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

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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 [^{3}H]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-att*80 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*$^-$ strains 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 endonuclease 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.

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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* φ80dII*lacBK*1 *thi*$^-$) (Fig. 1a, b) carries two copies of the *lac* region. This strain was constructed by introducing *lacMS286* and the defective prophage φ80dII*lacBK*1 into an Hfr Hayes strain (HfrH *proC*$^-$ Δ*tonB-trp thi*$^-$) by P1 transduction (12), utilizing the close linkage between *proC* and *lac* and the inclusion of the attachment site of prophage φ80 (*att*80) within the Δ*tonB-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 φ80dII*lac* was isolated by selecting melibiose-positive revertants of a strain carrying the prophage φ80dII*lac*2 (2, 20) and also a deletion of the *lac* region (Δ*lac-proX111*). Mapping showed the *lacBK1* deletion includes *lac-2* and *lacU118*, but not *lacX64* or *lacI* (6). Thus, at least one point mutation, *lacX64*, lies between *lacMS286* and φ80dII*lacBK*1. Therefore, deletions *lacMS286* and φ80dII*lacBK*1 do not overlap and would be expected to recombine with each other to form *lac*$^+$ or φ80dII*lac*$^+$ strains (see Results).

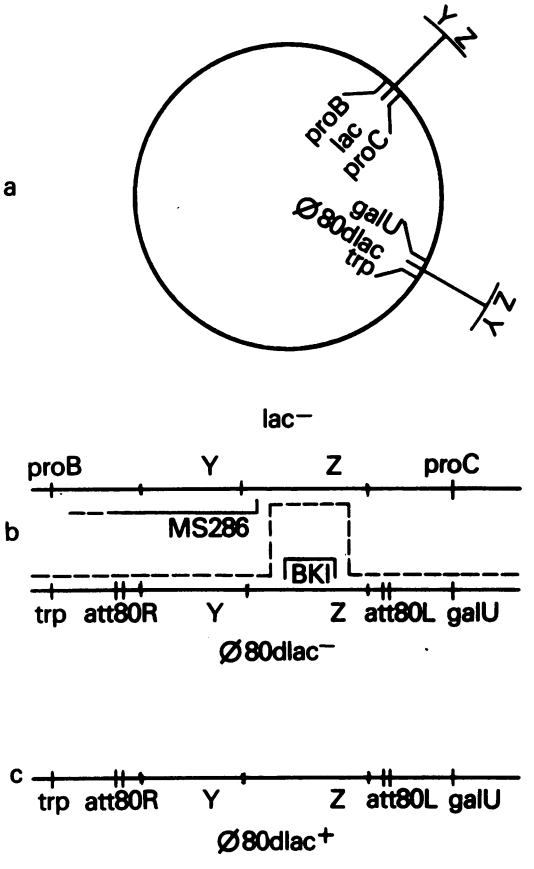

FIG. 1. *(a) Chromosome of lac-diploid strain (KS391) showing the normally present lac locus and the duplicate lac locus carried by prophage φ80dIIlac and inserted at the chromosomal attachment site for φ80 (att80) (1). A double-crossover event (b) between a lac deletion (BK1) on the prophage, and another (MS286) on the normal lac region to yield a lac⁺ region on φ80dlac (c), is shown. Z and Y are genes that code for β-galactosidase and lactose permease, respectively. The two hybrid φ80 attachment sites generated by prophage insertion are designated att80L and att80R. Loci proB and proC and trp and galU flank lac and the prophage φ80dlac, respectively (1).*

Media. Cells were grown in L-broth (12). Lactose tetrazolium plates, Xgal glucose + IPTG (isopropyl-β-D-thiogalactopyranoside) plates, and TY plates were prepared according to Miller (12). Methyl methane sulfonate (MMS) plates were prepared by adding 0.04% MMS to TY agar after autoclaving the agar.

Microbiological methods. Mutagenesis with ethyl methane sulfonate was done according to Miller (12). Sensitivity to ultraviolet radiation was measured as described in Konrad and Lehman (7). Transduction with phage P1*vir* and matings were done according to Miller (12). Mutation to resistance to BF23 phage was used to determine whether a strain had mutator activity. The strain was grown to saturation (about 2×10^9 cells/ml), and 0.1 ml was mixed with 10^{10} BF23 phage. After 10 min of incubation at 37°C, the mixture was plated on TY plates. The number of resistant colonies per cell plated was compared to the number obtained with a wild-type strain. Sensitivity to MMS was determined by streaking cells on TY MMS plates. MMS-sensitive strains did not grow on these plates. To determine whether a strain formed filaments, it was grown to exponential phase at 30°C and then shifted to 44°C for 3 h, chilled, and examined with a phase-contrast microscope. Strains were tested for superinfection curing of the φ80dII*lac*⁺ prophage by streaking onto lactose tetrazolium plates (on this indicator plate, lactose-negative colonies are red, whereas lactose-positive colonies are white) freshly spread with 10^{10} φ80 phage (20). Strains that were *lac*⁻/φ80dII*lac*⁺ showed a substantial number of red *lac*⁻/φ80dII*lac*° colonies that had lost the φ80dII*lac*⁺ prophage.

Enzyme assays. DNA ligase activity was measured as described by Modrich and Lehman (13); DNA polymerase I activity and its associated 5′ → 3′ exonuclease activity were measured as described in Konrad and Lehman (7). Methods for pulse-labeling DNA and determining whether labeled DNA fragments accumulate after short pulses were performed as described by Konrad and Lehman (7).

RESULTS

Isolation of hyper-rec mutants from strain KS391. A genetic map of strain KS391 (HfrH *lacMS286* φ80dII*lacBK*1 *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 φ80dII*lac* prophage at the attachment site (*att*80) 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 φ80dII*lacBK*1) 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 steroscopic 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 10^5 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 φ80dII*lac* (Fig. 1).

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

TABLE 1. *Characterization of three hyper-rec mutants*[a]

Strain	Tentative assignment	Accumulates DNA replicative fragments	Increased mutation frequency[b]	Sensitive[b] to:		Growth at 44°C[c]	P1 cotransduction with:	Other
				UV	MMS			
RS1	*lig*	Yes	No	Yes	Yes	Yes	*pts*	5% of wild-type levels of DNA ligase
RS3	*mutU* (19)	No	Yes	Yes	No	Yes	*ilv* and *metE*	
RS18	*dam*	No	Yes	Yes	Yes	Yes	*str* and *malA*	4% of wild-type levels of DNA adenine methylase[d]

[a] 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.

[b] 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.

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

[d] 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 ϕ80dII*lac* regions. However, such an inversion would preclude superinfection curing of the ϕ80dII*lac*$^+$ prophage since it disrupts the linkage between the two hybrid attachment sites (*att*80L and *att*80R) 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 KS391.

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 2. *Screening to isolate mutants that accumulate label in DNA fragments after a short pulse with [^{3}H]thymidine*[a]

Screening step	No.
Colonies examined for hyper-rec phenotype	~2 × 10^6
Hyper-rec mutants found	1,640
Conditional lethal at 44°C	140
Form filaments at 44°C	38
Accumulate labeled DNA fragments	17

[a] Screening steps were done sequentially.

TABLE 3. *Mutants that accumulate label in DNA fragments after short pulses with [^{3}H]thymidine*[a]

Mutant	Defect	Temperature sensitive
polAex1	5′ → 3′ Exo	Yes
polAex2	5′ → 3′ Exo	Yes
polAex3	5′ → 3′ Exo	No
polAex4	5′ → 3′ Exo	Yes
polA506	Polymerase and 5′ → 3′ Exo	Yes
polA507	Polymerase and 5′ → 3′ Exo	Yes
polA551	Polymerase and 5′ → 3′ Exo	Yes
polA580	Polymerase and 5′ → 3′ Exo	Yes
polA582	Polymerase and 5′ → 3′ Exo	?
polA587	Polymerase and 5′ → 3′ Exo	?
polA598	DNA ligase	?
lig-541	DNA ligase	?
lig-561	DNA ligase	?
lig-18	Unknown	?
dnaS386	Unknown	No
dnaS410	Unknown	No
dnaS559		No

[a] 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.

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.

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ADDENDUM IN PROOF

dnaS mutations have very recently been shown to be defective in deoxyuridine triphosphate diphosphohydrolase (deoxyuridine triphosphatase, EC 3.6.1.23) (B-K. Tye, P-O. Nyman, I. R. Lehman, Steven Hochhauser, and Bernard Weiss, Proc. Natl. Acad. Sci. U.S.A. 74:154-157, 1977).

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