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Method for the Isolation of Escherichia coli Mutants with Enhanced Recombination Between Chromosomal Duplications

E. BRUCE KONRAD

Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

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A method is described for the isolation of Escherichia coli mutants that show increased recombination between a pair of chromosomal duplications. These "hyper-rec" mutants display a variety of secondary phenotypes. I have isolated a large number of hyper-rec mutants and found them useful in screening for mutants that accumulate labeled DNA fragments after short pulses with [3H]thymidine. The mutants so recovered include ones that are defective in deoxyribonucleic acid ligase, deoxyribonucleic acid polymerase I and its associated 5' → 3' exonuclease, and a group of mutants, dnaS, that accumulate abnormally short Okazaki fragments. Evidence is presented that suggests that the lac-attB0 segment of the chromosome cannot be inverted.

Mutants of Escherichia coli unable to support general recombination have been an invaluable aid in efforts to clarify the mechanism of genetic recombination. A useful counterpart to such rec mutants would be mutants with an enhanced frequency of recombination (hyper-rec). In this paper I describe a method for the isolation of large numbers of E. coli mutants that show this phenotype. The recombination I have measured occurs between a pair of chromosomal duplications in a special strain I have constructed for isolating hyper-rec mutants.

A hyper-rec phenotype might result from a variety of mutational alterations. These include an increased frequency of single-strand breaks ("nicks") or gaps in the deoxyribonucleic acid (DNA), which could provide sites for the initiation of recombination, a higher than normal efficiency of pairing or synapsis due to an increased concentration of DNA binding protein, or an increased concentration of a specific exonuclease required in a postsynaptic event.

I describe here the isolation of a group of hyper-rec mutants in which the increased recombination is associated with a defect in a late step in discontinuous DNA replication, the joining of Okazaki fragments. These mutants include strains defective in DNA ligase and in DNA polymerase I and its associated 5' → 3' exonuclease, in addition to a third class, dnaS, in which abnormally short Okazaki fragments accumulate.

MATERIALS AND METHODS

Strains. Strains used in this work were derived from E. coli K-12. Nomenclature is from Bachmann et al. (1). A pts + strain (FF8040) was supplied by W. Epstein, an ilv "metE" strain by L. Soll, and a recA1 strain by S. Kushner. Phage BF23 was supplied by P. Fredericq. Other strains are from the collection of J. Beckwith.

Construction of the lac diploid strain KS391. Strain KS391 (Hfr Hayes lacMS286 φ80dIIlacBK1 thi') (Fig. 1a, b) carries two copies of the lac region. This strain was constructed by introducing lacMS286 and the defective prophage φ80dIIlacBK1 into an Hfr Hayes strain (HfrH proC ΔtonB-trp thi') by P1 transduction (12), utilizing the close linkage between proC and lac and the inclusion of the attachment site of prophage φ80 (att80) within the ΔtonB-trp deletion (1), respectively, for the selection of transductants. lacMS286 is a partial deletion of the lac region and was provided by M. Malamy. This deletion includes lacY and part of lacZ (Fig. 1b). Its end within the lacZ gene was mapped by introducing lacMS286 into a female (F-) strain and mating this strain with various F' lac strains carrying different lacZ point mutations. By this means, the end of deletion lacMS286, which lies within lacZ, was mapped between the point mutations lacX90 and lacX64 (6). The deletion lacBK1 on the prophage φ80dIIlac was isolated by selecting melibiose-positive revertants of a strain carrying the prophage φ80dIIlac2 (2, 20) and also a deletion of the lac region (Δlac-proXII1). Mapping showed the lacBK1 deletion includes lac-2 and lacU118, but not lacX64 or lacI (6). Thus, at least one point mutation, lacX4, lies between lacMS286 and φ80dIIlacBK1. Therefore, deletions lacMS286 and φ80dIIlacBK1 do not overlap and would be expected to recombine with each other to form lac + or φ80dIIlac + strains (see Results).
RESULTS

Isolation of hyper-rec mutants from strain KS391. A genetic map of strain KS391 (HfrH lacMS286 φ80dlIac BKI thi−) used to isolate hyper-rec mutants is shown in Fig. 1a and b. Details of the construction of this strain are given in Materials and Methods. The special feature of strain KS391 is a duplication of the region of the chromosome that includes genes specifically required to metabolize lactose. This duplication has been achieved by inserting a φ80dlIac prophage at the attachment site (att80) for this phage (20). Each of the two copies of the lac region in strain KS391 is partially deleted; thus, KS391 is lactose negative (Fig. 1b). However, the two deletions (lacMS286 and φ80dlIac BKI) do not overlap each other (see Materials and Methods) and would be expected to recombine to form an intact lac region, making the strain lactose positive (Fig. 1b, c). lac+ recombinants can, in fact, be observed by streaking strain KS391 to single colonies on a lactose tetrazolium plate. On this indicator plate, lactose-negative colonies are red, and lactose-positive colonies are white. Strain KS391 initially formed red colonies, but after prolonged incubation white papillae, each consisting of a clone of lactose-positive recombinants, appeared on the colo-
nies' surface. These papillae did not appear when KS391 was made recA-. This strongly indicated that the formation of lac+ papillae was due to recombination.

To isolate mutants that increase the frequency of these recombinational events, I have sought mutant derivatives of KS391 that show an increased formation of lac+ papillae. This was done as follows. A culture of KS391 was mutagenized with ethyl methane sulfonate and plated on lactose tetrazolium to give about 500 colonies on each plate. Under these conditions, most wild-type colonies showed one or two papillae when examined under a stereoscopic microscope at ×5 magnification, after incubation for 3 days at 30°C. Colonies were picked as candidates for hyper-rec when they showed six or more papillae, although the number of papillae on a mutant colony was occasionally as high as 100. The mutant phenotype was always confirmed by restreaking on lactose tetrazolium plates. In all cases the hyper-rec phenotype proved stable on restreaking.

To confirm that the increased number of lac+ papillae characteristic of hyper-rec strains growing on lactose tetrazolium plates correlated with an increased number of lac+ recombinants, the following experiment was done. A total of 10⁶ cells of two hyper-rec mutant strains, RS18 (Table 1) and RSS356, which yield approximately 20 and 100 papillae per colony, respectively, and of the parent strain (KS391) were each plated on Xgal glucose + IPTG plates. On this indicator plate, lac- colonies are colorless, whereas lac+ colonies are dark blue and easily distinguished. After 3 days at 30°C, the colonies were scored for the lac character with a stereoscopic microscope at ×12 magnification. The wild-type strain yielded one lac+ colony, whereas mutants RS18 and RSS356 yielded the expected increase, with 22 and 1,200 lac+ colonies, respectively.

Nature of the lac+ recombinants from strain KS391. There are two classes of double-crossover events that could yield lac+ recombinants from the diploid strain KS391. In each case, there must be one crossover between the two deletions. For a lac+ region to be restored to its normal chromosomal location, the second crossover event must be between the terminus of deletion MS286 and proB. However, this latter event would not be possible if the extent of deletion MS286 were such that there was no homology between the segment distal to the lacY gene on the prophage and the comparable segment of the normally located lac region. If the latter were the case, all recombinants should have their lac+ region in the prophage. This possibility can be tested by determining whether the lac+ character is lost with excision of the prophage.

The technique of superinfection curing (20) was used to test for the location of the lac+ region. Of 100 lac+ recombinants from strain KS391 infected with a wild-type φ80, all showed loss of the lac+ character. These results indicated that all recombinants appear to be due to the crossover events depicted in Fig. 1b and that one terminus of deletion lacMS286 extends beyond the region of mutant homology between the normal lac region and φ80dIIlac (Fig. 1).

It is also conceivable that lac+ recombinants could arise by a single crossover event between

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**Table 1. Characterization of three hyper-rec mutants**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Tentative assignment</th>
<th>Accumulates DNA replicative fragments</th>
<th>Increased mutation frequency</th>
<th>Sensitive to:</th>
<th>Growth at 44°C</th>
<th>P1 cotransduction with:</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS1</td>
<td>lig</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>pts</td>
</tr>
<tr>
<td>RS3</td>
<td>mutU (19)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>ilv and metE str and malA</td>
</tr>
<tr>
<td>RS18</td>
<td>dam</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>4% of wild-type levels of DNA adenylic methylase</td>
</tr>
</tbody>
</table>

*In each instance mapping was done by following the hyper-rec character. Association of this character with the other phenotypes during mapping indicates that all are due to the same lesion, or to very closely linked lesions.

Strains RS3 and RS18 showed, respectively, 10- and 40-fold greater levels of BF23-resistant mutants than did the wild type. Ultraviolet (UV)-sensitive strains showed a twofold or slightly greater slope than wild type in a UV-killing curve (7). MMS-sensitive strains failed to form colonies on TY-MMS plates at 30°C.

Growth at 44°C was assayed as ability to form colonies on TY plates at 44°C.

This assay was performed by M. Marinus (personal communication) (11).
the two deletions. Since the two lac regions are inverted relative to one another on the chromosome, such an event would lead to an inversion of the entire region of the chromosome between the lac and φ80dlac regions. However, such an inversion would preclude superinfection curing of the φ80dlac + prophage since it disrupts the linkage between the two hybrid attachment sites (att80L and att80R) of the prophage. Other evidence that strains with this large inversion cannot be recovered has been presented elsewhere (E. B. Konrad, Ph.D. thesis, Harvard University, Cambridge, Mass., 1970).

Identification of the lesion in several hyper-rec mutants. To gain some insight into the nature of hyper-rec mutants, seven of these mutants were chosen at random and tested for several characteristics associated with defects in DNA metabolism: sensitivity to ultraviolet irradiation and to MMS, mutator activity, and the capacity to accumulate labeled DNA fragments after short pulses with tritiated thymidine. Three of these seven hyper-rec mutants were tentatively identified as having lesions in known loci (Table 1). (The remaining four mutants were not identified.) Mutations in two of these loci, dam, the structural gene for DNA adenine methylase, and lig, the structural gene for DNA ligase, are known to accumulate nicks or gaps in their DNA (11, 17), suggesting that this might be the basis for the hyper-rec character in these otherwise dissimilar mutants.

Some hyper-rec mutants are defective in joining DNA replicative fragments. Joining replicative fragments to make continuous daughter strands is a late step in DNA replication (15) known to involve DNA ligase (4, 9, 18) and DNA polymerase I (10, 14). Deficiencies in either of these enzymes lead to an increased accumulation of nicks or gaps in the chromosome. This indicated that strains carrying these mutations might be hyper-rec. I have found, in fact, that the ligase mutation isolated by Pauling and Hamm (17), lig-7(Ts), and the polymerase I mutation, polA1, isolated by De Lucia and Cairns (3), are hyper-rec; both of these mutations show approximately 10 papillae per colony when introduced into strain KS91.

These results, taken together with the recovery of a lig mutant by randomly screening hyper-rec mutants (Table 1), suggested that the hyper-rec phenotype might be useful in isolating mutants defective in joining DNA replicative fragments. No procedure has been previously developed for obtaining this class of DNA replication mutant. To see whether the hyper-rec phenotype might serve this purpose, I devised a screening method (Table 2) in which

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Defect</th>
<th>Temperature sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>polAex1</td>
<td>5' → 3' Exo</td>
<td>Yes</td>
</tr>
<tr>
<td>polAex2</td>
<td>5' → 3' Exo</td>
<td>Yes</td>
</tr>
<tr>
<td>polAex3</td>
<td>5' → 3' Exo</td>
<td>No</td>
</tr>
<tr>
<td>polAex4</td>
<td>5' → 3' Exo</td>
<td>Yes</td>
</tr>
<tr>
<td>polA506</td>
<td>Polymerase and 5' → 3' Exo</td>
<td>Yes</td>
</tr>
<tr>
<td>polA507</td>
<td>Polymerase and 5' → 3' Exo</td>
<td>Yes</td>
</tr>
<tr>
<td>polA551</td>
<td>Polymerase and 5' → 3' Exo</td>
<td>Yes</td>
</tr>
<tr>
<td>polA580</td>
<td>Polymerase and 5' → 3' Exo</td>
<td>?</td>
</tr>
<tr>
<td>polA582</td>
<td>Polymerase and 5' → 3' Exo</td>
<td>?</td>
</tr>
<tr>
<td>polA587</td>
<td>Polymerase and 5' → 3' Exo</td>
<td>?</td>
</tr>
<tr>
<td>polA598</td>
<td>DNA ligase</td>
<td>?</td>
</tr>
<tr>
<td>lig-541</td>
<td>DNA ligase</td>
<td>?</td>
</tr>
<tr>
<td>lig-561</td>
<td>DNA ligase</td>
<td>?</td>
</tr>
<tr>
<td>lig-18</td>
<td>Unknown</td>
<td>?</td>
</tr>
<tr>
<td>dnaS386</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>dnaS410</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>dnaS559</td>
<td>Unknown</td>
<td>No</td>
</tr>
</tbody>
</table>

* Temperature sensitivity or its absence is indicated only for mutations transferred into a wild-type background. The mutants indicated with a question mark were isolated as temperature sensitive, but proved to be only partially or not temperature sensitive on subsequent testing, and thus may have undergone reversion or been only marginally temperature sensitive. For each temperature-sensitive strain, cotransduction of this character with the hyper-rec and filamenting, as well as the enzymatic deficiency, was shown. This was done exhaustively for polAex1, and it has been shown that the enzyme deficiency reverts to wild type, together with the temperature- and MMS-sensitive traits, and that the mutation was conditionally lethal in several different strains (6). Mutants were considered defective in 5' → 3' exonuclease (Exo) activity of polymerase I if they had 5% or less of the wild-type level, in DNA polymerase I if they had 1% or less of the wild-type level, and in DNA ligase if they had 5% or less of the wild-type level (11). dnaS mutants have normal levels of DNA ligase and DNA polymerase I.

Lucia and Cairns (3), are hyper-rec; both of these mutations show approximately 10 papillae per colony when introduced into strain KS91.

<table>
<thead>
<tr>
<th>Screening step</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies examined for hyper-rec phenotype</td>
<td>~2 × 10⁶</td>
</tr>
<tr>
<td>Type</td>
<td>4,640</td>
</tr>
<tr>
<td>Hyper-rec mutants found</td>
<td>1,640</td>
</tr>
<tr>
<td>Conditional lethal at 44°C</td>
<td>140</td>
</tr>
<tr>
<td>Form filaments at 44°C</td>
<td>38</td>
</tr>
<tr>
<td>Accumulate labeled DNA fragments</td>
<td>17</td>
</tr>
</tbody>
</table>

* Screening steps were done sequentially.
these mutants were successively tested for conditional lethality at 44°C, filamentation at 44°C, and the capacity to accumulate labeled DNA fragments after short pulses with tritiated thymidine. The requirement for temperature-sensitive conditional lethality was included, since this phenotype is useful in establishing whether the affected function is essential and in studying its enzymology and physiology. Filamentation has been found associated with residual growth at the nonpermissive temperature in known DNA replication mutants, including lig-7(Ts) (4, 5, 7, 9).

Seventeen of the 38 mutants isolated by this screening were found to accumulate labeled DNA fragments (Table 3). Of these, three mutants were defective in DNA ligase, four in the 5' → 3' exonuclease associated with DNA polymerase I (7), and seven in both the polymerase and 5' → 3' exonuclease of DNA polymerase I. The remaining three mutants, called dnaS, accumulated very small fragments (Table 3) (8). On examination, it was found that only 7 mutations among these 17 were conditional lethals, in which the lethality and the tendency to accumulate DNA fragments were due to the same lesion. These seven were all in the 5' → 3' exonuclease of DNA polymerase I or in both the polymerase and 5' → 3' exonuclease of this enzyme (Table 3).

DISCUSSION

I have described an easy procedure for isolating large numbers of mutants with increased recombination frequencies between duplicated regions of the chromosome. By screening these hyper-rec mutants for additional traits, I have isolated four classes of mutant that accumulate labeled DNA fragments after short pulses with \(^3\)H thymidine (Table 3). Two of these classes had not been described previously, dnaS mutants, which accumulate very small DNA fragments, and conditionally lethal polymerase I 5' → 3' exonuclease mutants. These mutants have been discussed elsewhere (7, 8). A conditionally lethal mutant similar to those described here, in which both the 5' → 3' exonuclease and the polymerase activity of DNA polymerase I are reduced, has recently been described by Olivera and Bonhoffer (16).

The failure to isolate a conditionally lethal DNA ligase mutation (Table 3) despite the fact that such a mutation has been reported (4, 9, 18) suggests that these mutations are sufficiently rare that none occurred among the 140 conditionally lethal hyper-rec mutants that were screened. The absence of conditionally lethal dnaS mutants may be similarly explained, or it might mean that the dnaS gene product does not play an essential role in the cell.

Hyper-rec mutants may have value in screening for other classes of mutants involving DNA metabolism. Thus, by examining seven randomly chosen hyper-rec mutants, I have isolated a mutant (dam) deficient in adenine methylase (Table 1). dam mutants have an abnormally large number of nicks or gaps in their DNA (11), a trait that they share with lig, polA, and dnaS mutants and that may be associated with the hyper-rec character of these mutants. Other mutants in which nicks or gaps are present in the chromosome might also be hyper-rec. This random screening also yielded a mutant (mutU) that is an ultraviolet-sensitive mutator (19). Too little is known about the effects of this mutation to suggest a basis for its hyper-rec phenotype.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF


LITERATURE CITED


