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Illegitimate Recombination in an *Escherichia coli* Plasmid: Modulation by DNA Damage and a New Bacterial Gene

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We studied DNA rearrangements in *Escherichia coli* by using a plasmid-based system with a transcriptionally silent *tet* gene and selecting for Tet^r isolates. The predominant activating event was a 1.3-kilobase-pair deletion in the plasmid between two sites, with 14 of 19 base pairs being identical. These deletions occurred equally frequently in a *recA*$^+$ strain and a *recA13* mutant. However, the frequency of Tet^r occurrence was stimulated 50-fold by treatment of the cells with UV light in a process that was at least partly independent of the SOS response. Bacterial mutants deleted for the *xth-pnc* region of the chromosome exhibited a strongly elevated spontaneous frequency of Tet^r isolates, all with the same 1.3-kilobase-pair deletion. This phenotype of high-frequency deletion could be complemented by an episome covering this region, but not by the cloned *xth* gene. These studies helped to define the role of different DNA damages in illegitimate recombination and identify a region of the *E. coli* chromosome that contains a gene whose product normally suppresses illegitimate deletions.

The mechanisms of nonhomologous, or illegitimate, recombination are still poorly understood in both procaryotes and eucaryotes. Illegitimate rearrangements are distinguished by their independence from extensive regions of sequence match and from proteins such as *Escherichia coli* RecA (19) and lambda Red (15) or specialized enzymes such as the bacteriophage integration proteins or yeast FLP recombinase (30). The site-specific recombinations mediated by these latter proteins are not usually referred to as illegitimate. A diverse array of DNA rearrangements are categorized as illegitimate, including deletions, insertions, inversions, duplications, and transpositions. Given the dissimilar features of these various products, it is likely that illegitimate rearrangements can occur by more than one mechanism.

Illegitimate recombination tends to occur at a low frequency and can have detrimental effects by disrupting gene regulation or function. The extensive chromosomal rearrangements associated with neoplastic cells constitute important examples of these consequences (5, 35). There is evidence that these rearrangements can play a key role in the progression of some tumors by activating cellular oncogenes, e.g., c-*myc* (7) and *trp-met* (8).

Stress can promote the incidence of some illegitimate recombination. Thus, treatment of bacteria with UV light, X rays, or nitrous acid enhanced the production of gene duplications (17) or deletions (32), although the extent of *recA* involvement in these processes was not always measured. Treatment of eucaryotic cells with ionizing radiation increased the frequency of aberrant rearrangements (6). These results support the view that illegitimate recombinations can arise through errors in the machinery that replicates, repairs, and recombines cellular DNA. Such errors are apparently minimized under ordinary circumstances. Nevertheless, up to 1% of growing *Salmonella typhimurium* or *E. coli* bacteria may have tandem duplications in some region of the genome (2).

Two models have been formulated to explain illegitimate recombination in bacteria. First, certain DNA rearrangements occur at short regions of sequence match that may be interrupted by short unmatched sequences (1, 12). This observation led Albertini et al. (1) to suggest that even large deletions are generated by slippage during replication, as proposed by Streisinger et al. (33) for small deletions. Second, Ikeda and co-workers (20, 21) observed that the addition of oxolinic acid or purified preparations of DNA gyrase to an in vitro lambda packaging system stimulated the formation of recombinant molecules between the phage and plasmid DNA. In a parallel to these observations, the DNA sequences at sites of simian virus 40 excision from a rat cell line were found to be similar to the known eucaryotic topoisomerase I cleavage sites (4). These data implicate DNA topoisomerases in at least some illegitimate recombination in both procaryotic and eucaryotic cells.

The object of the present study was to investigate the roles of different types of DNA damage and of specific bacterial genes in the formation of illegitimate recombinants. We approached these questions by using an *E. coli* plasmid containing a promoterless *tet* gene (3) that is apparently activated only by illegitimate rearrangements. A large number of the recombinant products were characterized by restriction mapping, and many were characterized by DNA sequencing. The results help to delineate illegitimate recombination mechanisms in *E. coli* and point to the existence of a previously unidentified bacterial gene that is involved in controlling this process.

MATERIALS AND METHODS

Strains and routine growth. The strains used in this study were derivatives of either AB1157 (9) [BW9109 Δ(*xth-pnc*), BW9091 (*xth-1*), and DM49 (*lexA3*) (26)] or RR1 [HB101 (*recA56*) (25)]. Strain GMS724 containing the F'500 episome was provided by Barbara Bachmann (*E. coli* Genetic Stock Center, Yale University, New Haven, Conn.). Streptomycin was added to a final concentration of 25 μg/ml in both liquid and solid media for the growth of AB1157 derivatives. Unless otherwise specified, ampicillin was added at 150 μg/ml for plasmid-bearing strains. Isolates carrying an activated

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pKK175-6 *tet* gene were selected on solid media containing 5 μg of tetracycline per ml (see below) and were cultured in liquid media containing 6 to 15 μg of tetracycline per ml. Routine culture and plating methods were done as described by Miller (27).

Plasmids. Plasmid pKK175-6 (Fig. 1) was derived from pBR322 with a few important alterations (3). The promoter for the tetracycline gene was deleted, and a transcriptional terminator (T1) was placed in front of the promoterless *tet* gene so that the plasmid did not confer tetracycline resistance. There was a second such insertion beyond the 3′ end of *tet* (data not shown). A provisional 4,370-base-pair (bp) DNA sequence for this plasmid was supplied by Harry Osterman (Pharmacia, Milwaukee, Wis.).

The plasmid pXth1 was constructed by replacing the 0.5-kbp *SalI-Hin*dIII fragment in pACYC184 (25) with the 0.6-kbp *SalI-Hin*dIII fragment from pKK175-6. This manipulation removed the *tet* promoter from pACYC184. The large *Bam*HI fragment containing the *xth* gene from the plasmid pSGR1 (29) was then inserted into the *Bam*HI site of the modified pACYC184 plasmid. This generated a Tet^s plasmid (pXth1) that was compatible with pKK175-6 and that expressed exonuclease III (see below).

Media and chemicals. K medium was M9 medium (27) supplemented with 1% glucose, 1% Casamino Acids (Difco Laboratories, Detroit, Mich.), 0.5 μg of thiamine hydrochloride per ml, 1 mM $MgSO_4$, and 0.1 mM $CaCl_2$. H_2O_2 (30%) was purchased from Fluka-Garantte. Catalase was purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and had a specific activity of 65,000 U/mg. Restriction enzymes, T4 DNA polymerase and polynucleotide kinase, and *Bal* 31 nuclease were from Bethesda Research Laboratories (Gaithersburg, Md.) or New England BioLabs, Inc. (Beverly, Mass.) and were used according to the instructions of the manufacturers. T7 DNA polymerase (Sequenase) was from US Biochemicals and was used as suggested by the supplier. The oligonucleotide used for dideoxy sequencing (5′-ATCCCGCAAGAGGCCCG-3′, corresponding to the counterclockwise 17 nucleotides from the pBR322–pKK175-6 *Eco*RV site) was synthesized by Operon Technologies Inc. (San Pablo, Calif.). The [α-^{32}P]dATP used in the sequencing experiments was from New England Nuclear Corp. (Boston, Mass.).

Nucleic acid analysis. The plasmid DNA used for routine purposes was prepared by a mini-cleared lysate method (31). Other general procedures, including the Maxam-Gilbert sequencing reactions and acrylamide-urea gel electrophoresis, were as described by Maniatis et al. (25). For Maxam-Gilbert sequencing, CsCl-purified plasmid DNA (25) was digested with *Eco*RV and labeled in a reaction with T4 DNA polymerase and [α-^{32}P]dATP. This material was digested with *Dde*I, and the smaller fragment was isolated after electrophoresis on a 5% acrylamide gel and used in the sequencing reactions. For dideoxy sequencing, CsCl-purified plasmid DNA (or miniprep DNA in some experiments) was denatured by the addition of 0.25 M NaOH and was neutralized by the addition of 0.25 M HCl and 0.1 M Tris hydrochloride (pH 7.5). Annealing of the primer oligonucleotide and the DNA synthesis steps with [α-^{32}P]dATP were carried out as described by the supplier of T7 DNA polymerase (US Biochemicals).

Isolation and quantitation of Tet^r derivatives of pKK175-6. Approximately 3×10^7 cells from an overnight culture were inoculated into 500 to 1,000 ml of K medium and grown to the early log phase (optical density at 600 nm, 0.2 to 0.4; 5×10^7 to 1×10^8 CFU/ml). The culture was chilled on ice; and the cells were spun down (2,500 × *g*, 10 min), washed once with M9 salts, and suspended in 5 to 10 ml of M9 salts. Samples of 0.25 ml of the concentrated cell suspension were added to 2.5 ml of top agar and poured onto plates containing 5 μg of tetracycline per ml. The tetracycline-containing plates were incubated for 28 to 32 h at 37°C. Typically, 1×10^9 to 2×10^9 bacteria were plated onto each tetracycline-containing plate. Colonies were picked from the plates and restreaked onto tetracycline-containing plates to verify the Tet^r phenotype. The number of Tet^r isolates observed was proportional to the number of cells plated for strains AB1157 and BW9109 (see below). The rate of Tet^r production per cell per generation was estimated as described by Drake (11).

Hydrogen peroxide treatment. Cells (1 to 2 liters) were grown as described above. H_2O_2 (5 mM) was added, and incubation was continued at 37°C for 30 min, at which time 50,000 U of catalase was added. The treated cells were incubated at 37°C for 90 min, collected by centrifugation, washed, and plated as described above. One third of the culture was plated onto selective medium before hydrogen peroxide treatment.

UV irradiation. Mid-log-phase bacteria (1 to 2 liters) were spun down and suspended in 50 ml of 0.1 M $MgSO_4$. The cells were irradiated with gentle agitation for 75 s (AB1157 and BW9109) or 5 s (DM49) in two 20-cm glass petri dishes with a germicidal UV lamp (100 J/m^2 per min). After UV irradiation, the bacteria were centrifuged, suspended in 1 to 2 liters of LB medium, and incubated at 37°C in the dark for 90 min. The cells were then concentrated and plated as described above. The plates were incubated in the dark to prevent photoreactivation. One-third of the culture was plated onto selective medium before UV treatment.

RESULTS

A plasmid system to detect illegitimate recombination. A general strategy for the selection of illegitimate recombinants is to employ a reporter gene that is not expressed efficiently unless an activating rearrangement occurs. Such an approach has been used to select deletions (32) or tandem duplications (2, 17) in the bacterial chromosome. A useful system must enable the screening of large numbers of cells and the rapid determination of the newly activated structures. We chose to use plasmid systems to meet these ends.

We constructed an Amp^s derivative of plasmid pBR322 with the *bla* gene promoter deleted, but >95% of the Amp^r cells that were recovered contained mutations in the bacterial chromosome rather than in the plasmid (data not shown). An alternative plasmid construction was then brought to our attention. This plasmid, pKK175-6 (3), is also a derivative of pBR322, but it has a deletion of the *tet* promoter, along with transcriptional terminators installed just 5′ to *tet*. Consequently, pKK175-6 confers the Amp^r but not the Tet^r phenotype (3); we found that AB1157 containing pKK175-6 would not grow on as little as 5 μg of tetracycline per ml. Tetracycline-resistant colonies did appear at a low frequency, however. The spontaneous reversion rate for the wild-type strain AB1157 was approximately 7×10^{-11} per cell per generation (Table 1). In every case examined, the Tet^r character was transferred by the plasmid DNA isolated from the Tet^r cells. Furthermore, restriction enzyme analysis of a number of Tet^r plasmids revealed that 100% (99 of 99) of the molecules underwent a substantial rearrangement (see below).

Independence from the *E. coli recA* gene. Results of previous work in other systems have indicated that recombination

TABLE 1. Spontaneous frequency of Tet^r derivatives of pKK175-6[a]

Strain	Total no. of cells plated (10^{10})	Total no. of Tet^r isolates	Frequency/cell (10^{-10})	
			Total	Per generation
RR1 (*recA*$^+$ *xth*$^+$)	10.6	58	5.5	1.3
HB101 (*recA13*)	5.5	75	14	4.5
AB1157 (*recA*$^+$*xth*$^+$)	45.6	88	1.9	0.7
BW9109 [Δ(*xth-pnc*)]	6.3	423	67	13
BW9109(pXth1)	4.8	279	58	12
BW9109(F'500)	4.0	24	6.0	1.8
BW9091 (*xth-1*)	1.94	0	<1	<1

[a] The number of independent experiments for each of the following strains for the data presented here were as follows: RR1, 2; HB101, 5; AB1157, 8; BW9109, 3; BW9109(pXth1) 3; BW9109(F'500), 2; BW9091, 2. Additional experiments for which data are not presented produced jackpots, in which the Tet^r frequency was >10-fold higher than the averages seen here; the normal variance was approximately twofold.

between even short stretches of sequence matches in *E. coli* can require the RecA protein (1, 12). For pKK175-6, the *tet* activation rate was certainly not diminished in the *recA* mutant HB101 compared with that in its *recA*$^+$ parent RR1, and was elevated slightly (Table 1). This observation provides strong evidence that the rearrangements arose through illegitimate recombination. This conclusion was confirmed by direct sequence analysis of the breakpoints of the rearrangements (see below).

A bacterial gene that reduces deletion frequency. An important goal was to determine the role of known gene products in promoting or preventing illegitimate recombination. As part of this study, the pKK175-6 *tet* activation frequency was measured in strains containing mutations in the *xth* gene (Table 1). The *xth* gene encodes exonuclease III, an enzyme that has been implicated in the repair of DNA lesions produced by oxygen free radicals that might be produced continuously in vivo (9, 10; J. T. Greenberg and B. Demple, submitted for publication). Strain BW9109, which has a deletion of the *xth* to *pnc* interval, exhibited a spontaneous frequency of *tet* activation that was between 18- and 35-fold higher than its wild-type parent AB1157 (Table 1). In contrast, strain BW9091 (bearing the *xth-1* point mutation) displayed the wild-type frequency of Tet^r isolates (Table 1).

To clarify the possible role of exonuclease III in *tet* activation, the cloned *xth* gene (in plasmid pXth1; see above) was introduced into strain BW9109. Exonuclease III was expressed in this strain, as demonstrated by a substantially increased resistance to H_2O_2 (9). After a 30-min challenge with 1 mM H_2O_2, BW9109(pXth1) showed a survival of 102%, while BW9109 showed a survival of only 0.5%. However, the *tet* activation frequency was unchanged by the introduction of pXth1 into BW9109 (Table 1).

The episome F'500 spans the *xth-pncA* region of the *E. coli* chromosome (18). Introduction of this episome into BW9109 (selected by complementation of the His$^-$ phenotype) reduced the pKK175-6 *tet* activation frequency to the wild-type level (Table 1). Since no gene has been mapped between *xth* and *pnc*, a new locus in this region evidently functions to reduce the normal rate of illegitimate rearrangements.

The possibility was addressed for AB1157 and BW9109 that the number of cells plated might affect the occurrence of Tet^r isolates. The spontaneous frequency of *tet* activation was constant for AB1157 over the range 1×10^8 to 5×10^9 CFU per plate, and for BW9109 from 2.5×10^8 to 2×10^9 CFU per plate.

TABLE 2. Effect of DNA damage on pKK175-6 Tet^r activation[a]

Strain (treatment)	% Cell survival	Tet^r frequency (per 10^{10} cells) in:		Fold increase
		Controls	Treated cells	
AB1157				
UV light	13	1.4	66	47
H_2O_2	23	1.9	2.5	1.3
DM49 (*lexA3*) (UV light)	16	1.5	7.7	5.1

[a] These data represent the results of at least three independent experiments for each strain and treatment. The total number of cells screened was at least 10^{11} in each experiment.

Effect of DNA damage on illegitimate recombination. The possible role of DNA damages in causing illegitimate recombination has received limited attention. We tested three agents that produce different classes of DNA damage. UV light forms mainly bulky lesions in DNA (e.g., pyrimidine dimers), H_2O_2 is a radiomimetic agent, and methylnitrosoguanidine methylates DNA bases and the phosphodiester chain (16). UV light (17, 32) has been reported to increase the frequency of illegitimate recombinations in other systems, while H_2O_2 and methylnitrosoguanidine have evidently not been examined for this property.

Treatment of AB1157 bacteria with H_2O_2 (Table 2) or methylnitrosoguanidine (data not shown) gave no significant increase in the pKK175-6 *tet* activation frequency. Similarly, H_2O_2 treatment of BW9109 produced no increase in the Tet^r frequency, even though these cells are hypersensitive to this agent (9). In contrast, exposure of the cells to UV light stimulated the *tet* activation frequency by more than 50-fold in strain AB1157 (Table 2). To determine whether this dramatic increase was dependent on the activation of the SOS response (16), the experiment was repeated in the SOS-deficient *lexA3* strain DM49 (26). Because of the heightened sensitivity of the DM49 cells to UV irradiation (16), a 15-fold lower dose was used to produce the same survival as that in wild-type bacteria. This treatment resulted in a fivefold increase in illegitimate rearrangements (Table 2). It appears that the UV-induced increase in illegitimate recombination is at least partially independent of the SOS response. The exact degree of independence is unclear from the results of these experiments, owing to the lower UV doses necessary to treat the *lexA3* strain. In contrast to the wild-type and the *lexA3* strains, UV irradiation of the *xth* mutant BW9109 gave no significant increase in the frequency of Tet^r isolates in several experiments (data not shown).

A deletion is the predominant rearrangement in pKK175-6. Restriction mapping of many Tet^r plasmids revealed that the principal illegitimate rearrangement under all conditions was a deletion of about 1.3 kbp extending from approximately bp 3300 within the *bla* gene to approximately bp 250 between the polylinker and the 5' end of the *tet* gene (Fig. 1). This deletion evidently activates *tet* expression by moving an active promoter (located near position 3100) to a site directly upstream of the promoterless *tet* gene. Transcription would then be initiated from the strong P4 promoter, which was previously identified in plasmid pBR322 (34). Such a deletion represented 84% of all rearrangements observed (Table 3). In strain BW9109, only this rearrangement was detected (Table 3). In keeping with the apparent extent of the dele-

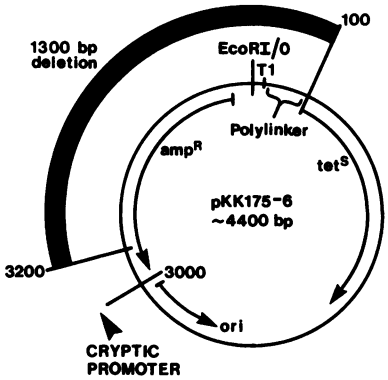

FIG. 1. Structure of pKK175-6 and extent of the 1.3-kbp deletion. The *tet* promoter was deleted during construction. T1 represents a segment of DNA from the *E. coli rrnB* gene containing transcriptional terminators (3). Another T1 terminator installed beyond the 3′ end of the *tet* gene is not shown. *amp*^{r} is *bla*$^+$; *tet*^{s} was deleted for the *tet* promoter.

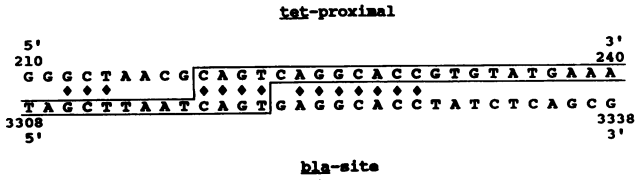

FIG. 2. The novel junction formed in the 1.3-kbp deletion derivatives of pKK175-6. The upper strand is the sequence near the 5′ end of the *tet* gene; the lower strand is within the 3′ end of the *bla* gene (25). The boxed regions indicate the sequence of the rearranged DNA. The exact point of crossover is not known within the CAGT box. Nucleotide matches of ≥2 bp between the sites are indicated by diamonds. The nucleotide numbering system is for pKK175-6.

tions, all of the monomeric 1.3-kbp deletion plasmids exhibited the Amp^s phenotype.

The remaining few rearrangements contained insertions directly upstream of the *tet* gene. These plasmids conferred resistance to both tetracycline and ampicillin. Restriction mapping indicated that no individual insertion was obtained more than once. Two of these insertions were analyzed by DNA sequencing (see below).

We attempted to enhance the selection procedure to obtain a greater fraction of insertions compared with the 1.3-kbp deletions. These experiments entailed the isolation of plasmid DNA from a relatively large collection of Tet^r colonies, retransformation of fresh cells with the DNA, and selection for both the Amp^r and the Tet^r phenotypes. Plasmids with insertions near *tet* would be expected to retain the Amp^r phenotype, unlike the 1.3-kbp deletion plasmids. Using strain AB1157 or BW9109, we observed only dimeric forms of pKK175-6, in which one of the copies contained the 1.3-kbp deletion. This approach was not tested in other strains.

A single novel junction predominates in rearranged plasmids. The novel junctions of 33 of the 1.3-kbp deletion plasmids were sequenced by the Maxam-Gilbert or the dideoxy method (25). These plasmids were generated spontaneously in all the strains listed in Table 1 and were recovered from UV- or H_2O_2-treated AB1157 and BW9109.

TABLE 3. Structures of Tet^r-activated plasmids

Strain (condition)	No. of plasmids with[a]:		Fraction with 1.3-kbp deletion[b]
	1.3-kbp deletion	Other structures	
AB1157			
Spontaneous	29	8	0.78
UV treated	10	1	0.91
H_2O_2 treated	12	0	≥0.92
BW9109 (spontaneous)	18	0	≥0.95
RR1 (spontaneous)	6	4	0.60
HB101 (spontaneous)	8	3	0.73

[a] The overall structures of the activated plasmids were determined by restriction mapping.

[b] Dimeric species containing one copy of the 1.3-kbp deletion and one copy of intact pKK175-6 are included.

These experiments revealed the same locus of recombination in every rearranged plasmid (Fig. 2). These novel junctions resulted from recombination between sites with a 14- of 19-bp identity involving positions 212 to 230 and 3310 to 3328 contained in three sections of 3, 4, and 7 identical bases and punctuated by unmatched sequences (Fig. 2). It is noteworthy that in every sample sequenced, the C at position 223 was retained in the recombinant. Thus, the crossover that led to these recombinants apparently occurred in the central section of the 4-bp match (Fig. 2) (see below).

Two Tet^r isolates containing insertions of approximately 1 kbp of additional DNA near the 5′ end of *tet* were also sequenced. The *tet*-proximal junctions in these plasmids did not bear any significant similarity to the frequent 1.3-kbp deletion junctions. One of the insertions was at position 213 of pKK175-6 and precisely matched residues 51 to 119 of the published sequence of the *E. coli* IS5 element (13) in the limited region that was sequenced. A possible transcriptional promoter was situated 23 bp 5′ of the translocation breakpoint in this recombinant. The sequence of the first 71 residues of the second insertion (at position 178) did not match any bacterial DNA sequence in a commercial data base (MicroGenie) and did not contain an obvious promoter (data not shown).

DISCUSSION

The selectable, plasmid-based system explored here provides a rapid and convenient method for determining the influence of genetic and environmental factors on the frequency of certain types of DNA rearrangements. The ease of plasmid isolation facilitates the structural and sequence analysis of the rearranged molecules. Thus, we found that 254-nm UV light, but not H_2O_2, causes a substantial increase in the frequency of events that activate the pKK175-6 *tet* gene. Genetic factors also had significant effects, with the product of a gene located near 38 min on the *E. coli* map inhibiting the production of at least some DNA rearrangements.

Alterations of pKK175-6 that activate the *tet* gene are usually rare, occurring at a rate of only a few in 10^{10} cells per generation in wild-type bacteria. Among the recombinant molecules analyzed to date, none have been identified that have the *tet* gene activated via a point mutation or even a relatively small sequence change (<1 kbp). Large changes in DNA structure were consistently observed; and in every strain and under every condition examined, the dominant rearrangement was a single 1.3-kbp deletion that involved a short region of near identity in sequences. Since those sequences were also present in pBR322, the same deletions might well have occurred there, but so rarely as to be

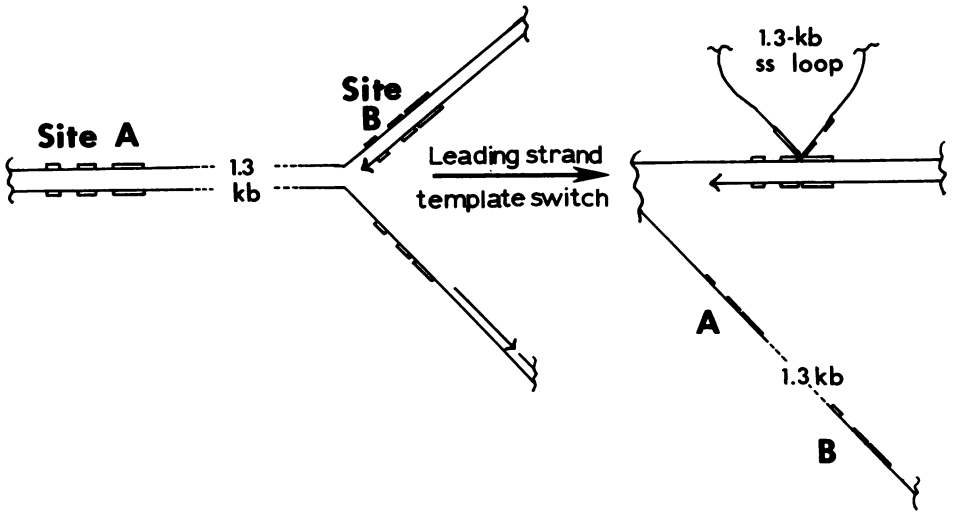

FIG. 3. Possible role of the leading strand in deletion formation during DNA replication of pKK175-6. The three perfectly matched segments (3, 4, and 7 bp, respectively; shown as three small boxes in the figure) between nucleotides 212 and 230 (site B) and nucleotides 3310 and 3328 (site A) would mediate the formation of a T structure with the template DNA looped out. DNA helicases and single-stranded (ss) binding proteins could facilitate this process. Extension of the 3′ end at this junction by DNA polymerase would stabilize the loop, leading to deletion after excision repair of the loop or another round of replication.

practically undetectable without a positive selection for the Amp^s phenotype. The less frequent rearrangements, including insertions, involved changes that were necessarily formed within the constraints of this system.

Plasmid rearrangements were dominated by the 1.3-kbp deletion species, in part because of the structure and transcriptional layout of pKK175-6 (34). The *tet*-proximal endpoint of any rearrangement must fall within the ~100 bp between the beginning of the *tet*-coding region and the transcriptional terminators installed just 5′ of the polylinker site (Fig. 1). The distal breakpoint for deletions appears to be determined by the location of the nearest correctly oriented promoter, P4 (34), and by the need for a functional origin of replication for the plasmid. Thus, deletions beyond position ~3200 would eliminate replication, while those extending only to positions between ~3500 and the 5′ end of *tet* might be too distant to give significant transcription of *tet* from P4. The short sequence (positions 3317 to 3328) with near-perfect identity to positions 219 to 230 near the 5′ end of *tet* appears to provide a viable target for illegitimate recombination.

There may be other recombinations that satisfy the criteria of retaining plasmid replicating ability while giving significant transcription of *tet*, but they must occur at <5% of the frequency seen for the dominant 1.3-kbp deletion. A computer search was conducted for other possible internally repeated sequences in pKK175-6 between the region including positions 50 to 350 compared with the region between positions 2600 and 4370 (also present in pBR322), which might recombine to generate deletions that would position an active promoter near the 5′ end of *tet*. This search revealed 15 additional pairs of sites with sequence matches containing a block of at least 7 contiguous bp of perfect match. In each of these other cases, however, the respective deletions would damage a known promoter, delete part of the *tet* gene, or interfere with DNA replication. Although the observed rearrangement sites therefore appear to present the best recombination sites within the constraints of this system, it cannot be ruled out that the actual sequences might be subject to a type of site-specific recombination.

The structures of the recombinants and the effects of genetic and environmental factors in their production place constraints on the mechanisms of DNA rearrangement in pKK175-6. It is noteworthy that, in every sequence determined, the C at position 223 in pKK175-6 was retained after the 1.3-kbp deletion (Fig. 2). Consequently, putative crossovers that formed these deletion junctions would have occurred in the central 4-bp box of sequence match (Fig. 2). With these results, it is not possible to determine the direction from which such crossovers might originate. This observation of multiple short sequence matches punctuated by unmatched sequences is reminiscent of the spontaneous deletions observed in the *lacI* gene, although those events exhibited a significant dependence on the *recA* gene (1).

The size of the most frequent deletion and the nature of the sequences involved suggest a possible role for DNA replication in this illegitimate recombination. Others (1) have proposed that slippage of DNA polymerase during replication could mediate recombination between sites of short sequence match. Such a process would involve translocation of the polymerase-primer-template complex in a kind of copy choice mechanism (Fig. 3). The gaps that apparently occur on the lagging strand during replication are of about the same size as the 1.3-kbp deletion (23, 28). These transient gaps might fold out to allow the juxtaposition of matching sequences. Such pairing would evidently occur without the RecA protein and would perhaps be promoted by another bacterial recombinase (14). The C at position 223 delineated two sections of 7 and 4 bp of sequence match, and these could serve to stabilize the loop that has been proposed as a predeletion intermediate (Fig. 3).

The increased production of *tet*-activating rearrangements following only some kinds of DNA damage suggests a specialized disruption of the replication-recombination apparatus (24). H_2O_2 treatment is known to inhibit DNA replication in *E. coli* (10), but it appeared to have no effect on

this illegitimate recombination. A related agent, ionizing radiation, also failed to increase the Tet^r frequency in HB101 bearing pKK175-6 (E. Shimony and E. Eisenstadt, personal communication). It is possible that the daughter strand gaps caused by stalled replication of UV-damaged DNA (16) could lead to predeletion intermediates like those described above. In such a case, peroxide or X-ray damage would evidently form such gaps less efficiently than would UV light. It seems unlikely that induction of SOS functions plays a role in UV-induced deletion, since the SOS-deficient *lexA3* strain was affected significantly by UV light. The apparent lack of a substantial UV effect in the Δ(*xth-pnc*) mutant could be ascribed to masking by the already high spontaneous Tet^r frequency in that strain. Alternatively, UV damage might mimic the phenotype of Δ(*xth-pnc*) by, for example, neutralizing the unknown gene product(s) that is absent from the Δ(*xth-pnc*) strain.

The high deletion frequency seen in the Δ(*xth-pnc*) mutant indicates a preventative role for the unknown gene product. A possible function, for example, would be as a helicase or a nuclease that removes potential deletion intermediates before they are resolved to deletions. The identity and function of the gene located in the *xth-pnc* region will be an important object of future studies. However, a simple genetic means of localizing such a gene is not obvious. The recent construction of a nearly complete ordered clone bank of the *E. coli* chromosome (22) should allow the physical isolation of this interesting gene for the ultimate dissection of its role in averting potentially deleterious illegitimate recombination.

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