REGION-SPECIFIC RECOMBINATION IN PHAGE T4. I. A SPECIAL GLUCOSYL-DEPENDENT RECOMBINATION SYSTEM

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ABSTRACT

In this communication, we describe a recombination mechanism in bacteriophage T4D that acts only on glucosylated phage, acts in some regions of the genome, but not others, and is heat sensitive, showing decreasing activity with increasing temperature.

STUDIES of recombination with bacterial viruses have begun to show that special features of DNA may influence local frequencies of genetic exchange. Such features include the "hair-pin" structure in the A cistron of ΦX174 (FIERS and SINSHEIMER 1962; BENBOW *et al.* 1971), molecular ends (MOSIG 1963; MICHALKE 1967; and DOERMANN and PARMA 1967) and local base sequences (RONEN and SALTS 1971; LAM *et al.* 1974). It has been known for some time that bacteriophage T4 contains several regions in which genetic recombination frequencies appear higher than one would expect from the physical size of these regions. The most impressive of these distortions is in the gene *34–35* region (MOSIG 1966). BECKENDORF and WILSON (1972) made careful comparisons of the peptide and genetic maps of gene *34* (the structural gene for the proximal portion of the tail fiber) for phages T2 and T4. They showed that the distortion is most pronounced at the right-hand end of gene *34* (*i.e.*, the end nearest to gene *35*) of T4, and is virtually absent from gene *34* of T2. Since LEHMAN and PRATT (1960) showed that these phages had different patterns of glucosyl residues on the hydroxymethylcytosine (HMC) bases of their DNA, MOSIG (1966) proposed that glucosylation patterns might affect recombination frequencies. L. A. MCNICOL, in this laboratory, first showed that recombination between T4 gene *34* mutations was indeed substantially higher when the phage were glucosylated than when they were not (unpublished data). We have investigated this phenomenon in further detail. In this paper, we document the phenomenon and present evidence that the recombination occurring in the tail fiber region of normally glucosylated T4 phage is qualitatively, as well as quantitatively, different from recombination occurring elsewhere. In companion articles, we deal in greater detail with the structure of these recombinants and with the enzymatic pathways responsible for their formation.

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MATERIALS AND METHODS

Media: Plain phage broth contains 10.0 g bactopeptone, 5.0 g NaCl, 3.0 g beef extract and 1.0 g dextrose per liter of distilled H_2O. For most experiments, this was supplemented with 2.0 μg/ml vitamin B1. Dilution fluid contains 1.0 g bactopeptone, 3.0 g NaCl and 0.25 g $MgSO_4$ per liter of distilled H_2O. Bottom agar contains 12.0 g bacto agar, 10.0 g bacto tryptone, 8.0 g NaCl, 2.0 g sodium citrate and 1.0 g dextrose per liter of distilled H_2O and was adjusted to neutral pH by addition of 1.5 ml of 0.1 N NaOH per liter after autoclaving. Top agar contains 6.0 g bacto-agar per liter and required only 1.0 ml 0.1 N NaOH per liter for neutralization, but otherwise was like bottom agar. T2 buffer contained, per liter, 5.0 g K_2SO_4, 1.5 g KH_2PO_4, 4.0 g NaCl, 5.7 g $Na_2HPO_4 \cdot 7H_2O$, 0.01 g gelatin and 1.0 ml each of 0.1 M $MgSO_4$ and 0.1 M $CaCl_2$.

Phage strains: Most mutant phage strains were obtained from the collections of R. EDGAR and W. WOOD. The genes in which these mutations map are mentioned in the text or figures where the individual mutations are cited. Exceptions are the *r*II mutations *r*61 (rIIA) and *r*73 (*r*IIB), which came from A. H. DOERMANN and the glucosyl transferase mutations αgt^{57}, αgt^{am8}, βgt^{14} and βgt^{am10} (denoted below as αgt, αgt^{am}, βgt and βgt^{am}, respectively), which were provided by C. GEORGOPOULOUS. Phage stocks were routinely prepared by the agar layer technique (SWANSTROM and ADAMS 1958), using overnight *Escherichia coli* CR63rgl⁻ as host. The number of phage that was required to give near-confluent lysis of such plates varied from about 10^5 to 10^8, depending on the genotype of the stock. Strains bearing multiple mutations were constructed by crossing single mutant or lower order multiple-mutant stocks. In most cases, the progeny of the desired genotype could be identified by spotting an appropriate series of indicator bacteria. In a few cases, it was necessary to backcross individual isolates in order to confirm the presence of mutations in a stock. Most phage were prepared at 30°, although some nonglucosylated stocks were prepared at room temperature.

Bacterial strains: *E. coli* B40suI, obtained from P. STRIGINI, was used as the su+ (rgl+) host in testing multiple mutant phage strains for the presence of mutations αgt and βgt. Strains CR63rgl⁻ and K172rgl⁻ were obtained from H. REVEL; the derivative strains CR63(λh)rgl⁻ and K172(λh)rgl⁻ were made from CR63rgl⁻ and K172rgl⁻, respectively, by challenge with phage λh, provided by I. HERSKOWITZ. These four strains are referred to below with the following designations: su+ = CR63rgl⁻, su⁻ = K172rgl⁻, (λh)su+ = CR63(λh)rgl⁻ and (λh)su⁻ = K172(λh)rgl⁻. Note that all of these strains are rgl⁻, although the rgl⁻ designation has been omitted throughout the text for the sake of simplicity. Strains that are rgl⁻ bear mutations in the genes $r_{2,4}$ and r_6 and are permissive for nonglucosylated T4 phage as a result of these mutations. Strains designated su⁻ are restrictive for the amber mutants used, and those designated (λh) are restrictive for T4 *r*II phage.

Plating bacteria were prepared by diluting overnight cultures about 150-fold into fresh medium, shaking at 37° until the cells had grown to an OD_{550} of about 0.2 and concentrating 10-fold by centrifugation in the cold and resuspension in fresh prechilled broth. Host bacteria for crosses were prepared by diluting overnight cultures 1000-fold into fresh broth, growing at the temperature at which the crosses were to be done until the cells reached an OD_{550} of about 0.1, chilling on ice, centrifuging in the cold and resuspending in fresh pre-chilled broth to an OD_{550} = 1.50 (gives 2×10^8 cells/ml for su+).

Procedure for crossing phage: Input phage stocks were adjusted to 6×10^9 plaque-forming units per milliliter (pfu/ml), and phage mixtures were made by combining equal volumes (usually 0.50 ml each) of the two genotypes to be crossed. Unless otherwise specified, host cells were grown and crosses were performed at 30°. To initiate crosses, a volume of input bacteria (see above) equal to that of the phage mixture was added, and this "adsorption mixture" was aerated gently. After 8.0 minutes (6.0 minutes for crosses at 42° or 44°), a small sample was removed and saturated with $CHCl_3$ to assay for unadsorbed phage, and anti-T4 serum was added to the remaining adsorption mixture to give a K between 1 and 2. After another equal period, the infected cells were diluted 4×10^4-fold into pre-warmed broth and a small sample plated from the final (growth) tube to assay for infective centers. After at least 110 minutes (90 minutes for crosses at

37° or above), $CHCl_3$ was added to the growth tubes. The chloroformed supsensions were then diluted appropriately and plated on su+ to measure total phage, on (λh)su+ to measure *r*II+ recombinants and on su⁻ to measure *am*+ recombinants. Recombination percentages are expressed as 200 times the ratio of progeny on the restrictive host (*i.e.*, (λh)su+ or su⁻) to progeny on the permissive host (su+). All stocks used in generating recombination data were self-crossed (*i.e.*, a standard cross performed in which the phage "mixture" contained 0.5 ml of the phage stock in question diluted to 6×10^9 pfu/ml and 0.5 ml of phage broth). The progeny from such infections were plated on the three indicator strains mentioned to assure that the fraction of revertants so produced was not high enough to contribute significantly to the measured recombination frequencies. Marker rescue crosses were performed in this same manner; the amount of irradiated input phage used in these crosses was based on the titer of the phage sample before irradiation. The host bacterial strain su+ does not adsorb T4 well, and usually only from 50% to 70% of the input phage adsorbed during the adsorption period.

Irradiation procedure: Phage preparations in T2 buffer were placed in a glass petri dish and swirled gently on a rotating apparatus. They were irradiated by a single General Electric 15 watt germicidal bulb placed 24 inches above the suspension. Irradiation was initiated and terminated by removal and replacement of the glass cover on the dish. Under these conditions, the time required to reduce the plaque forming titer of a phage stock by a factor of 10 was 12 sec for T4+ and 9 sec for T4 *αgt βgt*.

RESULTS

Region-specific effects of glucosylation: The amount of recombination in various subintervals of genes *34* and *35* was measured in crosses of the form:

(1) T4 am_1 $r\mathrm{II}_1$ × T4 am_2 $r\mathrm{II}_2$

(2) T4 *αgt βgt* am_1 $r\mathrm{II}_1$ × T4 *αgt βgt* am_2 $r\mathrm{II}_2$

where am_1 and am_2 are amber mutations in gene *34* and/or gene *35* and $r\mathrm{II}_1$ and rII_2 are the *r*II mutations *r*61 and *r*73. For convenience we will sometimes refer to crosses of type (1) as glucosylated crosses, and to crosses of type (2) as nonglucosylated crosses. The corresponding phage will be spoken of as glucosylated and nonglucosylated phage, respectively, with the understanding that it is actually the phage DNA that may be so modified. The results of these crosses are presented in Figure 1. The figure presents three maps of the gene *34*–gene *35* region. The first map gives the percent recombination between amber mutants in this region, as measured in crosses of type (2) above. The second shows the corresponding values as measured in crosses of type (1) above. The third presents the ratio of the values in crosses of type (2) to those in crosses of type (1), after normalizing each value for the amount of recombination between the *r*II mutants measured in the same cross. This value is referred to below as the "stimulation coefficient." Glucosylated phage show as much as four times more recombination than nonglucosylated phage, depending upon the interval in which the comparison is made.

Separate effect of α- and β-linked glucosyl residues on recombination: Despite the close homology between T2 and T4 (Cowie, Avery and Champe 1971; Russell 1974; Kim and Davidson 1974), T2 does not show high recombination in its tail-fiber region (Beckendorf and Wilson 1972; Russell 1974). Thus, the region-specific stimulation of recombination in T4 may depend upon the 30% of

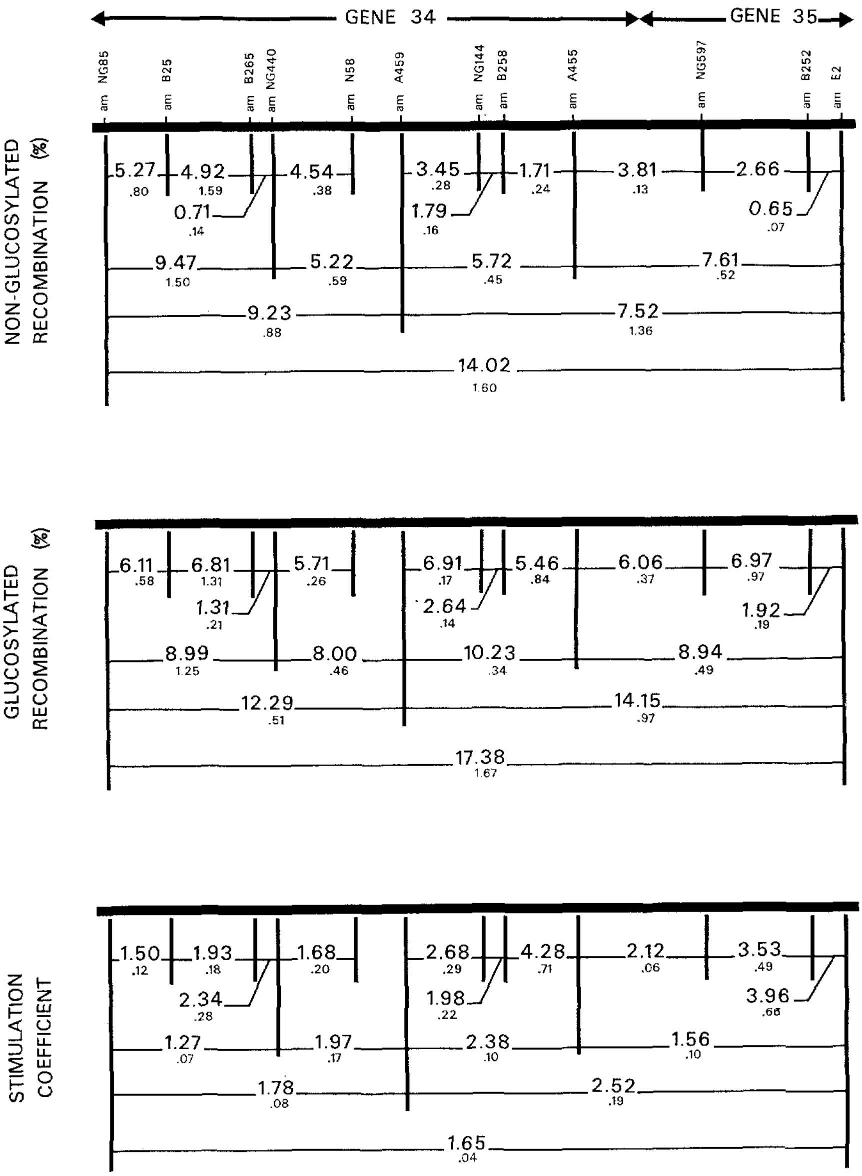

FIGURE 1.—The effect of glucosylation on recombination. This figure presents results of crosses done to measure the effect of glucosylation of T4 DNA on recombination in subintervals of the gene *34*–gene *35* region. At the top of the figure is a map of this region giving the order of the mutants used. The upper panel indicates the recombination (in percent) obtained between various pairs of these markers in nonglucosylated crosses (in which both parents carry the mutations αgt^{57} and βgt^{14}). Recombination frequencies are measured as described in METHODS. The measured recombination frequencies for each cross between the indicated markers has been normalized to the amount of recombination between the standard pair of *rII* markers measured in the same cross. This normalization has been done by dividing by the ratio of the percent *r*II+ recombination in the individual cross in question to the average percent *r*II+ recombination for

β-linked glucose residues unique to T4 (LEHMAN and PRATT 1960). In order to test this, we performed crosses of the following types:

(3) T4 $\alpha gt\ am_1\ r\mathrm{II}_1 \times$ T4 $\alpha gt\ am_2\ r\mathrm{II}_2$

(4) T4 $\beta gt\ am_1\ r\mathrm{II}_1 \times$ T4 $\beta gt\ am_2\ r\mathrm{II}_2$

in which the phage were only β-glucosylated (3) or only α-glucosylated (4). The results of such crosses for four genetic intervals at the righthand end of gene *34* are shown in Figure 2, where they are compared with the results of fully glucosylated and nonglucosylated crosses for the same intervals. When α-glucosylation alone is present, we would expect T4 to resemble T2 most closely, since 30% of the glucose residues remain nonglucosylated (GEORGOPOULOS and REVEL 1971). Nevertheless, the effect of glucosylation is still marked. However, full stimulation of recombination is observed only when both types of glucose residue are present. This is especially interesting because, in infections of T4 that have only the β-transferase, all of the HMC becomes β-glucosylated. Thus, it is not only the degree of glucosylation, but also the type of glucosylation, that is responsible for the stimulation of recombination in this region.

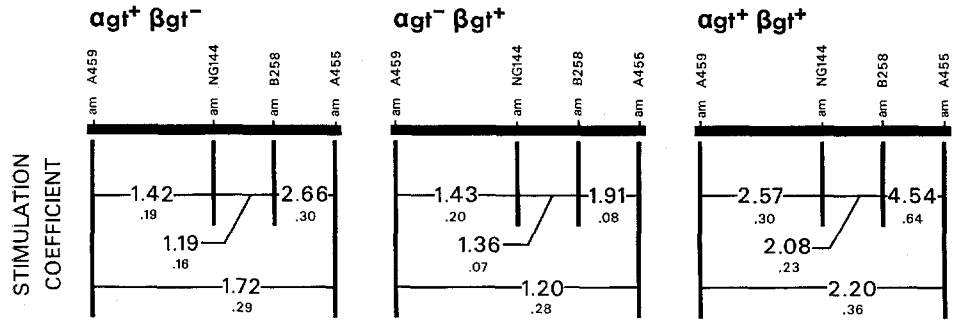

FIGURE 2.—The separate effects of α- and β-glucosylation on recombination. This figure presents stimulation coefficients (calculated as in Figure 1) for four genetic intervals at the righthand end of gene *34*. The three maps correspond to crosses in which the phage had only α-glucosyl residues on their DNA (left map), only β-linked glucosyl residues (right map). Each cross was done three to five times.

all (nonglucosylated) crosses. The larger numbers indicate the average of these normalized values for three to five repetitions of the cross indicated; the smaller numerals below each such number indicate the standard deviation about the average. The middle panel presents the results of glucosylated crosses in an analogous fashion. The bottom panel presents the average stimulation coefficients and standard deviations for the same genetic intervals. The stimulation coefficients are calculated as

$$\frac{(\%am+/\%r\mathrm{II}+)\ \alpha gt+\ \beta gt+}{(\%am+/\%r\mathrm{II}+)\ \alpha gt-\ \beta gt-}$$

where the numerator is the ratio of *am*+ to *r*II+ recombinants for a glucosylated cross and the denominator is the same ratio for an analogous nonglucosylated cross run in parallel. These numbers are slightly higher than the ratio of recombination values given in panel (B) to those in panel (A) because slightly higher recombination values are obtained between the *r*II markers in nonglucosylated crosses than in glucosylated crosses.

Glucosylation does not affect marker rescue: We were interested in finding out whether the stimulatory effects of glucosylation on recombination observed in the gene *34*–gene *35* region also occurred in other regions of the genome. It can be seen from Figure 1 that this stimulation is most apparent between closely linked markers, *i.e.*, that the stimulation between markers bounding a large interval (*e.g.*, *am*A455 and *am*E2) may be less than the amount of stimulation in the component subintervals. WOMACK (1965) found that rescue of markers from UV-irradiated T4 by unirradiated "helper" phage was more efficient for markers in the gene *34* region than for those in most other regions of the genome. It seemed likely that this was another manifestation of region-specific recombination and that marker rescue between nonglucosylated phages might, therefore, occur with equal efficiency for all markers. If this were the case, we could use this technique to search more easily for the other regions of glucosylation-dependent recombination. In marker-rescue crosses, as the dose increases, the effective interval in which recombination must occur becomes smaller; the size of these intervals is a function of dose and not of position (DOERMANN 1961). In addition, only the helper phage need be mutant, and the frequency of wild type alleles rescued from the irradiated phage can then be measured by selective plating. The results of marker-rescue crosses between glucosylated phages and nonglucosylated phages are given in Table 1. Even at a dose (2 min) that resulted in five-fold more rescue for a gene *34* marker (*am*B25) than for a gene *31* marker (*am*N54), there was no effect of glucosylation on the efficiency of rescue of either marker. Thus, marker rescue is not a result of the same kind of recombination as that observed in "standard" genetic crosses, and we could not use it to look for stimulatory effects of glucosylation. Though high marker-rescue frequencies are also observed in the tail fiber region of T4 when the damaged parent has been treated with X rays

TABLE 1

The effect of glucosylation on marker rescue

UV dose (min)	Relative rescue efficiency *am*N54 (gene *31*) +GLU	–GLU	*am*B25 (gene *34*) +GLU	–GLU
0	0.95	1.01	1.05	1.00
1	0.88	0.80	2.11	2.50
2	1.13	1.10	4.70	5.82
5	1.33	1.18	5.26	4.01

In this table we present the results of marker rescue crosses of the type:
(a) T4 am_a *r73* × uv-T4 *am*+ *r*II+
(b) T4 *αgt βgt* am_a *r73* × uv-T4 *αgt βgt am*+ *r*II+.
Cross procedures were similar to those for standard crosses (see MATERIALS AND METHODS). Crosses were performed and progeny were plated in subdued light to prevent host cell reactivation. The amber mutation used and the gene in which it maps are indicated at the top of each pair of columns. The columns are headed with +GLU for glucosylated crosses and –GLU for nonglucosylated crosses. The individual values represent the ratio of *am*+ to *r*II phage among the progeny. Results similar to those for *am*B25 were also obtained for the gene*34* mutants *am*B258 and *am*A455.

(CAMPBELL 1969) or by decay of incorporated ^{32}P (LEVY 1975), we have not measured marker rescue between nonglucosylated phage so treated.

Time course of recombinant formation: As part of a search for qualitative differences between the "special" recombination in the tail-fiber region of glucosylated phage and recombination that occurs independent of the state of glucosylation of the phage DNA, we determined the time-course of recombinant formation in glucosylated and nonglucosylated crosses for two genetic intervals: *r*61–*r*73 (in the rII region) and *am*B258–*am*A455 (at the right end of gene *34*). Aliquots of the diluted infected cells were lysed prematurely with chloroform so that total progeny and recombinants could be assayed at various times during the lytic cycle. Figure 3 shows that nearly maximum recombination frequencies are reached by the time that the first intracellular progeny phage appear. This is true for both genetic intervals studied, whether or not the phage were glucosylated. The "special" recombination cannot be distinguished from "general" recombination on the basis of these kinetics. However, only a rather gross difference in time of recombinant formation would be detectable by this method, since a large pool of DNA has been synthesized and recombination is well under way by the time the first DNA is packaged into mature phage. Recombinants present at the end

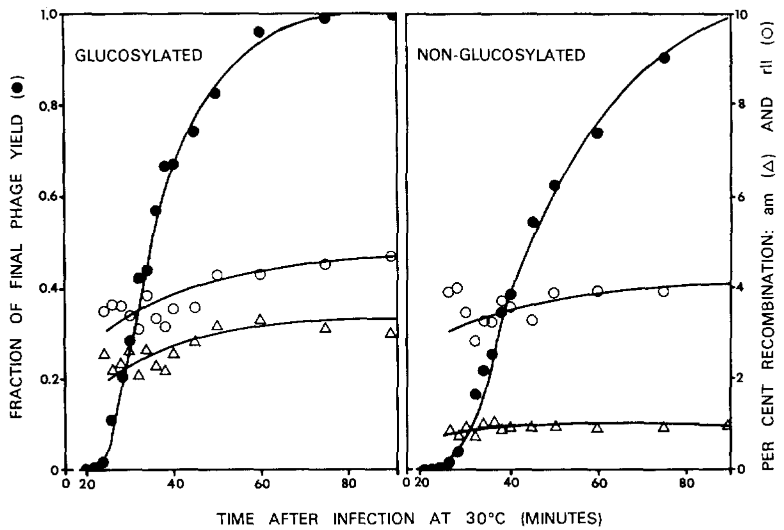

FIGURE 3.—Glucosylation does not affect the time-course of recombination. This figure presents a typical result of crosses in which samples of infected cells were lysed prematurely with $CHCl_3$ and the total progeny and *r*II+ and *am*+ recombinants assayed by selective plating as described in METHODS. Total phage (—●—) are plotted as the fraction of the final phage yield (at 90 min), which was 109 per infective center for the glucosylated cross and 51 per infective center for the nonglucosylated cross. The percent recombination between the gene *34* markers *am*B258 and *am*A455 (—Δ—) and between the standard *r*II markers *r*73 and *r*61 (—O—) are calculated as 200 times the fraction of progeny that are *am*+ and *r*II+, respectively. The lefthand panel gives the results of crosses between glucosylated ($\alpha gt+\beta gt+$) parents, and the righthand panel gives the results of crosses between nonglucosylated ($\alpha gt^{57}\beta gt^{14}$) parents. In both cases, recombination is nearly complete by the time there are enough progeny to measure recombinants.

of the eclipse influence the final recombination frequency much more than those formed late in infection, since early recombinants can still replicate several times before being removed from the DNA pool. It would, therefore, take a major perturbation of DNA metabolism to produce a major portion of the final recombination frequency by events occurring late in infection, and this was not found. While a slight delay in the eclipse period, which is characteristic of nonglucosylated phage (DHARMALINGAM and GOLDBERG 1979), can be seen, recombination in both genetic intervals and for glucosylated as well as nonglucosylated phage appears similar, as judged by the gross criterion of kinetics of recombinant formation.

Role of parental and progeny DNA molecules in recombination: Since recombination occurs very early during infection, we asked whether the ability to participate in special T4 recombination was an attribute unique to the parental phage DNA. By employing phage carrying suppressible mutations in the glucosyl transferase genes, we were able to design crosses in which only the parental DNA or only the progeny DNA was glucosylated. We thus performed crosses of the following types:

(5) T4 αgt^{am} βgt^{am} ts_1 $r\mathrm{II}_1 \cdot su^+$ × T4 αgt^{am} βgt^{am} ts_2 $r\mathrm{II}_2 \cdot su^+$

(6) T4 αgt^{am} βgt^{am} ts_1 $r\mathrm{II}_1 \cdot su^-$ × T4 αgt^{am} βgt^{am} ts_2 $r\mathrm{II}_2 \cdot su^-$

where ts_1 and ts_2 are two temperature-sensitive mutations at the right hand end of gene *34*, and the designations $\cdot su^+$ and $\cdot su^-$ refer to phage stocks prepared on su^+ and su^- hosts, respectively. By performing such crosses in both su^+ and su^- hosts, it was possible to measure recombination when both parents and progeny were glucosylated [cross (5), in su^+ host], when neither was glucosylated [cross (6), in su^- host], when only parental DNA was glucosylated [cross (5), in su^- host], and when only progeny DNA was glucosylated [cross (6), in su^+ host]. It was necessary to use temperature-sensitive mutants for measuring the recombination in gene *34*, rather than *am* mutants as used in other crosses, since *am* mutants in gene *34* will not grow on su^- hosts. Table 2 shows that both parental and progeny

TABLE 2

Participation of parental and progeny DNA in recombination

Glucosylation (?)		Relative recombination efficiency		
Parents	Progeny	*ts*A44—*ts*B63	*ts*B45—*ts*B63	*ts*A44—*ts*B45
Yes	Yes	0.93	0.16	1.02
No	Yes	0.84	0.12	0.82
Yes	No	0.51	0.10	0.50
No	No	0.30	0.06	0.33

This table shows the results of crosses between phages bearing temperature-sensitive markers at the righthand end of gene *34*, under conditions where both parent and progeny phage were glucosylated (line 1), only progeny were glucosylated (line 2), only parents were glucosylated (line 3) and neither parents nor progeny were glucosylated (line 4). Those conditions were arranged by using phage with amber mutants in both glucosyltransferase genes, and by appropriate choice of su^+ and su^- hosts for preparing stocks and doing crosses (see text for further details). The gene *34* mutants used probably map in the order gene *33*—tsB45—tsB63—tsA44—gene *35*. The values presented are the ratio ($\%ts^+/\%r\mathrm{II}^+$) for the indicated crosses.

TABLE 3

The effect of temperature on recombinant formation

Temperature	Glucosylation	Burst size	Recombination %rII+	Recombination %am+	%am+ / %rII+	Stimulation coefficient
25°	+GLU	128	6.1	6.2	1.02	4.1
	−GLU	33	5.1	1.3	.25	
30°	+GLU	167	8.0	7.3	.91	3.5
	−GLU	67	5.0	1.3	.26	
42°	+GLU	88	7.9	4.9	.62	2.3
	−GLU	23	5.1	1.4	.27	
44°	+GLU	27	7.4	2.1	.28	(1.07)

Crosses were performed as described in the section on MATERIALS AND METHODS, but at a variety of temperatures, using glucosylated (+GLU) and nonglucosylated (—GLU) phage bearing the segregating markers *am*B258 *r*73 and *am*A455 *r*61. Nonglucosylated phage were not able to carry out successful infections at 44° under the conditions used for these crosses, and the table therefore lacks the corresponding entries. However, since temperature had little effect on the ratio of *am*+ to *r*II+ recombinants for nonglucosylated phage in the range from 25° to 42°, we conclude that, were this cross possible, the stimulation coefficient at 44° would be close to 1.0. Burst sizes were calculated as the ratio of total progeny to infective centers (burst size for —GLU at 44° = 0.4).

DNA are involved in recombination in gene *34*, since both must be glucosylated in order for maximal recombination levels to be achieved. It is likely that when crosses of type (6) are performed in the su+ host, some of the parental DNA becomes glucosylated (McNICOL and GOLDBERG 1973). However, since we did see less recombination under these conditions than in similar infections by glucosylated parents, our conclusion that parental DNA molecules do play a significant role in "special" recombination is not weakened by this possibility. It seems, therefore, that the early formation of recombinants can be attributed to ordinary kinetics of recombinant formation; no unique role of parental phage in formation of the extra (gene *34*) recombinants need be invoked.

Effect of temperature on recombinant formation: As part of our search for conditions that would enhance the differences in recombination between glucosylated and nonglucosylated phages, we performed crosses of types (1) and (2) at several different temperatures. Table 3 shows that increasing the temperature reduces the amount of recombination between the gene *34* mutations in glucosylated crosses. There is no effect of temperature on recombination in nonglucosylated crosses or between the *r*II mutations in glucosylated crosses. The latter observation indicates that the reduction in recombination with increasing temperature (in the tail-fiber region) in glucosylated crosses is not merely a consequence of the reduced burst size. Thus, some component of the recombination mechanism is special to the recombinants that form in the tail-fiber region of glucosylated phage, and this component is heat sensitive.

DISCUSSION

In this report we have characterized a special region-specific recombination mechanism of T4 that operates preferentially in the gene *34*–gene *35* region. We have found that this special recombination differs from normal recombination in several respects: (1) it occurs only when the phage crossed are glucosylated; (2) it is active in producing recombinants in the gene *34*–gene*35* region, but not in *r*II region in crosses between glucosylated phages; and (3) it is heat sensitive.

The high recombination in the tail-fiber region of glucosylated phage cannot merely be due to high activity of the "apparatus" that accounts for the general recombination in response to local patterns of glucosylation, since only in this region is recombination temperature sensitive. Glucosylated phage produced at 30° show no measurable special recombination after crossing at 44°. Thus, the temperature sensitivity of "special" recombination can be attributed to glucosylating enzymes only if their presence is required during recombination and not merely during phage production. However, glucosylated phage do show significant levels of special recombination at 30°, even when the glucosylating enzymes are absent (and progeny DNA is not glucosylated). Therefore, it is not temperature sensitivity of the glucosyl transferases that reduces "special" recombination at high temperatures.

The results presented here are consistent with the idea that a specific endonucleolytic cleavage or cleavages (*e.g.*, ALTMAN and MESELSON 1970) initiate the special gene *34*–gene *35* recombination (BECKENDORF and WILSON 1972). There are several distinct mechanisms that depend upon site- or region-specific nicking of the DNA, any one of which might account for the special recombination reported here. These models include the following: (1) a specific nicking enzyme might act only on glucosylated DNA, and gaps or free single-stranded ends that result from specific nicks in the tail-fiber region might be recombinogenic (BROKER and DOERMANN 1975); (2) specific nicking enzyme might act on both glucosylated and nonglucosylated DNA, but the nick might be resealed more rapidly in the nonglucosylated DNA, so that a larger fraction of the nicks formed in glucosylated DNA lead to recombinogenic gaps or free ends; or (3) the nicks and resultant gaps or free single-strand ends might all be equally common in glucosylated and nonglucosylated DNA, but nonglucosylated gaps might be more sensitive to endonucleolytic cleavage (DHARMALINGAM and GOLDBERG 1976), with the consequence that presumptive nonglucosylated recombinants are often not matured. The data presented here do not allow us to distinguish between such models.

In summary, we have shown that recombination in T4 phage can proceed by more than one mechanism. The "special" mechanism, with which this paper has been primarily concerned, acts preferentially in the tail-fiber region of glucosylated phages, does not act in nonglucosylated phages and is shown to be distinct from the mechanisms of recombination acting in nonglucosylated crosses on the basis of its heat sensitivity.

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