RecA-mediated Strand Exchange Reactions between Duplex DNA Molecules Containing Damaged Bases, Deletions, and Insertions*

(Received for publication, November 20, 1987)

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RecA protein from Escherichia coli promotes homologous pairing and strand exchange between duplex DNA molecules if one is partially single-stranded. Using linear duplexes and circles with a single-stranded gap as the substrates, this reaction generates nicked circular heteroduplex DNA and linear molecules with single-stranded ends. The completion of strand exchange can be demonstrated by the production of nicked circular heteroduplex DNA detected by gel electrophoresis and autoradiography using radiolabeled linear molecules. When the effect of ultraviolet damage to the substrate DNA was tested, strand exchange was found to pass 30 or more pyrimidine dimers in each duplex. In contrast, exchanges were blocked or severely slowed by interstrand cross-links and monoaducts produced by psoralen and 360 nm light.

Deletions and insertions of from 4 to 38 base pairs in the DNA substrates had little effect on the production of nicked circular heteroduplex DNA. However, those of 120 base pairs, or greater, reduced the product yield to a level below the threshold of detection. These results contrast with those obtained in related three-stranded reactions (Bianchi, M. E., and Radding, C. M. (1984) Cell 35, 511-520), in which stable heteroduplex products with 500 or 1300 unpaired bases were obtained when the insert was located within a single-stranded circular substrate.

The recA gene of Escherichia coli plays an essential role in genetic recombination and in promoting cell survival following exposure to agents that damage chromosomal DNA. Studies in vitro on the action of RecA protein on appropriate DNA substrates suggest that this enzyme performs key steps in recombination.

The reactions of RecA protein have been investigated with various DNA substrates. The most widely used involves only three DNA strands and consists of single-stranded circular and duplex linear phage DNA. RecA catalyzes homologous pairing followed by the transfer of one strand of the linear duplex to the single-stranded circle forming nicked circular duplex molecules. Single-stranded binding protein is required for full efficiency. The substrates, intermediates, and the final products from distinct bands when analyzed by gel electrophoresis and can be followed during the reaction (1-4). Strand transfer is not hindered by topological constraints, and the products are free to separate at the end of the reaction. As a result, these substrates have been used in many key experiments on the reaction mechanism of RecA protein. RecA protein is able to tolerate some lack of perfect structural complementarity between the polynucleotide chains and still function. For example, it can drive the strand transfer past UV-induced pyrimidine dimers (5) or even heterologous inserts of up to 1300 heterologous bases in the circular single-stranded DNA (6).

Whereas only three DNA strands are involved in the studies just referred to, most forms of recombination in living cells take place between two chromosomal duplexes. Reactions involving four strands of DNA have been studied using circular duplex DNA with a single-stranded gap and linear duplex DNA. Again, RecA protein mediates homologous pairing and strand exchange between the two substrates (7-11). However, in this case, there is no requirement for single-stranded binding protein (12).

Since the three- and four-stranded reactions may differ with regard to the ability of RecA protein to overcome the effects of DNA damage, or insertions and deletions on the strand exchange reaction, we have investigated the efficiency of strand exchange under these conditions. Our results show that, while the four-stranded reaction can pass 30 or more pyrimidine dimers with little retardation, it is severely slowed or blocked by psoralen interstrand cross-links and monoaducts in the linear duplex. We have also explored the effects of deletions and insertions, ranging in size from 4 to 430 nucleotides. Our results show that RecA drives the strand exchange past the unpaired bases due to insertions or deletions ranging from 4 to 38 nucleotides. However, nicked circular heteroduplex products with 120 or more mismatched bases were not detected.

**EXPERIMENTAL PROCEDURES**

**Enzymes and Proteins**—RecA protein was purified by the method of Cox et al. (14) with minor modifications. Concentrations in the text refer to moles of monomeric protein. EcoRI and BamHI restriction endonucleases were purchased from International Biotechnologies, and exonuclease III from New England Biolabs. The materials for dideoxy sequencing were from Bethesda Research Laboratories.

**Bacterial Strains and Plasmids**—The plasmids pSW31 and pSW44 (13) (Fig. 1) were constructed from pACYC184 (17). pSW31 has 4 bp1 deleted at the BamHI site within the tetracycline resistance gene, while pSW44 has 430 bp deleted between the two PstI sites within the chromosomal resistance gene. All plasmid DNA was grown and labeled in a recA1 thyA derivative (AB2457) of E. coli K12 (AB1157) (15). E. coli strain JMN103 del(lac pro)F' traD36 proAB' lacF' lacZM15 (16) was used as host for M13. Plasmid DNA amplification, labeling, and purification were as described previously (13).

1 The abbreviation used is: bp, base pairs.
Construction of Derivatives of Phage M13mp8—The cloning vector phage M13mp8 consists of phage M13 with the BamHI site at 2220 removed, and the insertion of a multilinker and the α-fragment of lacZ. When plated with 5-bromo-4-chloro-3-indolyl and β-D-galactoside on the host cells (8M103) that carry λlacI' lacZΔM15, blue (lac+) plaques are obtained only if the correct reading frame is maintained (16).

We constructed a phage M13H, a derivative of M13mp8, in which the BamHI site of phage M13 was restored to position 2220. This site was necessary for the production of single-stranded gaps at a position distant from the polylinker (where deletions were to be made). To make the phage M13H, the BamHI site at the polylinker of M13mp8 was removed by cleaving it with BamHI, filling in the overhanging ends with DNA polymerase (large fragment), and ligating the blunt ends with T4 ligase. The form I DNA of the resulting Lac− phage was resistant to cutting by BamHI and found by dideoxy sequencing to carry the expected 4-bp repeat at this restriction site. The BamHI site at position 2220 was moved into our new phage vector by cleaving this phage and also wild type M13 with Clal to yield large and small fragments (3612 and 2896 bp). These were separated by gel electrophoresis, electroeluted, and the large fragment of the modified M13mp8 DNA was annealed to the small fragment of M13 and ligated. The resulting DNA was used to transform host cells (JM103), and ten transformed plaque-forming units were purified, grown in 10-mI cultures, and used to make mini prep of form I phage DNA. One isolate giving white plaques, designated M13H3, produced form II DNA that was sensitive to BamHI. The presence of the BamHI site at position 2220 was confirmed by restriction digestion. A restriction map and the nucleotide sequence in the polylinker region of M13H are shown in Fig. 1.

Three derivatives were made from M13H. Phage M13H-10 was prepared by cutting the multilinker region with PstI and HindIII. After extraction with phenol, the overhanging end of the HindIII restriction cut was filled in the DNA polymerase 1 fragment, joined with DNA ligase, and the DNA was transformed into a new host. A blue plaque-forming Lac− phage was purified, but when the nucleotide sequence was determined, it was found to be deleted by 10 instead of the expected 4 bases and was therefore designated M13H-10. The nucleotide sequence in the polylinker region is shown in Fig. 1.

To make phage M13H-38, M13H was deleted by treating form I DNA with HindIII and EcoRI, followed by S1 nuclease to digest the overhanging ends. The DNA was ligated and a Lac− phage M13H-38 was isolated. The 38-bp deletion was confirmed by the loss of restriction sites for HindIII, EcoRI, and Sall from the polylinker, and the sequence is also shown in Fig. 1.

One more phage designated M13H-120 was derived from M13H by cutting form I DNA with HindIII and PvuII. The overhanging ends were filled in with DNA polymerase large fragment, joined with DNA ligase, and the DNA was transformed into host cells. Form I DNA from this Lac− phage designated M13H-120 was resistant to PvuII and HindIII, confirming the loss of the HindIII-PvuII fragment. Moreover, the single-stranded DNA primer (shown in Fig. 1) used for nucleotide sequencing failed to bind and act as a primer for DNA polymerase, confirming the loss of the primer binding site near the HindIII end of the expected deletion.

Preparation of Plasmid DNA Substrates—Circular duplex plasmid DNA carrying a single-stranded gap was prepared from covalently closed (form I) H-labeled plasmid DNA by nicking with either EcoRI or HindIII in the presence of ethidium bromide, followed by treatment with a predetermined saturating amount of exonuclease III, until about 1% of the DNA was acid-soluble (11). The resulting gapped circular DNA was purified from gapped linear DNA by agarose gel electrophoresis. Each gapped molecule contained a single-stranded region of 100-160 bases in length in one of the two DNA strands. Linear duplex DNA was produced by cutting 32P-labeled covalently closed plasmid DNA with a restriction enzyme. All DNA substrates were phenol-extracted, ether-extracted, ethanol-precipitated, and dialyzed against 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. DNA concentration was determined as moles of nucleotides.

Preparation of Gapped M13 DNA—To prepare the circular gapped DNA, form I DNA was made from phage M13H and each of its derivatives, and was nicked at the BamHI site (position 2220), treated with exonuclease III to release about 1% acid-soluble nucleotides, and purified as described above. The linear duplex DNA was made by cutting form I DNA with BamHI.

DNA Damaging Treatments—To produce pyrimidine dimers in plasmid DNA, 400 μl of DNA (12 μM) in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA was irradiated with a low pressure mercury germicidal UV lamp at a dose rate of 1 J/m2/s, calibrated with a 254 nm UV light meter (General Electric). A dose of 27 J/m2 was calculated to produce an average of one dimer per plasmid molecule of approximately 4000 bp (18). To cross-link plasmid DNA, 400 μl (12 μM) in 10 mM Tris-HCl (pH 7.5) were supplemented with 20 μg/ml tri-methyl psoralen and irradiated with 10 J/m2/s of 360 nm light, as measured with a 360 nm light meter (J-22, Ultraviolet Products). Because irradiation of DNA in the presence of psoralen produces both cross-links and monoaducts, the dose required to produce an average of one cross-link per linear duplex was measured by alkali agarose gel electrophoresis (19).

Strand Exchange Reaction Mixtures and Assays—Reaction mixtures (30–50 μl) contained 8–10 μM gapped duplex DNA and 6–15 μM linear duplex DNA (20). Samples were allowed to incubate with 5% glycerol and 0.01% bromphenol blue for electrophoresis through 1% agarose, using 40 mM Tris-HCl (pH 7.8), 5 mM sodium acetate, and 1 mM EDTA as the buffer system. Gels were dried onto filter paper and exposed to Kodak X-OMat AR-5 film for 24–72 h. The autoradiograms were scanned with a Joyce-Loebl densitometer.

DNA Sequencing—The nucleotide sequences in the multilinker region of the phases of the phages M13H and derivatives were determined by the dideoxy sequencing method (20), using a 17-base universal oligonucleotide primer (Bethesda Research Laboratories), the position of which is shown in Fig. 1.

RESULTS

The Reaction between Gapped Circular and Linear Duplex DNA Substrates—The plasmids pACYC184, pSW31, and pSW44 (Fig. 1, top right) were used in the first set of experiments, and phage form I DNA from M13H and derivatives carrying deletions of 10, 38, and 120 bp (Fig. 1, top left) were used in the second series. Gapped circular plasmid or phage DNA was constructed from form I material, which was nicked in the presence of ethidium bromide in either strand at the respective EcoRI or BamHI sites. The nicked DNA was treated with exonuclease III to produce single-stranded gaps of 100–160 bases in length, and the resulting gapped DNA was incubated with the corresponding 32P-labeled homologous EcoRI or BamHI linear duplexes and RecA protein. In each case the linear duplexes had 3' ends complementary to the single strand in the gap, as required for the strand exchange reaction (11). Agarose gel electrophoresis and autoradiography were then used to test for the production of 32P-labeled nicked circular heteroduplex DNA under various experimental conditions. The overall reaction for the phage substrates is illustrated in Fig. 2, and the reactions with the plasmid substrates are similar.

Effect of Pyrimidine Dimers on Strand Exchange between Duplex Molecules—Circular plasmid DNA (pACYC184) carrying a gap at the EcoRI site was incubated with homologous 32P-labeled EcoRI linearized DNA and RecA protein in a series of reactions in which one DNA substrate was damaged by irradiation with 254 nm UV light. The results are shown in Fig. 3 with the negative and positive controls in lanes a and b. The exposure of the 32P-labeled linear duplex DNA to UV light (0, 30, 100, 250, and 1000 J/m2) sufficient to produce 0 (lane b), 1 (lane c), 4 (lane d), 9 (lane e), or 37 (lane f) pyrimidine dimers/molecule did not prevent efficient and complete strand exchange, as seen by the formation of heteroduplex 32P-labeled nicked circular product.

The exposure of both linear and circular substrates to a dose that produced approximately 37 dimers/strand (Fig. 3,
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pSW44 carries a 430 bp deletion between PvuII sites

pSW31 carries a small deletion (4bp) at the BamHI site

FIG. 1. Phage M13H showing positions of polylinker and restriction sites and plasmid pACYC184 showing the positions of the restriction sites and deletions. Left, three phages, M13H-10, M13H-38, and M13H-120, were derived from M13H and carry deletions in the polylinker region. The BamHI site at position 2200 was used to make the single-stranded gaps. Right, two plasmids were derived from pACYC184; pSW31 carried a 4-bp deletion at the BamHI site, while pSW44 carried a deletion of 430 bp between the two PvuII sites (13). The EcoRI site was nicked in either strand and digested with exonuclease III to make single-stranded gaps. Bottom, the nucleotide sequences in the polylinker region of M13H and M13H10, M13H-38, and M13H-120. The nucleotide sequences of the first three were verified by dideoxy nucleotide sequencing.

FIG. 2. Diagram of gapped circular and linear DNA from the M13H phages showing the relative positions of the initial pairing at the gap and the structures expected part way through the strand exchange. The upper or lower pairing may occur according to whether the gap was in the (+) or (-) strand.

Fig. 3 shows the results of reactions in which the 32P-labeled linear DNA was irradiated with 0, 0.4, 0.8, 1.7, or 3.4 kJ/m2 in the presence of psoralen, to produce an average of 0, 0.5, 1, 2, or 4 interstrand cross-links/molecule. Since no heteroduplex form II DNA was detected in lane p of Fig. 3, it demonstrated that one cross-link and associated monoadducts per duplex was sufficient to prevent strand exchange from reaching the end of the substrates.

Effect of Insertions and Deletions on Strand Exchange—The following experiments were designed to test whether RecA-mediated strand exchange between two duplexes could pass deletions and form full length heteroduplexes. We first used the plasmid pACYC184 and two derivatives of pACYC184 that carried either a 4 (pSW31)- or a 430 (pSW44)-bp deletion (13). When 3H-labeled gapped DNA from pACYC184 was reacted with linearized 32P-labeled duplex from pACYC184 or pSW31 in the presence of RecA protein, we observed the efficient formation of nicked circular heteroduplex products (Fig. 4, lanes b and d). Also seen in these lanes are faint bands of slow moving reaction intermediates that were unable to complete strand exchange and separate (11, 21).
protein. An ATP regeneration system was included in the reactions of heteroduplex nicked circular DNA. Lane a, RecA protein omitted; lanes b–g, complete, but irradiated with varying doses of 254 nm light to produce the indicated number of dimers per linear DNA molecule. It was assumed that 10 J/m² of 254 nm light produced approximately one pyrimidine dimer per kilobase of duplex DNA (18). The gapped circular DNA was not irradiated. Center, effect of pyrimidine dimers in both DNA substrates on the formation of heteroduplex nicked circular DNA. Lanes h and i, no dimers; lanes j and k, 37 dimers/molecule; lane l, form II (nicked circular) marker. RecA protein was present where indicated. Right, psoralen photoadducts and cross-links in the linear duplex DNA block strand exchange. Lane m, RecA protein omitted; lanes n–r, complete with varying doses of 360 nm UV light to produce the indicated average number of cross-links per linear duplex. Lanes s, control reaction, no psoralen or light treatment. In lanes o to r, the cross-linked linear duplex DNA migrates slightly slower through the agarose gel than noncross-linked linear duplex DNA (lane s).

**FIG. 3.** Effects of DNA damage on RecA protein-catalyzed strand exchanges between gapped duplex and linear duplex DNA by agarose gel electrophoresis. Left, effect of UV-induced pyrimidine dimers in the linear duplex DNA on the formation of heteroduplex nicked circular DNA. Lane a, RecA protein omitted; lanes b–g, complete, but irradiated with varying doses of 254 nm light to produce the indicated number of dimers per linear DNA molecule. It was assumed that 10 J/m² of 254 nm light produced approximately one pyrimidine dimer per kilobase of duplex DNA (18). The gapped circular DNA was not irradiated. Center, effect of pyrimidine dimers in both DNA substrates on the formation of heteroduplex nicked circular DNA. Lanes h and i, no dimers; lanes j and k, 37 dimers/molecule; lane l, form II (nicked circular) marker. RecA protein was present where indicated. Right, psoralen photoadducts and cross-links in the linear duplex DNA block strand exchange. Lane m, RecA protein omitted; lanes n–r, complete with varying doses of 360 nm UV light to produce the indicated average number of cross-links per linear duplex. Lanes s, control reaction, no psoralen or light treatment. In lanes o to r, the cross-linked linear duplex DNA migrates slightly slower through the agarose gel than noncross-linked linear duplex DNA (lane s).

**FIG. 4.** Analysis by agarose gel electrophoresis of RecA-mediated strand exchanges between gapped circular duplex and linear duplex DNA substrates carrying deletions or insertions of 4 or 430 bp. Left, strand exchanges pass 4-bp deletions in DNA substrates to generated heteroduplex form II DNA with high efficiency. Reaction of gapped circular plasmid DNA (pACYC184) with homologous linear plasmid DNA (lanes a and b) or plasmid DNA carrying a 4-bp deletion (pSW44) (lanes c and d) in the absence (lanes a and c) or presence of RecA protein (lanes b and d). Center, a 430-bp deletion in the linear duplex blocks strand exchange. H-labeled gapped plasmid DNA (pACYC184) was reacted with EcoRI linearized 32P-labeled homologous plasmid DNA (lanes e–g) or DNA from a plasmid carrying a 430-bp deletion (pSW44) (lanes h to j) in the absence (lanes e and h) or presence (lanes f and i and j) of RecA protein. An ATP regeneration system was included in the reactions of lanes g and j. Right, a 430-bp deletion in the gapped duplex DNA blocks strand exchange. H-Labeled gapped plasmid carrying a 430-bp deletion (pSW44) was reacted with EcoRI-linearized 32P-labeled homologous plasmid DNA (lanes k–m) or DNA from plasmid pACYC184 DNA (lanes n–p) in the absence (lanes k and n) or presence (lanes l and m and o and p) of RecA protein. An ATP regeneration system was included in the reactions of lanes m and p. H-Labeled gapped plasmid DNA was reacted with 32P-labeled linear DNA cut with EcoRI. The gapped plasmid DNA was prepared by nicking with EcoRI in the presence of ethidium bromide, followed by treatment with exonuclease III as described under "Experimental Procedures." Reaction conditions and gel assay followed by autoradiography were described as under "Experimental Procedures."

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These results show that although RecA-mediated strand exchanges between two duplexes can pass insertions or deletions to produce four unpaired bases, we were unable to detect the product of exchanges through insertions or deletions of 430 bp by these methods.

To investigate the effects of deletions of intermediate size, we created a set of deletions in the phage M13mp8 as described under "Experimental Procedures." M13H-10, M13H-38, and M13H-120 carry deletions of 10, 38, and 120 bases, respectively, in comparison with M13H (Fig. 1). Since certain pairs of these phages (e.g. M13H-10, M13H-120) carry nonoverlapping deletions, we will refer to the number of unpaired bases expected in the resulting heteroduplex. Thus, the combination of M13H-10 and M13H-38 which overlap (Fig. 1) is expected to produce only 28 unpaired bases, while the combination of M13H-10 and M13H-120 would produce approximately 130 unpaired bases (Fig. 1).

32P-Labeled and nonradioactive form I DNA was prepared from each of the new phages. Circular duplex DNA with a single-stranded gap at the BamHI site (at position 2220, see Fig. 1, top left) was prepared from unlabeled M13H-10, M13H-38, and M13H-120 DNA, and linear duplex DNA was prepared from the 32P-labeled form I DNA by BamHI digestion. Reaction mixtures were set up in each of the four linear duplexes was paired with each of three gapped circular DNA preparations. After incubation for 90 min, the DNA was examined by gel electrophoresis and autoradiography.

In the homologous controls, labeled heteroduplex products were seen at positions close to that of form II DNA (Fig. 6, lanes d, n, and x), while in reactions with substrates that differed by 10, 28, or 38 bases slightly reduced yields were obtained (Fig. 6, lanes b, j, f, and l). Reactions with substrates that differed by 120, 130, or 158 bases showed no material at the position of form II (Fig. 6, lanes h, p, r, t, and v). In this case, an increased amount of slow moving material was seen in the position of joint molecules (11). Indeed, with the larger insertions and deletions, up to 60% (+15% range) of total label was observed as reaction intermediates. In addition, we again observed a broadening of the linear band. This is particularly evident in Fig. 6, lanes r, t, and v, where there is an additional peak moving just behind the linear species. Densitometer traces of these lanes (Fig. 7) show the additional peak of lanes r, t, v, where linear DNA is at position 1 and form II DNA is at position 2. These results indicate that the larger insertions blocked the formation of heteroduplex products. Instead, an increased proportion of the DNA was detected as reaction intermediates that were unable to complete strand exchange to form heteroduplex DNA.

FIG. 6. Strand exchanges pass deletions or insertions of 10-38 bp but fail to generate heteroduplex form II DNA carrying 110 or 120 unpaired bases. Analysis by agarose gel electrophoresis of RecA-mediated strand exchanges between gapped circular duplex and linear duplex DNA. Lanes a, c, e, g, i, k, m, o, q, s, u, and w are controls without recA. Complete reactions: the mismatch numbers in the figure are the size of the deletion in the circular gapped DNA minus that in the linear duplex. Left, reactions with gapped DNA from M13H-10 (10-bp deletion) and linear DNA carrying the following insertions: b, 0; d, 10; f, 38; h, 120. Center, reactions with gapped DNA from M13H-38 (38-bp deletion) and linear DNA carrying the following deletions: j, 0; l, 10; n, 38; p, 120. Right, reactions with gapped DNA from M13H-120 (10-bp deletion) and linear DNA carrying the following deletions: r, 0; t, 10; v, 38; x, 120.

FIG. 7. Densitometer traces of Fig. 6, lanes r, t, v, and x. This shows the absence of significant amounts of form II DNA and the double peak at the position of linear DNA in lanes r, t, and v. Comparable amounts of form II and linear DNA are seen in lane x. The mismatches are r, 120; t, 130; v, 158; and x, 0. The numbers f, 2, and 3 in lane x indicate the positions of linear duplexes, form II, and heteroduplex intermediates, respectively. To compensate for the differing amounts of radioactivity in the lanes and facilitate comparison, the vertical scale of lanes r and t is twice that of lanes v and x.

DISCUSSION

The results presented here show that RecA protein can drive strand exchanges between duplex DNA molecules even when each 4 kilobases of duplex DNA contains as many as 30 UV-induced pyrimidine dimers. In previous studies it was shown that strand exchange between single-stranded and duplex molecules was not diminished by UV photoproducts in the single-stranded DNA (5). Since pairing and strand exchange reactions appear to take place along the axis of RecA helical polymers (22-24), these results indicate that RecA filaments have sufficient space and flexibility to accommodate both duplexes, even when distorted by bulky lesions.

The in vitro reactions studied here may be analogous to certain in vivo reactions. The resistance of bacteria to UV light depends in part upon recombinational repair, a process in which sister chromosome exchanges are initiated at post-replication gaps formed opposite pyrimidine dimers during DNA synthesis (25, 26). The present results indicate that RecA protein should function effectively at levels of damage up to 10 dimers/kb (or 1000 J/m2 of 254 nm UV light), which are higher than those likely to be employed in experiments on living bacteria.

As expected, psoralen interstrand cross-links and associated monoadducts were effective in blocking strand exchange. This result supports current theories which suggest that the repair of cross-linked DNA requires incision by UvrABC
endonuclease prior to RecA-mediated strand exchange (27–29).

In reactions between homologous gapped circular and linear duplex DNA, heteroduplex form II DNA was produced in good yield (approximately 60% efficiency), indicating that full length strand exchanges had taken place. Substrates that differed by a deletion of less than 40 bp also generated form II heteroduplex products, albeit with a lower yield. However, when the deletion or insertion was 120 bp or more, we failed to observe the product of complete strand exchange. Instead, we observed an increased number of joint molecules and also detected a new band that had an electrophoretic mobility slightly slower than the linear substrates. Our interpretation of these data is that the strand exchange reaction was blocked by insertions or deletions of 120 bp such that heteroduplex intermediates were generated. Many of these intermediates were unstable following removal of RecA protein, and were free to undergo branch migration during electrophoresis back to the starting substrates. Another possibility is that strand exchange passes the deletion to form heteroduplex products with looped out single strands. If the (+) and (−) strand loops then pair locally in the RecA reaction mixture, the heteroduplexes may, after deproteinization, undergo spontaneous branch migration to the more stable homoduplex conformation. The present results do not allow us to distinguish between these and other possibilities.

Our results on the four-stranded reaction differ somewhat from those previously obtained with the three-stranded reaction (6). In the latter reaction when the insertion was placed in the single strand, significant yields of strand exchange product were observed even with insertions as large as 500 and 1300 nucleotides. On the other hand, when insertions of 500 bp were present in the duplex, the yield was reduced almost to background level (6). In the present experiments which measure strand exchanges between two duplex substrates, we have found that insertions or deletions of 120 bp block the recovery of the products of complete strand exchange.

Since RecA protein can generate heteroduplex DNA containing many pyrimidine dimers or up to 38 unpaired bases, we conclude that RecA does not require precise local homology between the duplexes at the time of exchanging strands.

Acknowledgment—We thank Kathy Collins for determining the nucleotide sequences at the deletions in the M13 substrates.

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