RecA independent recombination of poly[d(GT)-d(CA)] in pBR322

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ABSTRACT

Short sequence tracts composed of alternating guanosine and thymidine nucleotide residues poly[d(GT)-d(CA)] carried in a derivative of pBR322 were recombinogenic in a *recA* host. Recombination brought about by poly[d(GT)-d(CA)] tracts displayed two interesting properties: (i) the reaction was quasi-sequence-specific in that while recombination usually occurred between two poly[d(GT)-d(CA)] tracts, recombination also occurred between sequences bordering the dinucleotide repeats. (ii) recombination was enhanced when two poly[d(GT)-d(CA)] tracts were clustered within 250 base pairs of each other, but not when the repeats were separated by 3 kilobase pairs. The mechanism by which poly[d(GT)-d(CA)] stimulated recombination remains to be determined, but the behavior of these sequences is consistent with the idea that general recombination in *E. coli* may involve formation of Z-DNA.

INTRODUCTION

Conformational polymorphism of DNA has been extensively studied in recent years. An interesting alternative to the B-form of DNA is the left-handed Z DNA form. Under the influence of torsional strain, DNA sequences composed of alternating purines and pyrimidines readily assume the Z conformation *in vitro* (1). The propensity of purine pyrimidine repeats to assume the Z conformation is particularly intriguing because large numbers of such sequences are found dispersed throughout the genomes of eucaryotes in the form of short runs of alternating dG-dC and dT-dA basepairs, (polyGT) (2,3). The function of polyGT sequences is not clear. They have been proposed to be recombinogenic elements in eucaryotic chromosomes, (3,4) and some support for this idea has been provided by experiments with SV40, (5) yeast (6) and mammalian cells (3). PolyGT sequences do not occur in the *E. coli* genome or in plasmids of *E. coli*, but studies on purified RecA protein and the Rec1 protein of *Ustilago Maydis* suggest that polyGT sequences might display unusual recombinational properties in *E. coli*. Both RecA and Rec1 mediate the formation of DNA joint molecules whose strands are paired but not interwound (paranemic joints) (7,8). Paranemic joints formed by Rec 1 protein of *Ustilago* bind antibody specific

for DNA in the Z conformation (8). Furthermore, the RecI protein has been shown to be a Z-DNA binding protein *in vitro* (9). These findings imply that polyGT sequences inserted into plasmids might be expected to be recombinogenic.

The experiments reported here show that even in the absence of *recA* function, polyGT sequences can be highly recombinogenic in plasmids. Recombination brought about by polyGT tracts displayed two interesting properties: (i) the reaction was quasi-sequence specific in that while there was a strong bias toward exchanges between polyGT sequences, recombination involving adjacent sequences also occurred, apparently induced by the presence of polyGT; (ii) enhanced *recA*-independent recombination occurred when polyGT tracts were clustered, but did not occur in a plasmid that bore two polyGT tracts separated by 3 kb. The mechanism by which polyGT sequences stimulated deletion of the plasmid DNA remains to be determined, but the observation of polyGT hyperrecombinogenicity is consistent with the idea that the polyGT sequences in supercoiled plasmid DNA were predisposed to assume a structure that normally forms via the intercession of *recA* protein.

MATERIALS AND METHODS

Strains

Strain DH-1 (*recA*⁻, *F*⁻, *endA*⁻, *gyrA*⁹⁶, *thi*⁻¹, *hsdR*¹⁷ (*r*⁻, *m*⁻), *supE*⁴⁴) was obtained from D. Hanahan. It was verified to be *recA*-deficient by sensitivity to ultraviolet light. Strain W3110 (*F*⁻, *hsdR*⁻, *hsdM*⁺) was verified as *recA*⁺ by resistance to ultraviolet light and by generation of recombinants in the *π*VX system developed by Seed and described in reference 10. Strains JTT1, RS2 and SD7 were obtained from R. Sternglanz. These were an isogenic set derived from strain PLK831 (*trpE*⁶³, *pyrF*²⁸⁷, *nirA*, *trpR*⁷², *iclR*⁷, *gal*²⁵, *rpsL*¹⁹⁵). The genotypes of JTT1, RS2 and SD7 were PLK831 *trp*⁺ for JTT1, PLK831 *trp*⁺, *top10* for RS2 and PLK831 *trp*⁺, *top10*, *gyrB*²²⁶ for SD7 (11). Plasmid DNAs from strains JTT1, RS2, and SD7 were analyzed by electrophoresis through 1% agarose in the presence of 7 μg/ml chloroquine in 40 mM Tris, 25 mM NaAc, 1 mM EDTA pH 8.3. This analysis confirmed a previous report (15) showing that strain RS2 maintained plasmid DNA at a higher negative superhelicity than wild type *E. coli*, and that SD7 maintained plasmid DNA at a negative superhelicity lower than that of wild type *E. coli*.

Plasmid Construction

Plasmid pGT2 was constructed by simultaneous ligation of 5 DNA fragments derived as follows: (i) a fragment from pBR322 encompassing nucleotides from the Hind III site at 29 to the EcoRI site at 4362, (ii) a fragment from a modified SV40 that contained a polyGT tract inserted at the HpaI site at nucleotide 2666 and

encompassing nucleotides from the EcoR1 site at 1782 to the Bcl1 site at 2770, (SV40 numbered as per reference 12 (iii) a fragment from a modified SV40 genome in which the HpaI site at 2666 had been changed to an XbaI site, and encompassing the nucleotides from the XbaI site to the BamHI site at 2533, (iv) an 80 basepair polyGT tract ending in XbaI and ClaI sites. (v) a fragment from a modified SV40 genome in which the HpaI site at 2666 had been changed to a ClaI site and encompassing nucleotides from the ClaI site to the Hind III site at 3476.

Plasmid pAB2 was constructed by simultaneous ligation of 3 fragments derived as follows: (i) a fragment from pBR322 encompassing nucleotides from the Hind III site at 29 to the EcoR1 site at 4362, (ii) a fragment from SV40 encompassing the nucleotides from the BamHI site at 2533 to the Hind III site at 3476, (iii) a fragment from SV40 encompassing nucleotides from the EcoR1 site at 1782 to the Bcl1 site at 2770. Plasmid pAB3 was made by simultaneous ligation of 3 DNA fragments: (i) a fragment from pBR322 encompassing nucleotides from the Hind III site at 29 to the EcoR1 site at 4362, (ii) a fragment from SV40 encompassing nucleotides from the EcoR1 site at 1782 to the Bcl1 site at 2770, (iii) a fragment from plasmid pAB2 encompassing nucleotides from the Bam site in SV40 sequence to the Hind III site that corresponds to the Hind III site at nucleotide number 3476 of the SV40 genome.

Plasmid pClaGT-d was constructed in two steps. First, a 90 bp polyGT tract was cloned into the ClaI site of a deleted version of pBR322, pML-1, constructed by Luskey and Botchan (13). Second, a head to tail dimer of this pML-GT plasmid was isolated from a gel and transfected into the DH-1 strain of *E. coli*.

DNA Transfection, Plasmid Preparation and Analysis

Transfection was by the method of Hanahan (14) using 30 pg DNA per transfection. Transformation efficiency ranged from 50 to 150 colonies per pg. Cultures from which plasmids were prepared were either grown to saturation or subjected to chloramphenicol when the cell number reached 5×10^8 /ml. The use of chloramphenicol did not influence the composition of the plasmid population. Plasmid was extracted from cells using the boiling lysis method of Holmes and Quigley as described in reference 10. Form I plasmid was isolated by equilibrium-CsCl gradient centrifugation. Use of restriction enzymes and klenow polymerase, and electrophoresis through agarose and polyacrylamide were as described by Maniatis, et al. (10).

RESULTS

Structure of pGT2

Plasmid pGT2 was constructed by the ligation of five DNA fragments, the cohesive ends of which favored the formation of a single circular DNA molecule.

Fig.1.

Structure of plasmid inserts. Each line represents the structure of the insert carried in the plasmids indicated in the left margin. The open rectangles represent blocks of sequence that are present in multiple copies. Blocks labeled A and B were derived from the SV40 genome. Blocks labeled GT were composed of polyGT. Restriction endonuclease cleavage sites were as indicated. Spaces between blocks indicate fragment junctions ligated in the construction of each plasmid.

The five DNA fragments employed in making pGT2 are described in the Methods section. Plasmid pGT2 contained a direct repeat, labelled A GT B in Figure 1. In each of these repeats two stretches of SV40 DNA, labelled A and B, flanked a simple-sequence composed of 80 to 90 base pairs of alternating G and T nucleotide residues, labelled GT. One GT sequence tract was bounded by the cleavage sites for the restriction endonucleases, XbaI and ClaI. This GT block was composed of 80 basepairs of pure polyGT sequence. The other GT block contained 90 base pairs of pure polyGT and 45 base pairs of non polyGT sequence. The sequence of the non-GT DNA has been described previously (15).

The 5-fragment ligation was transfected into the DH-1 strain of *E. coli* and colonies resistant to ampicillin were screened by hybridization to ³²P-labelled SV40 DNA. A colony was found that harbored a plasmid with the structure depicted schematically in Figure 1. This plasmid was named pGT2.

pGT2 was Unstable

The pGT2 plasmid population contained two size species of supercoiled DNA that differed in abundance. The major species migrated slightly slower than the minor species (Fig. 2A, lane 2). Figure 2 also shows the DNA fragments produced by cleavage of pGT2 DNA with PstI (lane 1). The digest contained equimolar amounts of three expected fragments, 3832, 1680, and 921 base pairs in size, but also present was a fourth fragment approximately 1350 bp in size and about one tenth as abundant as the three main fragments. The minor fragment was of a size consistent with what

Fig.2.**Size of pGT2, pAB2, and pAB3 plasmids isolated from rec A or wildtype strains.**

Cells were transformed with 30 pg of plasmid DNA. Colonies were picked, expanded, and plasmid DNA extracted. Plasmid DNA was electrophoresed through 0.7% agarose, stained with ethidium bromide, and photographed under UV light. (A) Lane 1, 1 μ g Pst I digested pGT2 DNA recovered from the five fragment ligation described in the text. Lane 2, 1 μ g of the pGT2 DNA sample in lane 1, undigested. OC indicates open circular DNA, SC indicates supercoiled DNA. The numbers in the right margin indicate fragment sizes in base pairs. Designation of the size and topological form of the DNA in bands was based on migration relative to standard DNA molecules of known size and conformation. (B) Plasmid DNAs isolated from strains DH-1 and W3110 that had been transfected with pGT2, pAB2, or pAB3 DNA. Each lane contained DNA from a separate colony. Lanes 1 and 2, pGT2 from W3110. Lanes 3-6, pGT2 from DH-1. Lane 7, input DNA used in experiments shown in lanes 3-6. Lane 8, pAB3 from W3110. Lanes 9 and 10, pAB3 from DH-1. Lane 11, pAB2 from W3110. Lanes 12 and 13, pAB2 from DH-1. The roman numerals in the left margin indicate the positions of supercoiled (form I) and open circle (form II) DNA.

would be expected if pGT2 were to delete one copy of the direct repeat. Digestion with Bam HI produced the same result (not shown).

The size heterogeneity seen in the plasmid DNA displayed in Figure 2A was not an artifact of the ligation transfection protocol employed to generate the original plasmid. This was shown by transferring individual pGT2 DNA molecules to new cells by transfection at a DNA:cell ratio low enough to effectly eliminate double transformation events. Control experiments showed that when cells were exposed to 100 pg of an equimolar mixture composed of two pBR322 derivatives encoding

Fig. 3.

Restriction sites in deleted pGT2 molecules. DNA was digested first with Pst I and subsequently as follows: lane 1, ClaI digestion, lane 2, XbaI digestion, lane 3, digestion with ClaI and XbaI, lanes 4-8, no further digestion. Lanes 1, 2 and 3 each received 1.5 µg DNA. The amounts of DNA loaded in lanes 4-8 were 1.5 µg, 0.75 µg, 0.30 µg, 0.15 µg, and 0.075 µg respectively. Numbers in the margins indicate fragment sizes in base pairs.

different antibiotic-resistance genes, less than 1% of the cells were doubly transformed.

The *recA* strain DH-1 was transformed with 30 µg of a pGT2 DNA preparation that contained approximately 10% deleted molecules (input DNA shown in Figure 2B, lane 7). Four colonies were picked and plasmid DNA prepared by the procedure described in the Methods Section. As shown in Figure 2B, (lanes 3 through 6) each colony yielded plasmid DNA that contained deleted DNA. These data demonstrated that deletion of pGT2 was not an artifact of the protocol employed to generate the original plasmid.

Heterogeneity of Deleted pGT2

The deleted molecules in the pGT2 population could have arisen by a single event early in the life of the colony. Alternatively, deleted plasmids could have been generated by numerous recombination events. If the population of deleted plasmids were formed by recruitment from the pool of intact pGT2 molecules, deleted plasmids would be expected to vary structurally because deletion of pGT2 can occur via three alternative homologous recombination reactions involving either A, B or GT sequence blocks (Figure 1). Each of these recombination reactions would produce a different plasmid. Deletion mediated by recombination between A sequences would produce a molecule containing both XbaI and ClaI restriction

endonuclease cleavage sites. Deletion mediated by recombination between B sequences would produce molecules lacking both of these restriction sites. Deletion via GT recombination would produce a plasmid that retained the site for *Cla*I, but not the site for *Xba*I. In addition to this restriction site polymorphism, unequal recombination between polyGT sequences would be expected to produce plasmids with varying lengths of polyGT.

The data shown in Figure 3 demonstrated heterogeneity in the deleted plasmid population with respect to the presence of *Xba*I and *Cla*I cleavage sites. Figure 3 shows an ethidium bromide stained agarose gel used to assess the presence or absence of *Cla*I and *Xba*I cleavage sites in deleted pGT2 DNA. Lane 4 shows the band pattern of pGT2 DNA cut with *Pst*I. The minor band at 1350 bp contained DNA from deleted plasmids. About 80% of the deleted plasmids were sensitive to *Cla*I digestion (lane 1), indicating that most deletions were mediated by recombination between either two A sequence blocks or two GT sequence blocks. Lane 2 shows that deleted plasmid DNA was rather insensitive to *Xba*I digestion, indicating that the contribution of A to A recombination was small. The complete cleavage of the 1680 bp *Pst*I fragment served as an internal control for *Xba*I activity, proving that the lack of cleavage of deleted plasmid DNA was due to the absence *Xba*I sites in the majority of the deleted plasmid DNA molecules. Lane 3 shows that 10 to 20% of the deleted DNA was insensitive to both *Cla*I and *Xba*I. Lanes 5 through 8 contained dilutions of the material in Lane 4 and were used to make quantitative determinations.

Because *Xba*I cleavage was barely detectable by this technique, the presence of deleted plasmids carrying an *Xba* site was confirmed by analysis of radioactively end-labelled DNA. In this procedure, the 1350 basepair *Pst*I fragment was purified by preparative gel electrophoresis. The purified DNA was digested with either *Cla*I or *Xba*I, or both, and the cleavage products were end-labelled by treatment with klenow polymerase. End-labelled DNA fragments were separated by gel electrophoresis and visualized by autoradiography. The results were consistent with those shown in Figure 3 and clearly showed that about 5% of the deleted plasmids contained an *Xba*I cleavage site (data not shown). This approach was extended to assess the contribution of gene conversion to the segregation of restriction sites among plasmid molecules. In this experiment, plasmid DNA was subjected to digestion with either *Cla*I or *Xba*I, end-labelled, electrophoresed through polyacrylamide and the fragments visualized by autoradiography. If gene conversion were operating upon the plasmid DNA at a significant level it would be expected to generate undeleted molecules carrying either two *Xba*I sites or two *Cla*I sites. We could detect no such molecules (data not shown).

To corroborate the results shown in Figure 3, we analyzed the distribution of restriction enzyme cleavage sites among 19 individual deleted pGT2 molecules. Fifteen of the 19 isolates contained only a C1aI site, 3 isolates lacked both restriction sites, and one of the 19 isolates contained both the C1aI and XbaI sites (data not shown). These findings are consistent with the analysis of the mixed plasmid population. The combined data indicated that the population of deleted plasmids contained molecules produced by recombination events involving all three of the repeated sequence blocks in pGT2 DNA. The predominant pathway was GT by GT recombination, which occurred about 3 times more frequently than would have been expected based on the length of GT sequence homology (80 bp) relative to the sequence homology available for A by A (133 bp) and B by B (104 bp) recombination.

Because GT by GT recombination was the major pathway to deletion, and because two polyGT tracts can be expected to recombine unequally, many individual deleted molecules would be expected to bear polyGT tracts of lengths different from those of intact pGT2 DNA. To assess polyGT tract lengths, PstI fragments from intact and deleted pGT2 plasmids were purified, digested with Sau3A, end-labelled by treatment with the klenow fragment of DNA polymerase I, and electrophoresed through polyacrylamide. As shown in Figure 4, Sau3A digestion of the PstI fragment from intact plasmids produced the expected five bands, four of which are shown in lane 2. The map positions of these fragments are shown in Figure 1. By contrast, Sau3A digestion of the PstI fragment from deleted plasmids produced a heterogeneous collection of bands ranging between 317 and 372 bp (lane 1). This heterogeneity is consistent with the idea that recombination between GT sequences occurred multiple times and that many such recombination events were unequal. Shortened polyGT tracts were not due to internal deletions within individual polyGT tracts. We did not see shortened polyGT tracts in cloned pGT2 molecules that had undergone deletion via recombination between SV40 sequences (not shown). Furthermore, polyGT tracts that resided in plasmids as single insertions proved to be quite stable in recA cells (Murphy and Stringer, unpublished).

Instability of pGT2 Was Attributable to PolyGT Sequences.

The data described above established that the pGT2 plasmid was an unstable molecule prone to undergo deletion via homologous recombination in the absence of recA function. The preponderance of GT by GT recombination suggested that the instability of pGT2 may be due to a recombinogenic activity associated with these simple sequences. To explore this possibility, we examined the behavior of two plasmids, pAB2 and pAB3, the structures of which are depicted in Figure 1. Plasmids pAB2 and pAB3 contained two and three copies respectively of the A and B sequences that flanked the polyGT sequence tracts in pGT2 DNA. The data in Figure

Fig. 4.**Heterogeneity in polyGT tracts from deleted pGT2 plasmids.**

Pst I fragments from intact and deleted pGT2 plasmids were purified, digested with Sau3a, end-labelled, and electrophoresed through 6% polyacrylamide. The figure shows an autoradiograph of the gel. The numbers in the right margin are fragment sizes in base pairs. Lane 1, DNA from deleted plasmids. Lane 2, DNA from intact plasmids.

2B show that plasmids lacking polyGT tracts did not produce a detectable amount of deleted DNA when transfected into the DH-1 strain (lanes 9, 10, 12 and 13). Plasmids pAB2 and pAB3 also failed to accumulate detectable levels of deleted derivatives in W3110 cells, a recombination proficient strain (Figure 2B, lanes 8 and 11). By contrast, pGT2 was highly unstable in W3110 cells, accumulating deleted molecules to levels exceeding those of intact plasmid (Figure 2B, lanes 1 and 2). Analysis with restriction endonucleases ClaI and XbaI showed that the population of deleted PGT2 molecules isolated from strain W3110 was structurally heterogeneous in a manner similar to the deleted plasmid population isolated from strain DH-1 (data not shown).

These data indicated that the polyGT elements in pGT2 DNA endowed the plasmid with a propensity to undergo deletion. Because deletion of pGT2 molecules occurred not only via GT recombination, but by recombination mediated by A or B sequence tracts which were stable in plasmids pAB2 and pAB3, it appears that in addition to being recombinogenic themselves, the polyGT elements in pGT2 potentiated recombination between neighboring DNA sequences.

A Plasmid with Distantly Spaced PolyGT Tracts was not Unstable in a Rec A Host.

The instability of pClaGT2 DNA was surprising because we had previously observed no such behavior in pClaGT-d, a plasmid that carried two polyGT tracts situated 3Kb apart. Plasmid pClaGT-d was a head to tail dimer of a pML-1 plasmid (13) into which a 90 bp polyGT tract had been inserted at the ClaI restriction site

(Figure 5A). The influence of polyGT on monomerization of this dimeric molecule was tested by transfection of the DNA into *recA* *E. coli*. The results of this experiment, shown in Figure 5B, were in marked contrast to our findings with pGT2 DNA. We could detect no monomeric plasmid DNA. We also examined the propensity of monomeric plasmids bearing polyGT to multimerize. PolyGT did not increase plasmid multimerization in *recA* cells (data not shown).

Stability of pGT2 Maintained Under Increased or Decreased Torsional Strain.

A possible explanation for the hyperrecombinogenicity of polyGT elements in pGT2 molecules is that these stretches of alternating purine and pyrimidine residues may be conformationally disposed to recombine. Bianchi and Radding showed that homologous recombination *in vitro* entailed noninterwound side-by-side DNA pairing promoted by purified Rec A protein (7). Kmeic and Holloman subsequently showed that the DNA in noninterwound recombination complexes formed by Rec 1 protein bound antibody specific for DNA in the Z conformation, (8) and that the Rec 1 protein is a Z-DNA binding protein *in vitro* (9). Since polyGT has been shown to adopt the Z conformation under the influence of torsional strain engendered by treatment of closed circular DNA with DNA gyrase, it seemed reasonable that this *in vitro* property might be involved in the behavior we observed *in vivo*. To assess the possible role of torsional strain in the deletion of pGT2, we introduced the plasmid into strains of *E. Coli* that were altered in the introduction and maintenance of torsional strain in plasmid DNA. Two mutant strains were used: RS2, which is deficient in topoisomerase 1 activity, and SD7, which is deficient in both topoisomerase 1 and gyrase activities. Plasmid DNA isolated from strain RS2 has been shown to be 12% more underwound than plasmid DNA from the wild type parent, strain JTT1, and plasmid DNA from strain SD7 has been shown to be 14% less underwound relative to wild type levels of supertwisting (16). We verified that the strains maintained plasmids at different superhelical densities by gel electrophoretic analysis of plasmids isolated from each strain (see Methods).

Figure 6 shows that pGT2 DNA was unstable in all three strains. Plasmid DNA from strain RS2 (lanes 2, 3 and 4) accumulated higher levels of deleted DNA than seen in wild type (lane 1) or in the gyrase mutant strain SD7 (lanes 6 and 7). However, the plasmid was not totally stabilized by the relaxation of torsional strain

Fig. 5.

Distantly spaced polyGT tracts were stable. (A) Structure of plasmid pClaGT-d. This plasmid was a head-to-tail dimer of plasmid pML-1 (a derivative of pBR322) into which had been inserted a 90 basepair polyGT tract. (B) Stability of pClaGT-d and dimeric pML-1 (pML-d) in strain DH-1. DNAs were electrophoresed through 1% agarose. The gel was stained with ethidium bromide and photographed in UV light. Margin abbreviations: mon., monomeric; dim, dimeric; sc, supercoll; oc, open circle; lin, linear. Lanes marked pClaGT-m and pML-m contained monomeric plasmids.

Fig. 6.**Plasmid stability as a function of torsional strain.**

The photograph shows an ethidium bromide stained 1% agarose gel through which uncut plasmid DNA samples were electrophoresed. The roman numerals in the left margins indicate the positions of supercoiled (form I) and open circle (form II) DNA molecules. Lane 1, strain JTT-1 (normal degree of underwinding). Lanes 2-4, strain RS-2 (more underwound). Lane 5, pAB2 marker. Lanes 6 and 7, strain SD-7 (less underwound).

in SD7 cells. It should be noted that all of the strains used in this experiment were wild type for RecA protein function, and that functional RecA appeared to potentiate deletion of pGT2 (Figure 3, lanes 1 and 2).

DISCUSSION

The presence of deleted molecules in pGT2 plasmid preparations indicates that either pGT2 molecules were prone to recombine, or that the products of recombination acquired a replicative advantage over pGT2. We have not examined the relative rates of replication of pGT2 and deleted plasmids, and cannot rule out a contribution of differentiation replication to the abundance of deleted molecules. However, two lines of evidence indicate that the abundance of deleted pGT2 molecules was primarily due to frequent recombination events. First, each plasmid preparation contained three different types of deleted plasmid. This structural heterogeneity of deleted pGT2 molecules indicates that multiple recombination events gave rise to the population of deleted plasmids. Furthermore, the three different deleted plasmids must have been produced by more than three recombination events because the relative abundance of the three types of deleted plasmids was not equimolar, but rather plasmids generated by recombination between two polyGT tracts predominated over plasmids formed by recombination between SV40 DNA tracts. Since all three types of deleted plasmids were of nearly

identical structure, and would not be expected to replicate differentially, the predominance of GT-type recombinants indicates that polyGT tracts were more likely to recombine. Moreover, deleted pGT2 molecules of the GT-type were structurally heterogeneous with respect to polyGT tract lengths. Since this variability in polyGT tract lengths was unique to GT-type recombinants, it appears that each GT-type plasmid was the product of a different unequal homologous recombination event between two polyGT tracts. It follows that the population of GT-type deleted plasmids was formed by recruitment from the pGT2 pool. A second reason to discount differential replication of deleted plasmids as a contributor to the abundance of deleted pGT2 is that strains with functional RecA contained deleted plasmids in greater abundance than did recA strains. The correlation between the abundance of deleted molecules and an optimally functional recombination system suggests that recombination was the major pathway to the accumulation of deleted molecules.

These data support the conclusion that polyGT tracts can act as potent recombinators. Consistent with this idea, removal of both polyGT tracts from pGT2 stabilized the plasmid to the point that deleted molecules no longer accumulated to detectable levels, even in cells with a functional RecA protein.

The recombinogenic activity of polyGT is presumably due to its structure which is unusual in two respects (i) as a dinucleotide repeat, polyGT presents to the cell an unusually uniform primary structure that could interact more efficiently with recombinases, (ii) As a purine-pyrimidine repeat polyGT readily adopts the Z conformation when under torsional strain in vitro. Thus, it could exert its effect by engendering localized changes in DNA secondary structure.

In considering how the structural features of polyGT might cause deletion of pGT2 DNA, three properties of polyGT recombination must be accommodated. First, as was shown by the data in Figure 3, pGT2 deletion was quasi-sequence-specific in that recombination was not limited to the polyGT tracts themselves, but also occurred between the A and B sequence tracts that flanked blocks of polyGT sequence. Second, we have observed that the recA-independent recombination that occurred in pGT2, where polyGT tracts were clustered, did not occur in a plasmid where the polyGT tracts were spaced 3 kb apart (see Figure 5). Third, as shown in Figure 4, blocks of polyGT sequence carried in undeleted pGT2 plasmids were relatively stable.

Quasi-specificity suggests that polyGT tracts may act as sites at which recombination is initiated, and that the resolution of recombination intermediates sometimes occurs by strand exchange outside the boundaries of the polyGT tract. One way in which this could happen would be by branch migration of structures

formed by strand exchange between polyGT sequences. However, this model seems inadequate to explain recombination between two A sequences because one GT-A junction is interrupted by 40 basepairs of sequence that might be expected to block branch migration (17,18). Furthermore, branch migration beyond the borders of polyGT might be expected to occur only in molecules in which the polyGTs were paired in the appropriate register. A caveat to these arguments, however, is that under some circumstances *in vitro*, Rec A protein has been shown to be capable of perpetuating strand exchange through regions of heterology (19). Another way to explain quasi-specificity would be to ascribe it to the action of a nuclease that cleaves at polyGT producing an end that invades the second polyGT sequence. PolyGT cleavage could also stimulate recombination in adjacent sequences if cleavage were followed by degradation that extended into an A or B sequence tract. However, nuclease hypersensitivity seems unlikely because we have observed that polyGT sequences are stable when carried as single inserts (Murphy and Stringer, unpublished). Furthermore, the involvement of an exonuclease might also be expected to lead to frequent gene-conversion events, (20) but such events were not in evidence.

Secondary structure offers an alternative explanation for the quasi-specificity of pGT2 deletion. Perhaps conformational distortion centered in the polyGT tracts is propagated to adjacent SV40 sequences. There is evidence *in vitro* that structural perturbation can spread from Z DNA to adjacent sequences, albeit the structural distortion was propagated only 4 basepairs beyond the B-Z junction (21). Another way in which secondary structure could lead to quasi-specificity would be if the polyGT sequences were to assume a conformation that bound recombination enzymes with greater affinity. *In vitro*, the Rec1 protein binds Z DNA 75 times tighter than B DNA (9). Perhaps the polyGT tracts in pGT2 act to nucleate a protein-DNA complex that can involve neighboring sequences.

PolyGT tracts did not exhibit rec-A independent hyperrecombination when spaced 3 kb apart suggesting that polyGT tracts must be near each other to be recombinogenic in the absence of recA. It is possible that this apparent proximity requirement reflects the action of a mechanism that matches close neighbors, e.g., non-reciprocal recombination mediated by slipped mispairing events. However, this seems unlikely because such slipped mispairing events would be expected to occur in single tracts of polyGT, which showed no sign of suffering internal deletions. Alternatively, secondary structure effects could explain the apparent requirement for proximity. Perhaps the clustering of 160 basepairs of polyGT sequences in a 450 basepair region facilitated conformational distortion that did not occur when polyGT blocks were spaced farther apart.

While our data are consistent with a role for secondary structure in the recombinogenic activity shown by polyGT, other data appear to support the idea that deletion of pGT2 DNA was simply a manifestation of the simple primary structure of polyGT. Brutlag *et al.* (22), reported that a *Drosophila* satellite DNA composed of a repeated AAGAG sequence could not be propagated as long (greater than 1 kb) insertions. However, satellite DNA tracts up to 500 bp in length were much less prone to suffer deletion. It is not known whether 80 bp tracts of polyAAGAG would be hyperrecombinogenic and stimulate recombination involving adjacent DNA of complex sequence. It would be interesting to test such sequences in plasmid molecules structured like pGT2. It should be noted, however, that if sequence tracts composed of AAGAG repeats should prove to be recombinogenic in the manner of polyGT, such activity could still be due to deformation since polypurine tracts have been shown to adopt a non-B conformation when torsionally strained (21). Furthermore, polypurine sequences have been shown to induce altered DNA conformation upon neighboring DNA sequences (23). To distinguish between primary and secondary structure effects it will be necessary to synthesize a DNA sequence that is nonrepetitive and composed of alternating purine and pyrimidine residues.

The relationship between torsional strain and conformational distortion presents another avenue through which to explore the mechanism of pGT2 deletion. Although pGT2 DNA was unstable in bacterial strain SD7, in which plasmid DNA has been shown to be less underwound than in wild type *E. Coli* (16), it is possible that the reduction in tension in SD7 cells was insufficient to prevent conformational distortion in pGT2 DNA. It is also possible that the instability of pGT2 in strain SD7 was due to the action of RecA protein, which appeared to potentiate deletion of pGT2 DNA in strains with normal supercoiling. It may be possible to stabilize pGT2 in a strain that both lacks RecA function and maintains plasmid under reduced torsional tension. Alternatively, it may be possible to demonstrate an effect of torsional strain by constructing plasmids that carry shorter polyGT tracts.

It is interesting to note that polyGT sequences behaved similarly to the naturally-occurring plasmid recombinators, Cer (24) and parB (25), which mediate the resolution of multimeric plasmids to monomer circles via site-specific recA-independent recombination. However, parB recombination differs from pGT2 deletion in that there is no known requirement that two parB elements be near each other. Thus, while polyGT is similar to parB in mediating site-specific recA-independent plasmid recombination, polyGT appears distinct in its proximity requirement. The parB sequence does not contain runs of poly purine-pyrimidine repeats longer than 7 basepairs (25).

PolyGT appears to be a recombinator with novel properties. When considered

in conjunction with the structure of DNA synapsed through the action of recA-like proteins, pGT2 deletion raises the possibility that polyGT tracts in supercoiled plasmid DNA frequently recombined in recA cells because these sequences were predisposed to assume a structure normally requiring the intercession of RecA protein.

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