DNA rearrangement mediated by inverted repeats

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ABSTRACT Inverted repeats of DNA are widespread in the genomes of eukaryotes and prokaryotes and can mediate genome rearrangement. We studied rearrangement mediated by plasmid-borne inverted repeats in Escherichia coli. We show that inverted repeats can mediate an efficient and recA-independent recombination event. Surprisingly, the product of this recombination is not that of simple inversion between the inverted repeats, but almost exclusively an unusual head-to-head dimer with complex DNA rearrangement. Moreover, this recombination is dramatically reduced by increasing the distance separating the repeats. These results can be readily explained by a model involving reciprocal switching of the leading and lagging strands of DNA replication within the inverted repeats, which leads to the formation of a Holliday junction. Reciprocal strand switching during DNA replication might be a common mechanism for genome rearrangement associated with inverted duplication.

Repetitive DNA sequences can mediate recombination via various mechanisms (1, 2). In Escherichia coli, the recA-dependent general recombination proceeds mainly through the RecBCD pathway (1, 2). RecA promotes homologous pairing of DNA molecules and catalyzes the strand exchange reaction leading to the formation of heteroduplex DNA in vitro (3). Despite the apparently central role of RecA in homologous recombination, recA-independent recombination between tandem direct repeats has been observed in plasmids (4, 5) and the chromosome of E. coli (4). This recombination is affected by structural factors such as the distance between the repeats (5–7).

Recombination between direct repeats can lead to deletion of one of the repeats and any intervening sequence, whereas that between inverted repeats can invert the intervening sequence. Relative to recombination between direct repeats, recombination between inverted repeats in E. coli has not been extensively studied. The few reported studies of inversion all made use of “genetic switches” of genes. When the promoter of a gene is bracketed by inverted repeats, its orientation can be changed by recombination (inversion) between the repeats, resulting in the reversible alteration of the “on” and “off” states of gene expression. Such a genetic switch was found in phage A mediated by the inverted insertion sequence elements of Tn10 and shown to be recA-dependent (8). This switching was later shown to occur efficiently via the RecBCD pathway and was suggested to be either intra- or intermolecular (9). Inversion of the lac promoter mediated by short inverted repeats in the chromosome of E. coli was also shown to be recA- and recBC-dependent (10). In Salmonella typhimurium, inversion between large inverted repeats (>5 kb) separated by large intervals (>60 kb) has been shown to be recA- and recB-dependent (13).

To further examine recombination between inverted repeats, we created a genetic switch for the tetA gene of the plasmid pBR322 by bracketing its promoter with inverted repeats. We show that recombination between inverted repeats in this plasmid system is recA- and recBC-independent and is dramatically reduced by lengthening the distance separating the repeats. Moreover, instead of the predicted product of simple intramolecular inversion, almost all of the products of this recombination are a special head-to-head dimer. Our results can be readily explained by a replicational model for recombination between inverted repeats.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. E. coli strains used in this study are listed in Table 1. Plasmid pSV8 was from Clontech. Plasmid pH8 (Fig. 1B) was derived from pBR322 (Fig. 1A) as follows. The Sal I site between the P and H fragments of pBR322 was used for cloning. The P and H fragments were first amplified by PCR with the addition of Sal I sites to both ends of them (accomplished by including restriction sites in the PCR primers). After being digested by Sal I, both fragments were incubated with pBR322 cut with Sal I in a ligation reaction. The product (named pH8) with double insertions of P and H in the directions as shown in pH8 was selected. Note that pH8 has another copy of the P fragment compared with pH8. This extra P fragment was then deleted to generate pH8. The pH8 series of plasmids were derived from pH8 as described in the legend to Fig. 4A.

Recombination Tests and Product Analysis. Plasmid recombination was examined as described by Bi and Liu (5). The plasmid substrate was transformed into a strain and the transformants were selected on LB plates containing Ap (100 µg/ml). After ≈20 hr of incubation at 37°C, 10 or more colonies were picked, and the plasmid was isolated and digested with the appropriate restriction endonuclease. The sizes of the recombinant plasmids were then determined by agarose gel electrophoresis.

Abbreviations: Ap, ampicillin; Tc, tetracycline; R, resistant; S, sensitive; RSS, reciprocal-strand-switching.

Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Designation</th>
<th>Mutations affecting recombination</th>
<th>Other mutations</th>
<th>Source or ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM294</td>
<td>None</td>
<td>F' endA1 hsdR17 (r&lt;sub&gt;5&lt;/sub&gt; m&lt;sub&gt;s&lt;/sub&gt;) supE44 thi1 relA1? rfbD? spoT?</td>
<td>11</td>
</tr>
<tr>
<td>MM294ΔrecA</td>
<td>ΔrecA</td>
<td>As for MM294</td>
<td>B. Weiss*</td>
</tr>
<tr>
<td>RR1</td>
<td>None</td>
<td>F' Δ(spr-pro4)66 leu supE44 ara14 galK2 Δ(mcrC-mrr) lacY lacY1 rpsL20 xyl-5 met-l</td>
<td>12</td>
</tr>
<tr>
<td>HB101</td>
<td>recA13</td>
<td>As for RR1</td>
<td></td>
</tr>
<tr>
<td>AB1157</td>
<td>None</td>
<td>F' thr1 leu6 thi1 lacY1 galK2 ara14 xyl5 metI proA2 his4 argE3 str31 tss33 supE44 kindK51</td>
<td>A. J. Clark†</td>
</tr>
<tr>
<td>JC10287</td>
<td>Δ(srlR-recA)304</td>
<td>As for AB1157</td>
<td>A. J. Clark†</td>
</tr>
<tr>
<td>JC5519</td>
<td>recB21 recC22</td>
<td>As for AB1157</td>
<td>A. J. Clark†</td>
</tr>
</tbody>
</table>

*University of Michigan Medical School.
†University of California at Berkeley.
colonies were collected and suspended in M9 minimal salts (14). Cells were plated on LB plates containing Tc (20 μg/ml) and Ap (referred to as the Tc plate) to select for cells containing recombined plasmids. Cells were also plated on plates containing only Ap (the Ap plate) to obtain the number of viable ApR cells in the suspension. The frequency of recombination was measured as the ratio of the number of colonies on the Tc plate to that on the Ap plate. For each substrate, 3–10 independent experiments were performed and the frequency was calculated as the mean of the data from all experiments. The standard deviation was also calculated. Note that the frequency reported is the overall frequency, not the rate of recombination. For the recombination tests in this work, the rate of recombination as calculated by the method of Luria and Delbrück (15) would be about one order of magnitude lower than the frequency. In addition, in calculation of the frequency, the multicopy number nature of the plasmid substrate is not taken into consideration. Taking the average copy number of pBR322 as 100, the real recombination rate (estimated as the number of events per copy of plasmid per cell per generation) would be about three orders of magnitude lower than the overall frequency.

To analyze the products of recombination of a plasmid substrate, 100 or more TcR colonies from the recombination tests were used to individually inoculate 2 ml of LB with Ap. After ≈15 hr of growth at 37°C, a quick phenol method (6) was used to isolate plasmid from each culture for analysis by agarose gel electrophoresis.

RESULTS

Construction of a Genetic Switch for the tetA Gene of pBR322 as a Model System for Studying Recombination Between Inverted Repeats. We constructed a series of plasmids (pHPH and its derivatives described in the legends to Fig. 1B and Fig. 4A) for studying recombination between inverted repeats in E. coli. The basic strategy was to create a genetic switch for the tetA gene of pBR322 (Fig. 1A) by placing its promoter (Ptet) between two inverted repeats. In pHPH (Fig. 1B), Ptet and part of tetA (together designated P) was bracketed with two inverted repeats (H). Note that H is also part of tetA. Ptet in fragment P of pHPH is in the “wrong” orientation so that tetA cannot be expressed. Recombination between the H repeats is expected to cause inversion of P so that the intact tetA can be regenerated (in pHPHR, Fig. 1B). In order to examine if the distance separating the inverted repeats affects recombination, we also inserted additional DNA fragments in between the H repeats of pHPH (Fig. 4A).

Table 2. The major product of recombination between inverted repeats in pHPH is a special dimer (pSWI)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Products*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Special dimer (pSWI), %</td>
</tr>
<tr>
<td>MM294 (wt)</td>
<td>98†</td>
</tr>
<tr>
<td>MM294ΔrecA</td>
<td>100†</td>
</tr>
<tr>
<td>RR1 (wt)</td>
<td>90†</td>
</tr>
<tr>
<td>HB101 (recA13)</td>
<td>100†</td>
</tr>
<tr>
<td>AB1157 (wt)</td>
<td>98†</td>
</tr>
<tr>
<td>JC10287 (ΔrecA)</td>
<td>100†</td>
</tr>
<tr>
<td>JC5519 (recBC)</td>
<td>98†</td>
</tr>
</tbody>
</table>

wt, Wild type.

*The products of recombination of pHPH were examined as described in the text. Each sample of plasmid was from an individual ApRTeR colony.

†For the recA+ strains MM294, RR1, AB1157, and JC5519, in some of the samples, besides pSWI, the major species, barely detectable amount of plasmid(s) of the size of pHPH is present. The plasmid may be pHPH or its product of simple inversion, pHPHR, or a mixture of them.

‡Samples in which there is a significant amount of a plasmid(s) of the size of the tetramer of pHPH besides pSWI.
Plasmid Recombination Between Inverted Repeats Is Efficient and Independent of RecA and RecBCD. Recombination between inverted repeats in pHPH (Fig. 1B) was examined in various strains of E. coli (Table 1). Surprisingly, recombination as scored by TcR is very efficient (with a frequency of about $0.5 \times 10^{-2}$) and is recA-independent (Fig. 1C, compare 1, 3, and 5 with 2, 4, and 6, respectively). Moreover, it is also recBC-independent (Fig. 1C, compare 5 with 7). Similar results were also obtained using another pBR322-based substrate in which P$_{559}$ was bracketed with inverted repeats of 559 bp in length (data not shown). These results indicate that there is a mechanism(s) other than conventional recA-dependent recombination for inverted repeats-mediated recombination.

The Product of recA-Independent Plasmid Recombination Between Inverted Repeats Is an Unusual Head-to-Head Dimer. The expected product of recombination between inverted repeats is that of a simple intramolecular inversion.
We analyzed the products of recombination of pHPH under various genetic backgrounds. One hundred or more ApF  
TcR  
 colonies from recombination tests of pHPH in each strain tested were analyzed. Unexpectedly, for all recA  
 strains tested, all of the ApR  
TcR  
 colonies examined contained a single species of plasmid of the size of dimeric pHPH as revealed by  
 ethidium bromide staining (Fig. 2 and Table 2). Restriction analysis (Fig. 3C) revealed that this plasmid is an unusual  
 dimer (designated pSWI) consisting of inverted duplications (Fig. 3A). Plasmid pSWI is certainly not the product of a simple intramolecular inversion between the H repeats of pHPH because such a product (pHPHR in Fig. 1B) has the size of pHPH. For the rec  
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 colonies also contained mainly or only the special dimer pSWI instead of the product of simple inversion (Table 2). Similar results were also obtained for the other pBR322-based substrate mentioned above (data not shown). These results indicate that recA  
-independent recombination between inverted repeats is not simple inversion.

**Plasmid Recombination Between Inverted Repeats Is Highly Sensitive to the Distance Separating the Repeats.** To examine if the distance separating the inverted repeats can affect recombination, we derived from pHPH the pHP\betaH series of plasmids (Fig. 4A) in which the H repeats were separated by intervening sequences of 751, 1049, 1593, 3319, and 4523 bp, respectively. Using these plasmids and pHPH, recombination between inverted repeats as a function of the length of the intervening sequence was examined. As the distance between the H repeats increased, the frequency of recA-independent recombination decreased exponentially (Fig. 4B). However, the pHP\betaH series of plasmids still yielded special dimers similar to pSWI (data not shown) indicating that lengthening the intervening sequence did not affect the type of recombination involved.

**DISCUSSION**

We have demonstrated that plasmid-borne inverted repeats can mediate an efficient and recA- and recBC-independent recombination which produces almost exclusively an unusual head-to-head dimer with complex DNA rearrangement. In theory, pSWI (Fig. 3A), the unusual dimeric product of pHPH, can be formed by an intermolecular reciprocal exchange between two pHPH monomers (Fig. 3D). However, this is unlikely to be the case for the following reasons. (i) Intermolecular recombination is rare in recA  
 strains. In recA mutants there is virtually no intermolecular conjugal recombination (16). Oligomer formation from monomeric plasmid is recA-dependent (17). It has been shown that recombination between two compatible plasmids is greatly reduced in a recA  
 strain, with a frequency of \( \approx 4 \times 10^{-6} \) (18), >100-fold lower than the frequency of pSWI formation (Fig. 1C). (ii) If the special dimer is formed by intermolecular recombination, increasing the distance between the inverted repeats should not affect its formation. However, its formation is greatly reduced as the intervening sequence increases (Fig. 4B).

It is striking that, even in recA  
 strains, very few, if any, products of pHPH detected are that of simple inversion between the H repeats (Table 2). One possible explanation is that the simple inversion product (pHPHR, Fig. 1B) is either toxic to, or cannot be stably maintained in, host cells. This was ruled out by the fact that pHPHR (constructed in vitro) was not affect its formation. However, its formation is greatly reduced because such a product (pHPHR in Fig. 1B) has the size of pHPH. For the rec  
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-independent recombination between inverted repeats is not simple inversion.
able to transform cells and be stably maintained on medium containing only Ap or medium containing both Tc and Ap (data not shown). Why simple intramolecular inversion is not the predominant recombination is unclear.

DNA replication has been invoked to explain recA-independent deletion between direct repeats and accompanying rearrangements (4–7). However, these models cannot explain simple inversion between inverted repeats or the formation of pSWI from pHPH. Here we propose a model for recA-independent recombination between inverted repeats, which involves reciprocal switching of the leading and lagging strands when the repeats are being replicated.

Our reciprocal-strand-switching (RSS) model is detailed in Fig. 5 using pHPH as an example. Fig. 5A indicates the substrate pHPH. At the replication fork, the leading strand DNA polymerase (19, 20) is copying the second repeat (proximal to the fork), while the lagging strand polymerase is copying the first repeat (Fig. 5B). Fig. 5C indicates reciprocal switching of the leading and lagging strands within the inverted repeats and continuation of replication. Note that the junction created by strand switching is formally a Holliday junction (21). It can be resolved by endonuclease and ligase activities. If the nascent strands (thin lines in the junction) are cut and religated after exchange, the original fork structure is regenerated as if nothing has happened. However, if the template strands are cut and religated after exchange as illustrated in Fig. 5C, a dumbbell-shaped replicating plasmid would be formed (Fig. 5D). Completion of replication would result in an unusual complex dimer (pSWI) consisting of inverted duplications (Fig. 5E). Plasmid pHPH has the unidirectional origin of replication (ori) of pBR322 (22). However, it is obvious that plasmid with a bidirectional ori will also generate the structure in Fig. 5E. Note that the sequence of the markers W, X, Y, and Z in pHPH is WXYZ (Fig. 5A). In the product (pSWI), two new joints, ZX and YW, are created, and the intact plasmid with a bidirectional ori will also generate the structure in Fig. 5E. In conclusion, we propose that RSS within the inverted repeats of pHPH during replication leads to the formation of the special dimer pSWI.

None of the processes of the RSS model involves RecA or RecBCD and thus the proposed recombination between inverted repeats is recA- and recBC-independent. The distance effect on recombination between inverted repeats (Fig. 4B) can also be explained by the RSS model. Since, in general, synthesis of the leading and lagging strands is coordinated spatially (19, 20), reciprocal strand switching might occur efficiently only when the two repeats are separated by a relatively short distance, perhaps in the range of an Okazaki fragment (1–2 kb in length; refs. 19 and 20).

Simple strand switching in replication has been first proposed to explain certain deletions in phage λ (23) and phage Mu excision by aborted transposition (24). It was also used to explain the generation of inverted duplications in certain gene amplification events in mammalian cells (refs. 25 and 26; reviewed in ref. 27). These models (23–26) all invoke that strand switching model and the RSS model can explain the formation of inverted duplications, only the RSS model can explain the inverted repeats-mediated complex rearrangement observed in this study.

It is not known if the complex rearrangement mediated by inverted repeats in plasmids also occurs in the chromosomes of cells. If it occurs in the E. coli chromosome, the product would be a head-to-head dimeric chromosome (structurally similar to pSWI), which might be lethal to the cell. This might be the reason why this kind of rearrangement has not been detected in E. coli. If the rearrangement occurs in a linear chromosome, the products would be two palindromic DNA molecules (as can be extrapolated from Fig. 5). Interestingly, palindromic chromosomes have been found in mammalian cells (for an example, see ref. 28). If two such events occur in the same chromosome, three products would be generated: two palindromic chromosomes and an extrachromosomal circle. The circle would contain mostly an inverted duplication separated by two unique DNA segments. Interestingly, this circle is structurally similar to the H circle in the protozoan parasite Leishmania (29, 30) and certain Ado plasmids derived from phage λ (23), as well as certain circular ampiclons in mammalian cells (31). It is noteworthy that the H circle is originated from the H locus which is flanked by two pairs of inverted repeats in the Leishmania chromosome (29, 30). In light of our RSS model, it is possible that the H circle is formed via RSS at both pairs of inverted repeats bracketing the H locus during bidirectional DNA replication. It will be interesting to investigate whether the RSS model underlies the mechanism of certain gene amplification and genome rearrangement events.