Isolation and Characterization of *Escherichia coli* Mutants With Altered Rates of Deletion Formation

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ABSTRACT

Using site-specific mutagenesis *in vitro* we constructed a genetic system to detect mutants with altered rates of deletion formation between short repeated sequences in *Escherichia coli*. After *in vivo* mutagenesis with chemical mutagens and transposons, the system allowed the identification of mutants with either increased or decreased deletion frequencies. One mutational locus, termed *mutR*, that results in an increase in deletion formation, was studied in detail. The *mutR* gene maps at 38.5 min on the *E. coli* genetic map. Since the precise excision of many transposable elements is also mediated at short repeated sequences, we investigated the effects of the mutant alleles, as well as *recA*, on precise excision of the transposon Tn*9*. Neither *mutR* nor *recA* affect precise excision of the transposon Tn*9*, from three different insertions in *lacI*, whereas these alleles do affect other spontaneous deletions in the same system. These results indicate that deletion events leading to precise excision occur principally via a different pathway than other random spontaneous deletions. It is suggested that, whereas precise excision occurs predominantly via a pathway involving replication enzymes (for instance template strand slippage), deletions on an F′ factor are stimulated by recombination enzymes.

WORK in several laboratories during the past 10 years has demonstrated that spontaneous deletions in bacteria (FARABAUGH *et al.* 1978; ALBERTINI *et al.* 1982; JONES, PRIMROSE and ERLICH 1982; YI, STEARNS and DEMPLE 1988) and bacteriophage (STUDIER *et al.* 1979; PRIBNOW *et al.* 1981) occur preferentially at short repeated sequences, which are often less than 10 base pairs. Short repeats appear to be involved in deletion formation in several higher cell systems (NALBANTOGLU *et al.* 1986; HENTHORN *et al.* 1986; OGIHARA, TERACHI and SASAKUMA 1988). Human chronic external ophthalmoplegia is associated with mitochondrial DNA deletions of 4.5–7.7 kb at 13–18-bp homologies (JOHNS *et al.* 1989; SHOFFNER *et al.* 1989). Also, the SV40 genome is excised from the mammalian chromosome at 2–3-bp repeats (BULLOCK, FORRESTER and BOTCHAN 1984; BULLOCK, CHAMPOUS and BOTCHAN 1985). An exception is *Caenorhabditis elegans*, where short homologies are not found at deletion end points (PULAK and ANDERSON 1988).

Duplications have also been analyzed at the molecular level. Some duplications are mediated by *recA* at large homologies (ANDERSON and ROTH 1981), whereas another class of duplications occurs at short homologies (EDLUND and NORMARK 1981; WHORISKEY *et al.* 1987). It is very possible that duplications and deletions at short homologies are occurring by a similar mechanism. Our goal is to understand enzyme pathways stimulating these events.

The role of RecA in deletion formation depends on the system used. Deletions in *lacI* carried on an F′ factor are reduced 25-fold in a *recA* strain, yet they still predominate at short repeats (ALBERTINI *et al.* 1982). Other studies have described *recA* independent deletion formation at short sequence repeats on multicopy plasmids (*e.g.*, JONES, PRIMROSE and ERLICH 1982; YI, STEARNS and DEMPLE 1988). The degree of homology required for RecA to stimulate recombination has been investigated in several systems. Recombination frequencies of cointegrate formation extrapolated to 0 when perfectly homologous substrate lengths fell below 23–27 base pairs (SHEN and HUANG 1986). This was consistent with the findings of RUTTER and co-workers (WATT *et al.* 1985), that a minimum of approximately 20 bp was required for a high stimulation of recombination. On the other hand, the stimulation of recombination at short homologies is still greater than at sequences with no homologies. It is possible that the RecA protein acts in combination with other enzyme systems to generate deletions and duplications.

To identify enzyme pathways in *E. coli* which promote deletions, we have exploited the advantages of the well characterized *lacI* system for deletions (ALBERTINI *et al.* 1982), to isolate mutants which are altered in deletion formation. Here we describe these mutants and their characterization. One of the loci identified, defined by both EMS-induced point muta-

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tions and mini-Tn*10* insertions, results in increased deletion formation and maps near 38.5 min on the *E. coli* genetic map, and is termed *mutR*. DEMPLE and coworkers (YI, STEARNS and DEMPLE 1988) have shown that a large deletion of 0.5–0.75 min of the chromosome, including the 38.5-min region, results in increased deletion formation on a multicopy plasmid. It seems likely that the removal of the gene we have identified as *mutR* is responsible for the phenotype of strains carrying this deletion.

MATERIALS AND METHODS

Bacterial strains and reagents: Episomes were in P90C strain background, *ara* (*lacproB*) *thi*. A *recA* derivative of this strain was constructed by P1 transduction of *srl*::Tn*10* linked to a partial deletion of *recA*, from a strain supplied by K. B. LOW. The *U118* episome is F′*lacI373 lacZU118am proB*$^+$. The *Gly461* episome is F′*lacI378 lacZ102 proB*$^+$ (CUPPLES and MILLER 1989). XA103 (COULONDRE and MILLER 1977) is *ara* (*lacproB*) *thi gyrA metB argE*$_{am}$ *supF rpoB* and the *I-Z40* episome is F′*lac 14 lacI*^{Q} *proB* (MUELLER-HILL and KANIA 1974). The following Hfr strains were used for genetic mapping: A *nupG511PB*::Tn*10* derivative (CABRERA, NGHIEM and MILLER 1988) of Hfr PK191 from K. B. LOW. The remaining three Hfr strains were provided by B. BACHMANN: CGSC #6754, CGSC #6755 *ilv-691*::Tn*10*, and CGSC #6762 *argA81*::Tn*10*. The Tn*9* insertions in *lacZ, 29, G47* and *G7*, have been previously described (JOHNSRUD, CALOS and MILLER 1978; GALAS, CALOS and MILLER 1980). The lambda phage 1098 and 1105 (WAY *et al.* 1984) were obtained from N. KLECKNER and R. SIMONS. Media and genetic manipulations are as described (MILLER 1972).

***In vitro* mutagenesis of *lacI* and subcloning:** An F$_1$ phage containing a 1.7-kb *lacI* insert (kindly provided by M. MOTT) was used as template for site directed mutagenesis using mutant oligonucleotides as described by ZOLLER and SMITH (1982). Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer and purified and kinased as described (NORMANLY *et al.* 1986). To identify the phage containing the correct mutation, the mutant oligonucleotides were 5′ end-labeled with [^{32}P]ATP (WHORISKEY *et al.* 1987) and used to probe the mutagenesis mixture by plaque hybridizations (HANAHAN and MESELSON 1980) on nylon filters (Gelman Sciences). All potential candidates were plaque purified twice prior to dideoxy sequencing by the method of SANGER, NICKLEN and COULSON (1977). To subclone the *lacI* insert containing all the desired mutations into a high copy plasmid vector, the RF form of the F$_1$ phage was digested with *Eco*RI and *Bam*HI. The *lacI Eco*RI insert was ligated into the unique *Eco*RI site of the Tet^r Cm^r plasmid pBR329 (New England Biolabs; see CUPPLES and MILLER 1988), inactivating the *cat* gene. A strain was transformed for Tet^r and screened for Cm^s. The recombinant plasmid was confirmed by restriction enzyme digestion and electrophoresis of the plasmid DNA.

***In vivo* recombination of *lacI* mutations onto an episome:** To transfer the mutations from the cloned *lacI* to an episome, *E. coli* strain S90C, containing the *lacI-Z* fusion episome *I-Z40*, was transformed with the recombinant plasmid. The transformants were grown up to allow recombination between the *lac* operon sequences on the plasmid with the episome. The *I-Z40* episome was than mated, by bacterial conjugation (MILLER 1972) to the Su3$^+$ strain XA103. The mating mixture was plated on selective glucose-Xgal plates supplemented with methionine and nalidixic acid (20 μg/ml). On this medium, strains containing the wild type *I-Z40* episome stain dark blue. Recombinants were identified by their white (Lac$^-$) color. The recombinants were probed by colony hybridization with each of the mutant oligonucleotides to confirm that all of the mutations had been crossed onto the episome.

Isolation of Lac$^+$ revertants: Overnight cultures of strains grown in LB medium (MILLER 1972) were plated on lactose-minimal medium and titered on glucose-minimal plates. Colonies were counted and picked for purification after 48 hr.

***In vivo* mutagenesis and screening:** Cultures were treated with 30 μl of ethyl methanesulfonate (EMS) for various times, washed, grown overnight and monitored for survival frequencies as previously described (COULONDRE and MILLER 1977). The cultures were screened for mutant colonies by plating on glucose-minimal medium containing phenyl β-D-galactoside (Pgal) and 5-bromo-4-chloro-3-indoyl β-D-galactoside (Xgal) and looking for colonies with altered papillation rates as described (NGHIEM *et al.* 1988). For transposon mutagenesis, the lambda phage 1098 and 1105, which carry the Tn*10* and Tn*10-kan* derivatives (WAY *et al.* 1984) were used to infect DB-1. After 30-min preadsorption at room temperature, the cells were grown for 2 hr at 37° and then plated on glucose minimal Xgal Pgal plates with either tetracycline (20 μg/ml) or kanamycin (50 μg/ml) and incubated at 39.5° for 48 hr before being transferred to 37°. Colonies with altered papillation rates were saved for further study.

Southern analysis: Total *E. coli* chromosomal/episomal DNA was prepared from cultures grown in selective media as described (WHORISKEY *et al.* 1987). Oligonucleotides (18 mers) were 5′-end labeled and purified as described (WHORISKEY *et al.* 1987). An aliquot of 10 μg of DNA was digested with *Hin*cII (Pharmacia), separated by electrophoresis on 1% agarose gels (agarose from Bio-Rad) in Tris, borate, EDTA buffer (DAVIS *et al.* 1980) at 100 V for 5 hr. Southern analysis on nylon membranes (Gelman) was as described by SOUTHERN (1975).

RESULTS

The genetic system: Spontaneous deletions in *lacI* occur at about 10^{-7} in the population, making it difficult to isolate a class of mutants with deletion rates lower than this. For this reason recombinant DNA techniques were used to construct a strain which displayed an increased frequency of deletions. This strain was then mutagenized *in vivo* with chemical mutations and transposons to generate mutants which lowered (or raised) the deletion frequency.

Figure 1 shows the genetic selection used. The starting strain, DB, contains the *lac* operon on an F′ factor (ALBERTINI *et al.* 1982), and is depicted in Figure 1a. A *lacI-lacZ* gene fusion places β-galactosidase expression under the control of the *lacI*^{Q} promoter (MUELLER-HILL and KANIA 1974). The *lacI* gene contains two frameshift mutations, alleles *S42* and *378*, separated by 697 bp, causing a Lac$^-$ phenotype (ALBERTINI *et al.* 1982). Large, spontaneous deletions which remove both frameshifts and restore the reading frame result in a Lac$^+$ character. A hotspot for deletions was identified at a short, incomplete homol-

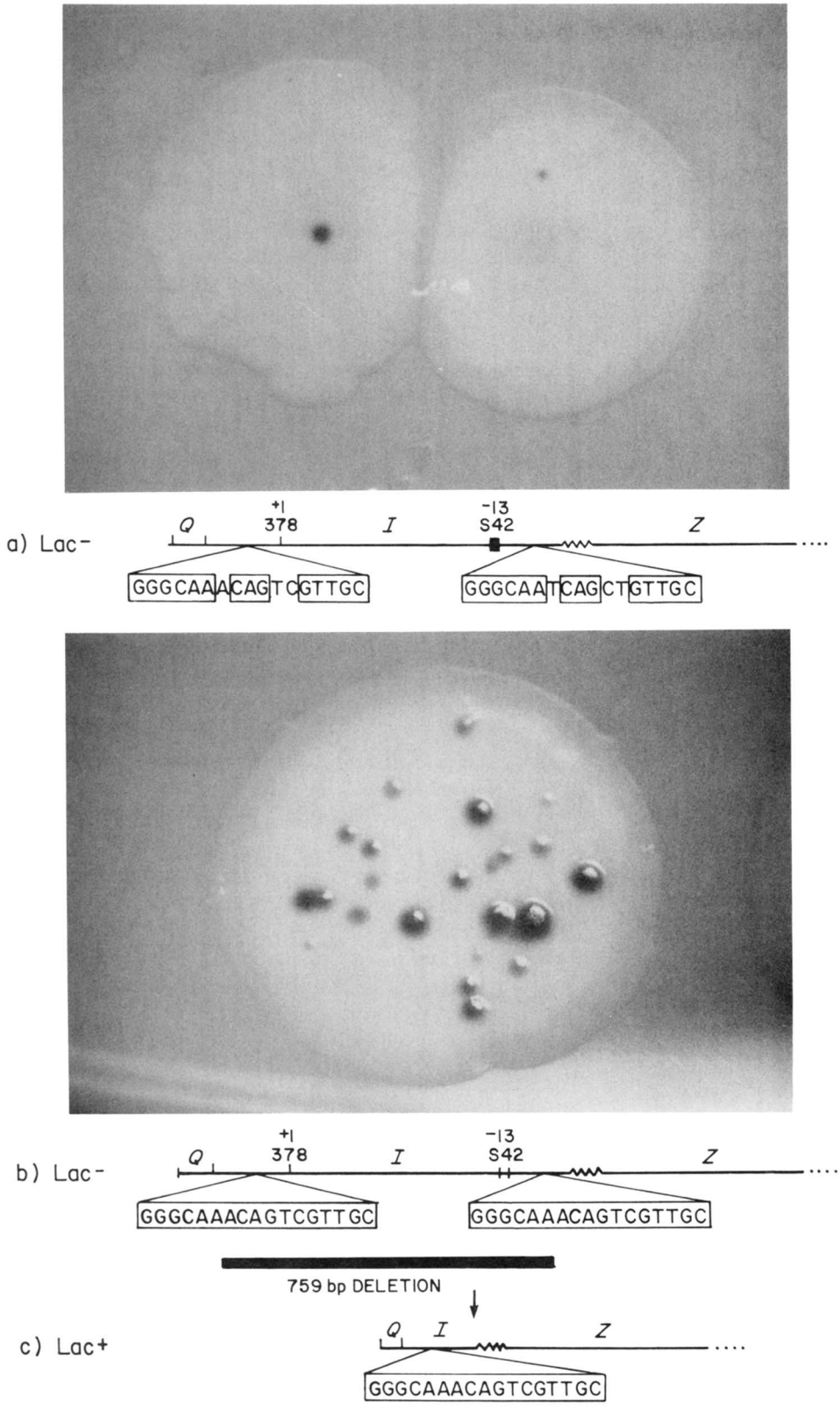

FIGURE 1.—Comparison of the reversion rate of strain DB to DB-1 by a papillation assay. The bacteria were grown on glucose minimal medium containing Pgal and Xgal (NGHIEM *et al.* 1988). The top photograph is a magnification of a colony of the Lac$^-$ DB strain. Lac$^+$ revertants are generated primarily by deletions at a hotspot site resulting from a 14/17 bp homology (see text). The relatively low level of spontaneous deletions results in the few blue papillae that are observed. An increase in the hotspot homology to a perfect 17/17 bp match increases the rate of deletions, resulting in an increased rate of papillation in strain DB-1, as seen in the bottom photograph.

ogy where 14 out of 17 bases are the same (ALBERTINI *et al.* 1982). Deletions at this site leave one copy of the homology at the endpoint of the deletion and restore the correct reading frame.

To increase the rate of deletions in this strain, the partial 14/17 bp homology was increased to a complete 17/17 bp match, as shown in Figure 1b. Because a decrease in the homology decreases the rate of deletions (ALBERTINI *et al.* 1982) we reasoned that an increase in the extent of the homology should increase the rate of that specific deletion, to generate a new *lacI-Z* fusion, as shown in Figure 1b.

TABLE 1

Lac$^+$ revertant frequencies for strains DB and DB-1 in a wild-type (+) or *recA* background

Strain	Background	Lac$^+$ revertant frequency × 10^8
DB	+	3.7 ± 0.8
DB-1	+	120 ± 11
DB	*recA*	0.6 ± 0.1
DB-1	*recA*	22 ± 3.0

Cultures were grown overnight in rich medium and plated on selective, lactose minimal medium. Frequencies are averages from several experiments. Strains DB and DB-1 are isogenic except for a 3-bp change which increases the hotspot homology site for deletions in *lacI* in strain DB-1.

TABLE 2

Lac$^+$ revertant frequencies in strains with altered papillation rates

Mutation in DB-1 background		Phenotypic classification	Lac$^+$ revertant frequency × 10^8
A[a]	+	Wild-type	120 ± 11
	61	Up	1100 ± 170
	66	Up	740 ± 90
	34	Up	170 ± 40
	22	Down	28 ± 3
	80	Down	29 ± 3
	93	Down	24 ± 5
B[b]	+	Wild-type	130 ± 11
	zdj84	Up	540 ± 30

[a] The strains are DB-1 carrying EMS-induced chromosomal mutations, which were originally identified by their altered rates of papillation on selective indicator media. An "up" or "down" classification indicates an increase or decrease, respectively, in papillation rates relative to DB-1. Frequencies are averaged from an analysis of several cultures.

[b] *zdj84* in a mini Tn*10* insertion which has been transduced into DB-1.

Since the sequences we wanted to alter to increase the hotspot homology were tightly linked to the frameshift mutations, it was necessary to first make the *S42*, *378* and *lacI-lacZ* fusion deletion and the increased homology all on one molecule *in vitro* and then recombine all the mutations together onto an episome *in vivo*. This was done in the following way: (1) Changes in *lacI* were introduced by site-directed mutagenesis *in vitro* using oligonucleotides containing the mutations. The single-stranded phage vector F1R1.7, which contains a cloned insert of the *lacI* gene (MOTT, VAN ARSDELL and PLATT 1984), was used as a template for single stranded synthesis using the mutation containing oligonucleotides as primers (ZOLLER and SMITH 1982). (2) The *lacI* gene containing the mutations was subcloned from the F1R.17 into a high copy plasmid vector (pBR329) and used to transform an *E. coli* strain containing a *lac* episome. The cells were grown to allow recombination of the *lac* region of the plasmid with the episome. (3) The episome was mated into a different strain background where the recombinant episome containing all the mutations was identified by using the appropriate seletions and screens (see MATERIALS AND METHODS). The recombinant strain was named DB-1.

Characterization of strain DB-1: The phenotypes of DB and DB-1 were compared on selective/indicator media and by a quantitative assay to determine if increasing the homology at the hot-spot site from a 14/17 to a 17/17 bp match increased the rate of deletions to a level allowing selection of mutants with decreased rates.

Figure 1 shows DB and DB-1 in a plate assay we have developed (see NGHIEM *et al.* 1988), to indicate the frequency of Lac$^+$ revertants of a Lac$^-$ strain. The strategy behind the plate is as follows: The bacterial minimal medium contains glucose-Pgal-Xgal (see MATERIALS AND METHODS). Lac$^-$ colonies metabolize the glucose. Lac$^+$ revertants have a growth advantage when the glucose is depleted because they are able to metabolize Pgal. They continue to grow out of the surface of a colony forming papillae and are easily identified by their blue stain in the presence of Xgal. The rate of formation of papillae therefore is an indicator of the reversion rate within the colony. This assay facilitates the screening of thousands of colonies to identify the rare strain with an altered reversion rate. This assay has been used to identify new mutator loci in *E. coli* (NGHIEM *et al.* 1988; CABRERA, NGHIEM and MILLER 1988). As seen in Figure 1, by this criteria DB-1 has an easily visualized, characteristic increased rate of reversion compared to DB.

The *lac* reversion frequency of DB-1 was quantitated by growing multiple cultures overnight in rich broth and plating them on lactose-minimal selective medium. The results, summarized in Table 1, show that DB-1 has a 30–35-fold increase in lac reversion compared to DB both in a wild-type background and *recA* background. This is consistent with the phenotype of this strain seen in the papillation assay.

To confirm that the increased *lac* reversion rate of DB-1 was due to an increase in deletions at the hot spot site, multiple, independently occurring Lac$^+$ revertants of DB-1 were purified and screened by colony hybridizations for the presence or absence of *lacI* sequences using 5′ end-labeled oligonucleotide probes (see MATERIALS AND METHODS), complementary to sequences internal to the hotspot deletion (data not shown). If a deletion occurred, the primer should not hybridize to the revertant. The starting Lac$^-$ strain, which had not undergone deletion, hybridized the primer under high stringency conditions, whereas the Lac$^+$ revertants did not. Analysis with other probes that were complementary to sequences that flank the hot spot homology outside of the deleted region still hybridized to these same revertants under stringent conditions. These results demonstrated that 60/62 Lac$^+$ revertants of DB-1 screened were deletions of

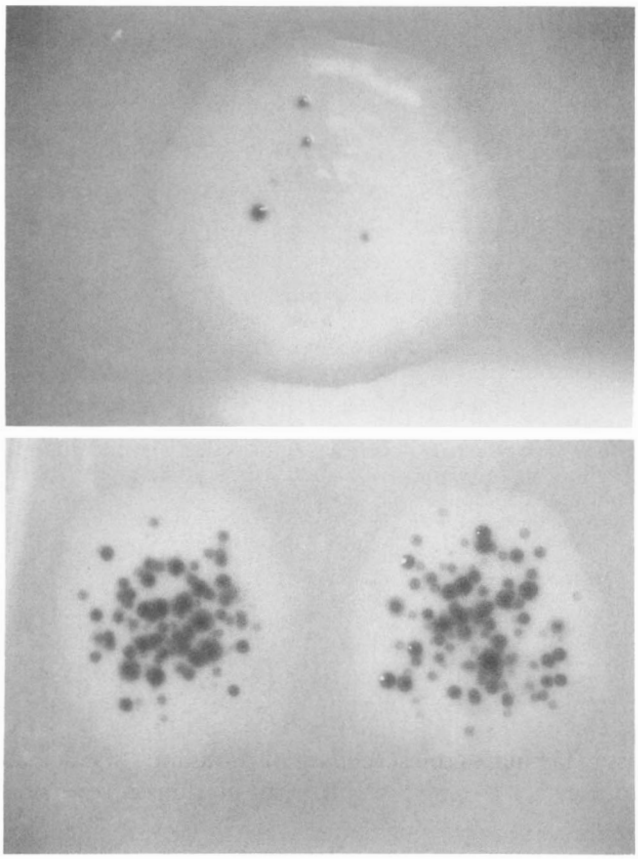

FIGURE 2.—Phenotype of *E. coli* mutants with altered rates of deletion formation in a papillation assay. Strain DB-1 was mutagenized *in vivo* to generate mutants with altered rates of deletion formation. Representatives of the two mutant classes identified are shown here grown on glucose-Pgal-Xgal plates (NGHIEM *et al.* 1988). Mutant 22 in the top photograph has a decreased rate of deletion formation (compare with the DB-1 strain at the bottom of Figure 1), and mutant 66 in the bottom picture has an increased rate of deletion formation compared to the starting DB-1 strain.

the DNA between the hotspot homology sites.

Isolation of mutants with altered deletion rates: To generate mutants affected in deletion formation, DB-1 was mutagenized *in vivo* with EMS (MATERIALS AND METHODS), followed by washing and outgrowth in rich medium overnight. The cultures were plated on glucose-Xgal-Pgal plates and screened for altered rates of papillation. Approximately 18,000 colonies were screened of which 10 were deemed potential candidates. Seven of these 10 were characterized further.

The candidates were re-screened on glucose-Xgal-Pgal and tested quantitatively for their Lac^+ reversion frequency. The results are summarized in Table 2A. By this initial analysis, the mutants were classified into two groups; those that had characteristic, increased reversion rates relative to DB-1 were classified as "up" mutants, whereas those with decreased rates were called "down" mutants. Figure 2 shows the phenotype of a representative mutant from each class on the papillation plate assay, which can be compared with the phenotype of the starting strain in Figure 1b.

Mutagenesis was also carried out by infecting cells with lambda-1098 carrying a mini-Tn*10* element (WAY *et al.* 1984). Following infection the mini-Tn*10* element, containing the gene encoding tetracycline resistance and parts of the ends of the original Tn*10*, frequently transposes from the lambda into the bacterial DNA. Since mini-Tn*10* does not encode transposase, once it inserts into a gene, it can no longer transpose to a new location (WAY *et al.* 1984). After preadsorption and outgrowth, cells were plated on the same indicator media described above, with tetracycline. Colonies with apparent increased rates of papillation were analyzed further. We detected four such colonies among 10,000 screened colonies. These were tested along with those derived by EMS mutagenesis. Table 2B shows the increase in deletions restoring the Lac^+ phenotype to strain DB-1 resulting from a representative of the mini-Tn*10* insertions. These data were taken after transduction of the Tet^r marker to an unmutagenized copy of DB-1.

Characterization of mutants: To determine the specificity of the mutant alleles, the reversion frequencies of defined mutations in the *lac* operon were analyzed in the different mutant backgrounds. Episomes containing mutant *lac* alleles were transferred by conjugation into the host mutants which had been cured of the initial episome. The frequencies of reversion of the *lac* episome mutations were analyzed and compared to the reversion rates in a wild type strain. Table 3 summarizes the results from this analysis. Mutants 61 and 66, which revert DB-1 at a high rate, also increase the reversion rate of DB. These host backgrounds however, do not affect the reversion of point mutations, as measured by the reversion of a *lacZ* amber mutation, and the reversion of a *lacZ* missense mutation. The two mutants derived by Tn*10* inserts behave similarly to mutants 61 and 66 in these tests (data not shown). Mutant 34, which has a 2-fold increase in DB-1 and DB reversion, does not affect reversion of any of the other mutant alleles. The down mutant 22, significantly affects deletions as seen by an approximate 10-fold reduction in the reversion of DB, but does not affect the reversion of one of the *lacZ* mutations and has only a slight affect on the reversion of the other. The remaining down mutants 80 and 93 also appear to specifically affect deletion formation. None of the up and down mutants tested affected the frequency of Rif^r mutants (which can only occur virtually exclusively via base substitutions; data not shown).

Genetic mapping: Mapping studies were undertaken to determine the genetic locus conferring an altered deletion rate in the mutants. Spontaneous Rif^r and Nal^r derivatives of the mutants were isolated in order to facilitate genetic selections. The derivatives

TABLE 3

Effect of different mutant backgrounds on Lac$^+$ revertant frequencies

Mutation carried on episome/strain	Reversion method	Lac$^+$ frequency × 10^8
DB/P90C	Deletion	2.6 ± 0.9
DB/61		18 ± 4.1
DB/66		14 ± 1.1
DB/34		5.1 ± 1.5
DB/22		0.2 ± 0.1
DB/80		0.3 ± 0.1
DB/93		0.1 ± 0.1
U118/P90C	Point mutation	5.3 ± 1.1
U118/61		6.4 ± 0.6
U118/66		4.8 ± 1.3
U118/34		
U118/22		4.5 ± 1.3
U118/80		3.3 ± 1.1
U118/93		5.3 ± 1.1
lacZ102/P90C	Point mutation	6.5 ± 1.3
lacZ102/61		4.6 ± 1.8
lacZ102/66		4.4 ± 1.4
lacZ102/34		
lacZ102/22		3.8 ± 1.2
lacZ102/80		
lacZ102/93		3.7 ± 1.5

The mutant alleles were present on an F'*lacproB* episome in strains deleted for the normal *lac-proA,B* region. *U118* is an amber mutation (converted from the *U118* ochre mutation) at the position encoding amino acid 17 in β-galactosidase. Lac$^+$ revertants can result from base substitutions either at the amber codon itself or at sites which create nonsense suppressors. The *lacZ102* mutation changes a residue in the active site of beta-galactosidase (CUPPLES and MILLER 1988), and can only revert via a G:C → A:T change at the mutational site (CUPPLES and MILLER 1989). Strains carrying the chromosomal mutations *61*, *66*, *22*, *80* and *93* are examined here.

were mated with four different Hfr strains. A selection was imposed for genetic markers donated by the respective Hfrs and the recombinants were screened for revision of the altered papillation frequencies using the papillation plate assay described above. The results indicated that the mutations resulting in an increased papillation mapped several minutes prior (clockwise) to 42 min, based on linkage with an antibiotic resistance marker at 42 min carried in an Hfr KL16 derivative. P1 crosses with different antibiotic resistance markers in that region, summarized in Figure 3, demonstrated with the mutations clustered near and probably clockwise to a marker at 38.25 min, suggesting a placement near 38.5 min. The phenotype of the mutants with a lowered deletion formation proved more difficult to score reliably on indicator plates after crosses, and definitive mapping will have to await the isolation of mutants carrying Tn*10* inserts. Therefore, we concentrated our studies on the mutants showing an increased deletion formation.

Distinguishing between precise excision of a transposon and other spontaneous deletions; the genetic system: Tn*9* is a 2800 bp transposable element (ALTON and VAPNEK 1979) which contains the genetic determinants for chloramphenicol (cm) resistance and is flanked by two 800-bp IS*1* elements in direct orientation. Allele *92* is an insertion of Tn*9* into *lacZ* between the sequence encoding amino acids 7 and 8 of β-galactosidase (JOHNSRUD, CALOS and MILLER 1978). This insertion results in a Lac$^-$ phenotype. We have shown that this strain reverts back to Lac$^+$ by one of the following three deletion events (diagrammed in Figure 4). (A) A precise excision deletion removes the Tn*9* element. (B) A spontaneous deletion that removes part of Tn*9* and a small part of the *lacZ* gene results in a *cat-lacZ* gene fusion placing *lacZ* under the control of the *cat* promoter. This event is limited in the size of the possible deletion as the *cat* promoter cannot be excised and the deletion cannot extent too far into *lacZ*, to maintain a Lac$^+$ phenotype. (C) A larger, but also limited deletion occurs that fuses *lacI* and *lacZ* in-frame, removing all of the Tn*9*. This deletion places *lacZ* expression under the control of the *lacI* promoter.

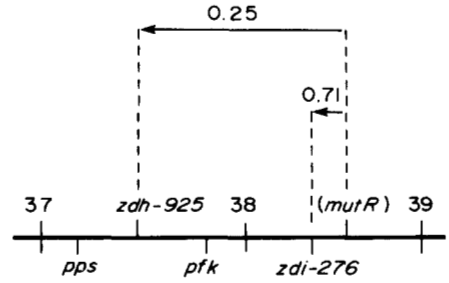

FIGURE 3.—Map position of the *mutR* locus. P1 cotransduction frequencies with antibiotic resistance markers mapping near 37.5 and 38.25 min (SINGER *et al.* 1989) are shown. *MutR* maps near the marker at 38.25 min, and has not been unambiguously ordered with respect to this marker and outside markers.

To determine if the deletions in the revertants represented a precise excision (A) or one of the other types of deletions (B or C), we screened for an inducible Lac$^+$ phenotype. In a precise excision deletion, expression of the *lac* enzymes are repressed on glucose-minimal-Xgal media by lac repressor, yielding white colonies, and induced on glucose-minimal-Xgal-isopropyl thiogalactoside (IPTG) media, giving rise to blue colonies. In a deletion that results in a fusion however, *lac* expression is constitutive (blue) on both media.

Molecular analysis of revertants: We confirmed that IPTG-inducible and constitutive revertants resulted from precise excision and fusion deletion events, respectively. DNA from revertants was examined by Southern analysis. The probe used was an oligonucleotide that hybridizes to *lacI* sequences of a restriction fragment that contains the 29 insertion. As seen in Figure 5 the probe hybridizes a 3.5 *Hin*cII fragment in the 29 insertion, a 789-bp fragment in the IPTG-inducible strains, representing a precise excision deletion and does not hybridize to the Lac$^+$

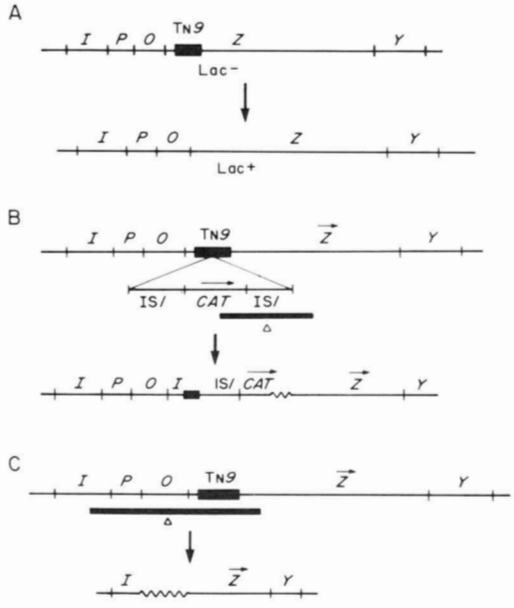

FIGURE 4.—Revertants of Tn*9* insertion *29* in *lacZ*. Reversion to Lac$^+$ can occur by (A) a precise excision of Tn*9*, (B) an in-frame deletion with one endpoint in the *cat* gene and one endpoint in *lacZ* resulting in *cat-lacZ* gene fusion or (C) a larger deletion resulting in a *lacI-lacZ* gene fusion.

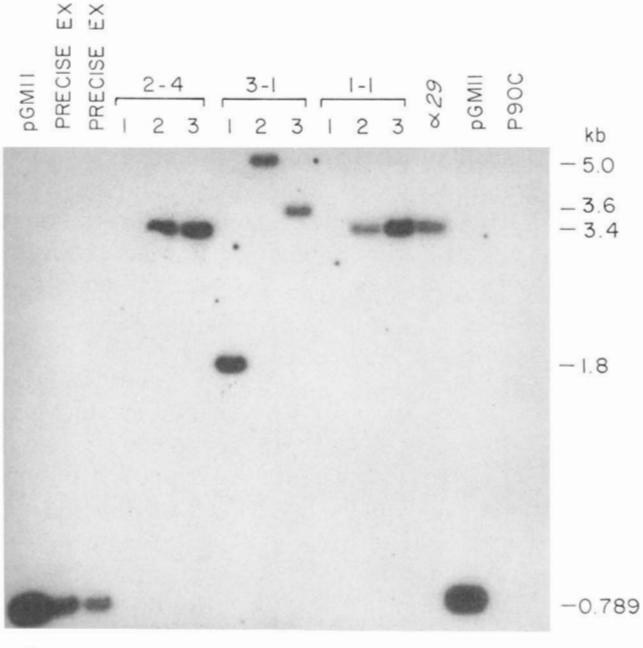

FIGURE 5.—Southern analysis of revertants of Tn*9* insertion *29*. DNA was isolated from each strain, digested with *Hin*cII, transferred following electrophoretic separation to nylon and probed with a 5′-end labeled probe complementary to a *lacI* sequence as described in MATERIALS AND METHODS. pGM11 is a plasmid containing the *lacI* gene on a 789-bp fragment. The lanes labeled precise excision are IPTG-inducible revertants. Samples 2-4, 3-1 and 1-1 are Lac$^+$ constitutive revertants, and are unstable. In each group, lane 1 is Lac$^+$Cm^s, lane 2 is Lac$^+$Cm^r and lane 3 is Lac$^-$Cm^r.

constitutive revertants. Analysis with several different probes (data not shown) confirmed that 2-4 and 1-1 are *lacI-lacZ* gene fusions and deletions of type C (as diagrammed in Figure 4), and 3-1 is a deletion of type B.

TABLE 4

Characteristics of Lac revertants from strains with Tn*9* insertions in *lacZ*, in a wild-type (+) or *recA* background

Tn*9* insertion	Background genotype	Lac$^+$ revertant frequency × 10^8	Ratio of IPTG inducible: constitutive Lac$^+$ revertants
29	+	5.6 ± 2.0	1:1
29	*recA*	2.1 ± 2.0	6:1
G74	+	1.7 ± 1.0	
G74	*recA*	1.0 ± 1.0	
G47	+	1.0 ± 0.5	
G47	*recA*	1.7 ± 1.5	

Lac$^+$ colonies were screened for the ratio of IPTG-inducible:constitutive revertants (see text). *29* is a Tn*9* insertion early in *lacZ*, whereas *G47* and *G74* are Tn*9* insertions later in *lacZ*. Frequencies reflect averages from 20 or more cultures.

Analysis of genetic requirements: Precise excision and fusion deletions were analyzed in a *recA* host. Table 4 shows the Lac$^+$ reversion frequency of three different Tn*9* insertions in *lacZ*. *G47* and *G74* are insertions in the middle and the end of the *lacZ* gene respectively. These insertions can only revert to Lac$^+$ by precise excision. Deletions of type B and C would not survive the genetic selection because deletion of *lacZ* sequences surrounding either of these insertions would disrupt β-galactosidase activity. The data indicate that precise excision is very slightly affected or unaffected in a *recA* host. The fusion deletions, however, are lowered in a *recA* strain. The nature of this system allows us to calculate the ratio of precise excision deletions to fusion deletions directly, independent of the actual calculated frequencies of each event. As can be seen from Table 4, this ratio changes from 1:1 in a wild-type strain to 6:1 in a *recA* background, reflecting the drop in fusion deletions relative to precise excision deletions. Note also that *recA* mutants do not stimulate precise excision of *G47* and *G74*, further showing that the increased proportion of IPTG-inducible revertants of insertion *29* in a recA strain is due to a decrease in fusion deletions.

We examined the mutant 66, described above, which increases deletion frequency, for the level of precise excision and fusion deletions. As Table 5 shows, there is no significant effect on precise excision of the Tn*9* insertions *G47* and *G74* in the mutant background. However, in the Tn*9* insertion *29*, where both precise excision and deletions can be scored, deletions are increased 5-fold in mutant 66 relative to precise excision, as evidenced by the shift in proportion from 1:1 in a wild-type background to 1:5 in a *recA* background.

DISCUSSION

We designed a system to identify *E. coli* mutants with altered rates of deletion formation. The system

TABLE 5

Behavior of *lacZ*::Tn*9* insertions in strains with altered rates of deletion formation

Insertion/strain	Lac$^+$ revertant frequency × 10^8	Ratio of IPTG-inducible to constitutive Lac$^+$ revertants
29/P90C	5.6 ± 2.0	1:1
29/66	8.8 ± 2.6	1:5
G47/P90	1.0 ± 0.5	
G47/66	1.0 ± 0.6	
G74/P90C	1.7 ± 1.0	
G74/66	2.5 ± 0.9	

Frequencies are averages from several cultures. *22* and *66* are mutants with decreased and increased rates of deletion formation, respectively. P90C is the wild-type background.

is based on the alteration of a previously characterized hot spot site for deletions in the *lacI* gene (ALBERTINI *et al.* 1982) and the ability to monitor specific *lacI* deletions by a well characterized, sensitive bacterial plate assay (NGHIEM *et al.* 1988). To identify mutants with either decreased or increased rates of deletions, the short incomplete 14/17 bp homology at the site of a hotspot for deletions was increased to a perfect 17/17 bp match. The 20-fold increase in deletion formation as a result of this change extends our previous data on the importance of the extent of the homology in deletion formation (ALBERTINI *et al.* 1982). In that study single base substitutions reducing the homology from 14/17 bp to 13/17 bp resulted in a 10–40-fold decrease in deletion frequency.

Since the increase in deletion frequency resulting from the 20/20-bp homology could be easily visualized by a bacterial plate assay it became possible to identify host mutants which lowered or raised this rate. We identified two classes of mutants after *in vivo* mutagenesis with EMS, and by the insertion of a mini-Tn*10*. These mutants had either a 2–9-fold increase or a 4–5-fold decrease in deletion rates. Tests with different *lac* mutations and with the induction of *rpoB* mutations showed that the loci specifically affected the formation of three types of deletions (see Figures 1, 4 and 5) and did not affect base substitutions. The "down" mutations proved difficult to map, although tests for sensitivity to MMS showed that they were not *recA* mutations. The "up" mutations clustered near 38.5 min. We have identified both EMS-induced mutations and mini-Tn*10* inserts at this locus, termed *mutR*. It seems likely that *mutR* is responsible for the phenotype observed for a deletion of the 38.25–38.5-min region of the chromosome (YI, STEARNS and DEMPLE 1988). The *mutR* locus is distinct from any described single gene. LUNDBLAD and KLECKNER (1984) detected "up" mutations for Tn*10* excision, also using a papillation assay. These mutations were in the mismatch repair genes (*mutH, mutL, mutS, uvrD*) or in *recB,C* (LUNDBLAD *et al.* 1984), and can be distinguished from *mutR* both by resulting phenotype and map position. The precise excision of three different Tn*9* insertions tested was not affected by *mutR*.

The finding that many rearrangements occur preferentially at short homologies has led to two basic models to explain the mechanism of deletion formation. One model, which relies on aberrant replication, suggests that slipped-mispairing during DNA synthesis can lead to deletions (FARABAUGH *et al.* 1978; see also EFSTRADIADIS *et al.* 1980 and BRUNIER, MICHEL and ERLICH 1988). An alternate model involves recombination by enzymes systems promoting rearrangements at short sequence repeats (FARABAUGH *et al.* 1978; ALBERTINI *et al.* 1982; WHORISKEY *et al.* 1978). Additional experimental evidence in favor of each mechanism has been presented (*e.g.*, DASGUPTA, WESTON-HAFER and BERG 1987; SINGER and WESTLYE 1988).

One can envision deletions and duplications at short repeated sequences as in fact being subject to both types of pathways, those involving replication enzymes and those involving recombination enzymes. Which mode will predominate depends on the nature of the replicating element, the experimental conditions, and even the DNA sequence surrounding the short homology involved. Thus, a significant fraction of deletions in T4, a highly recombinogenic phage, may be stimulated by recombination enzymes (SINGER and WESTLYE 1988), whereas deletions at short repeats flanked by extensive inverted repeat structures might occur predominantly by replicative slippage, especially when on a fast replicating plasmid (DASGUPTA, WESTON-HAFER and BERG 1987). In this respect, the precise excision of transposable elements at short, repeated sequences, would be expected to occur under normal conditions principally via replicative slippage, as has indeed been argued in the case of Tn*10* (BRUNIER, MICHEL and ERLICH 1988). A central question, however, is which mode is operating for the majority of rearrangements that occur at short sequence homologies on the chromosome or on a single copy F′ factor without the aid of significant inverted repeat structures? The finding that large duplications also occur at short sequence repeats (WHORISKEY *et al.* 1987) might be interpreted as evidence against slipped mispairing during replication in these cases, since the template strand would have to slip back and disrupt a significant stretch of duplex DNA. Although there are ways around this objection, it is reasonable to focus on recombination enzyme based mechanisms for deletions and duplications occurring on the chromosome and the episome, particularly in light of the finding that *recA* strains have a reduced frequency of deletions occurring on F′ factors (ALBERTINI *et al.* 1982), and that increased recombination can result in increased deletion formation (SYVANEN *et al.* 1986).

The role of RecA in deletion formation appears to differ in different systems. A 25 fold decrease in deletions is detected in *recA* derivatives of strain DB (ALBERTINI *et al.* 1982). A study on intermolecular recombination between a 12 bp perfect homology in the 16-bp termini of IS*5*, demonstrated that this small homology was not a good substrate for RecA (TIMMONS, LIEB and DONNIER 1986). Other studies have also demonstrated a *recA* independence of deletions (FRANKLIN 1967; INSELBURG 1967; JONES, PRIMROSE and ERLICH 1982; YI, STEARNS and DEMPLE 1988). One explanation of these differences stems from the work of SYVANNEN and co-workers, who showed that large F′ factors stimulate recombination (SYVANEN *et al.* 1986). It is conceivable that the level of deletions and duplications at short homologies seen on F′ factors is normally increased by the higher level of recombination, which is *recA* dependent. Thus, a significant lowering is seen in *recA* strains. However, deletions on non-conjugative plasmids do not benefit from this increase and have a lower level of deletions to start with. Therefore, a reduction in a *recA* strain is not observed. It is not clear whether the role of RecA is direct, or whether it is indirect, acting in concert with other systems. Three pathways for homologous recombination have been identified in *E. coli* (the *recB,C,D*, the *recE*, and the *recF* pathway; see review by SMITH 1989). It remains to be seen what role, if any, each of these pathways plays in deletion and duplication formation at short homologies.

Two additional mechanisms which have been proposed for deletion formation bear mention at this point. One theory proposes that deletions are created by the repair of double stranded breaks and the searching for short homologies by template-directed and post-repair ligation (PULAK and ANDERSON 1988). Alternatively, IKEDA and co-workers (IKEDA, KAZUKO and NAITO 1982; NAITO, NAITO and IKEDA 1984; IKEDA 1986) have proposed that DNA gyrase stimulates intermolecular recombination between nonhomologous sequences. The recombination joints that were studied *in vitro* contained not more than 3 bp homologies (NAITO, NAITO and IKEDA 1984).

In summary, in the work reported here we have defined a genetic locus by both point mutations and insertions that increases deletion formation on an F′ factor, and which does not correspond to any of the known loci which both stimulate mismatch repair and create a hyper-recombinogenic phenotype. This locus, *mutR*, which maps near 38.5 min, is very likely the locus deleted in a strain which displays a sharp increase in deletions at short homologies on a non-conjugative plasmid (YI, STEARNS and DEMPLE 1988). The *xth* locus, which maps in this deleted segment, is not the locus responsible for the increased deletion frequency (YI, STEARNS and DEMPLE 1988). *MutR* is the first locus which affects deletions in the same manner on both F′ factors and on small, multicopy plasmids. There is no response of Tn*9* precise excision to *mutR*, indicating that even though short direct repeats are involved, the deletions which result in precise excision occur by a different pathway than other spontaneous deletions on an F′ factor, a conclusion reinforced by the different response to *recA* of precise excision and other deletions (see Table 4). The characterization of the *mutR* encoded product should yield important insights into the mechanism of deletion formation.

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