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Parent-to-Progeny Transfer and Recombination of T4rII Bacteriophage[1]

KARIN CARLSON AND ANDRZEJ W. KOZINSKI

Department of Medical Genetics and Graduate Group on Molecular Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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Transfer of parental, light (not substituted with 5-bromodeoxyuridine) ^{32}P-deoxyribonucleic acid (DNA) from rII$^-$ mutants of T4 bacteriophage to heavy (5-bromodeoxyuridine-substituted) progeny in *Escherichia coli* B was less homogeneous than in wild phages. The net transfer was 5 to 20% of the value for wild T4 phage, and the parental contribution per progeny DNA molecule amounted to 7 to 100% of the genome. Three classes could be distinguished, based on the density distribution of parental label in CsCl analysis of the progeny phages. "Far recombined" phages contain parental material only in semiconservatively replicated subunits covalently attached to progeny DNA, amounting to 5 to 10% parental contribution per genome. "Intermediate recombinants" contain, aside from conventional recombinant DNA, parental DNA banding at the original, light density. This DNA may be unattached to heavy progeny DNA or attached by weak bonds which are very sensitive to shearing during the extraction procedure. The parental contribution is 10 to 50% per progeny DNA molecule in this class. "Conservative" phages band close to the parental, light density in CsCl; their DNA is purely light. When the parental phage is labeled with both ^{3}H-leucine (capsid) and ^{32}P (DNA), the specific activity of ^{3}H/^{32}P in the "conservative progeny" is 10 to 40% of that in the parental, showing that at least some of the ^{32}P in this area belongs to phages with parental DNA as the sole DNA component inside an unlabeled capsid, i.e., parental DNA which has been injected into the host and matured in a new capsid without replication or recombination. This phenomenon occurs to about the same extent in both single and multiple infection.

In wild T-even phages, 30 to 50% of the injected parental deoxyribonucleic acid (DNA) is transferred to the progeny (5, 14). The parent-to-progeny recombination is very efficient, resulting in a homogenous 5 to 10% average parental contribution per progeny DNA molecule, as a single, semiconservatively replicated fragment (9, 16). No hybrid (50% parental, 50% progeny DNA) or conservative (parental density, nonreplicated) phages are found among the progeny (9). Even in phages produced in the presence of 5-fluorodeoxyuridine (5-FUdR; which permits only limited synthesis of DNA), semiconservatively replicated parental DNA fragments are found incorporated into progeny phage as an average 15% contribution per DNA molecule (8, 9).

It was observed that after infection of *Escherichia coli* B by amber mutants of T4 deficient in polynucleotide ligase (gene 30), the DNA produced is very short, and no viable progeny phages are formed (7). If chloramphenicol is added at 5 min after infection, however, large DNA units are produced, which upon removal of the drug will mature into viable progeny phages (11). This suggested that ligase compensates for nucleases. If so, it would be expected that genetic mutants with restricted nucleolytic activity would not have a stringent requirement for ligase. It has been found that rII$^-$ ligase-negative double mutants reproduce in *E. coli* B, with yields of 80 to 100 infective phages per infected bacterium (1, 6). This would be expected if the rII gene directly or indirectly controls endonucleases. If these are responsible for recombination, it would be expected that rII mutants should display decreased frequency of molecular recombination. In the present paper, parent-to-progeny recombination of rII$^-$ phages in *E. coli* B was studied as the simplest way to follow molecular recombination. The paper documents

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significant differences between wild (rII^+) and rII^- mutants in the mode of transfer of parental DNA to the progeny. It will be demonstrated that rII^- parental DNA is not transferred to the progeny as a unique class but as fragments varying in size, including completely conservative, nonreplicated DNA.

MATERIALS AND METHODS

Strains. T4 phages r59 (rIIA), r2-19 (rIIA), r2-20 (rIIA), and rb50 (rIIB) were used in the experiments described. Similar results were obtained with all mutants. All r phages were T4D mutants obtained from H. Berger. The wild phage used was $T4BO_1{}^r$. Phage stocks and parental phages were prepared on *E. coli* CR63, whereas *E. coli* B23 was used in the experiments.

Analytical methods. Procedures for isotope labeling of phage DNA, DNA extraction, CsCl centrifugation, and estimation of net transfer from parent to progeny have been described previously (9). Pertinent details will be given in the text. 5-Bromodeoxyuridine (5-BU) labeling of the progeny in the transfer experiments was done by growing bacteria one to two generations in TCG medium containing 5-BU (0.2 mg/ml), FUdR (5 μg/ml), and uracil (20 μg/ml) to 3×10^8 cells per ml, before infecting with parental phages. Parental phages labeled in both capsid and DNA were prepared as follows. Bacteria were grown for two generations to 3×10^8 cells/ml in TAG medium (13) with 5 μg/ml of both phosphate and L-leucine. The culture was sedimented, resuspended in an equal volume of fresh TAG medium with 5 μg of phosphate per ml, ^{32}P, and 0.5 μg of L-leucine per ml. They were infected with cold r59 phages, and 10 min after infection ^3H-L-leucine was added. Eighty minutes later the infected culture was lysed with $CHCl_3$ and phages were purified by differential centrifugation.

RESULTS

Comparison of the mode of parent-to-progeny transfer in rII^+ and rII^- phages: net transfer. To estimate the net transfer of parental material to the progeny, *E. coli* B23 was grown for two generations in TCG medium and the culture was divided into two parts. Half was infected with wild, ^{32}P-labeled phage, and half was infected with ^{32}P-labeled rII^- phage, in both cases with seven phages per cell. At 5 min after infection, the suspensions were sedimented and resuspended in equal volumes of fresh medium. The trichloroacetic acid-precipitable counts after resuspension were considered as 100% of adsorbed phages. Both cultures were lysed with $CHCl_3$ 90 min after infection, although visible lysis started in the rII^--infected culture about 35 to 45 min after infection. The lysates were incubated with deoxyribonuclease (5 μg/ml) for 20 min at 37 C, and bacterial debris was removed by a low-speed centrifugation. The progeny phages were sedimented at high speed, washed once, and resuspended in a small volume of tris(hydroxymethyl)aminomethane (Tris)-salt (0.15 M NaCl, 0.01 M Tris-hydrochloride, *p*H 7.4). For both rII^+ and rII^- phages, 50 to 70% of the adsorbed parental phage DNA was recovered as trichloroacetic acid-precipitable, deoxyribonuclease-resistant counts in the supernatant from the low-speed centrifugation. In the case of rII^+, most of the counts were recovered in the purified progeny after sedimentation and washing, but in the case of rII^- the figure was substantially lower, usually 5 to 20% of the value found for wild phages. Annealing of phenol-extracted, sonically treated, and heat-denatured progeny DNA to isolated left and right strands (4) of T4 DNA showed that the parental contribution consists of equal proportions of both strands, as is the case in wild T4 phage (3). It was assumed that freshly prepared phages tend to be lost preferentially during the purification. To check this possibility, ^3H-labeled purified wild phages were added to the lysate before purification, and recovery of both labels (reference ^3H and parental ^{32}P in the lysate) was followed during purification. The results are shown in the top row of Table 1 and indicate that, indeed, the ^{32}P activity is preferentially decreasing during purification. This experiment does not exclude the possibility that the observed losses are restricted mostly to those progeny phages inheriting parental ^{32}P, which could be somehow different from the average progeny phage, because of, for instance, the association with bacterial cell debris. To check this hypothesis, bacteria were infected with ^{32}P-labeled parental phages, and ^3H-thymidine was added after infection to label the majority of the progeny phages. In this experiment, the specific activity of $^3H/^{32}P$ remained constant during purification

TABLE 1. *Net transfer of parental rII^- DNA to progeny phage in E. coli B*

Determination	Specific activity of $^3H/^{32}P$[a]	
	Lysate after deoxyribonuclease and low-speed centrifugation	Purified progeny phage
^3H-labeled purified phage added to the lysate from cells infected with ^{32}P-labeled parental phage	1.0	1.67
^3H-thymidine added after infection with ^{32}P-labeled parental phage	1.0	1.01

[a] Trichloroacetic acid-precipitable counts.

(Table 1, bottom row). The results prove that the observed losses are random with respect to the total progeny population (i.e., parent and progeny suffer similar losses during purification) and that those progeny phages inheriting the parental label are indeed representative of the majority of the progeny.

Parental contribution per progeny and its DNA. The mode of distribution of parental DNA in progeny phages and in their DNA was then compared for wild and rII$^-$ phages. Purified progeny obtained from heavy hosts infected with light radioactive rII$^-$ or rII$^+$ [multiplicity of infection (MOI) of 7] were analyzed in CsCl in several ways (Fig. 1). One part was analyzed as intact phage (top row). Another part of the purified phage was extracted with phenol, and its DNA was banded in 9.3 molal CsCl (bottom row). The phage gradients were collected in shell vials, part of each fraction was removed for counting and plating, and the remainder was used for further analysis, described below. The DNA fractions were collected directly on fiberglass filters in scintillation vials.

Comparison of the distribution of phage in CsCl reveals marked differences between wild and rII$^-$ phages. Although parental ^{32}P in the wild progeny formed a homogeneous peak of 7 to 10% average contribution per progeny phage, similar to that previously described (9), the parental ^{32}P in the rII$^-$ progeny phage was inhomogeneously distributed. Density classes ranged from heavy to light locations. While performing CsCl analysis, efficiencies of recovery were estimated. In both gradients, the added ^{3}H-reference phage was recovered completely in the collected fractions. Although in the wild progeny phage the transferred ^{32}P was recovered quantitatively in the collected fractions, in the rII$^-$ progeny up to 70% of the parental label was left in the centrifugation tube upon collection. Slicing the tube showed that most of the residual ^{32}P is located in the upper part of the tube, thus being lighter than the average phage. (It has been experienced in this laboratory that material floating on CsCl gradients does not move with the water-oil interphase during collection but tends to roll against the walls of the tube as a disc-shaped pellet and adheres to the upper parts of the tube.) A comparison of the unconnected stars in Fig. 1, representing the summed per cent of ^{32}P recovered, reveals the sizable differences in the distribution between wild and rII$^-$ phages.

Analysis of DNA extracted from the progeny phages (panel B in Fig. 1) was generally consistent with the patterns found in the phage centrifugations, with a notable additional observation. In the case of rII$^-$, the conservative peak of DNA was more pronounced than in phage centrifugation. At first glance this sounds paradoxical. However, if one keeps in mind that in phage CsCl ^{32}P in rII$^-$ progeny encounters up to 70% losses (material adhering to the tube walls during fraction collection), the straightforward hypothesis will be that the parental DNA associated with this floating moiety is mostly conservative. This is documented below.

Reanalysis of various density classes of rII$^-$ phages. Three density classes of phages were isolated from the CsCl gradient of rII$^-$ progeny (Fig. 1); A, far recombined (i.e., banding close to the majority of heavy progeny); B, intermediate density, slightly denser than hybrid; and C, light phages close to the original parental location. The relative proportions between these classes varied rather strongly from experiment to experiment; however, all three were always represented. In addition, residuals from the centrifugation tube were extracted with detergent for analysis of its DNA (class D). Isolated moieties, with the exception of D, were reanalyzed in phage CsCl together with heavy and light ^{3}H-labeled reference phages. In addition, their DNA was extracted with phenol, with caution taken to minimize shearing, and analyzed in CsCl in native, denatured, and sonically treated form, after supplementation with heavy and light ^{3}H-labeled reference DNA. DNA from moiety D was analyzed only in its native form.

Reanalysis of moieties A, B, C, and D is shown in Fig. 2. The upper panels show phage CsCl analysis, proving that the moieties are indeed unique and reband at the original locations when rerun. The second panels represent analysis of phenol-extracted DNA from those phages. The third panels represent this DNA in denatured form, whereas the bottom panels show DNA which has been subjected to shearing by sonic treatment. The insert in the bottom panels show the analysis of native DNA from moiety D.

Vertical comparison of panels from top to bottom reveals that recombinant phages (moiety A) behaved in a manner similar to the majority of the wild progeny phages (9). The native gradients showed virtually no conservative DNA. Upon denaturation, the parental contributions were found mostly between hybrid and heavy progeny locations in the gradient, showing that they are covalently associated with heavy progeny subunits. Sonic treatment of moiety A released only hybrid fragments, showing that all parental DNA in the progeny resides in semiconservatively replicated subunits.

DNA from the intermediate class of phages (B) was found in two locations, one intermediate between heavy and light, and one coinciding

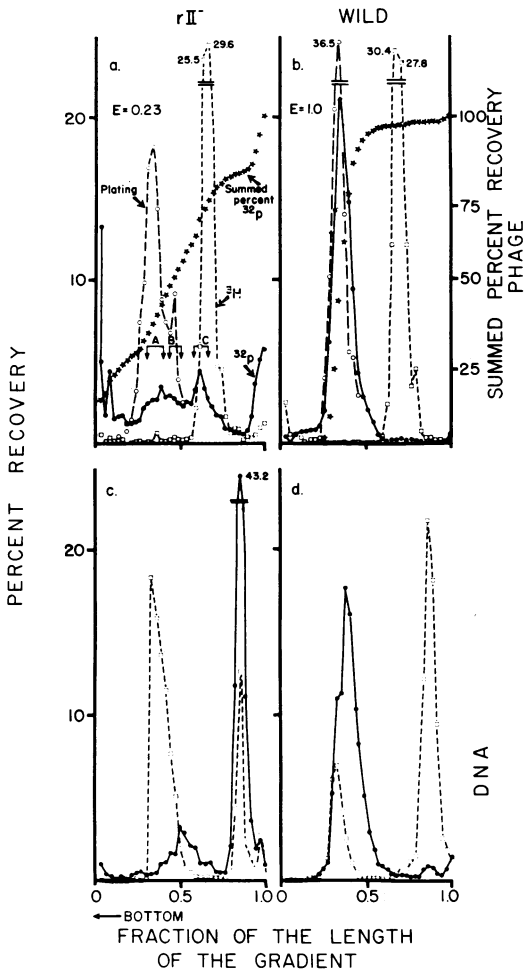

FIG. 1. *CsCl analysis of progeny phage (light parental in heavy host). E. coli B23 was grown for two generations in heavy medium and the culture was divided into two parts. Half was infected with light, ^{32}P-labeled r59, and half was infected with ^{32}P-labeled light wild phage (specific activity, 5 mCi/mg). At 5 min after infection, the cultures were sedimented and resuspended in fresh heavy medium. At 90 min after infection, the cells were lysed with $CHCl_3$. (Visible lysis started in the rII⁻-infected culture 50 min after infection.) Progeny phages were purified by differential centrifugation. Net transfer of parental ^{32}P to the purified progeny was 0.8% for the rII⁻ phage and 26% for the wild phage. Part of the phages was supplemented with light, ^{3}H-labeled wild reference phages and banded in CsCl (top row). These gradients were collected in shell vials. A part of each fraction was transferred to fiberglass filters for isotope estimation (Packard Tri-Carb scintillation spectrometer), and another part was diluted for plating to determine the location of the majority heavy progeny. From the remainder, fractions indicated with vertical arrows in the graph were pooled for reanalysis. Another portion of the purified progeny phages was phenol-extracted; the DNA was supplemented with light and heavy ^{3}H-reference DNA and banded in 9.3 molal CsCl (bottom row). These gradients were collected directly on fiberglass filters. Unconnected stars in the graphs represent summed per cent recovered ^{32}P. Efficiency of recovery (E) is calculated as $^{32}P/^{3}H$ recovered within the gradient divided by $^{32}P/^{3}H$ in the input to the gradient. Left panel, rII⁻ progeny; right panel, wild progeny.*

with the light reference. Denaturation yielded similar banding patterns; however, the light moiety was slightly enriched, as if some of the parental fragments are attached to progeny DNA by hydrogen bonding only, without covalent repair. After sonic treatment (not shown here), parental label moved from recombinant to hybrid position in the gradient, as was the case for moiety A, showing that the parental contributions reside in semiconservatively replicated subunits.

Since the phages, from which this DNA class was extracted, do not contain any significant conservative (parental density) moieties, the conservative DNA must reside together with progeny DNA inside the phage head. Whether indeed these phages contain two fragments of DNA physically unattached, or connected by a liable joint very sensitive to shearing, is presently not known.

Reanalysis of the moiety of phages banding close to parental density (class C) revealed that the DNA extracted from those phages overlaps with the light reference in all three permutations. Analysis of this DNA on alkaline sucrose gradients (not documented here) showed mostly integral DNA, with a slight shoulder corresponding to, at most, one to two nicks per strand.

Thus, in rII⁻ phages there is a sizable component of unreplicated phages which may represent truly conservative transfer, or, more trivially, contaminating unadsorbed or desorbed parental phages which were not removed by washing the infected cells. An experiment (*see below*) with parental phages labeled simultaneously with ^{32}P (DNA) and ^{3}H-leucine (capsid) discriminates between these possibilities.

The analysis of native DNA from residuals adsorbed to the centrifugation tube (moiety D; insert in bottom panel) shows that here parental DNA was largely conservative, unattached to progeny DNA.

In summary, this experiment indicated that in rII parental DNA tends to be transferred to the progeny as larger fragments, partially unrepaired, sometimes completely conservative. Three classes could be preparatively isolated. Class A consists of recombinant phages similar

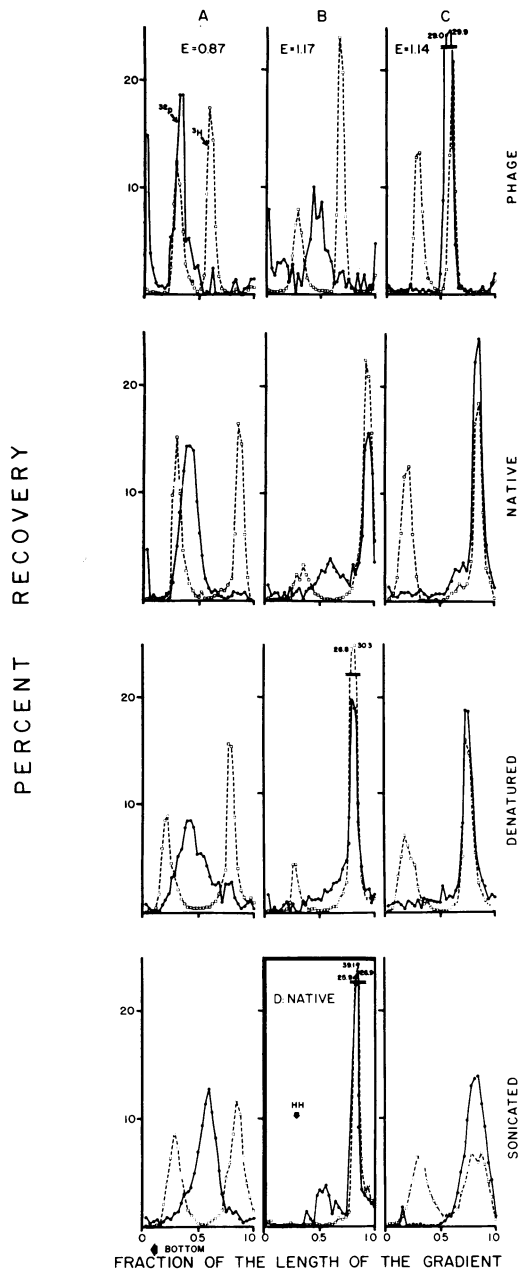

FIG. 2. *Reanalysis of various density classes from rII⁻ progeny (light parental in heavy host). Moieties A, B, and C from the previous experiment, indicated with arrows in Fig. 1, were pooled, dialyzed for 4 hr against Tris-salt (0.15 M NaCl, 0.01 M Tris-hydrochloride, pH 7.4) to remove the CsCl, and reanalyzed after supplementation with ³H-labeled heavy and light references. Part was banded in CsCl (top row). From another part, DNA was extracted with phenol. This DNA was analyzed in native form (second row), after heat-denaturation in the presence of 1% HCHO (the CsCl gradient in this case contained the same concentration HCHO (third row) and after sonic treatment to about 500,000 molecular weight (bottom row). Moiety D was extracted from the walls of the centrifugation tube with 1% sodium dodecyl sulfate in Tris-salt. Ninety-five per cent of the ³²P in the tube was recovered in the liquid. DNA was extracted with phenol and banded in CsCl in native form (insert in bottom row). The vertical arrow in this graph indicates the position of heavy DNA. Efficiency of recovery (E) in the phage gradients was estimated as described in the legend to Fig. 1.*

to the majority of the progeny phages produced in infection with wild T4, the composition of which is schematically shown in part A of Fig. 3. Class B consists of intermediate recombinants containing DNA with conservative (both strands light) and semiconservative subunits attached to progeny DNA without covalent repair, as well as conventional covalently linked recombinants (part B of the figure). The larger parental contribution per molecule in this class may be in the form of one or several pieces. The third class (C) consists of phages with parental DNA with not more than one or two nicks per strand. A fourth class, not illustrated in the scheme, consists of phages floating on the CsCl gradient, in which the parental DNA is largely conservative.

Transfer of nonreplicated parental genomes in rII⁻-infected E. coli B: proof of conservative parent-to-progeny transfer. In the previous experiment, the occurrence of conservative parental DNA as the exclusive DNA component of a class of progeny phages derived from the infection of heavy bacteria by light, radioactive phages was found. It was important to establish whether this was due to contaminating unadsorbed phages, phages desorbed without injecting their DNA, or true transfer involving injection and maturation of parental DNA into a new capsid. To discriminate between these possibilities, parental phages labeled with ³H-L-leucine (in the capsid) and ³²P (in the DNA) were used in a parent-to-progeny transfer experiment. Figure 4 shows the results of CsCl analysis of the parental phage and of the progeny obtained after infecting a heavy host with such parental phage (MOI of 6). The distribution of ³²P in the progeny gradient resembled that shown in Fig. 1, whereas ³H was found only in the parental location of the gradient. Figure 4 shows these areas of the gradients, with recoveries normalized to 100% within the areas included in the graphs. The straight lines above the graphs represent specific activities of ³H/³²P in each fraction. The total recovered counts per minute of ³H and ³²P in the light regions of the gradients were summed

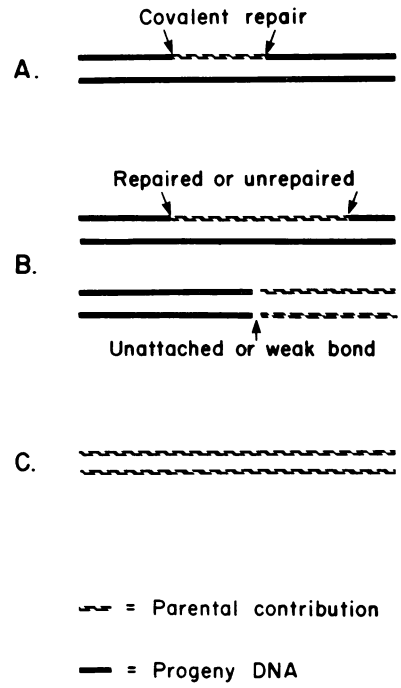

FIG. 3. *Models for molecular recombination in T4rII⁻ phage.*

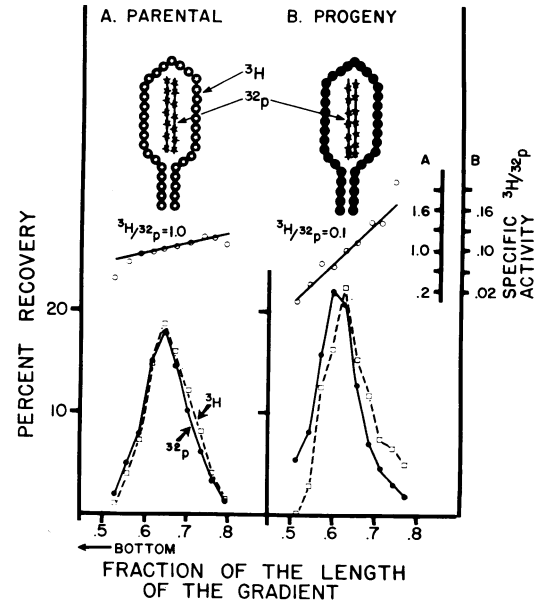

FIG. 4. *Conservative transfer in rII⁻-infected cells. Parental phages labeled with ^3H-L-leucine (specific activity, 80 mCi/mg) and ^{32}P (specific activity, 4 mCi/mg) were prepared as described in Materials and Methods. E. coli B23 was grown in heavy medium for one generation and infected with this parental phage (MOI 6). At 5 min after infection, the culture was sedimented and resuspended in fresh, heavy medium. At 45 min after infection, $CHCl_3$ was added and the progeny phages were purified. The net transfer of parental ^{32}P to the purified progeny was 4.5%. Parental and progeny phages were then banded in CsCl. For both phages, the amount of radioactivity between 50 and 80% of the length of the gradient, within which area the light parental banded, was summed and considered as 100% of recovered activities in constructing the graph. Specific activities of $^3H/^{32}P$ in each fraction are plotted above the graphs of recovered activities. The average specific activity of $^3H/^{32}P$ in the peak was obtained by summing the recovered activities between 50 and 80% of the length of the gradient and is expressed by the bold-faced numbers. Left panel, parental phage; right panel, progeny phage.*

and expressed as specific activities of $^3H/^{32}P$ in bold-faced numbers over the graphs.

Scrutiny of the figures reveals that, in the parental phage (left panel), ^3H and ^{32}P did not overlap perfectly, the ^{32}P peak being at a slightly denser location than ^3H, leading to an increased specific activity of $^3H/^{32}P$ towards the top of the gradient. Whether this was due to differences in the amount of protein or DNA between phages remains to be proven, as is the generality of this finding for other T4 phages. This phenomenon may well deserve further elaboration.

Analysis of the conservative moiety of the progeny (right panel) reveals a specific activity of $^3H/^{32}P$ which is 10% of that found in the parental phage. This small amount of ^3H may very well be accounted for by residual unadsorbed parental phages. The remainder of the conservative peak must indeed be due to conservative transfer of nonreplicated parental DNA, a phenomenon not previously described in T-even phages. It may be noted that the displacement between the ^3H and ^{32}P peaks is greater in the progeny than in the parental phage. This agrees with a general observation that rII⁻ phages prepared on *E. coli* B23 are slightly denser than those prepared on *E. coli* CR63 (and in both cases slightly denser than the wild reference phage) and further strengthens the argument that most of the progeny phages which band at the parental location contain DNA which has been injected and matured in a new capsid without replication, thereby receiving the density characteristics of the new host.

Effect of multiplicity on the conservative transfer. There are two mechanisms by which conservative transfer may occur: a nonreplicating DNA molecule may be "rescued" by enzymes coded for by simultaneously injecting, actively replicating phages, or it may, without replication, somehow be able to catalyze by itself all functions

necessary for production of mature progeny. If the former is the case, conservative transfer should be restricted to high multiplicities of infection only. The previously described experiment was performed with multiple infection and does not permit discrimination between the alternatives. An experiment allowing the direct comparison between phages from singly and multiply infected cells was therefore performed. With a poissonian distribution of infecting phages per cell, a multiplicity of 0.1 would result in single infection in 95% of the infected bacteria, double infection in 4.7%, and more than two phages per bacterium in 0.3% of the cells. Approximately 5% of the progeny, containing 10% of the parental ^{32}P, will therefore be derived from multiply infected cells. An average multiplicity of eight phages per cell, on the other hand, would lead to single infection in only 0.3% of the cells, contributing a negligible amount to the parental ^{32}P in the progeny. If conservative transfer is determined by the number of infecting phages, drastic differences should be expected in the density distribution of the progeny phages.

A culture of heavy bacteria was divided in two portions, which were infected with parental phages labeled with ^{3}H-leucine (capsid) and ^{32}P (DNA) at multiplicities of 0.1 and 8, respectively. Multiplicities were calculated from the ^{32}P content of the phage and confirmed by plating of infected centers. The viability of the parental phage, as estimated from ^{32}P content and plating, was 80%. The resulting progenies were analyzed in CsCl after purification (Fig. 5). Some ^{3}H was found in the light region of the gradient, probably belonging to the capsids of contaminating parental phages. The ^{3}H in the top of the gradient most likely resides in empty parental capsids which have not been washed away during the purification of the progeny. The distributions of ^{32}P in the two gradients shows that two clear peaks, corresponding to "recombined" and "conservative" phages, were found after multiple infection (left panel). With single infection, a more "smeared" distribution of parental label was found (right panel). Significantly, however, the total recovery of ^{32}P in the light region was quite similar, around 17% of the total recovery within the gradient, in both cases. The specific activity of $^{3}H/^{32}P$, calculated for the light regions of the gradients in the same way as in Fig. 4, was even slightly lower in the case of low multiplicity (1.0, 0.4, and 0.2, respectively, for parental, MOI 8 progeny, and MOI 0.1 progeny); this difference, however, may not be significant. The differences between the distribution patterns are far less striking than the similarities, and the

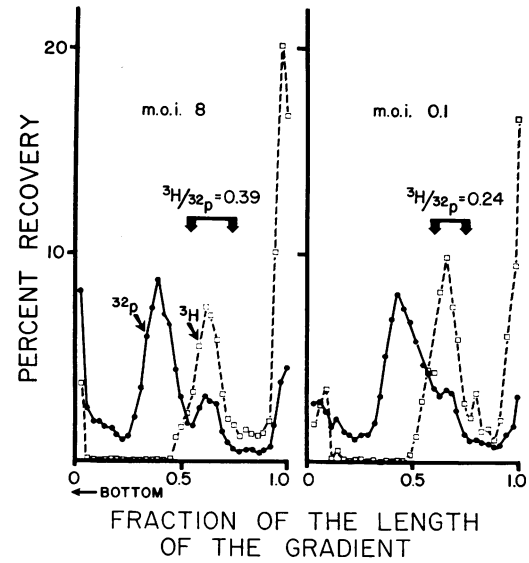

FIG. 5. *Effect of multiplicity on conservative transfer in rII-infected cells. E. coli B23 grown in heavy medium for two generations to 3 × 10⁸ cells/ml was infected with light r59 phages labeled with ^{3}H-L-leucine (specific activity, 266 mCi/mg) and ^{32}P (specific activity, 10 mCi/mg), part with an MOI of 8 (A) and part with an MOI of 0.1 (B). At 5 min after infection, the suspensions were sedimented and resuspended in equal volumes of fresh heavy medium. At 35 min after infection, the infected bacteria were superinfected with cold wild phage, (MOI of 30), and 5 min later they were lysed with $CHCl_3$. Progeny phages were purified and banded in CsCl. Net transfer of parental ^{32}P to the progeny was 0.5% for A and 0.9% for B. The specific activity of $^{3}H/^{32}P$ in the light region of the gradient, expressed in bold-face figures on the graph, was obtained by summing the activities of ^{3}H and ^{32}P recovered between the vertical arrows. The specific activity in the parental phage, calculated in the same way, was 1.0. Left panel, MOI of 8; right panel, MOI of 0.1.*

results strongly favor the second hypothesis: nonreplicating genomes are able to catalyze all functions necessary for maturation.

Similar results have been obtained in this laboratory with transfer of parental DNA from polymerase-negative amber phage to progeny in Cairns' polymerase-negative strain of *E. coli* (C. Howe and A. W. Kozinski, *unpublished data*). The results are at variance with several reports indicating the necessity for DNA replication before the transcription of late messenger ribonucleic acid (2, 12, 15). Recently, Riva et al. (Fed. Proc. 29:465, 1970) indicated an uncoupling between the transcription of late messenger ribonucleic acid and DNA replication.

DISCUSSION

When *E. coli* B is infected with wild T4 phage, parental DNA is invariably transferred to the progeny as short, semiconservatively replicated fragments even when recombination is studied under conditions of very low net DNA synthesis, i.e., in the presence of FUdR (8). In contrast to this, rII mutants transfer parental label to the progeny in a very inhomogeneous manner, ranging from short semiconservatively replicated fragments to fully conservative DNA molecules. Although the relative proportion between different classes obtained varies from experiment to experiment, maturation of nonreplicated conservative parental DNA as the sole DNA component of progeny phages is consistently found. This is true for both single and multiple infection. It is conceivable that nonreplicating genomes participate in recombination, leading to the formation of concatenates longer than the DNA molecule of mature phage. Since the "conservative progeny" contains integral parental DNA, the concatenates would have to be cut precisely at the "ends" of the original molecules participating in the concatenate formation, a rather unlikely mechanism. An intermediate concatenate stage is even more unlikely in the case of conservative progeny from singly infected cells, simply because there is no more DNA than one phage genome present in the infected cell during the latent period, namely the injected nonreplicating parental DNA molecule. It seems likely that concatenate formation is not an obligatory intermediate in the intracellular development of T4 bacteriophage.

In comparison with wild phage, less of the parental label transferred to the progeny is recovered in the purified phage. In contrast to wild T4, this purified progeny does not band quantitatively in CsCl, but 10 to 70% of the phages are located as a floating pellet which sticks to the wall of the centrifugation tube during collection. CsCl analysis of DNA and phages revealed that DNA contains more conservative material than do the phages. Consequently, there was reason to assume that apart from "conservative" and "semiconservative" phages, there are sizable amounts of phages which contain conservative DNA weakly or not at all attached to progeny DNA. The presented data are consistent with a much lower recombination frequency in rII than in wild phages, a recombination pattern in which the produced fragments are large and inhomogeneous, and a sizable proportion of conservative parental DNA is transferred as such to the progeny.

The experiments described here do not provide any clues to the mechanism(s) causing this reduced recombination frequency. The fact that rII mutants do not need phage polynucleotide ligase to produce viable progeny (1, 6) suggests that they may be deficient in nucleases counteracting ligase. It has been shown that chloramphenicol added at critical times after infection prevents both endonuclease activities and recombination, suggesting a correlation between the two phenomena (10). The reported data are in agreement with the hypothesis that rII mutants are deficient in a nucleolytic activity required for molecular recombination.

rI mutants form plaques on *E. coli* B phenotypically indistinguishable from those formed by rII mutants. The behavior of rI mutants in this system is therefore of interest. Berger and Kozinski (1) found that an rI mutant has a stringent requirement for ligase. In contrast to this, Karam (6) reported that among "revertants" of lig^- amber phage plated on a nonpermissive host, both rI^- lig^- and rII^- lig^- double mutants were found, indicating that the two r mutants are both capable of suppressing the ligase mutation. When an rI mutant (r48, obtained from H. Berger) was tested in the parent-to-progeny transfer system described in this paper, results very similar to those found for rII mutants were obtained. The possible role of rI mutations in ligase suppression will therefore be reinvestigated.

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