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Genetic Recombination of Bacteriophage T7 In Vivo Studied by Use of a Simple Physical Assay

DONALD LEE AND PAUL D. SADOWSKI*

Department of Medical Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

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A new physical method was developed to assay genetic recombination of phage T7 in vivo. The assay utilized T7 mutants that carry unique restriction sites and was based on the detection of a new restriction fragment generated by recombination. Using this assay, we reexamined the genetic requirements for recombination of T7 DNA. Our results were in total agreement with previous findings in that recombination required the products of genes 3 (endonuclease), 4 (primase), 5 (DNA polymerase), and 6 (exonuclease). Recombination was found to be independent of DNA ligase and of DNA packaging and maturation functions.

The process of genetic recombination of bacteriophages has been studied in the past by using two general approaches.

In the first of these, genetic recombination between two markers is measured and the system is studied by determining the effect of given mutations upon recombination frequencies. Typically, one uses conditional lethal mutations under semipermissive or nonpermissive conditions to study the role of certain gene products in recombination. This approach has been used to study recombination in the phage T4 system (1, 2) and in the phage T7 system (8, 15) but suffers from the drawback that one only looks at the phage progeny in which successful DNA packaging has occurred. Furthermore, since one is working with conditional lethal mutations under nonpermissive conditions, burst sizes are drastically reduced.

An alternative approach is to study the intracellular DNA by physical means in an effort to isolate recombination intermediates and thereby deduce what the pathways of recombination might be and what gene products might be needed. Since such experiments frequently involve the use of density-labeled parental phages under replication-blocked conditions (4, 29, 30), the yields of recombinant DNA are frequently low. Such experiments are further complicated by uncertainties created by the use of density-labeled DNA and by the fact that DNA replication must be inhibited. Finally, the major techniques used for such studies involved buoyant density centrifugation and electron microscopy, both of which are rather time-consuming.

We have been studying the mechanisms of genetic recombination promoted by bacteriophage T7 both in vivo (8) and in vitro (19, 22). Previous in vivo studies (8, 15) have implicated the T7 products of genes 3 (endonuclease), 4 (primase), 5 (DNA polymerase), and 6 (exonuclease) in the process of recombination, and in vitro studies have suggested the possible existence of three distinct pathways of recombination (19).

It is apparent that studies of genetic recombination would be greatly facilitated by the development of a simple physical assay which would be applicable to both in vivo and in vitro studies. In this paper, we report the development of such an assay which detects recombinant DNA by a change in the pattern of migration of DNA fragments after restriction enzyme digestion and agarose gel electrophoresis. We have applied this assay to studying the process of T7 genetic recombination in vivo.

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* B was obtained from F. W. Studier (Brookhaven National Laboratory) and was used as the nonpermissive host. *E. coli* BBW/1 is a *supF* strain obtained from R. Hausmann (University of Freiburg) and was used as the permissive host for all T7 amber mutants. *E. coli* BL2 is a DNA ligase-deficient sup^0 host (28) and was used as the nonpermissive host for the ligase-deficient (1.3^-) mutant (11). (Further details are given by Roeder and Sadowski [19]).

Bacteriophages. All amber phages have been described previously (17). These are *am*HA13 (1.3^-), *am*29 (3^-), *am*147 (6^-), *am*17 (9^-), and *am*10 (19^-). T7 4*am*20 and T7 15*am*114 were found by mass screening of T7 amber mutant DNAs to have *Hin*dIII digestion sites at 22 and 72%, respectively, from the left end of the T7 genome. Likewise, T7 5*am*28 was found by others (31) to have an *Eco*RI site at 46%. The phages bearing the *Hin*dIII sites were designated *sHin*dIII$_1$ and *sHin*dIII$_2$, respectively, and those bearing the *Eco*RI site were designated *s*RI (31). Wild-type DNA

is insensitive to both *Hin*dIII and *Eco*RI (20). All phage crosses were done in T broth plus $MgSO_4$ as described previously (8), and all phages were prepared in LB medium (18).

Restriction enzymes. *Eco*RI and *Hin*dIII enzymes were prepared by the methods of Bingham et al. (3) and Smith (24), respectively. *Bgl*I and *Bst*NI (*Eco*RII isoschizomer) were purchased from New England Biolabs, Inc.

Recombination assay. Cells were grown at 30°C to an optical density at 650 nm of 0.5 (ca. 5×10^8 cells per ml) in LB medium. A 1-ml amount of cells was then removed and infected with the appropriate phages at a multiplicity of 5 for each parent. The infected cells were then left on ice for 7 min, after which 95% of the phages had adsorbed. The cells were shaken vigorously at 30°C for 20 to 30 min. The cells were chilled and harvested by centrifugation (12,000 rpm, 5 min, 4°C) and then suspended in 50 μl of DNA buffer (10 mM Tris-hydrochloride [pH 7.5], 50 mM NaCl, and 1 mM EDTA). The cells were frozen and thawed once, adjusted to 50 mM EDTA, and treated with Lysozyme (400 μg/ml; Worthington Diagnostics) at 0°C for 15 min. Deproteinization was done with self-digested pronase (5 mg/ml, 37°C, 4 h; Calbiochem) and 1% Sarkosyl. The samples were then phenol extracted and concentrated by ethanol precipitation for restriction enzyme digestions. DNA samples were suspended in 30 μl of cutting buffer (10 mM Tris-hydrochloride [pH 7.4], 66 mM KCl, 10 mM $MgCl_2$, 100 μg of gelatin per ml, and 6 mM 2-mercaptoethanol), and 1 U of *Hin*dIII enzyme (and *Bgl*I and *Bst*NI if required) was added to a final volume of 40 μl. Incubation was done at 37°C for 2 h, after which 4 μl of 1 M Tris-hydrochloride and 1 U of *Eco*RI enzyme were added. Samples were further incubated at 37°C for 1 h. Digested samples were then adjusted to a final concentration of 5% glycerol, 0.025% bromophenol blue, and 50 mM EDTA in a final volume of 100 μl, and 35 μl was subjected to agarose gel electrophoresis.

Agarose (Sigma Chemical Co.) was made up to 0.5% in 40 mM Tris-hydrochloride (pH 7.8)–5 mM sodium acetate–1 mM EDTA. Electrophoresis was done at room temperature. Ethidium bromide-stained gels (1 μg/ml) were photographed over a UV transilluminator by using a Polaroid type 55 P/N film.

Labeling by nick translation. The T7 DNA fragment E of a *Dpn*II digestion was used as the probe for filter hybridization. This DNA was separated by polyacrylamide gel electrophoresis and recovered by electroelution (12). The DNA was then labeled with [α-^{32}P]dCTP or [α-^{32}P]dATP in vitro by the method of Rigby et al. (16) to a specific activity of 2×10^7 to 4×10^7 cpm per μg of DNA.

Detection of recombinant fragments by filter hybridization. Agarose gels were blotted onto nitrocellulose (Millipore Corp.) sheets by the method of Southern (25) and then hybridized by the procedure of Jeffreys et al. (7), modified slightly as follows. The filter paper was prehybridized at 65°C for 1 h in 10 ml of 3× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate) supplemented with 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.1% sodium dodecyl sulfate, and 50 μg of denatured calf thymus DNA per ml. The solution was then replaced with 1× SSC containing the same supplements plus 10% dextran sulfate (Pharmacia Fine Chemicals), and incubation was continued for 1 h. Heat-denatured ^{32}P-labeled DNA (1.5×10^6 cpm) was then added to the same solution, and hybridization was continued overnight. After hybridization, the filters were washed twice with 1× SSC–0.1% sodium dodecyl sulfate and twice with 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C until the nonspecific background, as detected by a radioactivity monitor, was low. The air-dried filter was then exposed overnight on Kodak XR1 film at −70°C with an intensifying screen.

RESULTS

General strategy. Our general goal was to develop a relatively simple and sensitive physical assay which would be applicable to in vivo and in vitro studies of phage T7-promoted recombination. Our strategy was to detect physical recombination in the DNA molecule between two unique restriction enzyme sites. Recombination could then be readily measured as a change in restriction enzyme digestion patterns by agarose gel electrophoresis. Such an assay has been exploited in the study of λ*int*-promoted recombination in vitro (13).

T7$^+$ DNA is resistant to digestion by restriction enzymes *Eco*RI and *Hin*dIII (20). We used three T7 mutants whose DNA is susceptible to either *Eco*RI or *Hin*dIII. The mutations are presumed to be caused by silent base pair changes, since they have no apparent effect on phage growth. Furthermore, none of the mutant restriction sites is in the amber site, since the two sites can be separated by recombination (unpublished observation). One of these mutants, T7 5*am* *s*RI (sensitive to *Eco*RI), contains an *Eco*RI restriction site at 46% of the T7 genome and is the same as that reported previously (31). Two additional mutants, T7 4*am* *s*Hin$dIII_1$ and T7 15*am* *s*Hin$dIII_2$, carry *Hin*dIII sites at 22 and 72%, respectively, of the DNA.

T7 *s*Hin$dIII_1$ DNA, when digested by the *Hin*dIII restriction enzyme, produces two DNA fragments, one 22% and the other 78% the length of mature T7 DNA. When the digest is subjected to agarose gel electrophoresis in the presence of ethidium bromide, the two separate bands will fluoresce under UV irradiation. *Eco*RI-digested T7 *s*RI DNA similarly generates two fragments, but of different sizes: 46 and 54%. However, T7 *s*Hin$dIII_1$ *s*RI DNA formed by recombination between the two sites would generate three fragments 22, 24, and 54% in length. It is thus possible to detect recombination by using these mutants and to monitor the formation of a recombinant band (Fig. 1) which would have a length of 24%. By coupling appropriate mutations to these unique restriction sites, we should

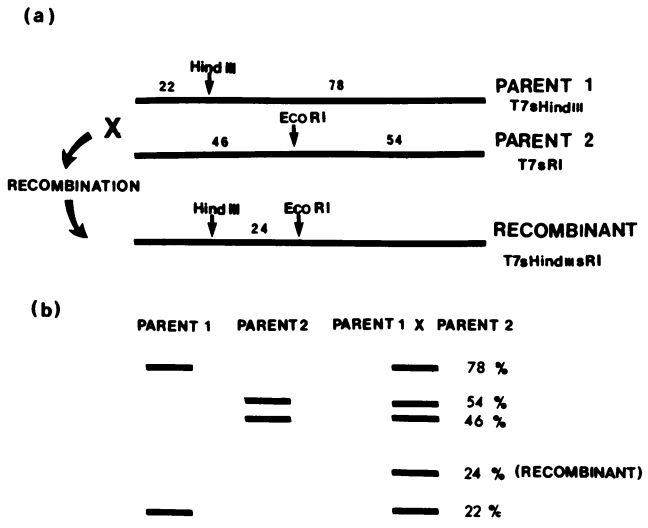

FIG. 1. *Schematic representation of strategy for restriction assay for T7 genetic recombination, based on detecting the formation of a 24% recombinant DNA fragment. (a) Relevant restriction maps of the parental DNAs, given with sizes expressed as percentages of T7 genome length. (b) Expected gel patterns after restriction digestion of parental and recombinant DNA with EcoRI and HindIII.*

then be in a position to ask what role specific genes play in T7 recombination.

Feasibility of approach. Since most T7 genes thought to be involved in recombination are also essential for phage production, it was first important to demonstrate that our assay allowed the detection of recombination in intracellular DNA. The results of such an experiment are shown in Fig. 2. *E. coli* BBW/1 was multiply infected with T7 *4am sHind*III$_1$ and T7 *5am s*RI, and cells were harvested just before lysis. Intracellular DNA was prepared and digested with *Hind*III and *Eco*RI as described above. At 30 min after infection, the bulk of the host DNA was degraded by the gene 3 endonuclease and the gene 6 exonuclease (21). The restriction enzymes *Eco*RI and *Hind*III also helped in eliminating the host DNA, though only to a limited extent. When both parental phages were present during infection, the 24% recombinant band (Fig. 2) was readily visible. It was also noticed that some larger DNA was present as bands migrating near the position of mature-length DNA. These were probably derived from restriction enzyme digestion of immature DNA, which is known to be joined together through the terminally redundant ends (10). Digestion of a concatemer formed by two molecules with the same restriction sites would yield a mature-length fragment of DNA as one of the products. Digestion of a mixed concatemer would result in the production of bands which were either 124 or 76% the length of mature T7 DNA. These bands would contain the junction of the terminal redundancies. Reciprocal recombinants containing no restriction sites would not be distinguished, since these would migrate close to the concatemer-derived DNA. These results demonstrate that recombination can be readily detected in intracellular DNA in this recombination assay system.

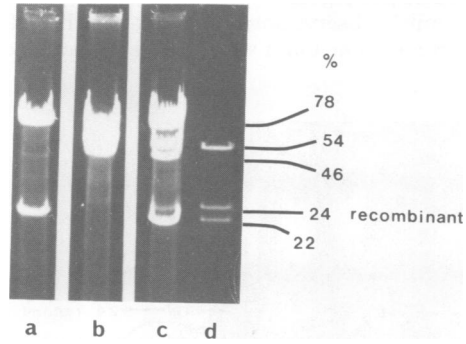

FIG. 2. *Restriction assay of infections with 4am sHindIII$_1$ and 5am sRI in E. coli BBW/1 (supF). (Lane a) Infection with 4am sHindIII$_1$ only. (Lane b) Infection with 5am sRI only. (Lane c) Infection with both parents. (Lane d) Reference DNA formed by digesting sRI sHindIII$_1$ DNA with EcoRI and HindIII. Numbers represent fragment sizes expressed as percentages of the unit length of T7 DNA (see also Fig. 1). Infections were accomplished as described in the text.*

Role of T7 genes in recombination. (i) Genes involved in DNA maturation and packaging. To investigate whether phage DNA maturation and packaging play a role in T7

recombination, we multiply infected *E. coli* B (*sup*^{0}) with T7 4*am* 9*am* *sHin*dIII$_1$ and T7 5*am* 9*am* *s*RI. The T7 gene 9 function is required for the formation of proheads (18) and is therefore absolutely essential for packaging (6). The 4*am* and 5*am* markers in these parents were necessary for the construction of restriction-sensitive mutants that also carry the gene 9 amber mutation, since we had no selection for these silent restriction markers. The presence of 4*am* and 5*am* should not interfere with the assay, since the two phages complement one another for these functions.

The results show that a single infection gave only a small amount of parental DNA (Fig. 3, lanes a and b) owing to the limitation of DNA replication imposed by mutation in gene 4 or 5, whereas double infection produced much more DNA, owing to complementation (Fig. 3, lane c). Recombination was apparent when both permissive and nonpermissive cells were infected, as indicated by the presence of the 24% recombinant band (Fig. 3, lanes c and d). This implies that recombination is unaffected by the absence of DNA packaging.

Similar observations were made when 4*am* 19*am* and 5*am* 19*am* were used to infect *E. coli* B (Fig. 3, lanes e and f). Since the gene 19 function is required for DNA maturation (6), we concluded that recombination proceeds independently of both DNA packaging and maturation.

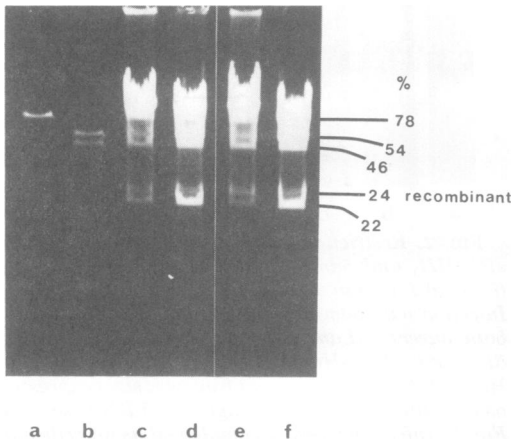

FIG. 3. *Role of gene 9 (lanes a through d) and gene 19 (lanes e and f) in recombination. For assaying the involvement of gene 9 in recombination, E. coli B was infected with 4am 9am sHindIII$_1$ only (lane 1), 5am 9am sRI only (lane b), or both (lane c). As a positive control, E. coli BBW/1 (sup$^+$) was infected with both 4am 9am sHindIII$_1$ and 5am 19am sRI (lane d). To assay for the involvement of gene 19, 4am 19am sHindIII$_1$ and 5am 19am sRI were used to infect E. coli B (lane e) and E. coli BBW/1 (lane f). Numbers represent fragment sizes expressed as percentages of the unit length of T7 DNA. Recombination was detected by the appearance of a 24% fragment.*

(ii) DNA metabolism genes. (a) Genes 4 and 5. T7 gene 4 (primase) and gene 5 (DNA polymerase) are both essential for DNA replication (27). The involvement of T7 primase (gene 4) in recombination was studied by multiply infecting *E. coli* B with T7 4*am* *sHin*dIII$_1$ and T7 4*am* 5*am* *s*RI. In the absence of the gene 4 product, DNA synthesis was reduced as expected (Fig. 4a, lanes a through c). Furthermore, no visible recombination band 24% the length of T7 DNA was present under nonpermissive conditions. For detecting the presence of an amount of the recombinant band that was not visible by staining with ethidium bromide, the agarose gel (Fig. 4a) was blotted onto a nitrocellulose filter and hybridized with ^{32}P-labeled T7 DNA by the method of Southern (25).

The probe chosen was fragment E of a *Dpn*II restriction digestion of T7 DNA which extends from 28.76 to 35.86% from the left end of the T7 genome (12). Because this region was within the recombinant fragment, this DNA probe should have been able to detect the presence of recombination. The results are shown in Fig. 4b. In the absence of the gene 4 product, no recombination was detected by ethidium bromide staining, whereas recombination was readily observed in the infection of permissive cells (Fig. 4b, lane d). A minute amount of recombination was detectable, however, by Southern hybridization (Fig. 4b, lane c). From reconstruction experiments we found that the Southern technique permitted the detection of 25 pg of DNA in a band. We estimate that the amount of recombination in the absence of the gene 4 protein is at least 1,000-fold less than is detectable under permissive conditions.

The role of gene 5 was studied in a similar fashion by using T7 5*am* *s*RI and T7 4*am* 5*am* *sHin*dIII$_1$ as the parents, and identical results were obtained (Fig. 4a, lanes e through f and Fig. 4b, lanes e through f). These findings, therefore, suggested that both DNA polymerase and primase are essential for recombination, in agreement with data reported previously (8, 15).

(b) Gene 1.3 (ligase). Since *E. coli* ligase can functionally substitute for the phage ligase (11), we chose to use *E. coli* BL2 (ligase deficient) as the nonpermissive host for the ligase$^-$ infection. Because of the position of the T7 ligase mutation, it was convenient for us to use a different combination of parents from those described previously. We therefore multiply infected the

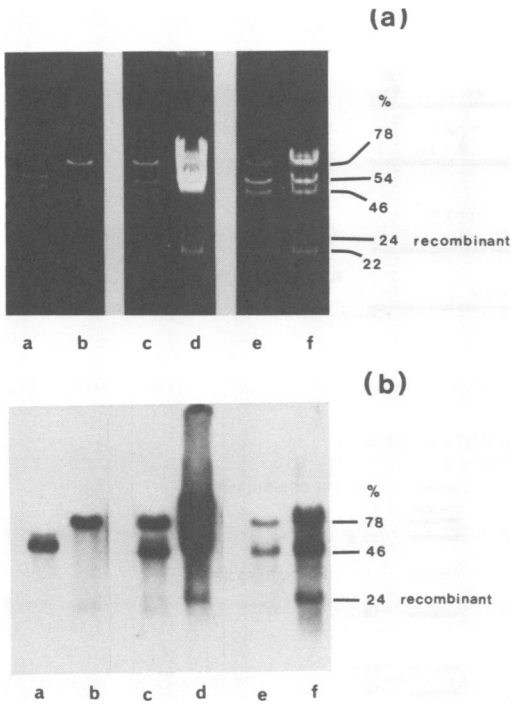

FIG. 4. *(a) Restriction assay for the involvement of gene 4 (primase) (lanes a through d) and gene 5 (DNA polymerase) (lanes e and f). To assay for the involvement of gene 4, E. coli was infected with 4am 5am sRI only (lane a) 4am sHindIII$_1$ only (lane b), or both (lane c). The permissive host E. coli BBW/1 was also infected with both phages as a positive control (lane d). Similarly, the involvement of gene 5 was assayed by infecting E. coli B (lane e) and E. coli BBW/1 (lane f) with 4am 5am 6am sHindIII$_1$ and 5am sRI. Numbers represent fragment sizes expressed as percentages of T7 DNA length. The 24% faint bands seen in lanes b, c, and e were due mainly to undigested host DNA, and not recombination, as shown by filter hybridization (see [b]). (b) Filter hybridization of the gel shown in (a). The gel was blotted onto filters as described in the text. T7 DNA fragment E from DpnII digestion was used as the probe. Since this DNA probe covers from 28.76 to 35.86% from the left end of the T7 genome (12), only the 78 and 46% parental bands and the 24% recombinant bands could be hybridized. Some faint bands, probably due to a slight contamination of the DNA probe, were also detected. The samples in each lane are in the same order as in (a).*

permissive and nonpermissive cells with T7 1.3*am* 5*am* *s*RI and T7 1.3*am* 15*am* *sHind*III$_2$. The *Hind*III site was positioned at 72%, and thus recombination would generate a 26% band upon restriction digestion (Fig. 5). Because of the poor resolution between the 26% recombinant band and the 28% parental band, we treated the DNA with *Bgl*I in addition to the *Eco*RI and *Hind*III restriction enzymes (Fig. 5). Since *Bgl*I restricts at positions 33.8 and 90.1% (20), we were able to completely prevent the 28% parental phage band from obscuring the recombinant (26%) band. The use of *Bgl*I also facilitated the visualization of a band 56% the length of T7 DNA which represented a band arising from the reciprocal recombinant DNA which had neither the *Eco*RI nor the *Hind*III site (Fig. 5).

The results of such an experiment are shown in Fig. 6. When ligase-deficient cells were infected with ligase-deficient T7, only parental DNAs accumulated since ligase is required for DNA synthesis. However, recombination between the parental DNAs was present, as the 26 (recombinant) and 56% (reciprocal recombinant) bands were readily visible. Ligase, therefore, did not appear to participate in the formation of the recombinant DNA bands. However, since these gels were run under nondenaturing conditions, it is possible that ligase would still be needed to repair single-strand breaks in the DNA that would go undetected in our assay.

(c) Genes 3 and 6. The products of genes 3 (endonuclease) and 6 (exonuclease) are responsible for the breakdown of host DNA after infection with T7 phage (21). The absence of either one of these gene products would lead to an accumulation of host DNA which would obscure the gel analysis in our recombination assays, since *Eco*RI and *Hind*III are not sufficient to degrade all of the host DNA. Therefore, to study the involvement of gene 3 or gene 6 in recombination, we used the *Bst*NI restriction enzyme as well as *Eco*RI, *Hind*III, and *Bgl*I. *Bst*NI (an isoschizomer of *Eco*RII) was able to completely degrade the host DNA to very small fragments. However, the same enzyme also cut T7 DNA at 5.8 and 20.6% (unpublished data). Fortunately, the cutting by this enzyme did not interfere with the detection of recombination: the assay can detect the formation of a 26% recombinant and a 56% reciprocal recombinant band (Fig. 7).

For studying the role of gene 3, cells were infected with 3*am* 5*am* *s*RI and 3*am* 15*am* *sHind*III$_2$. The results of this experiment are shown in Fig. 8. Recombination in a gene 3$^+$ infection was readily detected by the appearance of a 26% band in addition to the 56% reciprocal recombinant band (Fig. 8, lanes d through e). No recombination was apparent in a 3$^-$ infection (Fig. 8, lane c).

Similarly, cells were infected with 6*am* 9*am* *s*RI and 6*am* 15*am* *sHind*III. However we observed that when nonpermissive cells were singly infected with either of these 6*am* mutants, an anomalous DNA band appeared which was

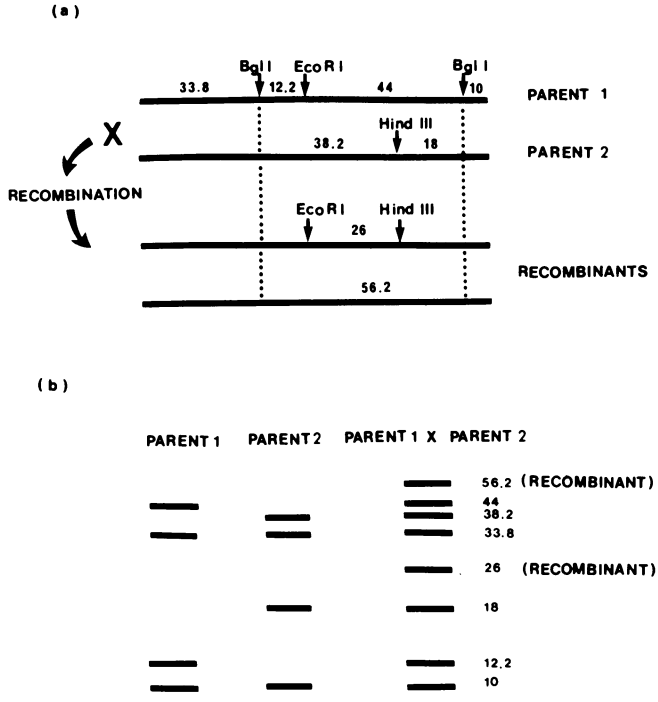

FIG. 5. *Schematic representation of strategy for assaying for the involvement of ligase in recombination. (a) Restriction maps of both parents (1.3am 5am sRI and 1.3am 15am sHindIII$_2$) and their recombinants. (b) Expected gel electrophoresis patterns after restriction digestions with EcoRI, HindIII, and BglI. Numbers represent the fragment sizes expressed as percentages of T7 DNA length. The assay was based on the detection of 26 and 56% fragments generated by recombination between the parents.*

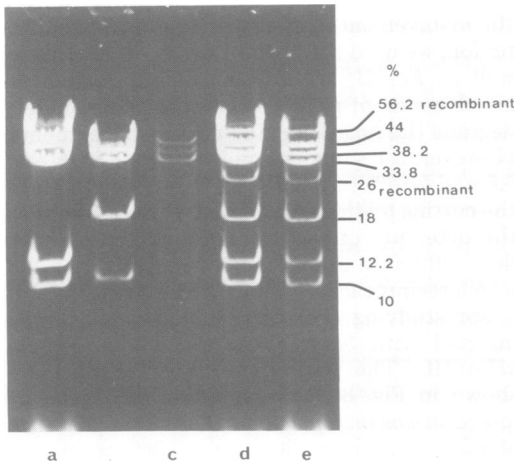

FIG. 6. *Restriction assay for recombination between 1.3am 5am sRI and 1.3am 15am sHindIII$_2$ in E. coli BL2 (lig$^-$ sup^0) and BBW/1 (sup$^+$). E. coli BBW/1 was infected with 1.3am 5am sRI only (lane a), 1.3am 15am sHindIII$_2$ only (lane b), or both (lane d). E. coli BL2 was infected with 1.3am 5am sRI and 1.3am 15am sHindIII$_2$ (lane c) or with 5am sRI and 15am sHindIII$_2$ (lane e). Numbers represent fragment sizes expressed as percentages of T7 DNA length.*

either 22 or 26% the length of mature T7 DNA, depending on the parent (Fig. 9, lanes a and b). Such anomalous bands could not be attributed to partial digestions, and their appearance was dependent on the presence of the 6*am* mutation, since no such bands were seen during the permissive infection (Fig. 9, lanes c and d). The origin of these anomalous bands is currently under investigation. Although the presence of an anomalous band 26% the length of T7 DNA interfered with our ability to detect a true recombinant band, we nonetheless could not detect the reciprocal recombinant band (56%) (Fig. 9, lanes e and f), and we therefore deduced that recombination was absent in the absence of gene 6 exonuclease. These experiments suggested that T7 nucleases coded for by genes 3 and 6 are required for recombination, in agreement with previous results.

The failure to detect recombination bands was not due to the decreased quantity of DNA in the infected cells, for the following reasons. Increasing the multiplicity of infection by fourfold had no effect, nor did loading as much as 10 times more DNA onto the agarose gel cause the recombinant DNA bands to become visible. These experiments, together with Southern blotting

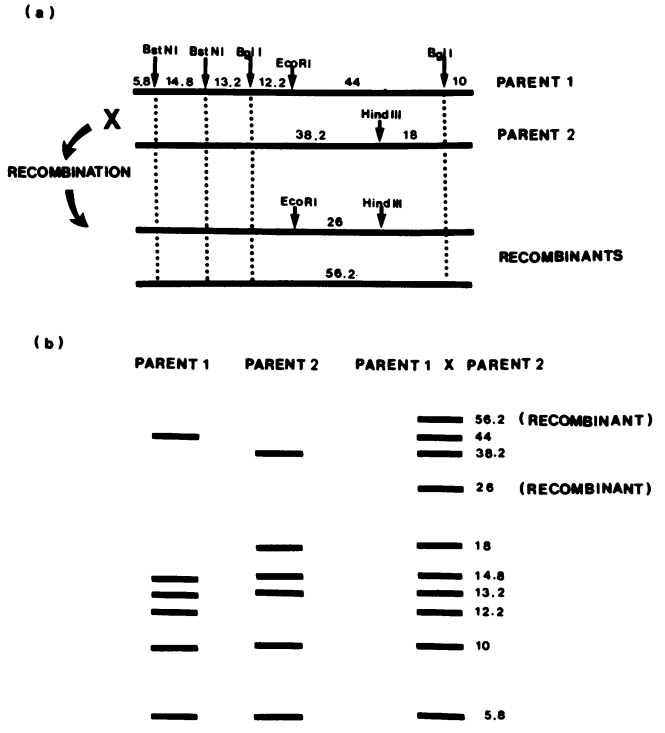

FIG. 7. *Schematic representation of strategy for assaying the involvement of endonuclease and exonuclease in recombination. (a) Restriction maps of both parents and their recombinants. (b) Expected gel electrophoresis patterns after restriction digestions with EcoRI, HindIII, BglI, and BstNI. Numbers represent fragment sizes expressed as percentages T7 DNA length. Recombination was detected by the formation of 26 and 56% fragments.*

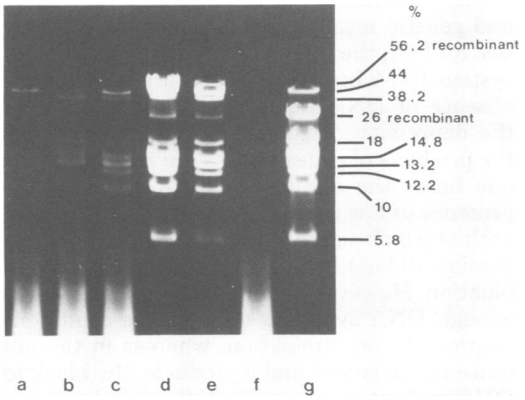

FIG. 8. *Restriction assay of infections with 3am 5am sRI and 3am 15am sHindIII$_2$. (lane a) Infection of E. coli B (sup^0) with 3am 5am sRI. (Lane b) Infection of E. coli B with 3am 15am sHindIII$_2$. (Lane c) Infection of E. coli B with both parents. (Lane d) Infection of E. coli BBW/1 (sup$^+$) with both parents. (Lane e) Infection of E. coli B with 5am sRI and 15am sHindIII$_2$. (Lane f) Uninfected cells. 5am 15am sRI sHindIII$_2$ DNA cut with EcoRI, HindIII, BglI, and BstNI was used as a reference (lane g). Numbers represent fragment sizes expressed as percentages of T7 DNA length.*

experiments, make us confident that little or no detectable recombination can be found when the products of genes 3, 4, 5, or 6 are absent.

Effects of nalidixic acid. Previous experiments have demonstrated that nalidixic acid has a minimal effect on recombination, whereas DNA synthesis is decreased significantly (8). In the present study, we reexamined the effects of nalidixic acid by using the restriction enzyme assay.

Recombination was assayed as described above. It was noticed (unpublished data) that nalidixic acid seemed to inhibit degradation of host DNA during T7 infection; therefore, recombination was assayed under conditions similar to those devised for examining the effects of mutations in genes 3 and 6. *E. coli* cells were infected with 5*am* sRI and 15*am* sHindIII$_2$, and nalidixic acid was added at the time of infection. As seen in Fig. 10, DNA synthesis was markedly reduced in the presence of as little as 5 μg of nalidixic acid per ml. However, recombination was not drastically affected until 30 to 50 μg of nalidixic acid per ml was present, as demonstrated by the appearance of both 26 and 56% recombinant bands.

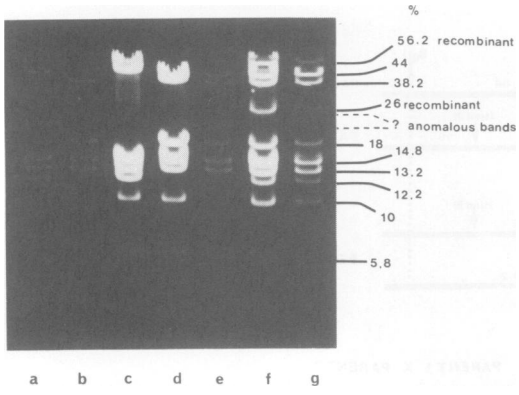

FIG. 9. *Restriction assay for the involvement of gene 6 (exonuclease). E. coli B (sup^0) was infected with 6am 9am sRI only (lane a), 6am 15am sHindIII$_2$ only (lane b), or both (lane e). E. coli BBW/1 (sup$^+$) was infected with 6am 9am sRI only (lane c), 6am 15am sHindIII$_2$ only (lane d), or both (lane f). As another positive control, E. coli B was infected with 5am sRI and 15am sHindIII$_2$ (lane g). Numbers represent fragment sizes expressed as percentages of T7 DNA length. The positions of the anomalous bands in lanes a, b, and e are also indicated.*

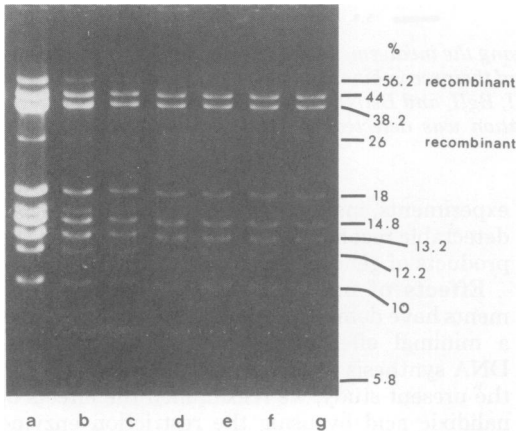

FIG. 10. *Effects of nalidixic acid on recombination. One-milliliter samples of a culture of E. coli B were multiply infected with 4am sRI and 15am sHindIII$_2$ in the presence of 0 μg (lane a), 5 μg (lane b), 10 μg (lane c), 20 μg (lane d), 30 μg (lane e), 50 μg (lane f), and 100 μg (lane g) of nalidixic acid. Recombination was detected by the presence of the 56 and 26% bands. The very faint 28% bands that migrated just above the 26% fragments in lanes f and g were due to incompletely digested parental DNA.*

DISCUSSION

This paper presents a relatively simple direct physical assay for the study of generalized genetic recombination. We show the utility of such an assay here by applying it to the study of T7-promoted recombination in vivo.

In addition to its simplicity, the assay has several other advantages. First, it is highly sensitive, in that with ethidium bromide staining, as few as 0.5% of the endogenous molecules which had undergone recombination to yield a 26% recombination band could be detected. The level of sensitivity can be increased severalfold by the use of Southern blotting. Second, one is able to study directly intracellular DNA without the need for the recombinant DNA to be packaged in phage heads. The interpretation of results of recombination experiments both in vivo and in vitro is sometimes confounded by the fact that the recombinant DNA must be packaged before detection as a viable recombinant phage particle (17, 26). The method does not require the use of density-labeled DNA and blockage of DNA replication, with their attendant uncertainties. The apparent frequencies of recombination in vivo that are obtained by using this simplified physical assay are consistent with frequencies measured by standard genetic crosses. This was estimated by comparing the frequencies of recombination measured optically by using this assay with those obtained from standard genetic crosses (27). Finally, the assay seems to be a reliable indication of in vivo recombination, since the present work confirms previous in vivo experiments (8, 15) which suggested that the products of genes 3 (endonuclease), 4 (DNA primase), 5 (DNA polymerase), and 6 (exonuclease) are needed for recombination.

The relationship between DNA replication and genetic recombination is obviously a close one for both the lambda system (26) and the T7 system (5). Thus, it could be argued that the absence of DNA replication is responsible for the depression of genetic recombination when the products of genes 4 and 5 are absent. On the one hand, the absence of DNA ligase or the presence of low concentrations of nalidixic acid, both of which significantly depressed DNA replication, did not have a marked effect on recombination. However, it is possible that there was enough DNA synthesis under these conditions to promote recombination, whereas in the absence of the gene 4 and 5 products, the block to DNA replication was sufficiently tight to impair recombination severely. On the other hand, the gene 4 and 5 proteins may have had a direct role in generating recombinants. With respect to the role of ligase, it is still possible that the recombinant molecules we observed contained single-strand breaks.

This assay enabled us to examine the role of DNA maturation and packaging in the generation of recombinants. Intracellular replicating DNA is found as large complex structures (14,

23). It is possible that such structures are generated by recombination and might have to be resolved by the DNA maturation and packaging apparatus. Our experiments show clearly that phage T7 recombination apparently occurs independently of DNA maturation and packaging.

Our ultimate aim is to understand generalized genetic recombination at the molecular level. This will require the use of simple physical assays to study the process in vitro, an approach which has been notably successful in dissecting the integration reaction of phage lambda (9). We are currently extending the physical assay described here to a study of T7 genetic recombination in vitro (19, 22).

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