental phage stocks were prepared as follows. The original (late) phage stock of each genotype was prepared by spontaneous lysis after infection of a bacterial culture with phage particles from a four-hour plaque (CHASE and DOERMANN 1958). It was used to infect a culture of *E. coli* B (DOERMANN and HILL 1953), about 5×10^8 /ml, with an average of three to four phage particles per bacterium. To obtain early phage, an aliquot of the culture was lysed by the addition of chloroform when the progeny particles numbered about ten per bacterium. Late phage were obtained when the remainder of the culture lysed spontaneously. The phage were passed through 0.47μ membrane filters, and were crossed as described by CHASE and DOERMANN (1958), with *E. coli* B or K(λ) (EDGAR 1958) as host bacteria.

Recombination frequencies were determined in the phage yields of single bacteria, most of which had been infected with only one phage particle of each

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parental type. Yields to be scored were selected according to criteria described previously (Mosig 1962). This procedure ensured a constant multiplicity of infection. Results are summarized in Table 1. They permit two conclusions: (1) Crosses between late phages yield more recombinants than crosses between early phages. (2) Crosses in $K(\lambda)$ yield more recombinants than those in B. The second result was also observed in other crosses not reported here.

Figure 1 shows the distributions of recombination frequencies among the individual single bursts from the crosses done in $K(\lambda)$. The distributions from crosses made in B were similar. In agreement with results of HERSHEY and ROTMAN (1949), fractional yields of recombinants in single bursts fluctuated widely when late phages were the parents. In crosses made with early phages, the percentages were more narrowly distributed, and very few mixed bursts without recombinants were found.

Differences in recombination potential must reflect physical differences among phage particles. As a sensitive test for such differences (DOERMANN and BOEHNER 1963), the buoyant densities of early and late phage were compared. Equal numbers of early phage particles of genotype $r_{73}h_{42}$ and late phage particles of genotype ac_{41} , both osmotic shock-resistant mutants obtained from Dr. A. H. DOERMANN, were mixed and heated at 45°C for 30 minutes before and 30 minutes

TABLE 1

Results of $++ \times r_{61} tu_{41}$ crosses made with different phage preparations

Phage preparation	Host B			Host $K(\lambda)$		
	Percent recombinants	Number of bursts	Burst size	Percent recombinants	Number of bursts	Burst size
Original (late lysis)	11.0	25	683	20.8	24	352
	11.9	15	405			
Late lysis	11.6	34	263	17.5	28	239
Early lysis	8.2	48	151	10.3	26	219

FIGURE 1.—Distributions of recombination frequencies among individual single bursts from crosses in $K(\lambda)$. Top: parents from original preparations (late lysates); middle: from late lysates; bottom: from early lysates.

after the addition of 0.75 g of CsCl per ml of fluid. Under these conditions little or no inactivation of phage occurred. Five ml of the final mixture was centrifuged for 36 hours at 28,000 rpm. Results are presented in Figure 2A; Figure 2B gives the corresponding results when the genotypes were reversed. Both sets of results show clearly that phage particles prepared by early lysis are denser than those prepared by late lysis. The same difference was observed in experiments with phage prepared in P broth (CHASE and DOERMANN 1958) instead of synthetic medium, or in *E. coli* strain BB (BENZER 1957) instead of B.

The correlation between density and recombination potential was confirmed in the following experiments. A mixture of two late lysates of genotypes $r_{73}h_{42}$ and ac_{41} (prepared in *E. coli* B in synthetic medium; HERSHEY and MELECHEN 1957) was fractionated by CsCl density gradient centrifugation. $K(\lambda)$ bacteria were infected at multiplicities of less than 0.1 with phage particles from the individual fractions. Since only bacteria that have been mixedly infected with rII and r^+ phages yield rII progeny (BENZER 1957), the percentage of rh^+ particles among the total r progeny was considered to be the frequency of recombination. The frequencies so measured are summarized in Table 2. They increase with decreasing density of the parental phage particles.

The results described above could be in error if dense phage particles were somehow able to escape the block to growth of rII mutants in $K(\lambda)$ bacteria.

FIGURE 2.—Difference in buoyant density in CsCl solution between phage particles prepared by early and late lysis. A: Phage of genotype ac_{41} from early lysis (4.6 phage particles per bacterium) and of genotype $r_{73}h_{42}$ from late lysis. B: Early- (8.0 per bacterium) and late-lysis phages of the reverse genotypes. Distributions were measured by plaque counts classified with respect of the r marker. Phage prepared in *E. coli* B in synthetic medium (HERSHEY and MELECHEN 1957).

TABLE 2

Frequencies of recombinants when particles from different fractions from a CsCl density gradient were crossed

CsCl gradient fraction no.	Burst size	Total <i>r</i> plaques counted	Percent <i>rh</i> ⁺ recombinants
18	63	51	11.8
21	77	256	18.8
	107	413	22.0
22	107	244	26.2
24	54	127	27.6
	94	123	25.2
	100	293	27.6
26	222	232	29.8
	93	250	24.8
28	113	231	26.8
30	176	134	30.6
32	122	225	30.2
34	135	161	31.1
36	132	77	39.0
40	62	43	37.2

Five ml of a phage suspension gave 60 fractions, numbered in order of decreasing density. Fractions Nos. 18-42 contained almost all the particles. Fraction No. 22 represents the peak of the distribution of viable phage particles. Crosses were performed as described by CHASE and DOERMANN (1958). Crosses between particles from fractions 21, 24, and 26 were repeated on different days.

However, two single-burst crosses in $K(\lambda)$, performed with heavy and light phages (prepared by spontaneous lysis in P broth and fractionated in CsCl), did not reveal any unmixed *r* yields.

The denser fractions of a spontaneous lysate contain more phosphorus per viable phage particle than do fractions of average density, as is shown in Figure 3. Both the higher phosphorus content and the higher density (WEIGLE, MESELSON, and PAIGEN 1959) suggest a higher DNA content per particle. It appears less likely, but has not been excluded, that the denser fractions contain more non-viable phage particles. Some fractions of low density in Figure 3 also show a high ratio of phosphorus per viable phage. They do contain nonviable particles, as will be reported elsewhere.

DISCUSSION

The results reported indicate that frequencies of recombination between given genetic markers depend on individual characteristics of the phage particles crossed. Phage particles matured early during the latent period of phage growth, have greater buoyant density, and yield fewer recombinants when crossed than do those finished late. As usually prepared, a population of phage particles is heterogeneous in both respects, and the two properties are correlated. It seems justifiable to assume that the variations in both properties depend on variation in DNA content, especially when the present results are considered together with DOERMANN and BOEHNER's (1963) finding that the dense particles in the progeny of a genetic cross are frequently heterozygous. Apparently, early phage particles and the dense particles from late lysates are partially diploid. They are homo-

FIGURE 3.—Distribution of viable phage and radioactivity in a CsCl density gradient. P^{32} -labeled phage (0.5 c/g P), prepared in *E. coli* B by spontaneous lysis in synthetic medium.

zygous in a phage stock of a single genotype, but may be heterozygous if they come from bacteria that were mixedly infected with different genotypes.

Why should partial diploidy reduce the ability to produce genetic recombinants? The following explanation is proposed. Recombination requires pairing between related structures. Homozygous diploids yield few recombinants because the redundant parts of their genomes are already paired and this interferes with heterologous pairing. Exchanges between the paired parts of the genome cannot appear as genetic recombination. If the diploids are heterozygous, however, recombination may be increased (CHASE and DOERMANN 1958; BARRICELLI and DOERMANN 1960; EDGAR 1961).

The possibility that early phage particles contain especially long, potentially heterozygous, diploid regions must be taken into account in genetic experiments. For instance, seeming discrepancies between the results of different experiments concerned with the role of heterozygotes in recombination (see STEINBERG and EDGAR 1962) may have arisen because STEINBERG and EDGAR investigated early phage whereas in previous experiments (LEVINTHAL 1954; TRAUTNER 1958; EDGAR 1961) particles from spontaneous lysates were examined.

SUMMARY

Frequencies of recombination between the same genetic markers depend on variable properties of the individual phage particles crossed. Early phage particles have on the average a slightly greater buoyant density in CsCl than late

particles, and generate fewer recombinants. Recombination also depends on the host bacteria: frequencies of recombination between the same parental stocks are higher in *E. coli* strain K(λ) than in strain B.

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