THE EFFECT ON RECOMBINATION OF MUTATIONAL DEFECTS IN THE DNA-POLYMERASE AND DEOXYCYTIDYLATE HYDROXYMETHYLASE OF PHAGE T4D

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EXPERIMENTS will be described which show that in the presence of mutations in genes 43 and 42 of phage T4D (EPSTEIN *et al.* 1963) recombination is increased several-fold. Gene 43 has been identified as the structural gene of DNA- polymerase (DEWAARD and LEHMAN 1965; WARNER and BARNES 1966). Gene 42 has been shown to specify dCMP[1]-hydroxymethylase (DIRKSEN, HUTSON and BUCHANAN 1963; WIBERG and BUCHANAN 1964).

MATERIALS AND METHODS

Phage and bacterial strains: The phage strains used in this study were: *rIIA* cistron mutants *r*ED35, *r*ED19, *r*ED144, *r*71, *r*64, *r*ED37, *r*ED44, *r*ED62, *r*ED41 and *r*ED220; gene 43 mutants *ts*L91 and *am*N101; gene 42 mutant *ts*L13. The *r*IIA mutants were originally described by EDGAR, FEYNMAN, KLEIN, LIELAUSIS and STEINBERG (1962). The origins of the other mutants were described by EPSTEIN *et al.* (1963). The bacterial strains employed were *E. coli* B/5, S/6/5, K112(λ) and CR63. All of the phage and bacterial strains used were kindly supplied by DR. R. S. EDGAR.

Standard cross procedure and preparation of stocks: In all crosses, except those involving the phage amber mutant *am*N101, *E. coli* B/5 was used as the permissive host and *E. coli* S/6/5 as the nonselective indicator strain. *E. coli* K112(λ) which supports the growth of wild-type T4, but not *rII* mutants, was used as the selective indicator strain in all $r \times r$ crosses. *E. coli* CR63 was used as the host and nonselective indicator in crosses involving *am*N101.

The media employed and the procedures used in performing crosses were the same as those described in STEINBERG and EDGAR (1962). All crosses were carried out in water baths having a temperature variation of less than 0.1 degree. Crosses were usually performed in sets of ten, each set at a different temperature. Since the latent period preceding normal lysis varies with temperature, as much as 240 minutes was allowed at low temperatures before chloroform was added and progeny assayed.

All phage stocks were clonally derived from single plaques and grown at 30°C. The procedure for growing stocks was the same as that in STEINBERG and EDGAR (1962), except that the centrifugation step was omitted .

Cultures of the indicator strains were prepared as in STEINBERG and EDGAR (1962). The efficiency of plating of wild-type r^+ phage was measured in each set of crosses and recombination percentages in each set corrected accordingly.

Acceptability of determinations: A total of 158 individual crosses were performed in obtaining the recombination percentages and burst sizes analysed in this study. Certain criteria of acceptability were applied to the data from a cross before its recombination percentage was considered

[1] Abbreviations used: dCMP = deoxycytidylate; dHMP = 5-hydroxymethyldeoxycytidylate.

reliable. First, the proportion of each parent among the input phage must have been greater than 33%, and, secondly, adsorption must have been greater than 90%.

Bacterial titer during phage adsorption was determined by direct colony count prior to addition of phage, and by infective center count after dilution from KCN. Burst size was calculated as total progeny phage over the bacterial titer determined by colony count. However, in any cross where the bacterial titer determined by colony count differed by at least a factor of two from the titer determined by infective center count, the burst size calculation was considered unacceptable.

By applying the above criteria a total of four recombination percentages and 21 burst sizes were classified as unacceptable. In addition, at high temperatures certain crosses gave such low burst sizes that, either recombination could not be measured (eight crosses), or both recombination and burst size could not be measured (three crosses).

RESULTS

Effect on recombination of a temperature sensitive mutational defect in the DNA-polymerase of phage T4: WARNER and BARNES (1966) and DE WAARD *et al.* (1965) showed that extracts prepared from *E. coli* infected with the temperature sensitive mutant *ts*L91 (gene 43) had lower DNA-polymerase activity at 35–37°C than wild-type phage infected cells. The latter investigators also showed that the DNA-polymerase extracted from *ts*L91 infected cells was more heat labile at 37° than the wild-type enzyme.

Each of the ten *rIIA* mutants shown in Figure 1 were crossed to *ts*L91, and the progeny plated on *E. coli* S/6/5 indicator bacteria. Morphologically *r* plaques were picked and stocks prepared. These were tested until a double mutant stock was found corresponding to each of the ten *rIIA* mutants.

The double mutants were crossed to each other in five pairwise combinations. These crosses are of the general type r_a,*ts*L91 × r_b,*ts*L91. The five pairs of *r* mutants included in these crosses are indicated in Figure 1. Corresponding crosses of the type $r_a \times r_b$ with wild-type DNA polymerase were also carried out. The recombination percentages at the standard temperature, 30°C, are shown in the

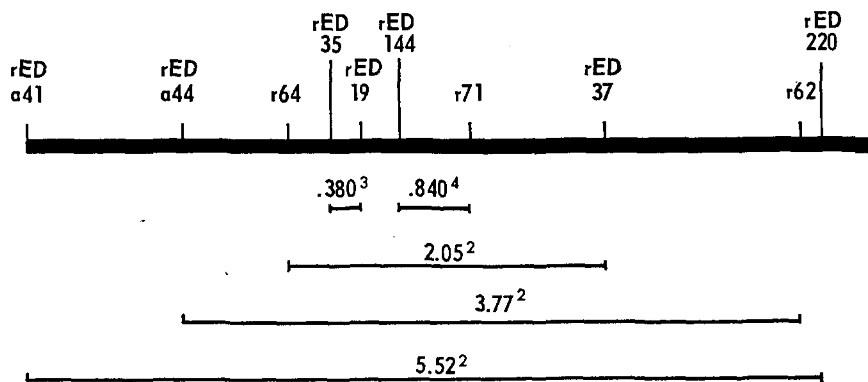


FIGURE 1.—Map of the *rIIA* cistron adapted from FISHER and BERNSTEIN (1965). The designations of the *rII* mutants used in this study are given above the solid bar and the standard interval lengths, determined at 30°C, are indicated below the bar. The superscripts signify the number of determinations entering into the indicated average values.

Figure. Also the locations of the intervals measured by these crosses within the *rIIA* cistron are shown in Figure 1.

All crosses were carried out at many different temperatures between 19.5° and 44.2°C. Recombination percentages are plotted in Figures 2 to 6. Each point represents the result of a single cross performed at the indicated temperature. In these figures the curve labeled "*ts*L91" gives the recombination percentages from r_a,*ts*L91 × r_b,*ts*L91 crosses in which the DNA-polymerase is mutant, and the curve labeled "wild type" gives the recombination percentages from the $r_a \times r_b$ crosses in which the polymerase is wild-type. The recombination percentages from a total of 45 r_a,*ts*L91 × r_b,*ts*L91 and 56 $r_a \times r_b$ crosses are recorded in Figures 2 to 6.

There are 41 instances in which an r_a,*ts*L91 × r_b,L91 cross is paired with an $r_a \times r_b$ cross performed at the same temperature and having the same *r* markers. In 40 of these 41 comparisons the former cross, in which the DNA-polymerase is mutant, gave the higher percent recombination. The sole exception is shown in Figure 4.

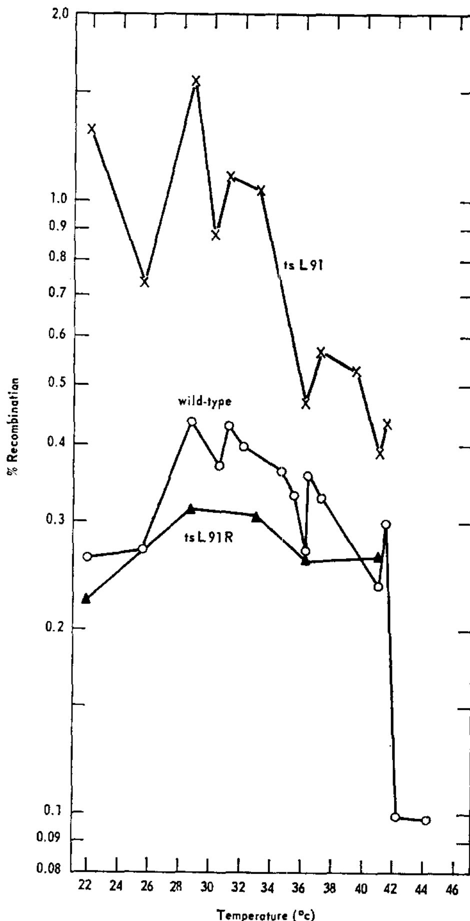
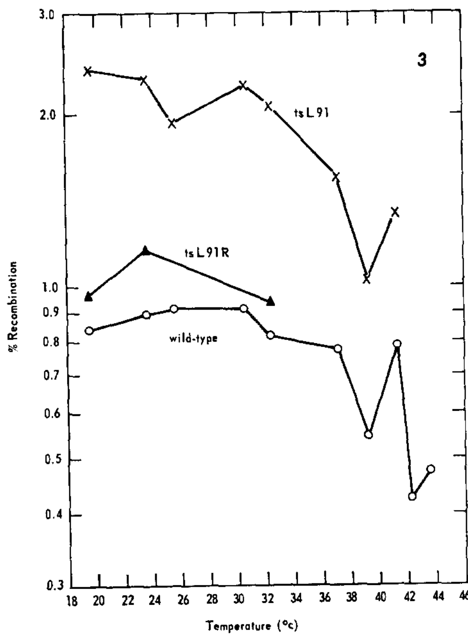
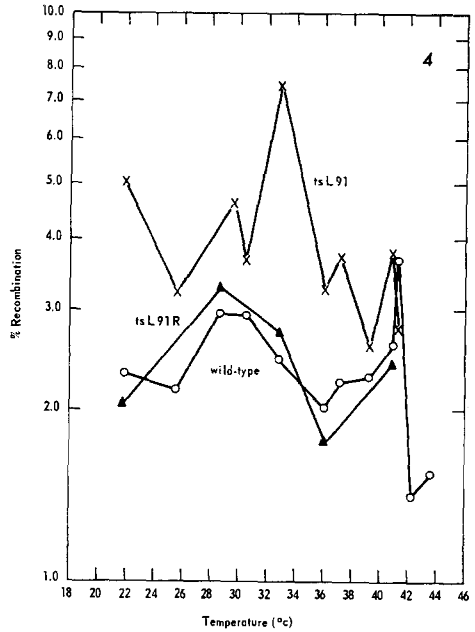
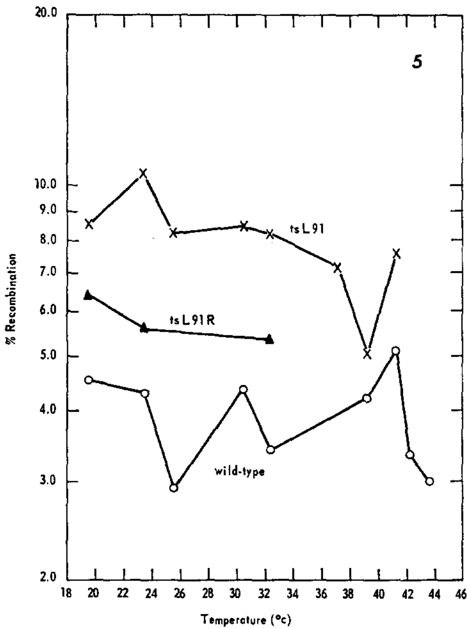


FIGURE 2.—Percent recombination from crosses: *r*ED35,*ts*L91 × *r*ED19,*ts*L91 (×), *r*ED35 × *r*ED19 (○) and *r*ED35,*ts*L91R × *r*ED19,*ts*L91R (▲).







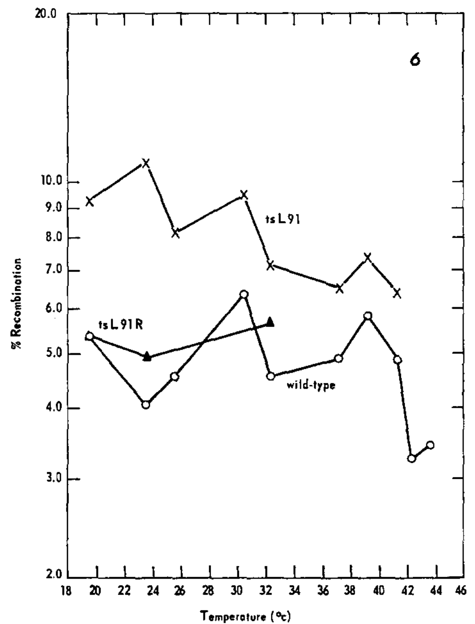


FIGURE 3.—Percent recombination from crosses: *r*ED144,*ts*L91 × *r*71,*ts*L91 (×), *r*ED144 × *r*71 (○) and *r*ED144,*ts*L91R × *r*71,*ts*L91R (▲). FIGURE 4.—Percent recombination from crosses: *r*64,*ts*L91 × *r*ED37,*ts*L91 (×), *r*64 × *r*ED37 (○) and *r*64,*ts*L91R × *r*ED37,*ts*L91R (▲). FIGURE 5.—Percent recombination from crosses: *r*ED44,*ts*L91 × *r*ED62,*ts*L91 (×), *r*ED44 × *r*ED62 (○) and *r*ED44,*ts*L91R × *r*ED62,*ts*L91R (▲) FIGURE 6.—Percent recombination from crosses: *r*ED41,*ts*L91 × *r*ED220,*ts*L91 (×), *r*ED41 × *r*ED220 (○) and *r*ED41,*ts*L91R × *r*ED220,*ts*L91R (▲).

Spontaneous ts^+ revertants were obtained from the ten *r,ts*L91 double stocks by plating at high titer on *E. coli* S/6/5 and incubating at 42°C. From the plaques which formed, stocks were prepared corresponding to each of the ten double mutants, and these were labeled with the appropriate *r* mutant symbols plus the designation *ts*L91R, the R standing for "revertant."

These ten revertant stocks were crossed in the same five $r_a \times r_b$ combinations as previously, and the resulting recombination percentages are plotted in Figures 2 to 6. A total of 19 r_a,*ts*L91R × r_b,*ts*L91R crosses were carried out. In 17 cases a corresponding $r_a \times r_b$ and r_a,*ts*L91 × r_b,*ts*L91 cross was also performed at the same temperature. In all such comparisons the percent recombination from the cross with revertant polymerase was less than the percent recombination from the corresponding cross with mutant polymerase, and generally close to the percent recombination obtained in the cross with wild-type polymerase. These results imply that most or all of the increase in recombination from the crosses with mutant polymerase can be attributed specifically to the mutant defect.

In Table 1 some significant characteristics of the data from Figures 2 to 6 are tabulated. For each temperature at which a comparison was possible, the ratio of recombination from the r_a,*ts*L91 × r_b,*ts*L91 cross to the $r_a \times r_b$ cross was calculated. In the first column the maximum ratios obtained at any temperature are given for each of the five different $r_a \times r_b$ crosses performed. The minimum and average ratios for the five different crosses are given in the next two columns. Also shown in the Table are the average maximum ratio (3.31) and the average minimum ratio (1.28) and the overall average ratio (2.03) for all five crosses. The latter ratio, 2.03 means that the presence of mutant DNA-polymerase caused increases in recombination averaging 103%.

In the last column of Table 1 the average ratios for five r_a,*ts*L91R × r_b,*ts*L91R crosses to the corresponding $r_a \times r_b$ crosses are listed, and the overall average of the five ratios is also calculated (1.13). This ratio means that in the presence of revertant polymerase recombination is increased on the average by 13%. It is uncertain whether this small increase is significant. However if it is, there are two possible interpretations. First, factors in the genetic background of these crosses, introduced in preparing the double mutant stocks, may have influenced recom-

TABLE 1

Proportional increases in recombination

Cross	*ts*L91			*ts*L91R
	Maximum	Minimum	Average	Average
*r*ED35 × *r*ED19	5.02	1.46	2.54	.915
*r*ED144 × *r*71	2.84	1.72	2.27	1.19
*r*64 × *r*ED37	3.02	.757	1.62	.982
*r*ED44 × *r*ED62	2.82	1.20	2.02	1.42
*r*ED41 × *r*ED220	2.86	1.27	1.70	1.16
Average	3.31	1.28	2.03	1.13

bination. The second possibility is that reversion of *ts*L91 may not in all cases have been back to true wild type.

To detect possible trends in recombination percentages that were common to all five $r_a \times r_b$ crosses in the presence of either mutant, revertant or wild-type polymerase, the data for these three classes of crosses were combined. First each recombination percentage was normalized, by dividing it by the appropriate standard recombination percentage shown in Figure 1, for that type of $r_a \times r_b$ cross. The resulting ratios were averaged within the three classes at each temperature. In Figure 7 the curve labeled "wild type" is the combined plot for all $r_a \times r_b$ crosses, the curve labeled "*ts*L91" is the combined plot for all r_a,*ts*L91 $\times$ r_b,*ts*L91 crosses and the curve labeled *ts*L91R is the combined plot for all r_a,*ts*L91R $\times$ r_b,*ts*L91R crosses. The wild-type curve fluctuates about unity, indicating that recombination in the presence of normal polymerase is relatively unaffected by temperature over the range 19.5° to 41.3°C. The *ts*L91 curve tends to vary about two, but there appears to be a trend with temperature, the lower average ratios occurring at higher temperatures. The *ts*L91R curve fluctuates about unity and approximates the wild-type curve.

Effect on burst size of a temperature sensitive mutational defect in the DNA-polymerase of phage T4: In Figure 8 average burst size per bacterium from the

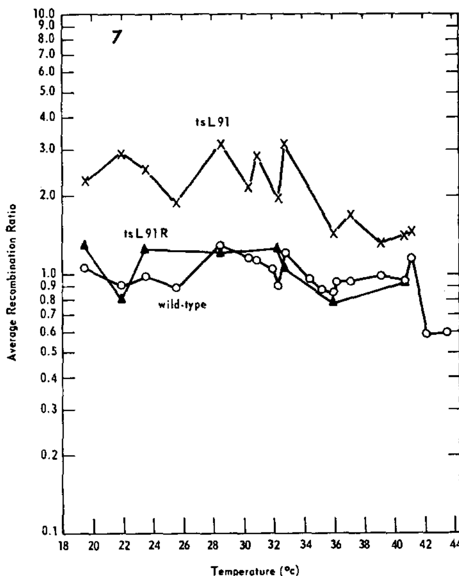


FIGURE 7.—Recombination percentages from the five different $r \times r$ crosses normalized against the appropriate standard recombination percentage in Figure 1 and averaged at each temperature: r_a, *ts*L91 $\times$ r_b, *ts*L91 crosses (×); $r_a \times r_b$ crosses (○) and r_a,*ts*L91R $\times$ r_b,*ts*L91R crosses (▲).

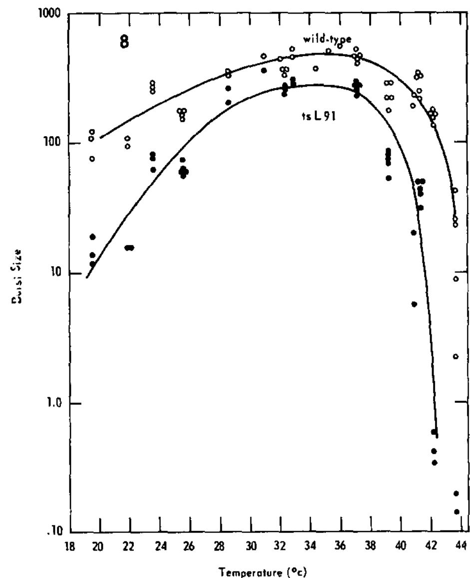


FIGURE 8.—Burst sizes from $r_a \times r_b$ crosses (○), and from r_a,*ts*L91 $\times$ r_b,*ts*L91 crosses (●).

$r_a \times r_b$ crosses and r_a,*ts*L91 $\times$ r_b,*ts*L91 crosses is plotted against temperature. The crosses in the presence of wild-type polymerase gave consistently higher burst sizes than the crosses in the presence of mutant polymerase. These results suggest that the *ts*L91 mutation has a deleterious effect over the entire range of temperatures studied.

The span from 41° to 43°C is particularly interesting since *ts*L91 is a member of a general class of temperature sensitive mutants defined by their inability to form plaques, in contrast to wild type, at these high temperatures. In Figure 8 the explanation for this differential plaquing ability at high temperatures is apparent. Above 40° the burst sizes from crosses either in the presence of mutant or wild-type polymerase decline drastically, but the drop in burst size of the crosses in the presence of mutant polymerase occurs at a temperature two or three degrees lower than the decline in burst size of the crosses in the presence of wild-type polymerase.

Reversion of the r *mutants used in the crosses in the presence of the* ts*L91 mutation:* SPEYER (1965) has shown that in the presence of a temperature sensitive DNA-polymerase mutation (*ts*L56) reversion of an *rII* mutation was increased about 3500-fold. Furthermore, SPEYER mentioned preliminary studies indicating that in the presence of the *ts*L56 mutation there was increased reversion of base analogue revertable *rII* mutations, but not of deletions or frame shift *rII* mutations.

The effect of the *ts*L91 mutation on reversion of the ten *rII* mutants used in this study was examined to determine if there were any contribution of r^+ revertants to the measured recombination percentages. Each of the original clonally derived *r* mutant stocks, and double mutant *r*,*ts*L91 stocks were tested for r^+ revertants. All of these stocks had been prepared at 30°C. The frequencies of revertants are given in Table 2.

In all cases these frequencies were negligible compared to the measured frequencies of r^+ recombinants. Furthermore there was no indication that the *ts*L91 mutation significantly increased the reversion frequency of any of the ten *rII*

TABLE 2

Proportion of r^+ *revertants per* 5×10^8 rII *phage*

rII marker	Polymerase *ts*L91+	Marker *ts*L91
*r*ED19	1	3
*r*ED35	52	50
*r*ED37	7784	701
*r*ED41	581	45
*r*ED44	97	3
*r*ED62	108	274
*r*64	0	0
*r*71	2	12
*r*ED144	49	65
*r*ED220	4	2

mutants tested. This finding is compatible with the results SPEYER obtained with *ts*L56. The *r* mutants used here were of spontaneous origin. BRENNER, BARNETT, CRICK and ORGEL (1961) showed that spontaneous mutations are mainly of the frame-shift type, and according to SPEYER such mutations are not revertable in the presence of the *ts*L56 mutation.

Since the growth conditions in preparing a stock are somewhat different than in performing a cross, a test for r^+ revertants was made by infecting *E. coli* B/5 under normal cross with conditions with phage of only one genotype. Single infections with *r*ED35,*ts*L91 and *r*ED19,*ts*L91 at 32.0°C gave no r^+ revertants among about 4×10^5 progeny in each case.

Complementation between tsL91 *and an amber mutant defective in DNA-polymerase:* The purpose of the experiments described in this section was to determine if the presence of an amber mutation, *am*N101, in the DNA-polymerase gene would affect recombination, and also to test complementation between this amber mutation and the temperature sensitive mutation *ts*L91.

WARNER and BARNES (1966), and DEWAARD, PAUL and LEHMAN (1965) have shown that amber mutant *am*N101 has lowered DNA-polymerase activity. The *rII* mutant, *r*ED19, was crossed to *am*N101 and a double mutant recombinant selected. A stock of this was prepared and used in two crosses: *r*ED35,*am*N101$^+$ $\times$ *r*ED19,*am*N101, and *r*ED35,*ts*L91 $\times$ *r*ED19,*am*N101.

The recombination percentages from these crosses are shown in Figure 9 under the labels "wild-type/*am*N101" and "*am*N101/*ts*L91" respectively. The two plots of recombination percentage shown previously in Figure 2 for the crosses *r*ED35 $\times$ *r*ED19 and *r*ED35,*ts*L91 $\times$ *r*ED19,*ts*L91 are included for comparison. These are labeled "wild-type" and "*ts*L91" respectively. Also shown in Figure 9 are recombination percentages from the cross *r*ED35,*ts*L91 $\times$ *r*ED19,*ts*L91$^+$. This plot is labeled "wild-type/*ts*L91".

The cross labeled "*am*N101/*ts*L91" tests complementation, and the ones labeled "wild-type/*am*N101" and "wild-type/*ts*L91" test dominance. As can be seen in Figure 9 there is a significant increase in recombination from the *r*ED35, *ts*L91 $\times$ *r*ED19,*am*N101 crosses compared to the corresponding *r*ED35 $\times$ *r*ED19 crosses. The average increase is 2.7-fold. The recombination percentages from the crosses *r*ED35,*am*N101$^+$ $\times$ *r*ED19,*am*N101 and *r*ED35,*ts*L91 $\times$ *r*ED19,*ts*L91$^+$ were respectively 1.3-fold and 1.02-fold higher than the *r*ED35 $\times$ *r*ED19 crosses. From these experiments it can be concluded that, with respect to their stimulatory effect on recombination, the mutation, *ts*L91 is recessive to wild-type, *am*N101 may be partially dominant, and together *ts*L91 and *am*N101 are noncomplementary.

Mutations *am*N101 and *ts*L91 are also noncomplementary with respect to burst size. When crosses were carried out under the restrictive conditions for both mutants (40.9°C with *E. coli* B/5 as host) the cross *r*ED35,*ts*L91 $\times$ *r*ED19, *am*N101 gave a burst size of 12 compared to a burst size from the cross *r*ED19, *am*N101$^+$ $\times$ *r*ED10,*am*N101 of 89, and from the cross *r*ED35, *ts*L91 $\times$ *r*ED19, *ts*L91$^+$ of 141.

The effect of the tsL91 *mutation on lysis time:* Using the technique of lysis

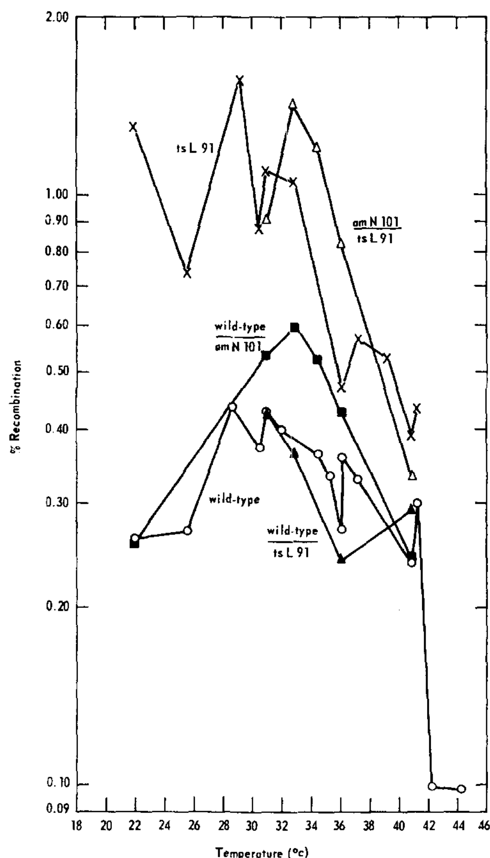


FIGURE 9.—Percent recombination from crosses: *r*ED35,*ts*L91 × *r*ED19,*ts*L91 (×), *r*ED35 × *r*ED10 (○), *r*ED35,*am*N101+ × *r*ED19,*am*N101 (■), *r*ED35,*ts*L91 × *r*ED19, *ts*L91+ (▲) and *r*ED35,*ts*L91 × *r*ED19, *am*N101 (△).

inhibition LEVINTHAL and VISCONTI (1953) showed that recombination between closely linked markers increased linearly with time of lysis. A possible explanation for the effect of *ts*L91 mutation on recombination is that it caused a delay in lysis. To test this possibility *E. coli* B/5 at 25°C was infected separately under the usual conditions for crosses with *r*ED19 and *r*ED19,*ts*L91. Infective centers were measured at frequent intervals to determine times of lysis.

The time between the onset of phage growth and the midpoint of the infective center rise in the one-step growth curve was measured in three separate experiments. In the *r*ED19 infections these times were 56, 61 and 59 minutes respectively. In the *r*ED19,*ts*L91 infections the times were 57, 70 and 80 minutes. On the average, lysis time at 25°C was increased 1.18-fold in the presence of mutant polymerase. The increase in recombination within the range 19.5°C to 32.9°C is about 2.6-fold. In the experiments of LEVINTHAL and VISCONTI (1953) over a similar frequency range, recombination was linearly related to lysis time and a 2.6-fold increase in recombination required approximately a 2.6-fold increase in lysis time. On the basis of this relationship, the increases in lysis time observed here could only account for about one tenth of the observed increase in recombination.

In addition in the LEVINTHAL and VISCONTI experiments, recombination frequency and burst size increased proportionately as lysis time was increased. In the present experiments, burst sizes in the presence of mutant polymerase were consistently lower than in the presence of wild-type polymerase.

The effect on recombination of a temperature sensitive mutational defect in dCMP-hydroxymethylase: WIEBERG and BUCHANAN (1964) have shown that temperature sensitive mutant *ts*L13 (gene 42) produces a dCMP-hydroxymethylase that differs from the wild-type enzyme. Whereas wild-type dCMP-hydroxymethylase can be reactivated after 40°C heat inactivation, the mutant enzyme cannot be reactivated.

Mutants *r*ED35 and *r*ED19 were crossed to *ts*L13, the double mutant recombinants selected, and stocks of these prepared. The double mutants *r*ED35,*ts*L13 and *r*ED19,*ts*L13 were crossed at various temperatures and the recombination percentages plotted in Figure 10. Also included for comparison in this figure is the plot of recombination percentages from the cross *r*ED35 × *r*ED19, shown previously in Figure 2. These curves are labeled "*ts*L13" and "wild type" respectively. Recombination percentages in the presence of the defective enzyme increase dramatically with temperature to values about fourfold higher than the percentages from the crosses in the presence of wild-type polymerase.

The burst sizes from the cross *r*ED35,*ts*L13 × *r*ED19,*ts*L13 are given in Figure 11 along with the burst size plots previously shown in Figure 8 for the crosses $r_a \times r_b$ and r_a,*ts*L91 × r_b,*ts*L91. The burst sizes from the crosses in the presence of the *ts*L13 mutation start to decline abruptly at 32°–34°C compared to the burst sizes represented by the other curves. At 40.9°C there are no detectable progeny. The temperature range over which burst size declines corresponds to the range over which recombination percentage increases (Figure 10).

It was also established that the rise in recombination was not complicated by reversion of *r* to r^+. *E. coli* B/5 cultures at 35.3°C were singly infected with *r*ED35 and *r*ED19 under conditions of a normal cross. From these infections no r^+ revertants were found among about 2×10^5 progeny in each case.

DISCUSSION

The effect of mutant DNA-polymerase on recombination: Before discussing possible specific mechanisms for the observed increase in recombination, it should be noted that indirect effects of the mutant enzyme on the phage mating population cannot easily be tested since the population dynamics of phage mating is not well understood.

The experiments on lysis times suggest that at least one important kinetic parameter, the number of rounds of mating, was not affected sufficiently to explain the increase in recombination.

Although it is difficult to test critically the possibility that blockage of DNA synthesis indirectly increases the frequency of intracellular matings, the following argument suggests that this is not the case. If it is assumed that the burst sizes observed at different temperatures are a valid measure of DNA synthesis, then

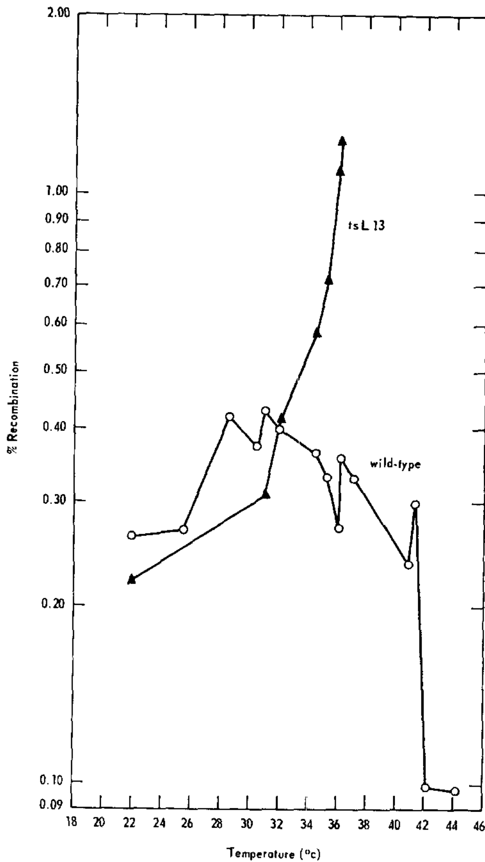


FIGURE 10.—Percent recombination from crosses: *r*ED35,*ts*L13 × *r*ED19,*ts*L13 (▲) and *r*ED35 × *r*ED19 (○).

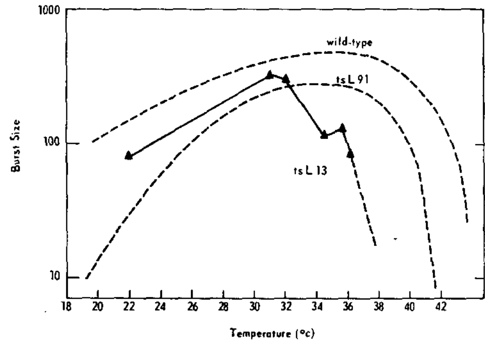


FIGURE 11.—Burst sizes from crosses *r*ED35, *ts*L13 × *r*ED19,*ts*L13 (▲). The dotted line extension of this curve is included to indicate that there was no detectable burst at 40.9°C. The dotted line plots labeled "*ts*L91" and "wild-type" are the burst size curves from r_a,*ts*L91 × r_b,*ts*L91 crosses and the $r_a \times r_b$ crosses respectively. These were shown previously in Figure 7 and are given here for comparison.

on the above possibility recombination and burst size should show an inverse relationship. In Figure 7 the average recombination percentage from the crosses with defective polymerase, normalized against the standard control percentages, were plotted against temperature. Between 32.9° and 19.5°C the increase varies around the factor 2.6. In this range burst sizes decrease from 293 to 15 (Figure 8). Above 32.9° to 41.3° the recombination values vary around the factor 1.5, whereas burst sizes decline from 293 to 43. Thus, in both the lower and upper range, recombination in the presence of the defective polymerase tends to stay roughly constant, while absolute burst size changes appreciably.

An inverse correlation between burst size and recombination was also sought by plotting versus temperature both recombination percentage and burst size from the r_a,*ts*L91 × r_b,*ts*L91 crosses normalized by dividing by recombination percentage and burst size respectively from the corresponding $r_a \times r_b$ crosses.

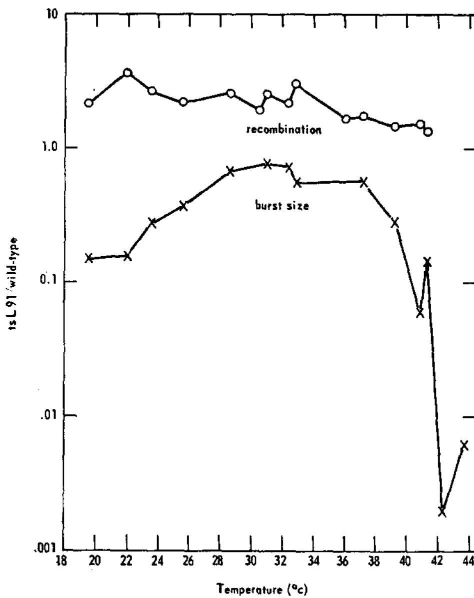


FIGURE 12.—Average ratio of recombination percentages from the r_a,*ts*L91 $\times$ r_b,*ts*L91 crosses to the corresponding $r_a \times r_b$ crosses (O). Average ratio of burst sizes from the r_a, *ts*L91 $\times$ r_b,*ts*L91 crosses to the corresponding $r_a \times r_b$ crosses ($\times$).

These proportions were then averaged at each temperature and the results plotted in Figure 12.

On comparing the two curves it is evident that there is no tendency for increases in normalized recombination to be correlated with decreases in normalized burst size. Since neither the absolute reduction in DNA synthesis nor the reduction in DNA synthesis relative to wild type correlate with increased recombination, it is probable that the blockage of DNA synthesis does not have a causal relationship to the increase in recombination.

Two further specific explanations will now be considered. (i) The defective enzyme in catalysing DNA synthesis may produce replication errors which are themselves recombination events. Such replication errors could be copy-choice occurrences as described in the model of BERNSTEIN (1962). (ii) The defective polymerase might introduce structural errors in DNA which stimulate recombination. Such errors could be either inheritable mutations, or defects that undergo repair. If the increases in recombination were mainly due to inheritable mutations, then one might expect these increases to occur even after the mutant polymerase had been reverted. However, in Table 1, the summarized data show that when the mutant polymerase is reverted, the increase in recombination declines from an average of 103% to 13%. Thus most of the increase in recombination is probably not attributable to inheritable mutation. This conclusion is subject to the qualification that the selective pressures on the revertant phage between the time they arose by mutation and their propagation into stocks are unknown. Thus secondary mutations influencing recombination might have been lost by selection.

Accepting the more likely possibility that repairable structural errors in DNA

are responsible for the increase in recombination, the following specific model can be offered. The defective polymerase may tend to leave gaps in the deoxyribose-phosphate backbone of DNA, weakening the double helix, and increasing the probability of breakage and rejoining with an homologous chromosome. The latter step could occur in the manner proposed by ANRAKU and TOMIZAWA (1965). In this case, repair and recombination would be concomitant.

The effect of mutant dCMP-hydroxymethylase on recombination: Whereas the effect of the DNA-polymerase mutation, *ts*L91, was tested in the five types of $r_a \times r_b$ cross indicated in Figure 1), the dCMP-hydromethylase mutation, *ts*L13, was tested only in the cross *r*ED35,*ts*L13 × *r*ED19,*ts*L13. As shown in Figure 10, in the presence of mutant dCMP-hydroxymethylase, recombination increases dramatically with temperature. In Figure 11 it is shown that burst size from these crosses declines in the range of temperatures in which recombination increases.

In Figure 13 the recombination percentages and burst sizes from the crosses *r*ED35,*ts*L13 × *r*ED19,*ts*L13, normalized against the corresponding recombination percentages and burst sizes from the crosses *r*ED35 × *r*ED19, are plotted versus temperature. This plot reveals that increases in the normalized recombination percentages coincide strikingly with the decreases in the normalized burst sizes. This observation suggests that there may be a cause and effect relationship between decreased dHMP synthesis and increased recombination.

Perhaps limited availability of dHMP leads to misincorporations into DNA

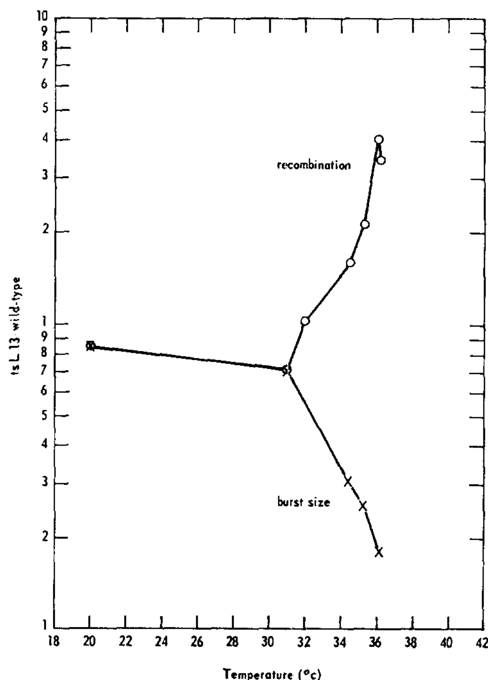


FIGURE 13.—Ratio of recombination percentages from the cross *r*ED35, *ts*L13 × *r*ED19, *ts*L13 to the cross *r*ED35 × *r*ED19 (○). Ratio of burst sizes from the cross *r*ED35,*ts*L13 × *r*ED19, *ts*L13 to the cross *r*ED35 × *r*ED19 (×).

of other bases, with consequent alteration in the pattern of DNA glucosylation. Such structural errors might then cause increased recombination.

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SUMMARY

Crosses of *rII* mutants were performed either in the presence of the temperature sensitive DNA-polymerase mutation *ts*L91 or its wild-type allele. In the presence of mutant polymerase, recombination was increased about twofold compared to the crosses in the presence of wild-type polymerase. The increase was observed at different temperatures over a wide range. After reversion of the DNA-polymerase mutation, recombination of the *rII* marker became very nearly normal. In mixed infections with *ts*L91 and *am*N101, a DNA-polymerase amber mutant, increases in recombination of the *rII* markers were again observed over a range of temperatures. In the presence of a temperature sensitive deoxycytidylate hydroxymethase mutation, *ts*L13, recombination was also increased several-fold compared to the crosses in the presence of wild-type polymerase. However here the increase occurred only at higher temperatures. In addition there was a clear correlation between burst size decrease and recombination increase. With the temperature sensitive polymerase mutant, no such correlation was apparent.—The favored explanations for the effect of the mutant polymerase are that it either enhances the probability of copy-choice events, or of DNA structural defects leading to breakage and exchange.

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