Efficient Double-Strand Break-Stimulated Recombination Promoted by the General Recombination Systems of Phages \(\lambda\) and P22

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Manuscript received October 26, 1992
Accepted for publication April 12, 1993

ABSTRACT

To examine bacteriophage recombination in vivo, independent of such other processes as replication and packaging, substituted \(\lambda\) phages bearing restriction site polymorphisms were employed in a direct physical assay. Bacteria were infected with two phage variants; DNA was extracted from the infected cells and cut with a restriction endonuclease. The production of a unique recombinant fragment was measured by Southern blotting and hybridization with a substitution sequence-specific probe. High frequency recombination was observed under the following conditions: the substituted \(\lambda\) phages infected a wild-type host cell bearing a \(\lambda\) repressor-expressing plasmid designed to shut down phage DNA replication as well. The same plasmid expressed the \(\lambda\) red and gam genes. In addition, the host cell bore a second plasmid which expressed the EcoRI restriction-modification system. Both phage chromosomes possessed a single EcoRI site in the middle of the marked substitution sequence; however, as the site was modified in one of the parent phages, only the other partner was cut. Recombination was found to be dependent upon (1) \(\textit{red}\), (2) \(\textit{recA}\), (3) inactivation of the host \(\textit{recBCD}\) function, either by Gam protein or by mutation and (4) double-strand breaks. The homologous recombination system of phage P22 could substitute for that of \(\lambda\).

ONE impediment to the study of general recombination is that the process typically occurs at low frequency at unpredictable locations throughout the recombining chromosomes. Especially for biochemical experiments, it would be advantageous if a general recombination system could be made to operate at high efficiency, at a specified location and time, on nonreplicating DNA. Studies by Stahl and co-workers (Stahl, Kobayashi and Stahl 1985; Thaler, Stahl and Stahl 1987a,b) suggest that the Red general recombination system of bacteriophage \(\lambda\) might be capable of functioning in this way. By analyzing recombinant progeny phage, produced in small numbers under conditions of severely limited DNA replication, these investigators concluded that the Red system promotes recombination events at high efficiency at the sites of double-strand breaks made by the phage DNA packaging apparatus, or by restriction endonucleases. To account for the high level of Red-mediated recombination occurring throughout the \(\lambda\) chromosome that is observed when phage replication takes place, these authors speculated that the phage replication apparatus might generate double-strand ends that initiate exchanges.

Indications of the activity of the Red system in promoting double-strand break repair have come from a number of additional studies. Takahashi and Kobayashi (1990) showed that Red could promote the repair, \textit{in vivo}, of plasmid DNA cut at restriction sites \textit{in vitro}, if the cut plasmid were provided with an uncut homologue in the transformed cell. Eddy and Gold (1992) showed that the Red (and RecE) systems could promote an event much like the "homing" of mobile group I introns, in which an artificially inserted DNA segment would transfer from one chromosome to a cut--and previously noninsertion-containing--homolog. Glyman and Belfort (1992) showed that Red, as well as the recombination system of bacteriophage T4, could promote the homing of the T4 \textit{id} intron.

As a step toward biochemical study, we describe methods for direct characterization of a Red-mediated recombination event \textit{in vivo}. The procedures combine the use of a restriction endonuclease to generate a double-strand break in one of the nonreplicating parental chromosomes (Thaler, Stahl and Stahl 1987a,b), with recombination functions supplied by plasmids (Poteete and Fenton 1984) and a direct physical assay of recombinants among intracellular DNA molecules (Poteete, Fenton and Murphy 1988). Results from the application of these methods to the \(\lambda\) Red system generally support the model advanced by Stahl, Kobayashi and Stahl (1985), under conditions in which the yield of recombinants (DNA molecules, rather than completed virions) is much higher. Extension of the methods to the P22 homologous recombination system reveals that its activity, too, is stimulated by double-strand breaks.

**MATERIALS AND METHODS**

**Bacteria:** *Escherichia coli* AB1157 (argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacI1 mit-1 xyl-5 thi-1 rpsL31 iss-33 supF48) and the otherwise isogenic strains JC10287 (Δsr1R-recA304), JC5519 (recB21 recC22), JC5547 (recE13 recB1 recC22), JC1629 (Δsr1R-recA306): Tn10 recB2 recC22 sbcB15), JC11451 (sbcB15), JC12190 (recB21 recC22 sbcB15 recI153), JC9299 (recF143), JC8111 (recB21 recC22 sbcB15 recF143), JC11830 (recB21 recC22 sbcB15), and JC12123 (recF284::Tn10) were obtained from A. J. Clark. A derivative of AB1157, KM535, in which the allele recD1901::Tn10 was installed by transduction, with subsequent selection for a tetracycline-sensitive variant, has been described, as has KM534, a tetracycline-sensitive derivative of JC12123 (MURPHY 1991).

Plasmid-bearing host bacteria for the experiments described below were not stored. Rather, freshly transformed clones were obtained and purified for each set of experiments. It was found that this procedure resulted in more efficient retention of pMB4 (data not shown).

**Plasmids:** Plasmid pMB4, encoding the EcoRI restriction and modification system, has been described (BETLACH et al. 1976; NEWMAN et al. 1981), as have pTP297, pTP302, and pTP304 (POTTEET, FENTON and MURPHY 1988). Plasmid pTP657, used for generating RNA hybridization probes, was constructed by insertion of an 1875-bp EcoRI-BamHI fragment containing P22 gene sequences, between the EcoRI and BamHI sites of pGEM®-4Z (Promega).

Plasmids pTP178, pTP184, pTP223, pTP224 and pTP232, in which various λ and P22 recombination genes are fused to PUV5 and inserted into the lacI-expressing, tetracycline resistance-conferring vector pMC7 (a derivative of pMB9), have been described (FENTON and POTTEETE 1984; POTTEETE and FENTON 1984). Plasmid pTP553 was constructed by inverting the recombination gene-containing EcoRI fragment of pTP178, thus giving it the same orientation as the corresponding segments in the other plasmids (data not shown). The λ cl gene was inserted into each of these recombination function-expressing plasmids (except pTP178), by ligating a 2300 bp DraI fragment from λ 25a into their filled-in EcoRI sites, generating plasmids pTP692, pTP666, pTP699, pTP645, and pTP667, respectively. The control plasmid pTP648 was made from pTP645 by deletion of PUV4 and red genes with EcoRI.

Several plasmids were constructed as intermediates in the construction of RFLP phages. Plasmid pTP354 contains, in order: phage λ sequences from the EcoRI site at λ coordinate 21,226 to the HindIII site at 23,130, a 1300-bp segment of pTP302 bounded by HindIII and BamHI sites (including phage λ sequences from the XhoI site at 55,498 to the BamHI site at 34,499), and the origin-containing segment of pBR322 converted by PouII and EcoRI sites (with the PouII site converted to a BamHI site by ligation of the PouII end to a filled-in BamHI end). Plasmid pTP356 was constructed by insertion of a KpnI linker (5'-CTCTAGAG-3') into the filled-in HindIII site of pTP354. Plasmid pTP367 was constructed by insertion of a SalI linker (5'-GGGTACCC-3') into the filled-in XhoI site of pTP356. Plasmid pTP368 was constructed by ligation of the large fragment generated by digestion of pTP367 with KpnI and SalI, the 1875-bp KpnI-EcoRI fragment containing phage P22 gene 9 sequences from pTP304, and the 3339-bp SalI-EcoRI fragment containing lac sequences from pTP997. Plasmid pTP378 was constructed by insertion of a BglII linker (5’-CAGATCT-3’) into the KpnI site of pTP368 (with the KpnI ends made flush by incubation with T4 DNA polymerase and dNTP’s). pTP379 was similarly constructed by insertion of an XhoI linker (5’-CTCTAGAG-3’) into the KpnI site of pTP367. Plasmids pTP381 and pTP382 were constructed by insertion of XbaI and BglII linkers into the filled-in SalI sites of pTP378 and pTP379, respectively.

**Phage:** In λ strains bearing restriction fragment length polymorphism (RFLP) substitutions, λ sequences including genes int, xis, red and gsm are replaced with sequences from the tail gene of phage P22 and the lac operon of *E. coli*. Methods for constructing and propagating them were as described previously (POTTEETE, FENTON and MURPHY 1988), except that most of the phage stocks used in this study were grown in *E. coli* 594 (sup’) Stocks of EcoRI-modified phages were grown in strain 594 bearing plasmid pMB4. λ sr1 Δ527sr3 sr4* Pus80 sr5* (MMS1723) was obtained from M. M. STAHL. A P” revertant was selected by plating on *E. coli* 594, and crossed with plasmids pTP381 and pTP382 to introduce the 5400-bp RFLP substitutions. Recombinants, in which the deletion Δ527 is replaced with the overlapping RFLP deletion-substitution, were selected on the basis of their formation of blue plaques on a lac-indicator strain in the presence of 5-bromo-4-chloro-3-indolyl β-d-galactopyranoside (Sigma). The genotypes of the resulting phages are sr1 Δ527 sr3 sr4* sr5* and sr1 RFLP 381 sr3* sr4* sr5*, but they are referred to as λRFLP381 and λRFLP382 below. λRFLP382 imm21 cl-ts was constructed by crossing imm21 cl-ts with pTP382. It was used to test for expression of λ red genes in plasmid-bearing cells. (In most of the *E. coli* strains used in these studies, expression of the red genes allows phages bearing the RFLP substitution to form larger plaques.)

**Crossovers:** Cultures of host bacteria were grown overnight at 37° in LB broth supplemented with 0.2% maltose and antibiotics (tetracycline at 10 μg/ml, ampicillin at 100 μg/ml). Overnight cultures were diluted 100-fold into fresh medium and grown with aeration by swirling at 37° to a density of approximately 1 x 10^8/ml. Isopropyl thiogalactoside (IPTG) was added to a final concentration of 1 mM and cultures were aerated at 37° for an additional 30 min, then placed on ice. Phage were added to 10%/ml each, typically resulting in a multiplicity of 5 each; MgSO_4_ was added along with the phage, to a final concentration of 1 mM. Following a 15–30 min period of adsorption, 10–μl portions were removed for determination of unadsorbed phage titers. In all cases reported, adsorption was >85%. Cultures were then diluted 5-fold with prewarmed medium containing antibiotics and IPTG and aerated at 37°. Immediately before the addition of phage, host bacteria were tested for retention of pMB4. Small portions of the cultures were streaked on LB-tetracycline plates. Following overnight incubation at 37°, 30 colonies from each culture were tested for growth on LB-ampicillin agar. Only experiments in which pMB4 was retained in a minimum of 28/30 were analyzed further; in practice, retention was generally 100%, except in *recA* strains.

**Preparation and analysis of intracellular DNA:** The extraction procedure employed was a modified version of that of SILBERSTEIN et al. (1990). At various times following addition of phage, 10-μl portions of culture were chilled by pipetting into tubes on ice, and infected cells were collected by centrifugation at 8000 rpm in a Sorvall SS-34 rotor at 4°C. Cell pellets were frozen by immersion in a dry ice-ethanol bath, then thawed and resuspended in 0.5 ml 50 mM Tris-HCl, pH 8.0, 10 mM Na_2EDTA, 100 mM NaCl, 0.2% SDS. Hen egg white lysozyme (Sigma) was added, from a freshly prepared stock at 10 mg/ml in 50 mM Tris-HCl, pH 8.0, to a concentration of 100 μg/ml, and the preparations were incubated for 10 min at room temperature. DNA was extracted twice with equal volumes of...
phenol:chloroform:isoamyl alcohol (24:24:1), and once with diethyl ether, then precipitated with ethanol in the presence of 1.5 M ammonium acetate and 45 μg tRNA. Dried pellets were redissolved with 100 μl 10 mM Tris-HCl, pH 8.0, 1 mM Na$_2$EDTA. λ DNA concentrations were roughly quantitated by electrophoresis in a 0.7% agarose gel, followed by staining with ethidium bromide. In most experiments, the amounts of λ DNA extracted from infected cells were compared with amounts extracted from the same mixture of infecting phages, in the absence of bacteria, processed in parallel. Typical yields of intracellular DNA were in the range of 25–50% of the input. Occasionally, yields of phage DNA were lower, especially in samples taken at early time points (less than 30 min). Other than this, there was no apparent systematic variation of yield with time.

Samples containing approximately 20 ng of phage DNA (quantitated as described above) were digested overnight at 37° with BglII endonuclease (Boehringer Mannheim) and 100 μg/ml RNase A (Sigma). On occasion, DNA preparations were refractory to complete digestion with BglII. In these cases, satisfactory results were obtained by dialyzing 50-μl portions of the DNA preparations for 30 min on a cellulose acetate-cellulose nitrate filter (VSWP, Millipore) floating on 10 mM Tris-HCl, pH 8.0, 1 mM Na$_2$EDTA. Following electrophoresis through 0.7% agarose, the DNA was fragmented and transferred onto a membrane by overnight capillary transfer as described previously (POTTEETE, FENTON, and MURPHY, 1988), except that Hybond N nylon filters (Amersham) were employed. A Stratagene UV cross-linker was used to immobilize the DNA on the filters. Filters were incubated for 45 min at 50° in a prehybridization mixture (volume 1 ml/10cm$^2$) consisting of 50% (v/v) formamide, 6 × SSC, 0.1% (w/v) sodium dodecyl sulfate (SDS), 1 mM EDTA, 100 μg/ml yeast RNA, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 33P-Labeled RNA probe was added, and incubated with the filter overnight at 50°. Probe was synthesized in vitro by using T7 RNA polymerase on EcoRI-linearized pTP657, according to the instructions of the supplier of pGEM®-4Z (Promega). Sequences represented in the probe are RFLP substitution sequences in the left of the EcoRI site indicated in Figure 1. Filters were washed at room temperature for 0.5 hr in 1 liter 0.1 × SSC, 0.1% SDS, and then at 68° for 1 hr in 9 liters of the same solution. Filters were exposed to film at −80° with Dupont Lightning Plus fluorescence enhancing screens, for periods of time determined empirically to give the optimal visibility of any recombinant bands present.

Relative amounts of parental and recombinant bands were quantitated with the integrator of a Quick Scan R and D Electrophoresis/TLC densitometer (Helena Laboratories). The optical density of the film at a point slightly below the position of the 5400-bp recombinant band was taken as the background and subtracted from values measured in the zones of the various bands in the gel track. Recombination frequencies reported below represent the amount of 5400-bp recombinant band divided by the total of parental and recombinant bands, measured from the same film. The upper limit of the sensitivity of these determinations was assessed with measurements conducted on samples that contained no recombinants, as follows. DNA was extracted from singly infected cultures, mixed, and analyzed as described above. In several such tests, values of the "recombination frequency" (in the absence of recombination) thus measured ranged up to 0.3%. In the tables that follow, the cases in which no recombinant band was visible are thus reported as <0.5%.

Experimental system: The experimental system was designed for direct physical detection of general recombination events, without significant amplification of the recombinant DNA via replication. To this end, derivatives of phage λ were used to infect λ repressor-containing host cells, in which injection and circularization of the phage chromosome could occur, but in which transcription of phage genes was blocked. This blockade of transcription indirectly inhibited phage DNA replication. (For a review of λ replication, see FURTH and WICKNER, 1983). Both λ and P22 recombination-promoting genes were expressed at high levels from plasmids. The technique of STAHL, STAHL and FRANKLIN (1976) was employed to stimulate and focus recombination events: the phages bore single EcoRI restriction sites; in each cross, one parent's EcoRI site was modified, and the other's was unmodified. The host cells carried the EcoRI restriction-modification system on a plasmid, pMB4 (BETLACH et al. 1976).
polymorphisms. This feature permitted detection of phages with BglI1, electrophoresed in an agarose gel, transferred to a membrane and hybridized with a RFLP substitution-specific probe. Plasmid pTP666, EcoRI by plasmid pMB4.

Recombination events were directed to a region in the substituted λ phages flanked by restriction site polymorphisms. This feature permitted detection of recombinants among DNA molecules extracted from the infected cells, by the use of restriction endonucleases, Southern blots, and hybridization with specific probes, as described previously (POTEETE, FENTON and MURPHY 1988). The structures of the parent and recombinant phages are shown in Figure 1. According to this scheme, the substituted λ chromosomes would enter the cell and circularize, then stop at that point in the phage life cycle. One phage’s chromosome would be cut at its single EcoRI site. The double-stranded end thus produced would serve as a substrate for the proteins of the phage recombination system, which would use it to invade the unbroken duplex of its partner.

The behavior of the experimental system is illustrated in Figures 2 and 3, and quantitated in Figure 4. In the experiment shown in Figure 2, the host was wild type, and the λ recombination functions reda (exonuclease), redβ, and gam were supplied by a plasmid. Cutting by the EcoRI endonuclease is shown by the appearance of a 1880-bp fragment, and, in theory, by a relative depletion of a 7590-bp DNA fragment from the unmodified parent. In practice, the 1880-bp fragment produced by in vivo digestion with EcoRI endonuclease and in vitro digestion with BglIII never amounted to more than one percent of the total DNA hybridizable with the probe, and relative depletion of the 7590-bp fragment from the unmodified parent was partial (Figure 2 and other experiments not shown).

Results of control experiments indicated that the evidently low steady-state level of EcoRI-digested phage DNA was not due to lack of EcoRI activity. As described under MATERIALS AND METHODS, retention of the EcoRI-bearing plasmid pMB4 was tested and found to be nearly complete in all experiments. Moreover, cultures used in some of the experiments were tested directly for their ability to restrict plaque formation by unmodified heterimmune λ. In the case of the recA recB recC strain bearing pMB4 and a plasmid expressing cl, red and gam, the efficiency of plating of unmodified phage (bearing multiple EcoRI sites) was 0.23; in all other strains it was less than 0.04.

Recombinant bands were measurable in most experiments by 15 min postinfection (Figures 4 and 5), though they are not highly visible in the photograph of an autoradiogram shown in Figure 2 until 30 min postinfection. Recombinant bands continued to increase in amount for at least an hour (Figures 4 and 5); the two reciprocal recombinants were produced in approximately equal amounts, approaching 20% of the total in some experiments. Phage DNA replication could not be accurately quantitated in these experiments, but was apparently held to low levels at most. The efficiency of extraction of DNA varied from one sample to another, but there was little tendency of samples extracted from cells at later time points to contain more phage DNA than those at earlier time points. In contrast, because cellular replication was unimpeded in the infected cells, recovered bacterial DNA increased in amount during the time course. In otherwise similar infections of non-repressor-containing cells, phage DNA was greatly amplified (data not shown). The plasmid-encoded repressor apparently inhibited replication more strongly than lysogen-encoded repressor (presumably due to its presence at higher levels in the plasmid-bearing cells); some replication of λ DNA was evident in similar experiments involving the infection of λ lysogens (data not shown).
The 1880-bp fragment produced by EcoRI digestion in vivo and BglII digestion in vitro appeared in samples taken five minutes after infection, and persisted, at a low level, during the entire period in which recombinants were produced. It is shown in Figure 2, and quantitated in Figure 4a.

Requirements for high frequency recombination: The effects of changes and omissions on the efficiency of recombination were tested in an experiment summarized in Table 1. Switching the genotypes of the modified and unmodified phages had little effect, except that the 1880-bp fragment was replaced by a 2580 bp fragment (see the diagram in Figure 1). Omitting red, the EcoRI restriction system, or gam made recombinants undetectable.

The requirement for gam suggested that inactivation of RecBCD might have a role in the recombination reaction. If so, the need for Gam might be bypassed in a recB recC mutant host. This was observed to be the case, as shown in Figures 3 and 4(b), and Table 1. As in the wild type host, the observed recombination was dependent upon red and EcoRI and was not significantly affected by switching the modified and unmodified phages. The observed recombination frequency in the recB recC host was reproducibly lower than in wild type, however, suggesting that Gam has a second role in this process, in addition to inactivating RecBCD function. This notion was strengthened by the experiment illustrated in Figure 4b; it was found that Gam stimulated recombinant formation in a recB recC host.

The observed frequency of red and gam-promoted recombination in the wild-type host could be doubled by infecting the wild-type host first with the unmodified phage, then, after ten minutes at 37°C, infecting with the modified phage, as shown in Figure 5a. The opposite result was found in the recB recC host in the absence of Gam (Figure 5b). Sequential infection, in this case, produced fewer recombinants than simultaneous infection. When Gam was supplied in the recB recC host, however, the wild-type pattern of recombination was observed (Figure 5c). As in the previous experiments, there was relatively little variation in the yields of recombinants from cells infected simultaneously with both parent phages: slightly more from wild type than from the recBC mutant, with the yield from the latter increased slightly when gam function was supplied by a plasmid. These effects associated with switching between simultaneous and sequential infection suggest that the hypothetical (non-RecBCD) second cellular target of Gam might destroy the recombinogenic potential of double-strand ends if pairing with a homologue does not occur quickly after their generation.

P22 and host recombination functions: The homologous recombination system of bacteriophage P22 consists of genes arf (accessory recombination function; Poteete, Fenton and Semerjian 1991), erf (essential recombination function; Botstein and Matz 1970), abcl and abc2 (anti-RecBCD; Murphy, Fenton and Poteete 1987). It could substitute for its λ counterpart, as shown by the experiment summarized in Table 2. A plasmid expressing these four P22 genes stimulated recombination that was, as in the case of λ functions, dependent upon double-strand breaks and upon the RecBCD-modifying proteins of the phage (Abcl and Abc2 of P22).

The question of the involvement of host recombi-
nation functions in red-mediated recombination in this system was investigated in the experiment summarized in Table 3. Recombination was not observed in a recA strain expressing red and gam, regardless of whether the strain was additionally mutant in recB and recC as well. In contrast, recombination was seen not to depend upon recB or recC, as observed previously, or upon recD, recF or recJ.

Fates of EcoRI-digested DNA in vivo: Blocking recombination in this system does not lead to accumulation of EcoRI-digested DNA in the infected cell, as shown in Figure 6. The first five lanes show the time course of infection of a recA host. As in the wild type host, the 1880-bp fragment resulting from EcoRI digestion in vivo and BglII digestion in vitro appears early in the infection, and is seen, in gradually decreasing amounts, thereafter; and there is a gradual depletion of the band corresponding to the unmodified parent. No recombinant bands appear. The last three lanes show 30-min time points from infections of wild-type cells bearing plasmids that supply all the necessary components but one: EcoRI, red and gam, respectively. In samples taken from the EcoRI-less infection, the unmodified parent band is not depleted, and no 1880-bp fragment appears. In the red-less infection, a small amount of the 1880-bp fragment, and a slight depletion of the unmodified parent band are seen, as in the recA-less infection. In the gam-less infection, depletion of the unmodified parent band is more pronounced, and no 1880-bp fragment is detectable; these latter observations presumably reflect the destructive activity of unmodified RecBCD nuclease.

DISCUSSION

The λ Red system, acting in concert with RecA, in the absence or near-absence of normal phage DNA replication, can promote recombination events at a high frequency, if one partner in the cross has a double-strand break. This finding is consistent with the mechanism of Red-mediated recombination proposed by STAHL, KOBAYASHI and STAHL (1985). Subsequent research by THALER, STAHL and STAHL (1987a,b) provided support for this mechanism through studies of the properties of recombinant progeny phage. The present study extends these earlier results by direct analysis of intracellular DNA substrates, possible intermediates, and products of recombination.

Intracellular digestion of the unmodified parent phage chromosome by the EcoRI endonuclease could be monitored by the production of the 1880-bp DNA fragment upon digestion with BglII in vitro (Figures 2 and 4). This fragment was present, in low amounts, in all cases in which recombinant formation could be detected. It was additionally detectable in cases in which recombinant formation was blocked by omission of red or recA function, but not when gam was omitted (thus leaving RecBCD nuclease active), or when EcoRI was omitted (Figure 6). We were initially surprised by the apparently sluggish activity of the EcoRI nuclease in cutting the unmodified parent
TABLE 1

Requirements for high frequency recombination in wild-type and recBC mutant E. coli

<table>
<thead>
<tr>
<th>Host</th>
<th>Plasmids</th>
<th>Functions(^a)</th>
<th>Phages(^b)</th>
<th>Percent recombination (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (AB1157)</td>
<td>pTP666 + pMB4</td>
<td>red + gam + EcoRI</td>
<td>(\lambda RFLP381) m(^+)</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^-)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pTP666 + pMB4</td>
<td>red + gam + EcoRI</td>
<td>(\lambda RFLP381) m(^-)</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pTP645 + pMB4</td>
<td>red + EcoRI</td>
<td>(\lambda RFLP381) m(^-)</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pTP699 + pMB4</td>
<td>gam + EcoRI</td>
<td>(\lambda RFLP381) m(^-)</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pTP666</td>
<td>red + gam</td>
<td>(\lambda RFLP381) m(^-)</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^+)</td>
<td></td>
</tr>
<tr>
<td>recBC (IC5519)</td>
<td>pTP645 + pMB4</td>
<td>red + EcoRI</td>
<td>(\lambda RFLP381) m(^+)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^-)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pTP645 + pMB4</td>
<td>red + EcoRI</td>
<td>(\lambda RFLP381) m(^-)</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pTP648 + pMB4</td>
<td>EcoRI</td>
<td>(\lambda RFLP381) m(^-)</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pTP648</td>
<td>None</td>
<td>(\lambda RFLP381) m(^-)</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\lambda RFLP382) m(^+)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) \(\lambda\) repressor was present in every case.  
\(^b\) m\(^+\) = EcoRI-modified, m\(^-\) = EcoRI-unmodified.  
\(^c\) 5370-bp recombinant band as percentage of total recombinant + parental bands at 30 min after infection.

TABLE 2

Double-strand break-stimulated recombination by \(\lambda\) and P22 systems

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Functions(^a)</th>
<th>Percent recombination (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTP666 + pMB4</td>
<td>red + gam + EcoRI</td>
<td>3.0</td>
</tr>
<tr>
<td>pTP666</td>
<td>red + gam</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>pTP645 + pMB4</td>
<td>red + EcoRI</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>pTP648 + pMB4</td>
<td>EcoRI</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>pTP667 + pMB4</td>
<td>arf + erf + abc1 + abc2 + EcoRI</td>
<td>1.1</td>
</tr>
<tr>
<td>pTP692 + pMB4</td>
<td>arf + erf + EcoRI</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>pTP667</td>
<td>arf + erf + abc1 + abc2</td>
<td>&lt;0.3</td>
</tr>
</tbody>
</table>

Host was AB1157 bearing the indicated plasmids. Phages were \(\lambda RFLP381\) m\(^-\) and \(\lambda RFLP382\) m\(^+\).  
\(^a\) The \(\lambda\) repressor was present in every case.  
\(^b\) 5370-bp recombinant band as percentage of total recombinant + parental bands at 30 min after infection.

phage chromosomes in vivo. This observation contrasted with the efficiency of the same host cells in restricting plaque formation by unmodified \(\lambda\), as described in RESULTS. On the other hand, a study by HEITMAN, ZINDER and MODEL (1989) has indicated that E. coli strains with normal ligase activity have a great capacity to repair EcoRI scissions in their chromosomes. We surmise that the low level of EcoRI-cut DNA observed in our experiments reflects a nearly steady state, with a substantial rate of turnover mediated by the processes of endonucleolytic digestion, recombination, ligation, and exonucleolytic destruction. The present study leaves unanswered the question of whether the EcoRI-ended 1880-bp fragment detectable in vitro is part of an actual recombination intermediate. The kinetics of its appearance in the cell, shortly before and during the period in which recombinants accumulate, suggest that it is. On the other hand, the 1880-bp fragment does not accumulate appreciably when recombination is blocked by omission of recA or red; it is possible that the actual intermediate has too short a lifetime for detection by these methods, and that the only EcoRI ends we observe are those that are destined for exonucleolytic degradation, for instance. Investigation of this and related questions, by the use of biochemical techniques in vitro, is needed.

Experiments described above show that the homol-
ogous recombination system of phage P22, like that of $\lambda$, is stimulated by double-strand breaks. This finding is unsurprising in light of the functional interchangeability of the recombination systems of the two related phages (POTEETE and FENTON 1984). However, it is intriguing in light of the apparent structural unrelatedness of the various parts of the two recombination systems (PROTEETE, FENTON and SEMERJIAN 1991).

The RecE recombination pathway of *E. coli* resembles the Red pathway of phage $\lambda$ in some respects (GILLEN, WILLIS and CLARK 1981). It depends upon activation of recombination genes in the cryptic prophage Rac. SYMINGTON, MORRISON and KOLODNER (1985) showed that *E. coli* strains in which the RecE pathway was active could circularize linear plasmid dimers by recombination following transformation. KOBAYASHI and TAKAHASHI (1988) found that such cells could carry out double-stranded gap repair on plasmid DNA introduced by transformation. NUSBAUM, SHALIT and COHEN (1992) have described experiments, similar in concept to those reported here, demonstrating double-strand break-stimulated homologous recombination of plasmids by the *E. coli* RecE pathway. Their experiments differed, though, in that recombination was slower, and was accompanied by extensive DNA replication in the linear multimer synthesis mode (COHEN and CLARK 1986).

High frequency Red-mediated double-strand break-stimulated recombination depends upon inactivation of RecBCD, either by mutation of *recB* or *recC* or by expression of phage-encoded RecBCD-modulating proteins. This observation is consistent with the idea that RecBCD is destructive to ends of double-stranded DNA lacking properly oriented Chi sites (STAHL et al. 1990). However, the Gam protein of $\lambda$ evidently has a second role in the process, since supplying it stimulates recombination in a *recB recC* mutant infected sequentially with the two test phages. KULKARNI and STAHL (1989) have shown that the gam gene is essential for the growth of $\lambda$ derivatives bearing long DNA palindromes in *sbcC*+, but not in *sbcC−* hosts. Possibly, the product of *sbcC*, or some other function under its control, interferes mildly with recombination in the crosses described here, and Gam prevents this interference via an interaction with *sbcC*.

It is formally possible that the effects attributed to gam in these studies are wholly or partially due to some other, as yet unknown $\lambda$ function expressed (in addition to gam) by plasmid pTP666 but not by pTP645. The former differs from the latter only in containing $\lambda$ sequences between positions 32,966 and 33,248 (SANGER et al. 1982). Computer-assisted inspection of this sequence does not reveal any likely protein-encoding reading frames other than those of *gam* and *red* (not shown). However, as noted by FRIEDMAN and HAYS (1986), there are two feasible translation initiation sites in the *gam* open reading frame; both are contained in pTP666. MURPHY (1991) has shown that Gam protein has an amino acid sequence consistent with initiation at the second of these sites, but these data do not rule out the possibility that a second protein is encoded by the longer open reading frame.

An unexpected finding in these experiments was the apparent enhancement, in the wild type host, of the recombinogenic potential of the cuttable phage chromosome when it was injected before that of the uncuttable partner. This observation suggests that some recombination-promoting component, one that operates only on the double-strand end-bearing partner, is partially sequestered by the first (uncut) phage chromosomes injected into the cell.

The requirement for RecA in this system illustrates the complexity of its mechanistic relationship with Red$.\beta$. On one hand, when $\lambda$ replicates freely, it recombines efficiently in the absence of RecA (ECHOCS and GINGERY 1968; SINGER and WEIL 1968). In addition, the single strand-pairing properties of Red$.\beta$ resemble those of RecA, although RecA can promote strand exchange, while Red$.\beta$ apparently cannot (KMIIEC and HOLLOMAN 1981; MUNIVAPPA and RADDING 1986). Moreover, Red$.\beta$ can suppress the deficiency in plasmid recombination of a *recA* mutant, operating via the RecF pathway (BERGER and COHEN 1989). On the other hand, recA strains containing
Redβ are in other ways phenotypically RecA−: they are UV-sensitive, and incapable of forming stable recombinants following conjugation (our unpublished observations). When DNA replication is blocked, the λ recombination system is nearly inactive in the absence of RecA (Stahl, Stahl and Malone 1978); overexpression of red and gam by a plasmid does not remedy this deficiency (A. R. Poteete and F. W. Stahl, unpublished observations). It would appear that RecA has an activity, not shared with Redβ, that is essential specifically for recombination of nonreplicating DNA. Clyman and Belfort (1992) reported a similar requirement for recA in red-mediated intron transfer; they speculated that the special role of RecA protein in the process might be to direct strand invasion.

We thank Frank and Mary Stahl for advice and strains. This research was supported by National Institutes of Health grant AI18234.

LITERATURE CITED


Communicating editor: G. R. Smith