MULTIPLE INTERACTIONS OF A DNA-BINDING PROTEIN *IN VIVO*. III. PHAGE T4 GENE-*32* MUTATIONS DIFFERENTIALLY AFFECT INSERTION-TYPE RECOMBINATION AND MEMBRANE PROPERTIES

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ABSTRACT

We have investigated the *in vivo* roles of T4 gene-*32* protein in recombination. We have studied the effects of gene-*32* mutations under conditions that allow normal DNA replication and are permissive for progeny production. Under these conditions, certain gene-*32* mutations specifically reduce insertion-type (short-interval) recombination but none affect crossover-type (long-interval) recombination (see Figure 5). Heterozygote frequencies in all gene-*32* mutants are similar to or higher than in a gene-32^+ background and are not correlated with recombination deficiencies. "Recombination-deficient" alleles are dominant or codominant over the "recombination-proficient" gene-*32* mutation *tsL171*. This explains apparent discrepancies between a gene-*32* map deduced from two-factor crosses and the map derived from three-factor crosses.

We have also found that the "recombination proficient" mutation *tsL171* and its homoalleles suppress the characteristic plaque morphology of *rII* mutants. Under restrictive conditions, *tsL171* is partially suppressed by *rII* mutations, which allow the use of host ligase in recombination.

Our present and previous results are discussed in terms of current recombination models. We conclude that gene-*32* protein functions in recombination by forming a complex with DNA, with recombination enzymes and with membrane components. Since gene-*32* protein interacts with many components of this recombination complex, gene-*32* mutations may differentially affect various recombination steps.

GENE *32* of phage T4 codes for a DNA-binding protein (ALBERTS and FREY 1970). It is essential for genetic recombination (TOMIZAWA 1967; KOZINSKI and FELGENHAUER 1967; BERGER, WARREN and FREY 1969; BROKER 1973) as well as for DNA replication (EPSTEIN *et al.* 1963) and repair of radiation damage (MAYNARD-SMITH and SYMONDS 1973; WU and YEH 1973). We have further investigated the *in vivo* roles of this protein in various steps of recombination. It has been thought that gene-*32* mutants are defective in DNA replication as well as in formation of "joint" recombinational intermediates (*i.e.*, in heteroduplex formation) mainly because their gene-*32* peptides cannot unwind DNA and because potential single-stranded DNA is excessively degraded (ALBERTS 1973; MESELSON and RADDING 1975; MILLER 1975). This hypothesis is based

on apparent lack of DNA synthesis and of "joint" intermediates late after infection with gene-*32 am* mutants (TOMIZAWA 1967; KOZINSKI and FELGENHAUER 1967; WARNER and HOBBS 1967; BROKER 1973) and on the failure of a gene-*32* mutant peptide (from *tsP7*) to bind to DNA at elevated temperatures (CURTIS and ALBERTS 1976).

We have recently shown, however, that gene-*32* mutants are *differentially* defective in various steps of recombination or DNA replication under conditions *that do not allow progeny production in any of the mutants.* The *am* mutants synthesize considerable amounts of DNA, which is subsequently degraded. The *ts* mutant *P7*, on the other hand, synthesizes little DNA but its DNA is not degraded. From these and other results we have concluded that gene-*32* protein is multifunctional: different domains interact with DNA, DNA ligase, DNA polymerase, recombination nucleases and probably other proteins to form a "recombination complex" (MOSIG and BRESCHKIN 1973, 1975; MOSIG and BOCK 1976; BRESCHKIN and MOSIG 1977*a,b*).

While mapping the gene-*32* mutations, we found discrepancies between the marker orders deduced from two-factor crosses and the order derived from three-factor crosses. These discrepancies suggested that certain mutations differentially affect recombination frequencies. "Marker effects" on recombination have been observed both in prokaryotes and eukaryotes (TESSMAN 1965; EPHRUSSI-TAYLOR 1966; NORKIN 1970; RONEN and SALTS 1971; STADLER and KARIYA 1973; MOORE and SHERMAN 1975; DUCK and CHOVNICK 1975; for reviews see STADLER 1973 and HASTINGS 1975). They are attributed to local base sequence effects, *e.g.*, either to altered frequencies in initiation of recombination, perhaps due to specific nuclease susceptibility (MOSIG 1966, 1968; GUTZ 1971; BECKENDORF and WILSON 1972; LAM *et al.* 1974; HENDERSON and WEIL 1975), and/or to preferential repair of certain heteroduplex mismatches (TIRABY, FOX and BERNHEIMER 1975; WHITE and FOX 1975; WILDENBERG and MESELSON 1975). It is not understood which changes in base sequence or other modifications of DNA are responsible for these marker effects; it is clear, however, that there is no single simple explanation for them.

On the other hand, mutations that alter recombination proteins affect recombination in general (BERNSTEIN 1968; BERGER, WARREN and FRY 1969; ALLEN, ALBRECHT and DRAKE 1970; MUFTI and BERNSTEIN 1974; HAMLETT and BERGER 1975). According to our model, gene-*32* protein interacts with many components of a recombination complex. This predicts that gene-*32* mutations might also differentially affect certain recombination steps under conditions that are permissive for progeny production. Thus, we measured the effects of different gene-*32* mutations on recombination elsewhere on the T4 genome. To differentiate between various steps in recombination (see Figure 5), we measured exchanges in short intervals as a test for insertion-type recombination and exchanges in large intervals as a test for crossover-type recombination (MOSIG 1974). We have also measured heterozygote frequencies.

We show here that under permissive conditions certain gene-*32* mutations reduce insertion-type recombination without affecting crossover-type recombina-

tion. Heterozygote frequencies are not reduced or enhanced in concert with recombination proficiencies. In addition, we show that certain gene-*32* mutations affect the plaque morphology of *rII* mutants, *i.e.*, some membrane properties, and that host ligase is involved in this membrane change. These results further define the functions of gene *32* in recombination and explain certain mapping anomalies within gene *32*. The relationship between deficiencies in recombination and in repair of UV damages will be discussed in a forthcoming paper.

MATERIALS AND METHODS

Bacteria: Escherichia coli strains B and S/6 (permissive for *rII* mutants, restrictive for *am* mutants), CR63 (permissive for *am* and *rII* mutants), K(λ) (restrictive for *rII* and *am* mutants), and streptomycin-resistant derivatives of these strains have been maintained in our laboratory. The isogenic ligase-deficient strains *ligts7 N2668* and *lig4 N1626* (GOTTESMAN, HICKS and GELLERT 1973) were obtained from M. GELLERT.

Phage: T4D wild type, the *am* mutants *N134* (gene *33*) and *E1* (gene *36*), the gene-*32* mutants (Figure 1), the *rII* mutants (Figure 2), the *rI* mutant *r48* and the *rIII* mutant *r67* from our stock collection were originally isolated and kindly supplied by R. S. EDGAR, A. H. DOERMANN, H. BERGER, S. MUFTI, J. LITTLE and H. REVEL. The gene-*32* mutant *tsP40* and the *rII* deletion *rdb52* each contained additional, unlinked *ts* mutations. We isolated the single gene-*32* and *rII* mutants from appropriate crosses with wild type T4 and named them *P401* and *rdb521*. The *rII* deletion *r1272* (BENZER 1961) in the genetic background of T4D was obtained from T. HOMYK. Multiple mutants were isolated from progenies of appropriate crosses. Their genotypes were confirmed by backcrosses to the single mutants. Five to ten double mutants were independently isolated from each cross and tested for phenotypic and genetic identity with other mutants containing the same mutations by plating at various temperatures between 25° and 42° and by stab crosses according to the method of DOERMANN and BOEHNER (1963).

We consider it unlikely that any of the single gene-*32* mutants analyzed here contain important secondary mutations in *other* genes. We have tested many independently isolated double mutants for genetic identity. By virtue of such tests we recognized and eliminated secondary mutations in the original mutants *P40* and *rdb52*. By this method we also recognized spontaneous secondary mutations in other genes, including the adjacent gene *59* (unpublished observations). In contrast, we have not been able to detect genetic heterogeneity in *L171*, *L170* or *L67* lysates which were grown at 25° or 30°, in spite of analyzing more than 200 independently isolated double mutants, containing *L171* in combination with other mutations in 8 different genes. In particular, the *L171* lysates do not contain additional ligase-defective mutations. They give normal recombination and complementation with ligase⁻ (gene *30*) mutants (MOSIG and BRESCHKIN 1975).

All lysates were grown from single young plaques (*ts* strains at 25°; *ts*+ strains at 30°); *am* strains were grown and assayed in CR63; *am*+ strains were grown in B and routinely assayed in S/6. (Occasional assays in B gave identical results.)

Crosses and burst-size measurements: General growth and assay procedures and media described in ADAMS (1959) were used. Bacteria were grown to a concentration of 2–4 × 10^7 per ml in H broth at 30°, spun down, resuspended in H broth at 1 × 10^9 per ml (adjusted after counting in the microscope) and assayed for colony production. They were infected with 4–5 particles of each genotype in an equal volume of H broth. The parental phage mixtures contained 5 × 10^9 particles of each genotype per ml (*i.e.*, exactly equal proportions). We used these precautions to improve the reproducibility of recombinant frequencies which depend on the multiplicities of infection (MOSIG 1962). We did not use cyanide, since in its absence recombinant frequencies and burst sizes were more reproducible and adsorption was faster. Three min after infection the bacteria were diluted to 2 × 10^3 per ml and assayed for infective centers. Unadsorbed particles were measured by the chloroform method (ADAMS 1959). Under our conditions of a high concentration of exponential-phase bacteria in the adsorption tube, more than 99%

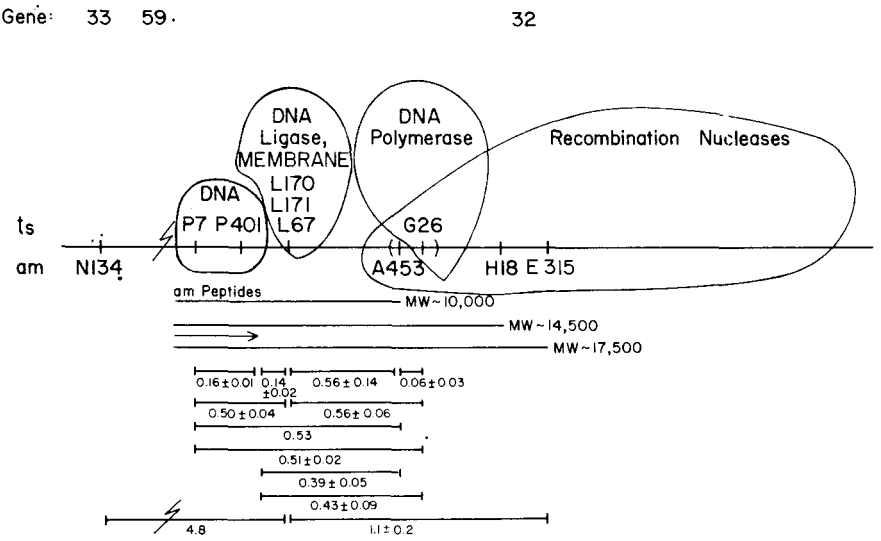

FIGURE 1.—Map of gene *32*. Relative positions of most gene-*32* mutations (except for *H18*, *E315* and *HL618*) were determined from three-factor crosses (Table 3). The data shown here are percentages of wild-type recombinants obtained in our two-factor crosses. Approximately 10% of the wild-type recombinants from these crosses (incubated under restrictive conditions at 42°) produced small plaques and were apparently heterozygotes: of 100 small plaques tested we found 95 to yield both *ts* and *ts*+ particles. This is probably an underestimate of heterozygote frequencies since, on the average, the small plaques contained > 80% wild-type particles. Most likely, the heterozygotes were able to produce wild-type recombinants because they were at semipermissive temperatures for a short time after plating. Such heterozygotes were not seen in the "reconstruction crosses" with single parents. Some of our recombinant frequencies, particularly from crosses with *P401*, are different from those reported by BERNSTEIN *et al.* (1972). We believe that most of the discrepancies are due to the fact that the original *P40* mutant (used by BERNSTEIN *et al.*), from which we derived *P401*, contained an additional *ts* mutation. We have confirmed the identity of their *A453* (erroneously labeled *P453* in their map) with our *A453*. Relative positions of the other *am* mutations were taken from SINHA and SNUSTAD (1971). Four *amE315* isolates, independently obtained from R. S. EDGAR, S. MUFTI, H. REVEL and D. P. SNUSTAD gave no wild type recombinants when they were crossed with each other or with *amHL618* (from D. P. SNUSTAD). In addition, their *am* peptides were indistinguishable in polyacrylamide gels. By these criteria the *E315* isolates were also identical with an *amH18* isolate obtained from J. LITTLE but they were different from SNUSTAD's *H18* isolate. The direction of translation (arrow) is deduced from the sizes of the *am* peptides. Our estimates of apparent molecular weights (WEBER and OSBORN 1969) are higher for the *am* peptides of *A453* and *H18* than the estimates of KRISCH, BOLLE and EPSTEIN (1974). Wild type gene-*32* protein has been reported to have a molecular weight of 35,000 (ALBERTS and FREY, 1970). The map also shows interactions that are affected by different mutations under restrictive conditions (MOSIG and BRESCHKIN 1975; MOSIG and BOCK 1976; BRESCHKIN and MOSIG 1977a,b).

of the phage particles adsorbed within two min after infection. Ninety min after infection, all bacteria were lysed by the addition of chloroform. Total phage progeny, parental types and wild-type recombinants were assayed by selective platings on appropriate hosts. The data were corrected, if necessary, for differences in efficiencies of plating and for revertants. Burst sizes of the single mutants and revertant frequencies were determined in "reconstruction crosses" after infection with 5 particles of one parental type per bacterium.

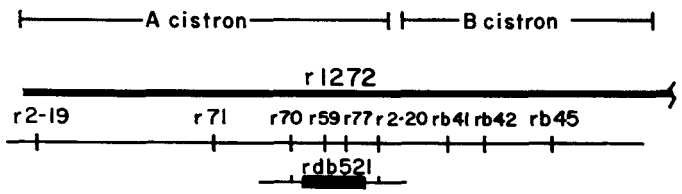

FIGURE 2.—Map of the *rII* mutations used in the present studies Relative positions were determined by BERGER (1965).

To test for heterozygosity of *ts* markers, small daughter plaques, grown at 42°, from crosses between *ts* mutants were resuspended in broth and replated under conditions that were permissive for both parents. Ten or more "granddaughter plaques" were then tested for growth under permissive and restrictive conditions by replica-stabbing with glass rods. Heterozygosity of *rII* markers was determined by testing aliquots from *r* or mottled plaques for the presence of *rII*+ particles, *i.e.*, for the ability to grow in lambda lysogens. We tested *r*/*r*+ heterozygotes by this method because the detection of heterozygotes as mottled plaques depends on plating conditions (G. STREISINGER, personal communication; and our own experience). We actually detected more heterozygotes (approximately 3% of the total progeny, Table 6) as compared with 2% reported by other investigators (for summary see MOSIG 1970). The difference may be due to detection of nonmottled heterozygotes by our method and/or to the omission of cyanide in our crosses.

Standard deviations and coefficients of variation were determined by standard procedures (STEEL and TORRIE 1960).

RESULTS

General properties of the gene-32 mutants: We assume that the gene-*32* mutants used in our studies (Figure 1) are nondeletions since they revert spontaneously; revertant frequencies ranged from 10^{-7} (*P7*) through 10^{-5} (*L171*) to 10^{-3} (in some lysates of *P401*). Unusually high revertant frequencies in some lysates are due to a combination of (1) mutator activities of gene-*32* mutations (BERNSTEIN *et al.* 1972; KOCH, MCGAW and DRAKE 1976; our unpublished observations), (2) "jack-pot" effects and (3) (at least in the case of *P401*) selective advantage of the revertants. For the experiments reported below, we chose lysates with the lowest available proportions of revertants.

The different gene-*32 ts* mutants differ considerably in their growth patterns. This is evident both from their efficiencies of plating (Table 1) and from their average burst sizes (Table 2) at different temperatures. The *ts* mutants *L171*, *L170* and *L67* had the highest and *P401* the lowest "maximum permissive temperature." DNA replication of *P401* was defective at all temperatures tested: in density-shift experiments we detected less than 30% replication of parental DNA at 25° and less than 10% at 42° (data not shown). This indicated that many of the *P401* particles could not establish infection at the "permissive" temperature of 25°. Apparently these particles could adsorb; the bacteria-killing titer of *P401* lysates was three times as high as the plaque-forming titer at 25°. Since we wanted to investigate recombination deficiencies under conditions permissive for DNA replication and progeny production, we do not emphasize the *P401* data here.

TABLE 1

*Efficiencies of plating on S/6 at different incubation temperatures as percent of 30° value**

	25°	30°	34°	37°	39°	42°
Wild type	98 ± 6	100 ± 6	101 ± 6	98 ± 6	110 ± 6	98 ± 6
r71	93 ± 7	100 ± 7	89 ± 8	98 ± 7	107 ± 7	102 ± 7
rb41	81 ± 9	100 ± 8	117 ± 8	93 ± 9	91 ± 9	98 ± 6
tsP7	96 ± 6	100 ± 6	54 ± 8	0	0	0
tsP401†	100 ± 3	47 ± 5	0	0	0	0
tsL171	95 ± 3	100 ± 3	104 ± 3	81 ± 4	76 ± 4	0
tsG26	105 ± 3	100 ± 3	93 ± 3	52 ± 4	0	0
amA453‡	89 ± 4	100 ± 4	93 ± 4	92 ± 4	98 ± 4	92 ± 4

* Data are the averages of 3–5 independent platings from fresh lysates, expressed as percent of the 30° platings. "0" means less than 0.1%. Standard deviations were converted into coefficients of variation.

† For *P401* the e.o.p. was determined relative to the 25° value.

‡ *amA453* was plated on CR63.

TABLE 2

Average burst sizes after multiple infection of E. coli *B at different temperatures**

Mutant	25°	30°	35°	37°	39°	41°	42°
rb41	244	278	317	-	251	-	90
tsP7	137	145	6	-	0	0	-
tsP401	23	6	0	-	0	0	-
tsL171	146	188	202	202	7	0.8	0.2
tsL171-r71	156	182	178	230	26	10	5
tsL171-r77	-	297	316	228	-	5	3
tsL171-rb41	150	203	234	239	30	8	4
amA453	0	0	0	-	-	-	0
tsG26	172	161	67	-	4	-	0
amE315	0	-	0	-	-	-	0

* Data are the average of 2–5 independent experiments, each with 2–3 measurements; "—" means not determined and "0" means less than unadsorbed particles.

The other mutants grew well at 25° or 30°, consistent with the observation that their DNA metabolism under permissive conditions is similar to that of wild-type T4 (BRESCHKIN and MOSIG 1977a).

The *ts* mutants *L67*, *L170* and *L171* had similar growth patterns. In crosses with each other, they gave no wild-type recombinants. We agree with BERNSTEIN *et al.* (1972) that they map at the same site and therefore report the combined data as *ts171* data. Further evidence that these mutations are similar or identical comes from the observation that all three (but no other gene-*32* mutations) are partially suppressed by *rII* mutations and in turn suppress the characteristic plaque morphology of *rII* mutants (MOSIG and BRESCHKIN 1975, and results illustrated in Figure 3).

Recombination analyses: Two-factor crosses between the single gene-*32* mutants gave the wild-type recombinant frequencies summarized in Figure 1. Three-factor crosses involving gene-*32* mutations and the gene-*33* mutation *amN134* gave the data summarized in Table 3. Standard evaluation of these recombinant frequencies establishes the marker order shown in Figure 1. The *tsG26* and *amA453* sites are so close that their relative positions cannot unambiguously be determined by genetic crosses because of high negative interference (CHASE and DOERMANN 1958); therefore, these sites are bracketed in Figure 1. Although recombination frequencies do not necessarily measure physical distances (MOSIG 1966, 1968), it is clear from these data that all *ts* mutations map within a promoter-proximal segment spanning approximately one-third of the gene-*32* length, since the *amA453* peptide comprises less than one-third of the gene-*32* protein.

The marker order in Figure 1 is deduced from the data in Table 3. The two-factor-cross data given in the lower part of Figure 1 would indicate that the positions of *P7* and *L171* are reversed, and that order has been shown in Figure 2 of MOSIG and BRESCHKIN 1973. To test whether the apparent discrepancies could be explained by differential recombination deficiencies of gene-*32* mutants, we measured recombination between closely linked *rIIA* sites in the background of different gene-*32* mutations under permissive conditions. The *rII* distances were chosen to be comparable to the relevant gene-*32* distances. It can be seen in Table 4 that the *ts* mutations *P7* and *G26* reduce recombination between *rII* markers to approximately one-half of the level seen in a gene-32^+ background (Table 4, lines 2 and 4) while *L171* crosses gave nearly wild-type proportions of r^+ recombinants (Table 4, line 3). In crosses with *P7* and *P401*, the high recombination potential of *L171* is recessive (Table 4, lines 6 and 7) and crosses of *L171* with *G26* or *A453* show intermediate recombination proficiencies (Table 4, lines 8 and 9). Crosses not involving *L171* show generally low recombination proficiencies (Table 4, lines 5 and 10–14). The reduction factors in recombination (R) caused by certain combinations of gene-*32* mutations are similar in two different *rII* intervals. We conclude that proficiencies for short-interval recombination are differentially affected by gene-*32* mutations.

In contrast, exchanges between *r59* and the loosely linked *amE1* mutation (in gene *36*) were not reduced by gene-*32* mutations (Table 5). Thus, short-interval recombination is selectively affected. In Figure 3 we have corrected the two-factor

TABLE 3

Percent of wild-type recombinants in three-factor crosses involving gene-32 mutations*

N134-P7 x P401	0.16 ± 0.02
P7 x P401†	0.16 ± 0.01
N134-P7 x L171	0.34 ± 0.08
N134-L171 x P7	0.12 ± 0.03
N134-L171 x P401	0.044 ± 0.009
L171 x P401†	0.14 ± 0.02
N134-P7 x A453	0.45
N134-A453 x P7	0.21
N134-A453 x G26	0.029 ± 0.004
N134-G26 x A453	0.012 ± 0.004
N134-L171 x G26	0.62 ± 0.02
N134-G26 x L171	0.42 ± 0.06
N134-G26 x P401	0.14 ± 0.04
G26 x P401†	0.43 ± 0.09
N134-P7 x G26	0.35 ± 0.06
N134-G26 x P7	0.14 ± 0.01

* Approximately 10% or more of the wild-type recombinants were actually heterozygotes, and double-mutant recombinants were not found with equal frequencies (see footnote to Figure 1). Therefore, we report only the percentages of wild-type recombinants (including heterozygotes). Data are the averages and standard deviations of 3–5 independent crosses, except for the *P7–A453* comparison, which is based on a single cross.

† All lysates of the double mutant *amN134–tsP401* contained $> 10\%$ $ts+$ revertants, and therefore could not be used to measure recombination. For comparison we give instead the two-factor-cross data.

cross data (shown in Figure 1) using the average reduction factor R (see Table 4) from the corresponding *rII* crosses (*e.g.*, the percentage of ts^+ recombinants from the cross *tsL171* × *tsP7* was divided by 0.5, etc.). It can be seen that the marker order deduced from two- and three-factor crosses becomes identical after this correction is made. Distances, however, do not become additive. This is not surprising, since (1) there is high negative interference and (2) the wild-type

TABLE 4

Percent of r+ recombinants in crosses at 30° in the absence or presence of gene-32 mutations

	Cross	Percent r^+ recombinants*	R†	Burst size range	Cross	Percent r^+ recombinants‡	R†	Burst size‡
1)	*r59 × r77*	0.35 ± 0.023		270–311	*r77 × r220*	0.49, 0.51		232, 278
2)	*P7–r59 × P7–r77*	0.15 ± 0.014	0.43	142–187				
3)	*L171–r59 × L171–r77*	0.30 ± 0.038	0.86	172–258				
4)	*G26–r59 × G26–r77*	0.17 ± 0.017	0.49	114–127				
5)	*P7–r59 × P401–r77*	0.17 ± 0.031	0.49	132–161				
6)	*L171–r59 × P7–r77*	0.16 ± 0.021	0.46	126–148	*L171–r77 × P7–r220*	0.26, 0.28	0.54	224, 267
7)	*L171–r59 × P401–r77*	0.19 ± 0.029	0.54	112–178	*L171–r77 × P401–r220*	0.22, 0.22	0.44	190, 249
8)	*L171–r59 × G26–r77*	0.23 ± 0.014	0.66	148–232	*L171–r77 × G26–r220*	0.29, 0.33	0.62	203, 251
9)	*L171–r59 × A453–r77*	0.21 ± 0.014	0.60	120–190	*L171–r77 × A453–r220*	0.29, 0.31	0.60	169, 199
10)	*P7–r59 × G26–r77*	0.19 ± 0.02	0.54	175–238	*P7–r77 × G26–r220*	0.20, 0.23	0.44	120, 164
11)	*P7–r59 × A453–r77*	0.18, 0.21	0.56	141–167	*P7–r77 × A453–r220*	0.20, 0.21	0.40	82, 86
12)	*G26–r59 × A453–r77*	0.16, 0.19	0.49	36–45	*G26–r77 × A453–r220*	0.21, 0.28	0.48	54, 57
13)					*P401–r77 × A453–r220*	0.22, 0.22	0.44	31, 46
14)					*P401–r77 × G26–r220*	0.21, 0.23	0.44	140, 167

* Average ± standard deviation of 3–5 independent crosses.
† R = (% r^+ recombinants in gene-*32* mutant background)/(% r^+ recombinants in gene-*32*+ background).
‡ Data from two independent crosses.

TABLE 5

Percent of r+ am+ *recombinants in 30° crosses between a gene* 36(E1) *and* rII *mutants in the presence or absence of gene-*32 ts *mutations*

Cross	Percent $\underline{r}^+$ recombinants*	Cross	Percent $\underline{r}^+$ recombinants*
r59 x E1	14.2 ± 0.3	r77 x E1	13.7 ± 0.6
P7-r59 x P7-E1	14.3 ± 1.1	P7-r77 x P7-E1	13.7 ± 0.8
L171-r59 x L171-E1	13.6 ± 0.4	L171-r77 x L171-E1	13.2 ± 0.7
G26-r59 x G26-E1	13.7 ± 0.03	G26-r77 x G26-E1	14.6 ± 0.5

* Average ± standard deviation of 2–4 independent crosses.

recombinants that we scored included more than 10% heterozygotes (see footnotes to Figure 1 and Table 3).

HERSHEY and CHASE (1951) first described partial heterozygotes as intermediates in recombination. They found that approximately 2% of the total progeny from $r \times r^+$ crosses formed mottled plaques because they contained both r and r^+ alleles. Subsequent efforts of many independent investigators established that in T4 approximately ¾ of such heterozygotes are heteroduplexes, while ¼ are terminal redundancy heterozygotes. Deletions are rarely found in mature heteroduplexes because they interfere with heteroduplex formation and/or cause preferential repair. Deletions may, however, increase the frequencies of terminal redundancy heterozygotes elsewhere on the T4 genome (for reviews see MOSIG 1970 and BROKER and DOERMANN 1975). There is no reason to assume that the gene-*32 ts* or *am* mutations are deletions. Thus we determined their effects on heteroduplex frequencies by measuring proportions of r/r^+ heterozygotes. Table 6 shows that proportions of such heterozygotes in *G26* or *P7* crosses were definitely *not* lower than in gene-32^+ crosses; in *P7* crosses they were probably

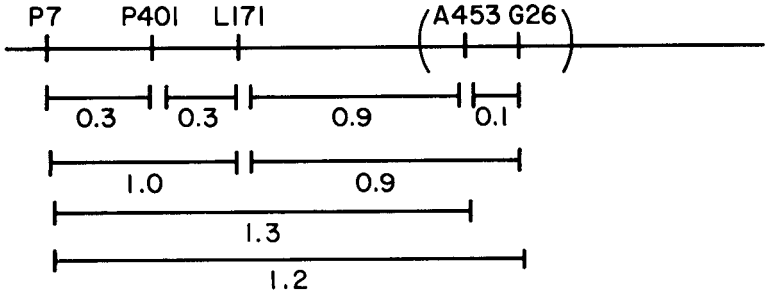

FIGURE 3.—Map of the gene-*32* mutations constructed from two-factor-cross data after percentages of recombinants are corrected for the recombination deficiencies that are shown by the specific combinations of gene-*32* mutations in crosses between *rII*-mutations (Table 4).

TABLE 6

Frequencies of heterozygotes in rII × r+ *crosses in B at 30°*

Parents	Percent r+† in: Parents	Progeny	Percent heterozygotes† in: Total progeny	Sum of r and mottled progeny	Hetero-zygotes/total plaques tested*	Burst size
r77 × wild type	50.9 ± 2.9	56.8 ± 3.8	2.9	6.6 ± 0.8	66/1000	227
P7–r77 × *P7*	47.2 ± 3.0	44.7 ± 3.7	5.2	9.4 ± 1.0	79/839	83
L171–r77 × *L171*	46.2 ± 2.1	43.3 ± 2.9	6.5	11.5 ± 1.0	122/1058	180
G26–r77 × *G26*	50.2 ± 2.6	49.9 ± 2.6	3.6	7.1 ± 1.0	55/775	93

* Based on sum of *r* and mottled plaques tested.
† Standard deviations were converted into coefficients of variation.

higher and in *L171* crosses they were significantly higher. Thus, the recombination deficiencies of *G26* and *P7* are not related in a simple way to heteroduplex formation and cannot be readily explained as deficiencies in potential heteroduplex repair.

Mutual suppression of the gene-32 mutation L171 *and* rII *mutations*: The mutation *L171* and its homoalleles, but none of the other gene-*32* mutations, are partially suppressed by additional *rII* mutations. We have measured DNA metabolism of the suppressed and unsuppressed mutant under conditions which are restrictive for progeny production in ligase-proficient and ligase-deficient hosts. From a comparison of these results we have concluded that the *L171* mutation under restrictive conditions interferes with the action of T4 ligase during recombination, but not during DNA replication, and that *rII* mutations mainly facilitate the use of host ligase. At 42°, the single *L171* mutant showed extensive DNA replication, but gave burst sizes of < 0.3 (MOSIG and BRESCHKIN 1975).

L171 stocks generate spontaneous *rII* mutations with high frequencies, particularly when the stocks are grown near the maximum permissive temperature. When grown at 40°, up to 10% of the progeny contained additional *rII* mutations, apparently because of selective advantage. All *L171-rII* double mutants, *i.e.*, those containing one of the *rII* mutations shown in Figure 2 together with *tsL171*, as well as newly isolated *rII-L171* mutants, gave burst sizes between 3 and 5 after multiple infection at 42°. Some representative data are included in Table 2. Slight differences between different *rII* point mutations are not statistically significant. The *rII* deletion *r1272* consistently caused slightly higher burst sizes (8–10) than the *rII* point mutations or short deletions, probably because *r1272* also eliminates the *D1* region and part of *denB* (endonuclease IV) (VETTER and SADOWSKI 1974). Neither the *rI* mutation *r48* nor the *rIII* mutation *r67* suppresses *L171*.

From our early experiments (MOSIG and BRESCHKIN 1973) we had erroneously concluded that not all *rII* mutations suppress the *L171* mutation. This error was caused by an unexpected effect of *L171* on DNA repair which we shall describe in detail in a forthcoming paper: at 30° (as well as at higher temperatures) *L171* is more radiation-sensitive than wild-type or *rII* mutants. This radiation sensitivity of *L171* is *not* suppressed by *rII* mutations. Some of the *rII-L171*

lysates used in our first experiments had experienced radiation damage from fluorescent light and for this reason *appeared* not to be suppressed. After we detected this effect, we repeated all experiments with freshly prepared lysates which were kept in the dark and we report here only experiments in which we used undamaged lysates.

Suppression by rII *mutations depends on functioning host ligase*: In the *ts lig7* host (GOTTESMAN, HICKS and GELLERT 1973) there is no suppression of *L171*, and unsuppressed *L171* is more defective than in *E. coli* B at temperatures above 37° (MOSIG and BRESCHKIN 1975). However, in the isogenic *lig4* host, which has reduced but measurable ligase activity, *L171* grows as well and *rII* suppression is as good as in *E. coli* B. This is noteworthy because the *lig4* host does not support the growth of *rII*-suppressed T4 ligase (gene *30*) mutants (GOTTESMANN, HICKS and GELLERT 1973). We interpret this to mean that the low residual ligase level in *lig4* suffices to substitute for ligase activity in recombination (which is impaired in the gene-*32* mutant *L171*) but not when T4 ligase is inactive in recombination *and* replication because of gene-*30* (*lig*$^-$) mutations.

At 37° the *L171* mutation in turn suppresses the characteristic plaque morphology of *rII* mutants in *E. coli* B (without restoring growth in λ lysogens) (Figure 4), although at this temperature *rII*-suppressed and unsuppressed *L171* give normal burst sizes (Table 2).

It appears that the host ligase also participates in lysis inhibition. Single *rII* mutants exhibit wild-type plaque morphology (*i.e.*, lysis inhibition) when they are plated on nonlysogenic K bacteria (BENZER 1957). Surprisingly, we found that all single *rII* mutants formed *r* plaques when they were plated on the non-

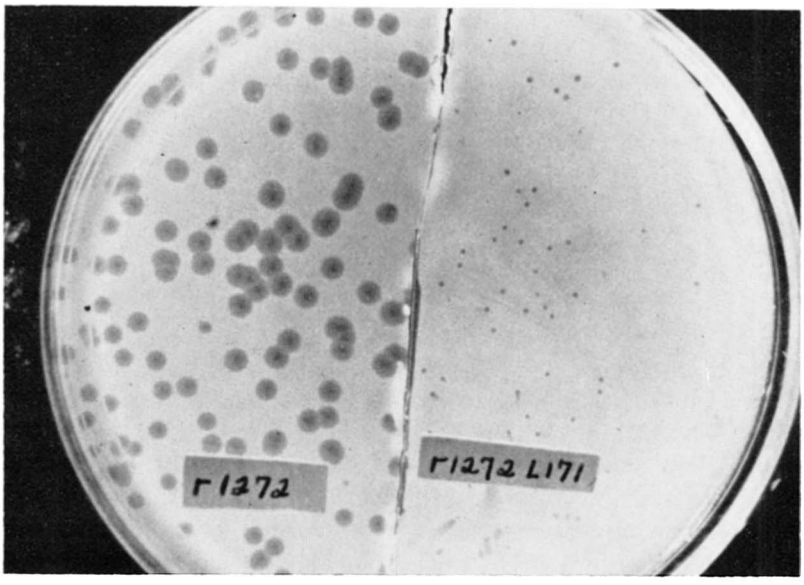

FIGURE 4.—Plaque morphologies of *r1272* and *L171-r1272* on S/6 at 37°. All other single *rII* mutants and *L171-rII* double mutants showed similar differences.

lysogenic K strain *lig ts7* above 37° (and only above 37°, when the host ligase becomes partially inactive but the bacteria are still able to grow). Moreover, the gene-*32* mutation *L171* suppresses this phenotype of *rII* mutants in *lig ts7* (as well as in *E. coli* B). On *lig ts7*, r^+ plaques are larger than on S/6 or B bacteria, but they are smaller than *rII* plaques and do not have their characteristic sharp edges. In contrast, *r* as well as r^+ phages produce wild-type plaques on the isogenic *lig 4* host, which has higher residual ligase activity than *lig ts7* (GOTTESMAN, HICKS and GELLERT 1973).

DISCUSSION

Our experiments show that all known gene-*32 ts* mutations map within a promoter-proximal segment spanning approximately one-third of gene *32* (Figure 1). Probably, mutations in the promoter-distal segment are less likely to be temperature-sensitive, because *in vivo* the *am* peptides, including the short *A453* peptide, suffice for DNA replication at high temperatures (BRESCHKIN and MOSIG 1977a).

Under conditions that are *permissive* for progeny production and DNA replication (30°), gene-*32 ts* mutants show different defects in various steps of recombination and in other properties. The following four points should be noted:

(1) *P7* and *G26* (but not *L171*) reduce recombination between closely linked *rII* markers twofold. The recombination deficiency of *P7* is dominant and the deficiency of *G26* is codominant to the "recombination proficiency" of *L171* (Table 4). These effects distort map distances and preclude ordering of gene-*32* mutations by two-factor crosses.

(2) The recombination-deficient gene-*32* mutations affect exchanges only in short, not in long intervals (compare Tables 4 and 5).

(3) None of the known gene-*32* mutations reduce heterozygote frequencies. Surprisingly, *P7* slightly enhances them, and *L171* also enhances them even though its recombination proficiency is nearly normal (Table 6).

(4) *L171* alone among gene-*32* mutations tested suppresses the characteristic plaque morphology of *rII* mutants.

How do these mutations exert their specific effects? This question has some bearing on current models of genetic recombination (for reviews see MOSIG 1970; CLARK 1973; RADDING 1973; HOTCHKISS 1974; MILLER 1975; and BROKER and DOERMANN 1975). Such models assume that "joint" or branched recombinational intermediates are formed first (Figure 5, step A). These intermediates are resolved to give double-exchange (insertion-type) or single-exchange (crossover-type) recombinants (WHITEHOUSE 1963; HOLLIDAY 1964, see Figure 5, mode I and II respectively). Heteroduplex regions may be partially repaired (step B) before or after ligation (step C), before replication resolves heteroduplexes into true recombinants (step D). DNA polymerases, ligases and nucleases are thought to participate in various steps of such recombination pathways and most models assume that gene-*32* mutations affect recombination because they affect potential unwinding of the DNA by the gene-*32* protein.

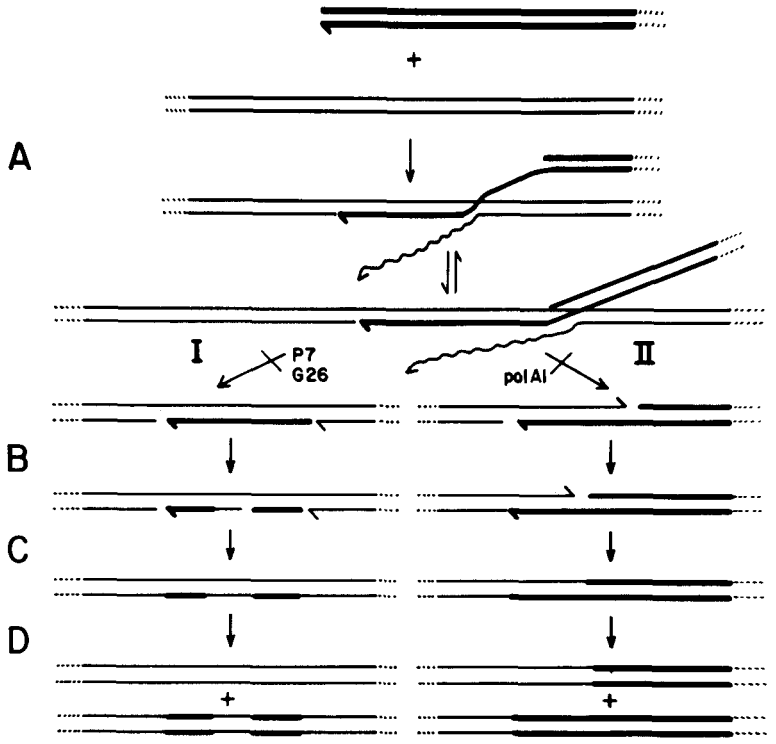

FIGURE 5.—A model for T4 recombination. The formation of "joint" recombinational intermediates (step A) is based on recombination patterns near chromosomal ends (MOSIG *et al.* 1971) and on electron micrographs of replication-proficient recombining T4 DNA (R. DANNENBERG and G. MOSIG, manuscript in preparation). Branched molecules show extensive branch migration as indicated in A. The temporal sequence of the other steps (described in the text) is not well defined. In fact, we assume that most steps do not occur independently but that they are coordinated by binding of the respective enzymes to gene-*32* protein. Half-arrows indicate 5′ termini. DNA from different parents is distinguished by thick or thin lines.

Our previous studies with gene-*32* mutants have shown, however, that under restrictive conditions most mutations affect *in vivo* interactions of gene-*32* protein with recombination enzymes (MOSIG and BRESCHKIN 1975; MOSIG and BOCK 1976; BRESCHKIN and MOSIG 1977a,b). Our present results support and extend this model. They indicate that under permissive conditions *P7* and *G26* are specifically defective in insertion-type recombination (see Figure 5, mode I). The normal frequencies of crossover-type recombination in these mutants (Table 5) and the observation that heterozygote frequencies are not reduced as compared with gene-*32*$^+$ crosses (Table 6) indicate that formation of branched recombinational intermediates (Figure 5A) is normal.

This indicates that under permissive conditions, the mutants are defective in other recombination steps. There are several possible explanations for these specific defects in insertion-type recombination. Branched intermediates (Figure 5A) would yield lower proportions of insertion-type recombinants (Figure 5,

mode I) with decreasing frequencies of nicks in the same strand. One plausible explanation for the recombination deficiencies of *P7* and *G26* is as follows: their peptides overstimulate ligase activity and/or understimulate endonuclease activity in the recombination complex so that effective frequencies of secondary nicks in these same DNA strands are reduced. This hypothesis would readily explain why *L171* is not recombination deficient. Under restrictive conditions, the *L171* peptide is specifically defective in interacting with T4 ligase during recombination (MOSIG and BRESCHKIN 1975). Thus, it might not be expected to overstimulate ligase under permissive conditions. It is also possible that there is an additional mechanism for recombinational insertion of single-stranded DNA (*e.g.*, the mechanism suggested by HOLLOMAN *et al.* 1975). *P7* and *G26* might be defective in this mechanism.

We have considered the possibility that a deficiency in crossover-type exchanges would not be detectable as a deficiency of long-interval recombination, but would be lethal and only reduce phage yield (because only crossover-type exchanges would give concatemers and presumably only concatemeric DNA could be packaged). In its simplest form this interpretation cannot be correct, because we have detected (MOSIG 1974) a specific deficiency in long-interval recombination (in the same *rII*-gene *36* interval used here) caused by the *E. coli polA1* mutation (DE LUCIA and CAIRNS 1969). More involved hypotheses are not excluded.

The observation that heterozygote frequencies in *L171* and *P7* crosses are higher than in gene-*32*$^+$ *crosses* (Table 6) suggests that heteroduplex regions are longer in these mutants. This could be explained if these mutants were deficient in heteroduplex repair or if longer heteroduplex regions were formed (*e.g.*, by more extensive branch migration). We shall discuss these possibilities together with deficiencies in repair of UV damages in a forthcoming paper. Note, however, that longer heteroduplex regions cannot be the only reason for the recombination deficiencies of *P7* and *G26*, because heterozygote frequencies are not related in a simple manner to recombination deficiencies, and because we have detected recombination deficiencies although we have scored heterozygotes as wild-type recombinants (see footnotes to Figure 1 and Table 3).

Additional support that gene-*32* protein is multifunctional and that different mutations affect different interactions comes from the observation that *L171* alone suppresses the *r*-plaque morphology of *rII* mutants. This plaque morphology on B or S/6 bacteria results from a membrane change in *rII*-infected cells that prevents lysis inhibition (DOERMANN 1948; BENZER 1957). The *rII* proteins are membrane proteins (WEINTRAUB and FRANKEL 1972; PETERSON, KIEVITT and ENNIS 1972; ENNIS and KIEVITT 1973). Suppression of *rII* plaque morphology by the gene-*32* mutant *L171* suggests that gene-*32* protein is involved in the membrane change. It should be noted that under restrictive conditions, *rII* mutations partially suppress *tsL171*. Under restrictive conditions, the single *tsL171* mutant can form "joint" but not covalently linked concatemers. Suppressing *rII* mutations facilitate the use of host ligase to convert "joint" into covalently linked concatemers (MOSIG and BRESCHKIN 1975). The mutual sup-

pression of *rII* and *L171* mutations suggests that ligation of recombining DNA occurs at the membrane.

The various phenotypic expressions of different gene-*32* mutations cannot simply be explained by differential binding of the mutant proteins to DNA, but are readily explained by differential effects on interactions with other proteins. We suggest that gene-*32* protein facilitates the coordinated action of these proteins on DNA. Thus, the analysis of several mutations in gene *32* under permissive as well as restrictive conditions provides information essential to understanding the specific functions of gene-*32* protein in recombination as well as in DNA replication.

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LITERATURE CITED

ADAMS, H., 1959 *Bacteriophages.* Interscience Publishers, Inc., New York.

ALBERTS, B., 1973 Studies on the replication of DNA. pp. 233–251. In: *Molecular Cytogenetics.* Edited by B. A. HAMKALO and J. PAPACONSTANTINOU. Plenum Press, New York.

ALBERTS, B. and L. FREY, 1970 T4 Bacteriophage gene 32: A structural protein in the replication and recombination of DNA. Nature **227:** 1313–1318.

ALLEN, E. F., I. ALBRECHT and J. W. DRAKE, 1970 Properties of bacteriophage T4 mutants defective in DNA polymerase. Genetics **65:** 187–200.

BECKENDORF, S. K. and J. H. WILSON, 1972 A recombination gradient in bacteriophage T4 gene 34. Virology **50:** 315–321.

BENZER, S., 1957 The elementary units of heredity. pp. 70–93. In: *The Chemical Basis of Heredity.* Edited by W. D. MCELROY and B. GLASS. Johns Hopkins Press, Baltimore. ——, 1961 On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U. S. **47:** 403–415.

BERGER, H., 1965 Genetic analysis of *T4D* phage heterozygotes produced in the presence of 5-fluorodeoxyuridine. Genetics **52:** 729–746.

BERGER, H., A. J. WARREN and K. E. FRY, 1969 Variations in genetic recombination due to amber mutations in T4D bacteriophage. J. Virol. **3:** 171–175.

BERNSTEIN, H., 1968 Repair and recombination in phage T4. I. Genes affecting recombination. Cold Spring Harbor Symp. Quant. Biol. **33:** 325–331.

BERNSTEIN, C., H. BERNSTEIN, S. MUFTI and B. STROM, 1972 Stimulation of mutation in phage T4 by lesions in gene *32* and by thymidine imbalance. Mutat. Res. **16:** 113–119.

BRESCHKIN, A. M. and G. MOSIG, 1977a Multiple interactions of a DNA-binding protein *in vivo.* I. Gene 32 Mutations of phage T4 inactivate different steps in DNA replication and recombination. J. Mol. Biol. (In press.) ——, 1977b Multiple interactions of a DNA-binding protein *in vivo.* II. Effects of host mutations on DNA replication of phage T4 gene *32* mutants. J. Mol. Biol. (In press.)

BROKER, T. R., 1973 An electron microscopic analysis of pathways for bacteriophage T4 DNA recombination. J. Mol. Biol. **81:** 1–16.

BROKER, T. and A. H. DOERMANN, 1975 Molecular and genetic recombination of bacteriophage T4. Ann. Rev. Genet. **9**: 213–244.

CHASE, M. and A. H. DOERMANN, 1958 High negative interference over short segments of the genetic structure of bacteriophage *T4*. Genetics **43**: 332–353.

CLARK, A. J., 1973 Recombination deficient mutants of *E. coli* and other bacteria. Ann. Rev. Genet. **7**: 67–86.

CURTIS, M. J. and B. ALBERTS, 1976 Studies on the structure of intracellular bacteriophage T4 DNA. J. Mol. Biol. **102**: 793–816.

DE LUCIA, P. and J. CAIRNS, 1969 Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature **224**: 1164–1166.

DOERMANN, A. H., 1948 Lysis and lysis inhibition with *Escherichia coli* bacteriophage. J. Bacteriol. **55**: 257–276.

DOERMANN, A. H. and L. BOEHNER, 1963 An experimental analysis of bacteriophage T4 heterozygotes. I. Mottled plaques from crosses involving six rII loci. Virology **21**: 551–567.

DUCK, P. and A. CHOVNICK, 1975 Resolution of an equivocal genetic element in *Drosophila melanogaster*: Organization of the maroon-like locus. Genetics **79**: 459–466.

ENNIS, H. L. and K. D. KIEVITT, 1973 Association of the *rIIA* protein with the bacterial membrane. Proc. Natl. Acad. Sci. U. S. **70**: 1468–1472.

EPHRUSSI-TAYLOR, H., 1966 Genetic recombination in pneumococcus. IV. The pattern of transmission and phenotypic expression of high and low-efficiency donor sites in the *ami* A locus. Genetics **54**: 211–222.

EPSTEIN, R. H., A. BOLLE, C. M. STEINBERG, E. KELLENBERGER, E. BOY DE LA TOUR, R. CHEVALLEY, R. S. EDGAR, M. SUSMAN, G. H. DENHARDT and A. LIELAULIS, 1963 Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28**: 375–394.

GOTTESMAN, M. M., M. L. HICKS and M. GELLERT, 1973 Genetics and physiology of DNA ligase mutants of *Escherichia coli*. pp. 107–121. In: *The Second Annual Harry Steenbock Symposium*. Edited by R. D. WELLS and R. B. INMAN. University Park Press, Baltimore, Md.

GUTZ, H., 1971 Site specific induction of gene conversion in *Schizosaccharomyces pombe*. Genetics **69**: 317–337.

HAMLETT, N. and H. BERGER, 1975 Mutations altering genetic recombination and repair of DNA in Bacteriophage T4. Virology **63**: 539–567.

HASTINGS, P. J., 1975 Some aspects of recombination in eukaryotic organisms. Ann. Rev. Genet. **9**: 129–144.

HENDERSON, D. and J. WEIL, 1975 Recombination-deficient deletions in bacteriophage λ and their interaction with chi mutations. Genetics **79**: 143–174.

HERSHEY, A. D. and M. CHASE, 1951 Genetic recombination and heterozygosis in bacteriophage. Cold Spring Harbor Symp. Quant. Biol. **21**: 91–101.

HOLLIDAY, R., 1964 A mechanism for gene conversion in fungi. Genet. Res. **5**: 282–304.

HOLLOMAN, W. K., R. WIEGAND, C. HOESSLI and C. M. RADDING, 1975 Uptake of homologous single-stranded fragments by superhelical DNA: A possible mechanism for initiation of genetic recombination. Proc. Natl. Acad. Sci. U.S.A. **72**: 2394–2398.

HOTCHKISS, R. D., 1974 Models of genetic recombination. Ann. Rev. Microbiol. **28**: 445–468.

KOCH, R. E., M. K. MCGAW and J. W. DRAKE, 1976 Mutator mutations in bacteriophage T4 gene *32* (DNA unwinding protein). J. Virol. **19**: 490–494.

KOZINSKI, A. and Z. FELGENHAUER, 1967 Molecular recombination in T4 bacteriophage deoxyribonucleic acid. II. Single-strand breaks and exposure of uncomplemented areas as a prerequisite for recombination. J. Virol. **1**: 1193–1202.

KRISCH, H. M., A. BOLLE and R. H. EPSTEIN, 1974 Regulation of the synthesis of bacteriophage T4 gene *32* protein. J. Mol. Biol. **88**: 89–104.

LAM, S. T., M. M. STAHL, K. D. MCMILIN, and F. W. STAHL, 1974 Rec-mediated recombinational hot spot activity in bacteriophage lambda. II. A mutation which causes hot spot activity. Genetics **77**: 425–433.

MAYNARD-SMITH, S. and N. SYMONDS, 1973 Involvement of bacteriophage T4 genes in radiation repair. J. Mol. Biol. **74**: 33–44.

MESELSON, M. S. and C. M. RADDING, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci. U.S. **72**: 358–361.

MILLER, R. C., JR., 1975 Replication and molecular recombination of T-phage. Ann. Rev. Microbiol. **29**: 355–376.

MOORE, C. W. and F. SHERMAN, 1975 Role of DNA sequences in genetic recombination in the iso-1-cytochrome *c* gene of yeast. I. Discrepancies between physical distances and genetic distances determined by five mapping procedures. Genetics **79**: 397–419.

MOSIG, G., 1962 The effect of multiplicity of infection on recombination values in bacteriophage T4D. Zeitschrift für Vererbungslehre **93**: 280–286. ——, 1966 Distances separating genetic markers in T4 DNA. Proc. Natl. Acad. Sci. U.S. **56**: 1177–1183. ——, 1968 A map of distances along the DNA molecule of phage T4. Genetics **59**: 137–151. ——, 1970 Recombination in bacteriophage T4. pp. 1–53. In: *Advances in Genetics*, Vol. 15. Academic Press, Inc., New York. ——, 1974 On the role of *Escherichia coli* DNA polymerase I and of T4 gene *32* protein in recombination of phage T4. pp. 29–39. In: *Mechanisms in Recombination*. Edited by R. F. GRELL. Plenum Publishing Corp., New York.

MOSIG, G. and S. BOCK, 1976 Gene *32* protein of phage T4 moderates the activities of the T4 gene 46/47 controlled nuclease and of the *E. coli* Rec BC nuclease *in vivo*. J. Virol. **17**: 756–761.

MOSIG, G. and A. BRESCHKIN, 1973 Interaction in DNA replication of the gene 32 and *rII* products of phage T4 with the *dna C* product of the host. Chapter 23, pp. 285–291. In: *Molecular Cytogenetics*. Edited by B. A. HAMKALO and J. PAPACONSTANTINOU. Plenum Press, New York. ——, 1975 Genetic evidence for an additional function of phage T4 gene *32* protein: interaction with ligase. Proc. Natl. Acad. Sci. U.S. **72**: 1226–1230.

MOSIG, G., R. EHRING, W. SCHLIEWEN and S. BOCK, 1971 The patterns of recombination and segregation in terminal regions of T4 DNA molecules. Molec. Gen. Genet. **113**: 51–91.

MUFTI, S. and H. BERNSTEIN, 1974 The DNA-delay mutants of bacteriophage T4. J. Virol. **14**: 860–871.

NORKIN, L. C., 1970 Marker-specific effects in genetic recombination. J. Mol. Biol. **51**: 633–655.

PETERSON, R. F., K. D. KIEVITT and H. E. ENNIS, 1972 Membrane protein synthesis after infection of *Escherichia coli* B with phage T4: The *rII* B protein. Virology **50**: 520–527.

RADDING, C. M., 1973 Molecular mechanisms in genetic recombination. Ann. Rev. Genet. **7**: 87–111.

RONEN, A. and Y. SALTS, 1971 Genetic distances separating adjacent base pairs in bacteriophage T4. Virology **45**: 496–502.

SINHA, N. K. and D. P. SNUSTAD, 1971 DNA synthesis in bacteriophage T4-infected *Escherichia coli*: Evidence supporting a stoichiometric role for gene *32*-product. J. Mol. Biol. **62**: 267–271.

STADLER, D. R., 1973 The mechanism of intragenic recombination. Ann. Rev. Genet. **7:** 113–127.

STADLER, D. and B. KARIYA, 1973 Marker effects in the genetic transduction of tryptophan mutants of *E. coli*. Genetics **75:** 423–439.

STEEL, R. G. D. and J. H. TORRIE, 1960 *Principles and Procedures of Statistics*. McGraw-Hill Book Co., Inc., New York.

TESSMAN, I., 1965 Genetic ultrafine structure in the T4 *rII* region. Genetics **51:** 63–75.

TIRABY, G., M. S. FOX and H. BERNHEIMER, 1975 Marker discrimination in deoxyribonucleic acid-mediated transformation of various *Pneumococcus* strains. J. Bacteriol. **121:** 608–618.

TOMIZAWA, J.-I., 1967 Molecular mechanisms of genetic recombination in bacteriophage: Joint molecules and their conversion to recombinant molecules. J. Cell. Physiol. **70:** suppl. 1, 201–214.

VETTER, D. and P. D. SADOWSKI, 1974 Point mutants in the *D2a* region of bacteriophage T4 fail to induce T4 endonuclease IV. J. Virol. **14:** 207–213.

WARNER, H. R. and M. D. HOBBS, 1967 Incorporation of uracil-^{14} C into nucleic acids in *Escherichia coli* infected with bacteriophage T4 and T4 amber mutants. Virology **33:** 376–384.

WEBER, K. and M. OSBORN, 1969 The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244:** 4406–4412.

WEINTRAUB, S. B. and F. R. FRANKEL, 1972 Identification of the T4*rIIB* gene product as a membrane protein. J. Mol. Biol. **70:** 589–615.

WHITE, R. L. and M. S. FOX, 1975 Genetic consequences of transfection with heteroduplex bacteriophage λ DNA. Mol. Gen. Genet. **141:** 163–171.

WHITEHOUSE, H. L. K., 1963 A theory of crossing-over by means of hybrid deoxyribonucleic acid. Nature **199:** 1034–1040.

WILDENBERG, J. and M. MESELSON, 1975 Mismatch repair in heteroduplex DNA. Proc. Natl. Acad. Sci. U. S. **72:** 2202–2206.

WU, J.-R and Y.-C. YEH, 1973 Requirement of a functional gene *32* product of bacteriophage T4 and UV repair. J. Virol. **12:** 758–765.

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