Deletions in Plasmid pBR322: Replication Slippage Involving Leading and Lagging Strands

Kathleen Weston-Hafer and Douglas E. Berg

Departments of Molecular Microbiology and of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110

Manuscript received July 6, 1990
Accepted for publication December 8, 1990

ABSTRACT

We test here whether a class of deletions likely to result from errors during DNA replication arise preferentially during synthesis of either the leading or the lagging DNA strand. Deletions were obtained by reversion of particular insertion mutant alleles of the pBR322 *amp* gene. The alleles contain insertions of palindromic DNAs bracketed by 9-bp direct repeats of *amp* sequence; in addition, bp 2 to 5 in one arm of the palindrome form a direct repeat with 4 bp of adjoining *amp* sequence. Prior work had shown that reversion to Amp^r results from deletions with endpoints in the 9- or 4-bp repeats, and that the 4-bp repeats are used preferentially because one of them is in the palindrome. To test the role of leading and lagging strand synthesis in deletion formation, we reversed the direction of replication of the *amp* gene by inverting the pBR322 replication origin, and also constructed new mutant alleles with a 4-bp repeat starting counterclockwise rather than clockwise of the insertion. In both cases the 4-bp repeats were used preferentially as deletion endpoints. A model is presented in which deletions arise during elongation of the strand that copies the palindrome before the adjoining 4-bp repeat, and in which preferential use of the 4-bp repeats independent of the overall direction of replication implies that deletions arise during syntheses of both leading and lagging strands.

DELETIONS are frequent among spontaneous mutations in both prokaryotes and eukaryotes (for reviews, see ALLGOOD and SILHAVY 1988; ROTH and WILSON 1988; EHRLICH 1989; MEUTH 1989). Many studies have shown that their endpoints tend to occur in short direct repeats found sporadically in any naturally occurring DNA sequence (FARABAUGH *et al.* 1978; GHOSAL and SAEDLER 1979; ALBERTINI *et al.* 1982; COLLINS, VOLCKAERT and NEVERS 1982; JONES, PRIMROSE and EHRLICH 1982; MARVO, KING and JASKUNAS 1983; DASGUPTA, WESTON-HAFER and BERG 1987), and that their formation is stimulated by palindromy (ALBERTINI *et al.* 1982; GLICKMAN and RIPLEY 1984; SCHAAPER, DANFORTH and GLICKMAN 1986). Such deletions are believed to result from errors in DNA replication: transient pausing, slippage of the tip of a growing DNA strand between the first and second copies of direct repeats, and then the priming of further synthesis without duplication of interstitial sequences. Palindromes could facilitate such slipped mispairing by forming hairpin structures that impede elongation of the nascent DNA strands. Reannealing of separated template strands in the hairpin would cause extrusion of the stalled nascent strand and free it for pairing with other complementary sequences.

Support for this model came from reversion tests with particular insertion mutant alleles of the *amp* gene of pBR322: these alleles contain insertions of palindromic DNAs bracketed by 9-bp direct repeats at a site where 4 bp in one arm of the palindrome form a direct repeat with adjoining pBR322 sequence (Figure 1a). Reversion to Amp^r results from deletions that, formally, end in either the 9-bp or the 4-bp direct repeats (WESTON-HAFER and BERG 1989a). With most DNAs the frequency of endpoints in direct repeats is related to repeat length (FARABAUGH *et al.* 1978; ALBERTINI *et al.* 1982; WESTON-HAFER and BERG 1989b), and it was thus striking that with these alleles the shorter (4 bp) repeats were used preferentially. Figure 1b sketches a replication error model that accounts for these results. It is proposed that deletions ending in 4-bp repeats arise during synthesis of the strand that first copies the 4-bp direct repeat within the palindrome so that its slippage to the second 4-bp segment and resumption of synthesis could fix the deletion of interstitial sequences.

It has not been clear whether deletions result from synthesis of both leading and lagging strands (CHOW, DAVIDSON and BERG 1974; WEISBERG and ADHYA 1977; SINGER and WESTLYE 1988; BALBINDER, MACVEAN and WILLIAMS 1989). Strand slippage might reflect the intrinsic error frequency of the DNA polymerases that act on each DNA strand. Alternatively, the chance of slippage might reflect the distribution of other proteins which affect processivity, or unique structural features in the DNA, for example associated with opening the parental duplex, or the

extent of single-strandedness of template strands.

Plasmid pBR322 provides a good opportunity to test the contributions of leading and lagging strand synthesis to deletion events. Its replication is inferred to be unidirectional and counterclockwise on the standard map, based on the homology between its replication origin and that of plasmid ColE1 (TOMIZAWA, OHMORI and BIRD 1977; SUTCLIFFE 1978), whose replication is well known to be unidirectional *in vitro* and *in vivo* (INSELBURG 1974; LOVETT, KATZ and HELINSKI 1974; TOMIZAWA, SAKAKIBARA, and KAKEFUDA 1974; LACATENA and CESARENI 1983). Figure 2 illustrates that reversing the orientation of the replication origin, and thus the direction of replication fork movement through a region interchanges leading and lagging strands in that region. We report here that the direction of replication does not strongly affect the frequency of deletions, nor the distribution of endpoints. Assuming that deletions ending in the 4-bp repeats arise during growth of the strand that first copies the palindrome (Figure 1b), these results suggest that leading and lagging strand syntheses contribute equivalently to the deletion process.

MATERIALS AND METHODS

General procedures: Media, bacterial growth, plasmid DNA extractions, recombinant DNA cloning and restriction endonuclease analyses have been described elsewhere (MANIATIS, FRITSCH and SAMBROOK 1982; NAG *et al.* 1985). DNA sequences were determined using the chain termination method (SANGER, NICKLEN and COULSON 1977) adapted for double-stranded DNA (ZAGURSKY *et al.* 1985). The primer used in sequencing new insertion mutant alleles and representative revertants corresponds to pBR322 positions 3677–3693 (5′GGCGAGTTACATGATCC). Synthetic oligonucleotides were made on an Applied Biosystems 380A DNA synthesizer.

Bacterial strains: *Escherichia coli* K-12 derivative MC1061 (CASADABAN and COHEN 1980) was the host for both plasmid constructions and reversion tests. A *dam*::Tn*9* derivative of MC1061 (constructed by K. DODSON, personal communication) was used for DNAs to be cleaved with *Bcl*I. All plasmids used are derivatives of pBR322 (Amp^r Tet^r).

Plasmid constructions: The "rep-reverse #1" and "rep-reverse #2" plasmids (Figure 3) were constructed by U. DASGUPTA as follows: *Xho*I linkers were spliced into plasmid pBR322 between the *Dra*I sites at positions 3232 and 3251, and also the *Eco*RI site (4361; in the case of rep-reverse #1) or the *Pvu*II site (2066; in the case of rep-reverse #2). these pBR322 derivatives were digested with *Xho*I and religated, and derivative plasmids in which the *Xho*I fragments had been inverted were isolated. Amp^s insertion alleles (Figure 4a) were incorporated into these plasmids by replacing the *Pst*I to *Sca*I fragment with the corresponding fragment from a non-rearranged plasmid containing a 32-bp palindromic insertion (DASGUPTA, WESTON-HAFER and BERG 1987; Figure 4b). These plasmids are designated "C" (Figures 2 and 4) because 4 bp in the palindrome matches 4 bp in pBR322 clockwise of the insertion allele.

The "CC" series of plasmids (in which the palindromic insertion contains a match to 4 bp counterclockwise of the insertion; Figure 4c) was made by a series of oligonucleotide

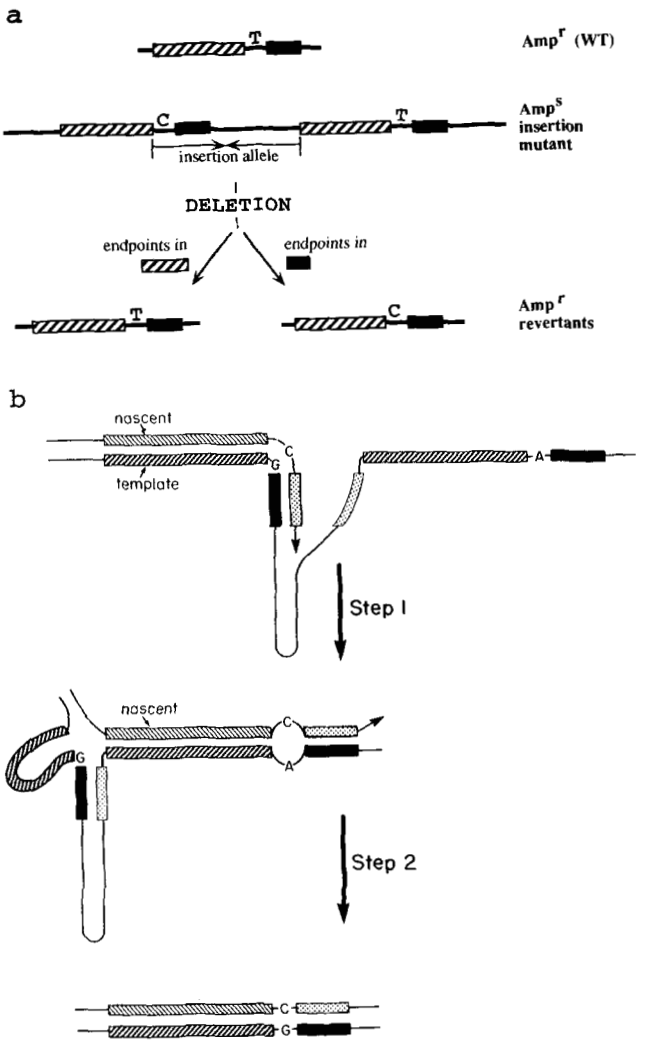

FIGURE 1.—Deletions obtained by reversion of insertion mutations. (a) Sequences of revertants indicate location of deletion endpoints. Hatched boxes, 9-bp direct repeats; filled boxes, 4-bp direct repeats. Horizontal arrows, palindromic insert (note that one 4-bp repeat is part of the palindrome). Endpoints in the 9-bp repeats (hatched) result in T (wild-type) at pBR322 position 3740, whereas endpoints in the 4-bp repeats (filled) result in C at position 3740 (adapted from WESTON-HAFER and BERG 1989a). (b) DNA synthesis error model of deletion formation. ▨ and ▧, and ■ and □ represent complementary strands of the 9-bp direct repeats and of the 4-bp repeats, respectively. Replication is shown 5′ (left) to 3′. Step 1: the nascent DNA strand stalls within the hairpin (formed in the palindrome), is displaced from the hairpin (branch migration), and pairs with the second copy of the direct repeat. Step 2: any unpaired bases at the end of the nascent strand are trimmed, and synthesis resumes, thereby fixing the deletion. The deleted strand is copied during the next round of replication (adapted from WESTON-HAFER and BERG 1989a). The inferred preferential use of the shorter (4 bp) repeats as deletion endpoints (see Tables 1, 2) depends on the assumption that heteroduplex structures are not repaired preferentially giving TA to CG transitions. This assumption is supported by findings that the first base pair of the palindrome was also recovered preferentially in revertants after reversing the positions of the critical TA and CG pairs in the *amp*^{r} parental alleles (WESTON-HAFER and BERG 1989a), and effects of palindrome length on sequences of revertants, as detailed in WESTON-HAFER and BERG (1989a, 1991).

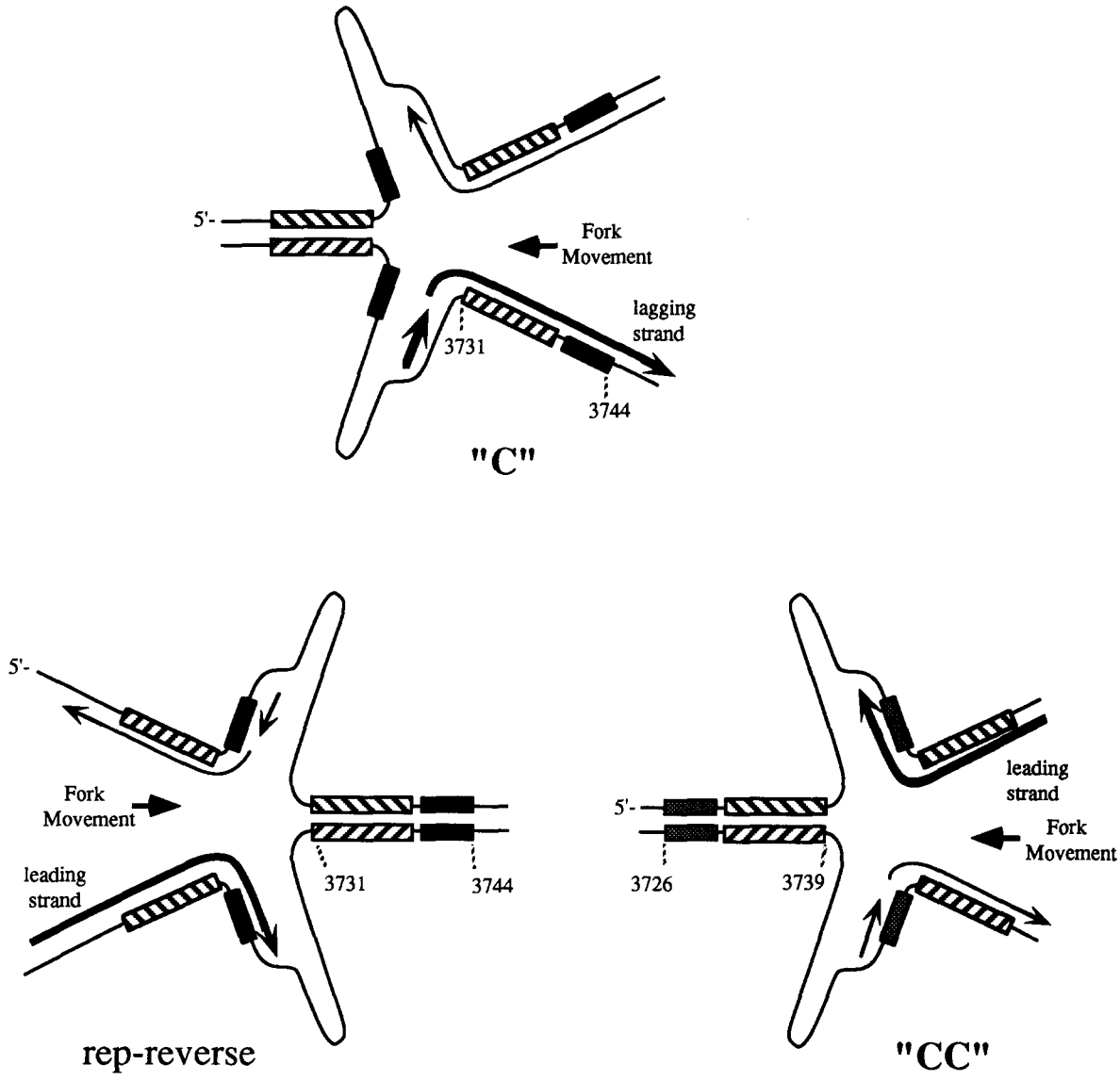

FIGURE 2.—Patterns of DNA synthesis postulated to result in deletion. The nascent strand whose slippage is likely to result in deletion events is indicated as a thickened line (see text). Hatched and filled boxes indicate 9-bp and 4-bp repeats, respectively.

mutageneses. First, a change from C to T at pBR322 position 3730 was made by the double strand break mutagenesis procedure as described by MANDECKI (1986). This entailed cleaving pBR322 plasmid DNA with *Pvu*I and mixing it with a bridging but mutagenic oligonucleotide which matches sequences on both sides of the cutting site: 5′GCTCCTTCGGTXCTCCGATCGTTGTCAGAAGT (*X* at 3730, an equimolar mixture of G, A and T; *Pvu*I site underlined). This mixture was used to transform recipient cells to Tet^r, transformants were scored for Amp phenotype, and representative mutants were sequenced. A plasmid with a C to A change at position 3730 (Gly-142 to Val) was Amp^s, whereas a plasmid with a C to T change at the same site (Gly-142 to Glu) was Amp^r, and thus suitable for our tests. A 16-bp palindromic insertion allele with 4 bp of the insert identical to pBR322 bases 3726–3729 [counterclockwise (cc) rather than clockwise (c) of the palindromic insert] was introduced into the *Pvu*I site of the plasmid with a C to T change at 3730 using the double-stranded oligonucleotide

5′-CGGACCGAGATCTCGGTCCTCCGAT
TAGCCTGGCTCTAGAGCCAGGAGGC-5.

Insertion-containing plasmids were found by their Amp^s phenotype, and those containing the insert in the desired orientation were identified by sequencing. A longer palindrome (Figure 4c) was created by splicing a *Bgl*II fragment containing 45-bp inverted repeats bracketing a *kan* marker segment (from WESTON-HAFER and BERG 1989a) into the *Bgl*II site (AGATCT) in the center of the 16 bp palindrome. A 74-bp perfect palindrome (Figure 4d) was then made by *Xho*I digestion, which removed the *kan* gene and part of the inverted repeats (as in DASGUPTA, WESTON-HAFER and BERG 1987).

Reversion tests: Amp^r revertant frequencies were measured in clones grown to about 10^{10} cells (~32-cell doublings) in L-broth prior to plating on L-agar with ampicillin (250 μg/ml), as described previously (DASGUPTA, WESTON-HAFER and BERG 1987; WESTON-HAFER and BERG 1989a). Each frequency reported represents the median of determinations with at least five separate single cell clones, and is based on counts of about 100–300 revertant colonies from each clone. The frequencies generally varied by less than a factor of three in repeated determinations.

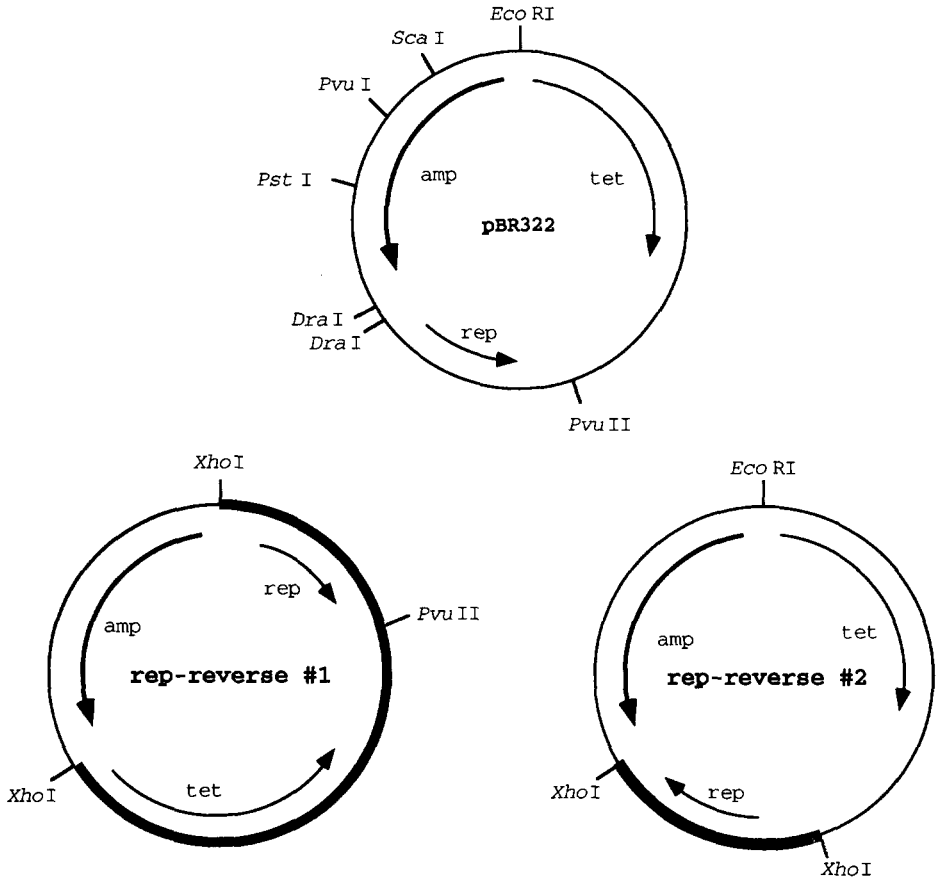

FIGURE 3.—Orientation of replication fork movement through the *amp* gene. Structures of pBR322 derivatives made by inverting a segment containing the replication origin, with one inverted segment thickened. The positions of the restriction sites used (pBR322 coordinates) are: *Eco*RI 4361; *Pvu*II 2066; *Dra*I 3232 and 3251; *Pst*I 3609; *Pvu*I 3735; *Sca*I 3846.

RESULTS

Overview: Deletions resulting in Amp^r revertants in our test plasmids are presumed to result from slippage of the strand that copies the palindromic insert before, not after, copying the 4-bp repeat in adjoining pBR322 sequences (Figure 1b). Two complementary experiments, detailed below, were designed to assess whether the growth of leading and lagging DNA strands each results in deletions, based on the unidirectionality of pBR322 replication.

Effect of reversing direction of replication: Amp^s insertion alleles were introduced into pBR322 derivatives in which the orientation of the origin of replication relative to the *amp* gene had been reversed (Figure 3). Two types of insertion alleles were analyzed: relatively short (16–90 bp) uninterrupted palindromes, and palindromes interrupted by a 2-kb unique *kan* sequence (Figure 4, a and b). Table 1a shows that the orientation of the replication origin did not strongly (at most, fivefold) affect the deletion frequency.

Reversion of these Amp^s insertion alleles results from deletions with endpoints in either 9-bp or 4-bp direct repeats. These endpoint positions were distinguished by colony hybridization, based on the presence of T *vs.* C in the diagnostic position between the 9- and 4-bp segments (Figure 1a). Table 1 shows that 4-bp repeats were used preferentially as deletion endpoints, independent of the direction of replication of the *amp* gene. For example, 65% of deletions of the 22-bp palindrome ended in the 4-bp repeats in plasmids with the normal pBR322 sequence arrangement, whereas 54% and 59% of deletions ended in these same direct repeats in plasmids in which *amp* is replicated in the opposite direction.

Effect of position of 4-bp direct repeats: In the second set of experiments, we constructed new Amp^s alleles which differed in sequence from those analyzed above, such that 4 bp in the palindrome matched 4 bp of pBR322 counterclockwise, rather than clockwise of the mutant allele ("CC" *vs.* "C," respectively; Figure 4). The "C" and "CC" alleles exhibited rather similar frequencies of deletion, for example only a 7-fold difference in the case of the 16-bp palindromes (Table 2), despite their different sequences. More important, the 4-bp direct repeats were also used preferentially as deletion endpoints with each of these "CC" alleles (Table 2).

We suggest that use of the 4-bp direct repeats as endpoints in only 17% of deletions of the 16-bp palindrome "C" allele is separate from the issue of the roles of leading and lagging strands in deletion formation. In complementary experiments, we had found that lengthening this "C" palindrome to 18 bp resulted in preferential use of the 4-bp direct repeats as deletion endpoints, whereas reducing it to 10 bp

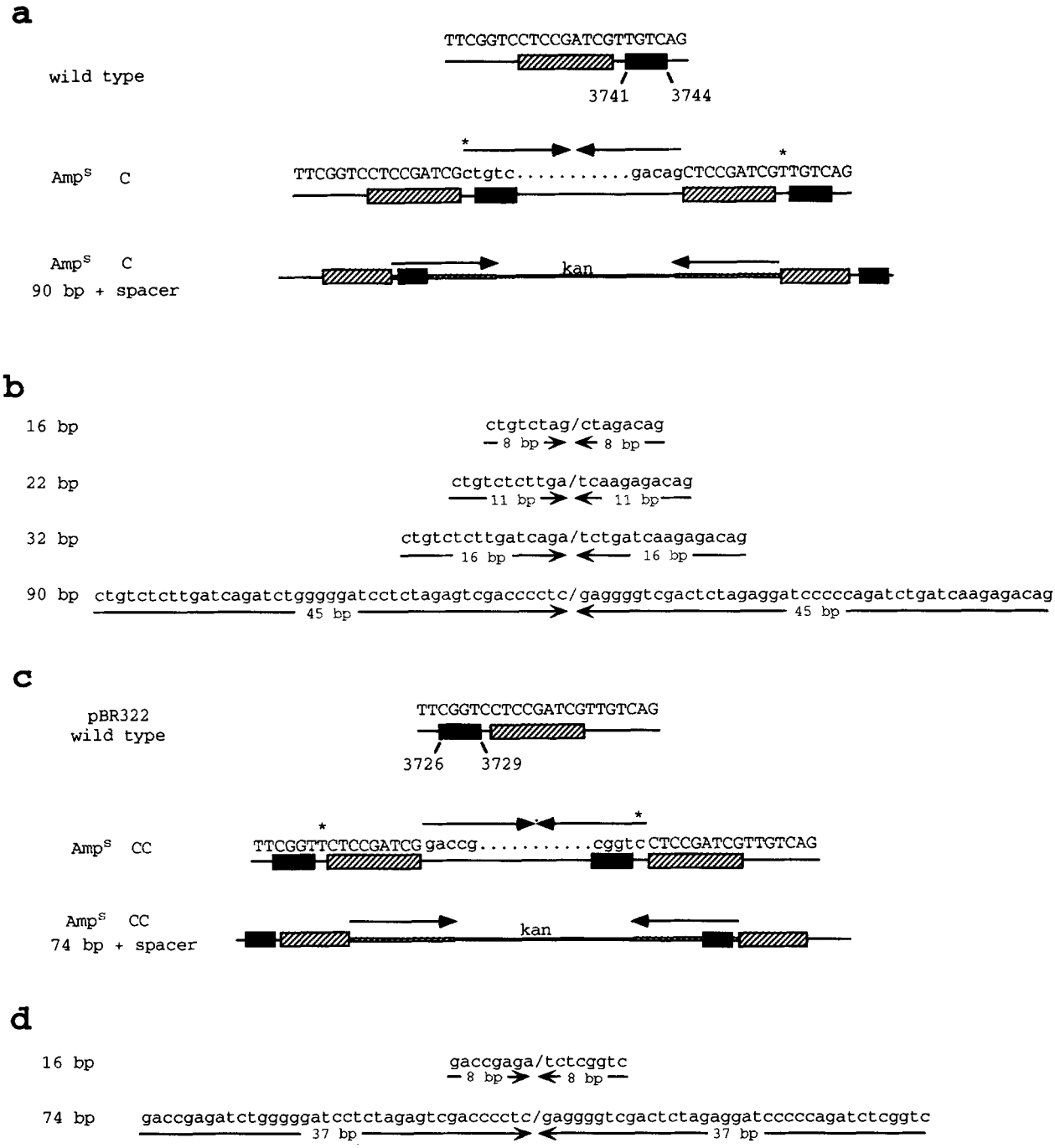

FIGURE 4.—Structures and sequences of insertion alleles. (a) Structures of "C" alleles (4-bp repeat clockwise of the palindromic insert). Top line, pBR322 sequence from 3724 to 3746. Second line, structure of alleles with insertion of uninterrupted palindromes and location of the adjoining 4-bp repeats. Third line, structure of the allele with 90 bp + spacer (spacer is 2-kb *kan* segment, WESTON-HAFER and BERG 1989a). Hatched boxes, 9-bp direct repeats; filled boxes, 4-bp direct repeats; arrows, palindromic insert; asterisks, bases that identify the location of deletion endpoints in revertants. (b) Detailed sequence of uninterrupted palindromes of "C" insertion alleles. Arrows under sequences indicate inverted repeats, with lengths shown. (c) Structures of "CC" insertion alleles (4-bp repeat counterclockwise of the palindromic insert). Boxes, arrows and asterisks as in part a. (d) Detailed sequences of uninterrupted palindromes of "CC" insertion alleles.

did not further reduce usage of the 4-bp repeats (WESTON-HAFER and BERG 1991). Accordingly, 16 bp would appear to be just below the threshold at which palindromy is important in determining endpoint position for the "C" allele sequence; the threshold length for the "CC" allele sequence would then seem to be even lower. But, additional experiments will be needed to determine how the different sequences of the "C" and "CC" alleles determine both the probability of undergoing deletion and the positioning of deletion endpoints.

DISCUSSION

In a DNA synthesis error model of deletion formation (Figure 1b), deletions result from slippage of

TABLE 1

Effect of relative orientation of replication origin and *amp* on deletion formation

Palindrome length (bp)[a]	Plasmid structure[b]	Amp^r reversion frequency	Endpoints in 4-bp repeats
22	wt	9.5×10^{-7} [c]	51/78 (0.65)[c]
22	Rep-reverse #1	3.1×10^{-7}	32/54 (0.59)
22	Rep-reverse #2	5.0×10^{-6}	31/58 (0.54)
32	wt	1.1×10^{-4} [c]	67/72 (0.93)[c]
32	Rep-reverse #1	2.5×10^{-5}	69/83 (0.83)
32	Rep-reverse #2	1.3×10^{-4}	109/114 (0.96)
90 + spacer	wt	6.0×10^{-8} [c]	44/48 (0.92)[c]
90 + spacer	Rep-reverse #1	1.5×10^{-8}	33/61 (0.54)
90 + spacer	Rep-reverse #2	3.1×10^{-8}	45/53 (0.85)

The alleles used are those designated "C" in Figure 2. wt = wild type.

[a] See Figure 4, a and b, for structure and precise sequence of palindromes.

[b] Rep-reverse plasmids diagrammed in Figure 3.

[c] Data from WESTON-HAFER and BERG (1989a).

TABLE 2

Effect of position of 4-bp repeats on deletion formation

Palindrome length (bp)[a]	Position of 4-bp repeat[b]	Amp^r reversion frequency	Endpoints in 4-bp repeats
16	CC	4.2×10^{-6}	27/47 (0.57)
74	CC	7.7×10^{-3}	64/64 (1.0)
74 + spacer	CC	6.9×10^{-9}	36/40 (0.90)
16	C	6.1×10^{-7} [c]	12/71 (0.17)[c]
22	C	9.5×10^{-7} [d]	51/78 (0.65)[d]
90	C	1.7×10^{-2} [d]	62/65 (0.95)[d]
90 + spacer	C	6.0×10^{-8} [d]	44/48 (0.92)[d]

[a] See Figure 4 for structure and sequence of palindromes.

[b] CC (counterclockwise) denotes that the 4-bp direct repeat consists of 4 bp within the insert and pBR322 bases 3726–3729. C (clockwise) denotes that the 4-bp direct repeat consists of 4 bp within the insert and pBR322 bases 3741–3744 (see Figure 4).

[c] Data from WESTON-HAFER and BERG (1991).

[d] Data from WESTON-HAFER and BERG (1989a).

the nascent DNA strand and failure to copy part of the template (STREISINGER *et al.* 1966; FARABAUGH *et al.* 1978; ALBERTINI *et al.* 1982; DASGUPTA, WESTON-HAFER and BERG 1987; WESTON-HAFER and BERG 1989a). Direct repeats would facilitate this process by providing new sites at which a displaced nascent strand can be anchored and used to prime further synthesis. Palindromy would also facilitate slippage by leading to hairpins in single-stranded templates, which in turn would impede DNA synthesis and tend to extrude the stalled nascent strand.

The results presented here extend previous conclusions that palindromy stimulates deletion formation and affects the positions of deletion endpoints. In two complementary tests, 4-bp direct repeats, one copy of which is within a palindrome, were used as deletion endpoints in preference to 9-bp direct repeats flanking the palindrome. This unusual pattern apparently reflects the location of one of the shorter repeats within the palindrome. Decreases in palindrome length to a limit of about 16 bp decreased the use of these shorter repeats, independent of the direction of replication. In the context of the DNA synthesis error model of deletion formation shown in Figure 1b, slippage after copying the first 5 bp of the hairpin would allow pairing of the nascent strand with the second of the 4-bp repeats, and then resumption of DNA synthesis. If more than 5 bp had been copied, exonucleolytic trimming of unpaired nucleotides would be needed for synthesis to resume and the deletion to be fixed.

The conclusion that sequence per se also affects the deletion process emerged most strongly in comparing the 16-bp palindromes of the "C" and "CC" alleles. These palindromes differ in sequence (see Figure 4, b and d), and the TCTC 4-bp repeats of the "C" allele were used only about one-fourth as frequently as the CGGT 4-bp repeats of the "CC" allele. These results may reflect effects of hairpin sequence or neighboring sequences on the rate or processivity of DNA synthesis (WEISMAN-SHOMER *et al.* 1989; BEDINGER, MUNN and ALBERTS 1989).

Assuming a model such as in Figure 1b, the deletions we analyzed should arise during elongation of the strand that copies the palindrome before the adjoining 4-bp repeat: the lagging strand in the case of the "C" alleles in non-rearranged pBR322, and the leading strand in the cases of the rearranged "rep-reverse" and "CC" plasmids (Figure 2). Our finding that the direction of replication fork movement through the region undergoing deletion does not strongly affect the frequency or the choice of deletion endpoints (given palindromes longer than 16 bp) indicates that deletions can arise during the synthesis of either leading or lagging strands. We propose that deletions result from occasional nonprocessivity in the tracking of replication enzymes along any template DNA strand, and that the frequencies of these rare errors are determined by subtle features of both DNA sequence and conformation.

We thank U. DASGUPTA for participation in early experiments, particularly construction of the rep-reverse plasmids, L. HENRY for expert technical assistance, and G. R. SMITH and anonymous reviewers for critical reading of the manuscript. This research was supported by National Science Foundation grant DMB-8608193, U.S. Public Health Service grant GM-37138, and Department of Energy grant DEFG02–89ER60862 to D.E.B.

LITERATURE CITED

ALBERTINI, A. M., M. HOFFER, M. P. CALOS and J. H. MILLER, 1982 On the formation of spontaneous deletions: the importance of short sequence homologies in the generation of large deletions. Cell **29:** 319–328.

ALLGOOD, N. D., and T. J. SILHAVY, 1988 Illegitimate recombination in bacteria, pp. 309–330 in *Genetic Recombination*, edited by R. KUCHERLAPATI and G. R. SMITH. American Society for Microbiology, Washington, D.C.

BALBINDER, E., C. MACVEAN and R. E. WILLIAMS,

1989 Overlapping direct repeats stimulate deletions in specially designed derivatives of plasmid pBR325 in *Escherichia coli*. Mutat. Res. **214:** 233–252.

BEDINGER, P., M. MUNN and B. A. ALBERTS, 1989 Sequence-specific pausing during *in vitro* DNA replication on double-stranded DNA templates. J. Biol. Chem. **264:** 16880–16886.

CASADABAN, M. J., and S. N. COHEN, 1980 Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138:** 179–207.

CHOW, L. T., N. DAVIDSON and D. E. BERG, 1974 Electron microscope study of the structures of λ *dv* DNAs. J. Mol. Biol. **86:** 69–89.

COLLINS, J., G. VOLCKAERT and P. NEVERS, 1982 Precise and nearly-precise excision of the symmetrical inverted repeats of Tn*5*; common features of the *recA*-independent deletion events in *Escherichia coli*. Gene **19:** 139–146.

DASGUPTA, U., K. WESTON-HAFER and D. E. BERG, 1987 Local DNA sequence control of deletion formation in *Escherichia coli* plasmid pBR322. Genetics **115:** 41–49.

EHRLICH, S. D., 1989 Illegitimate recombination in bacteria, pp. 799–832 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

FARABAUGH, P. I., U. SCHMEISSNER, M. HOFFER and J. MILLER, 1978 Genetic studies on the lac repressor. VII. On the molecular nature of spontaneous hotspots in the *lacI* gene of *Escherichia coli*. J. Mol. Biol. **126:** 847–863.

GHOSAL, D., and H. SAEDLER, 1979 IS*2–61* and IS*2–611* arise by illegitimate recombination from IS*2–6*. Mol. Gen. Genet. **176:** 233–238.

GLICKMAN, B. W., and L. S. RIPLEY, 1984 Structural intermediates of deletion mutagenesis: a role for palindromic DNA. Proc. Natl. Acad. Sci. USA **81:** 512–516.

INSELBURG, J., 1974 Replication of ColE1 plasmid DNA in minicells from a unique replication initiation site. Proc. Natl. Acad. Sci. USA **71:** 2256–2259.

JONES, I. M., S. B. PRIMROSE and S. D. EHRLICH, 1982 Recombination between short direct repeats in a RecA host. Mol. Gen. Genet. **188:** 486–489.

LACATENA R. M., and G. CESARENI, 1983 Interaction between RNA1 and the primer precursor in the regulation of ColE1 replication. J. Mol. Biol. **170:** 635–650.

LOVETT, M. A., L. KATZ and D. HELINSKI, 1974 Unidirectional replication of plasmid ColE1 DNA and relation of the origin-terminus to the nicked site in the ColE1 relaxation complex. Nature **251:** 337–340.

MANDECKI, W., 1986 Oligonucleotide-directed double-strand break repair in plasmids of *Escherichia coli*: a method for site-specific mutagenesis. Proc. Natl. Acad. Sci. USA **83:** 7177–7181.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MARVO, S. L., S. R. KING and S. R. JASKUNAS, 1983 Role of short regions of homology in intermolecular illegitimate recombination events. Proc. Natl. Acad. Sci. USA **80:** 2452–2456.

MEUTH, M., 1989 Illegitimate recombination in mammalian cells, pp. 833–860 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

NAG, D. K., U. DASGUPTA, G. ADELT and D. E. BERG, 1985 IS*50* mediated reverse transposition: specificity and precision. Gene **34:** 17–26.

ROTH, D., and J. WILSON, 1988 Illegitimate recombination in mammalian cells, pp. 621–653 in *Genetic Recombination*, edited by R. KUCHERLAPATI and G. R. SMITH. American Society for Microbiology, Washington, D.C.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SCHAAPER, R. M., B. N. DANFORTH and B. W. GLICKMAN, 1986 Mechanisms of spontaneous mutagenesis: an analysis of the spectrum of spontaneous mutation in the *Escherichia coli lacI* gene. J. Mol. Biol. **189:** 273–284.

SINGER B. S., and J. WESTLYE, 1988 Deletion formation in bacteriophage T4. J. Mol. Biol. **202:** 233–243.

STREISINGER, G., Y. OKADA, J. EMRICH, J. NEWTON, A. TSUGITA, E. TERZAGHI and M. INOUYE, 1966 Frameshift mutations and the genetic code. Cold Spring Harbor Symp. Quant. Biol. **31:** 77–84.

SUTCLIFFE, J. G., 1978 Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant Biol. **42:** 77–90.

TOMIZAWA, J.-I., H. OHMORI and R. E. BIRD, 1977 Origin of replication of colicin E1 plasmid DNA. Proc. Natl. Acad. Sci. USA **74:** 1865–1869.

TOMIZAWA, J.-I., Y. SAKAKIBARA and T. KAKEFUDA, 1974 Replication of colicin E1 plasmid DNA in cell extracts. Origin and direction of replication. Proc. Natl. Acad. Sci. USA **71:** 2260–2264.

WEISBERG, R. W., and S. ADHYA, 1977 Illegitimate recombination in bacteria and bacteriophage. Annu. Rev. Genet. **11:** 451–473.

WEISMAN-SHOMER, P., D. K. DUBE, F. W. PERRINO, K. STOKES, L. A. LOEB and M. FRY, 1989 Sequence specificity of pausing by DNA polymerases. Biochem. Biophys. Res. Commun. **164:** 1149–1159.

WESTON-HAFER, K., and D. E. BERG, 1989a Palindromy and the location of deletion endpoints in *Escherichia coli*. Genetics **121:** 651–658.

WESTON-HAFER, K., and D. E. BERG, 1989b Specificity of deletion events in pBR322. Plasmid **21:** 251–253.

WESTON-HAFER, K., and D. E. BERG, 1991 Limit to the role of palindromy in deletion formation. J. Bacteriol. **173:** 315–318.

ZAGURSKY, R. J., K. BAUMEISTER, N. LOMAX and M. L. BERMAN, 1985 Rapid and easy sequencing of large linear double-stranded DNA and supercoiled plasmid DNA. Gene Anal. Tech. **2:** 89–94.

Communicating editor: G. R. SMITH