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Mismatch repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs

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ABSTRACT **Bacterial *mutS* and *mutL* mutations confer large increases in recombination between sequences that are divergent by several percent at the nucleotide level, an effect attributed to a role for products of these genes in control of recombination fidelity. Since MutS and MutL are proteins involved in the earliest steps of mismatch repair, including mismatch recognition by MutS, we have tested the possibility that they may affect strand exchange in response to occurrence of mispairs within the recombination heteroduplex. We show that MutS abolishes RecA-catalyzed strand transfer between fd and M13 bacteriophage DNAs, which vary by 3% at the nucleotide level, but is without effect on M13–M13 or fd–fd exchange. Although MutL alone has no effect on M13–fd heteroduplex formation, the protein dramatically enhances the inhibition of strand transfer mediated by MutS. Analysis of strand-transfer intermediates that accumulate in the presence of MutS and MutL indicates that the proteins block branch migration, presumably in response to occurrence of mispairs within newly formed heteroduplex.**

Methyl-directed mismatch repair ensures the fidelity of *Escherichia coli* chromosome replication by correcting DNA biosynthetic errors that escape nucleotide selection and proofreading functions of DNA polymerase III holoenzyme. Previous reports described a complex excision repair reaction that depends on 10 activities [MutH, MutL, MutS, DNA helicase II, single-strand binding protein (SSB), exonuclease I, exonuclease VII, RecJ exonuclease, DNA polymerase III holoenzyme, and DNA ligase] and that can account for function of mismatch repair in replication fidelity (1–3). In addition to this role in genome stabilization, components of the methyl-directed system have also been implicated in the process of homologous recombination, where they act to reduce recombinant yield when crossovers are selected in the vicinity of genetic differences (4–8). Similar effects on recombination outcomes have also been extensively documented for the related mismatch repair system of *Streptococcus pneumoniae* (9).

Analysis of recombination in *E. coli* and *Salmonella typhimurium* has demonstrated that the yield of crossovers depends greatly on the degree of homology within the region in which a crossover is selected. Reduction of homology from 100% to 80% or 90% reduces the yield of crossovers by several orders of magnitude (6, 10). This effect is in large part due to the action of mismatch repair activities, since recombination between such quasi-homologous (homeologous) sequences is increased dramatically in strains deficient in *mutL* or *mutS* function, although *mutH* or *mutU* mutations have little or no effect on recombinant yield when the sequences involved differ to this degree (6, 8). This antirecombination activity of MutS and MutL is the basis of the barrier to genetic recombination between *E. coli* and *S. typhimurium*, which are 20% divergent at the sequence level (6), and also controls the frequency of chromosomal rearrangements occurring in *E. coli* as a consequence of recombination between *rhs* repeat elements, which are about 1% divergent at the sequence level (8). Recombination between homeologous sequences scored in either of these systems is increased 1–2 orders of magnitude in *mutL* or *mutS* mutants.

In regular genetic crosses between isogenic genomes, where the only sources of mismatches are the genetic markers used to score recombinants, one can observe so-called marker effects, which depend on the activity of mismatch-repair systems, but appear only when the markers are close enough to be included in the heteroduplex joint region (11). An active MutHLSU mismatch repair system appears to decrease recombinant yield [i.e., *mut*⁻ mutants are hyper-Rec (4)] because of co-repair of mismatched genetic markers on the same strand (5).

Two mechanisms which are not mutually exclusive have been proposed to explain the effects of mismatch repair activities on the outcome of homeologous recombination events. One postulates early interruption, or abortion, of initiated heteroduplex intermediates (12), while the other postulates destruction of the recombination product by the mismatch repair system (7, 11). As an initial approach to study of the molecular basis of the effects of mismatch repair activities on recombination outcomes, we have examined the effects of MutS and MutL on RecA-catalyzed heteroduplex formation. These experiments are based on the finding of Radding and colleagues (13, 14) that RecA-promoted branch migration will proceed through regions of imperfect homology. We show that while MutS is without effect on RecA-catalyzed fd–fd or M13–M13 strand transfer, the protein inhibits strand exchange between fd and M13 phage DNAs, which differ by 3% at the nucleotide level, and this effect is potentiated by MutL.

MATERIALS AND METHODS

Proteins and DNA. RecA protein was purified from an overproducing strain as described (15) except that an exonuclease I-deficient strain [SK4642, F⁻ *lac301 argA*::Tn*10 leu307am trpE9829am rpsL321 supD* Δ(*sbcB–his*), kindly provided by S. Kushner, University of Georgia] was used as host for the overproducing plasmid. SSB (16), MutS (17), and MutL (18) were purified as described. Molar concentrations for these proteins are expressed as monomer equivalents. Endonucleases S1, *Hpa* I and *Acc* I were from United States Biochemical. Proteinase K was obtained from GIBCO/BRL.

M13 and fd phage DNAs were gifts from Robert Webster (Duke University, Durham, NC). Single-stranded DNA (ssDNA) and the covalently closed replicative form (RFI) of M13 and fd DNAs were prepared as described (19). Phage

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Abbreviations: SSB, single-strand binding protein; ssDNA, single-stranded DNA.

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identities were confirmed by partial sequence analysis using the dideoxy method. Uniformly ^{32}P- or ^{3}H-labeled RFI DNA was prepared according to Cunningham *et al.* (20). RFI DNA was linearized by cleavage with *Hpa* I followed by extraction with phenol and diethyl ether, and dialysis against 10 mM Tris·HCl, pH 8.0/1 mM EDTA.

Other Materials. Phosphocreatine and creatine kinase were purchased from Sigma. [^{32}P]Orthophosphate (8500–9120 Ci/mmol; 1 Ci = 37 GBq) and [*methyl*-^{3}H]thymidine (6.7 Ci/mmol) were obtained from NEN.

Strand-Exchange Assays. Reaction mixtures contained 0.6 nM single-stranded circular DNA, 4 μM RecA, 0.6 μM SSB, 50 mM Hepes·KOH (pH 7.5), 12 mM $MgCl_2$, 2 mM ATP, 0.4 mM dithiothreitol, 6 mM phosphocreatine, and creatine kinase at 10 units/ml. Reaction mixtures were preincubated at 37°C for 10 min and strand exchange was initiated by addition of linear duplex DNA to a final concentration of 0.6–0.9 nM. Samples (50 μl) were taken as indicated and strand transfer was terminated by addition of EDTA to 25 mM, SDS to 0.1%, and proteinase K to 150 μg/ml. After incubation at 42°C for 30 min, DNA was analyzed by electrophoresis through 0.8% agarose in 40 mM Tris acetate, pH 8.0/10 mM EDTA at 6 V/cm for 3 hr. Gels were analyzed by staining with ethidium bromide (0.5 μg/ml) and quantitated with a cooled, photometric grade charge-coupled-device imager (Photometrics, Tucson, AZ) or, in the case of ^{32}P-labeled molecules, by autoradiography and use of a Molecular Dynamics Phosphor- Imager.

Strand transfer in the presence of MutS and/or MutL was performed in the same manner, with MutS and MutL added 1 min prior to the addition of duplex DNA.

RESULTS

MutS, but Not MutL, Interferes with Homeologous Strand Exchange. In the presence of SSB and an ATP-regenerating system, RecA protein catalyzes extensive heteroduplex formation between fd and M13 DNAs (14), molecules that differ by about 3% at the sequence level. The protein can therefore support branch migration through regions of imperfect homology, in the case of these DNAs generating all eight base–base mismatches with the vast majority being single mispairs. We have exploited this system to test effects of MutS and MutL proteins on RecA-catalyzed strand transfer between homeologous sequences. The experiments described below score strand transfer between linear duplex DNA and circular single strands to yield an open circular, heteroduplex product (21, 22). To ensure efficient exchange between homeologous fd and M13 sequences, convalently closed, circular duplex fd or M13 DNA was linearized with *Hpa* I, which cleaves within a region in which the two DNAs are highly homologous. Based on the known directionality of strand transfer in this system (23) and the sequences of fd and M13 DNAs (24, 25), only one mismatch is expected to occur within the first 200 base pairs (bp) of heteroduplex.

Fig. 1 *Upper*, lane 1, illustrates the efficiency of formation of open circular heteroduplex formation when strand transfer was scored between linear duplex ^{32}P-labeled fd DNA and M13 single-stranded circles. The yield of this product after 70 min of incubation was about 60% of that observed in otherwise identical fd–fd strand transfer reactions, findings similar to those of Bianchi and Radding (14). Addition of increasing amounts of MutS reduced the amount of open circular product, with concomitant production of material with reduced electrophoretic mobility, much of which migrated as a discrete species just behind open circular molecules (Fig. 1). MutL by itself was without effect on the homeologous reaction, and neither MutS nor MutL had a detectable effect on homologous fd–fd strand transfer. Results identical to those obtained with duplex fd and M13 ssDNA (Fig. 1) were

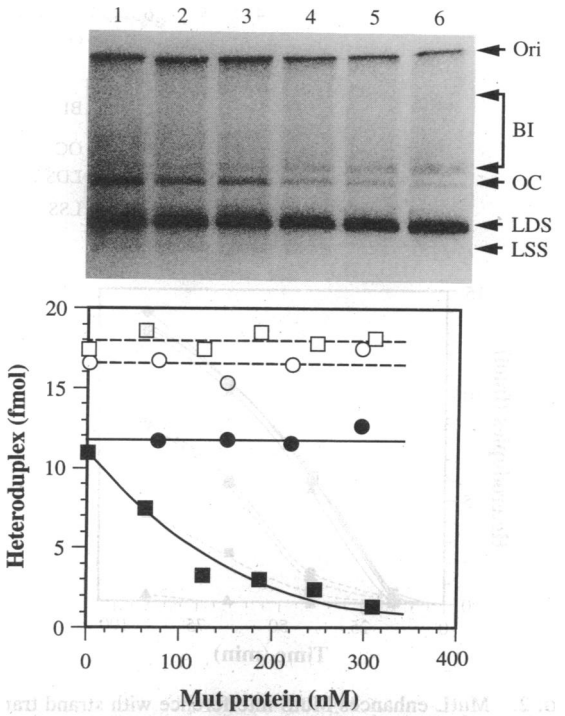

FIG. 1. MutS, but not MutL, interferes with RecA-catalyzed homeologous strand-transfer reactions. Strand-transfer reaction mixtures (50 μl; see *Materials and Methods*) were incubated for 70 min. (*Upper*) Strand exchange between linear duplex ^{32}P-labeled fd DNA and M13 ssDNA as a function of MutS concentration (lanes 1–6: 0, 61, 120, 190, 250, and 310 nM MutS). BI, OC, LDS, and LSS designate branched intermediates, open circular heteroduplex, linear double strands, and linear single strands, respectively. Ori, origin of electrophoresis. (*Lower*) Strand exchange between linear duplex ^{32}P-labeled fd DNA and M13 ssDNA (filled symbols) or ^{32}P-labeled fd DNA and fd ssDNA (open symbols) in presence of MutS (■, □) or MutL (●, ○).

observed when strand transfer was monitored between M13 duplexes and fd single strands (data not shown).

MutL Potentiates MutS Inhibition of Homeologous Strand Transfer. The failure of MutL to affect *in vitro* homeologous strand exchange is not surprising, since the protein adds to a heteroduplex subsequent to the binding of MutS at a mismatch (18). We therefore tested possible concerted action of MutL and MutS under conditions where MutS by itself only partially blocks strand transfer. At the concentration used in these experiments, MutS inhibited homeologous exchange by about 60%. MutL enhanced MutS inhibition of strand transfer between duplex M13 and fd ssDNA (Fig. 2 *Upper*, lanes 4–6) but was without effect on M13–M13 exchange (lanes 1–3). As observed when homeologous strand transfer was blocked by MutS alone (Fig. 1 *Upper*), inhibition of exchange by MutS and MutL was associated with production of material that migrated more slowly than open circular heteroduplex.

The cooperative effect of MutS and MutL in blocking M13–fd exchange is more dramatically illustrated by the kinetic experiment shown in Fig. 2 *Lower*. At a MutS concentration that reduced the rate of formation of homeologous, open circular heteroduplex by a factor of about 2, supplementation with MutL further reduced the rate by almost an order of magnitude. MutL alone had no effect on the rate of homeologous strand transfer, and neither MutS, MutL, nor the pair of proteins significantly altered the rate of the homologous control reaction.

MutS and MutL Block Branch Migration During Homeologous Strand Transfer. With the three-strand assay used here,

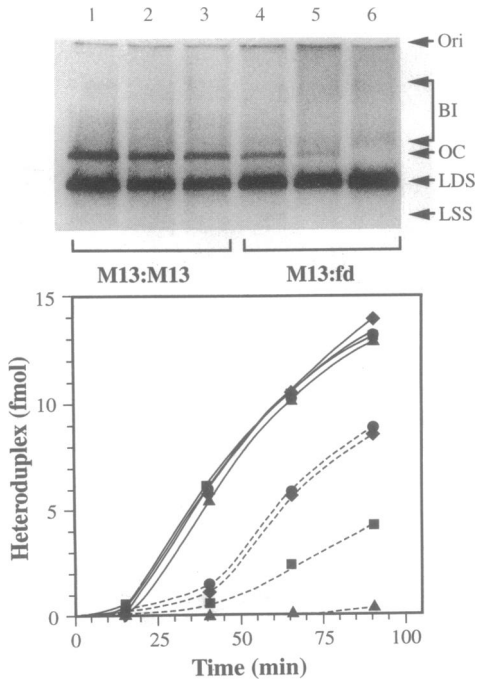

FIG. 2. MutL enhances MutS interference with strand transfer. Strand transfer reactions were carried out between linear duplex DNA and homologous or homeologous single strands. Reaction mixtures were supplemented with MutS (140 nM) and/or MutL (95 nM) as indicated. (*Upper*) Strand exchange between ^{32}P-labeled linear duplex M13 DNA and M13 (lanes 1–3) or fd (lanes 4–6) ssDNA in the presence of RecA and SSB alone (lanes 1 and 4) supplemented with MutS (lanes 2 and 5) or MutS and MutL (lanes 3 and 6). Incubation was for 70 min. Designation of DNA species is as in Fig. 1. (*Lower*) Reaction mixtures containing linear duplex fd DNA and fd or M13 single-strands (210 μl) were sampled (50 μl) and open circular heteroduplex was quantitated with a photometric grade charge-coupled-device camera after staining with ethidium bromide. ●, RecA only; ◆, plus MutL; ■, plus MutS; ▲, plus MutS and MutL; ——, fd–fd exchange; - - -, fd–M13 exchange.

the expected products are open circular heteroduplex and a single strand displaced from the linear duplex substrate (21, 22). The latter product can be observed in homeologous exchange reactions after prolonged incubation (Fig. 3, lane 1). Production of linear single strands in homeologous reactions was unaffected by the presence of MutL, but this product was not observed when both MutS and MutL were present (Fig. 3, lanes 2 and 3). The failure to observe displaced single strands was not due to degradation of the product by nucleases in the protein preparations used: <1%

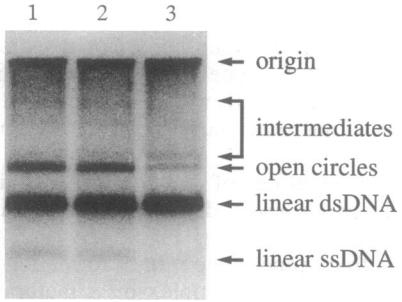

FIG. 3. The MutS–MutL couple prevents displacement of the leaving, linear single-stranded product in a homeologous exchange. Exchange reactions between ^{32}P-labeled linear duplex fd DNA and M13 ssDNA were performed in the absence of added Mut protein (lane 1), in the presence of MutL (95 nM, lane 2) or in the presence of both MutL and MutS (95 nM and 140 nM, respectively, lane 3). Incubation was for 90 min. dsDNA, double-stranded DNA.

of input radiolabeled linear duplex substrate was converted to an acid-soluble form in either homeologous or homologous strand-exchange reactions. Since linear single strands were generated more efficiently in the latter reactions, it is clear that such products were stable in the presence of all the proteins used.

These findings suggested that MutS and MutL may interfere with the branch-migration phase of the RecA reaction when mismatched base pairs occur within the heteroduplex. This idea is also consistent with the production of DNA species that migrated more slowly than open circular heteroduplex when homeologous strand transfer was performed in the presence of MutS, or MutS and MutL (Figs. 1–3). Material with reduced electrophoretic mobility has been previously observed in RecA-mediated reactions and has been attributed to intermediates in the strand-transfer reac-

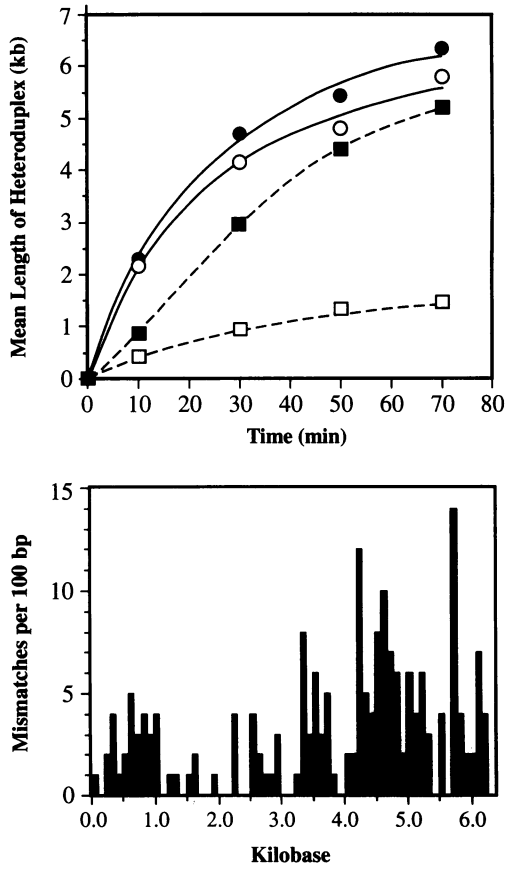

FIG. 4. MutS and MutL limit RecA-catalyzed branch migration in response to mismatched base pairs as judged by the mean length of heteroduplex formation. (*Upper*) Strand exchange between 0.6 nM ^{3}H-labeled M13 linear duplex DNA and M13 (●, ○) or fd (■, □) ssDNA was performed in 510-μl reaction mixtures in the presence (open symbols) or absence (closed symbols) of 140 nM MutS and 95 nM MutL. Samples (100 μl) were taken at indicated times, quenched with SDS (0.8%), and extracted with an equal volume of phenol/chloroform/isoamyl alcohol (24:24:1, vol/vol) equilibrated with 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA. The organic phase was back-extracted with 0.5 volume of 50 mM Hepes (pH 5.5) and the aqueous layers were combined, washed with water-saturated diethyl ether, and relieved of residual organic solvent by incubation for 30 min at 37°C. The mean length of stable heteroduplex was then determined by S1 nuclease (10 units/ml) assay according to Cox and Lehman (22). In this procedure, stable joint molecules are scored by virtue of the ssDNA-dependent retention of ^{3}H-labeled linear duplexes on nitrocellulose membranes, with S1-sensitive ^{3}H label corresponding to the extent of strand exchange. Heteroduplex resulting from complete strand transfer is 6.4 kb long. (*Lower*) Expected distribution of mismatches in M13–fd hybrid DNA when strand exchange initiates at the *Hpa* I site (24, 25).

tion (26). The possibility that MutS and MutL might interfere with branch migration during homeologous strand transfer was confirmed by using S1 nuclease assay to assess the mean length of heteroduplex formation in those molecules that had undergone synapsis and initiated strand exchange (22). Full-length heteroduplex (6.4 kb) was produced in M13–M13 exchange reactions, and the presence of MutS and MutL had little effect on the kinetics of branch migration with perfectly homologous substrates (Fig. 4). As previously observed by Bianchi and Radding (14), branch migration occurred more slowly with homeologous M13 and fd DNAs, but the extent of heteroduplex formation after prolonged incubation was nearly equivalent to that observed in the homologous reaction. In this case, however, the presence of MutS and MutL dramatically reduced both the rate and extent of heteroduplex formation, implying that these proteins interfere with the branch-migration step of the RecA-catalyzed exchange reaction. Addition of MutS and MutL to homeologous strand-exchange reactions that had gone to completion did not alter the amount of previously formed heteroduplex (data not shown). The two proteins therefore interfere with the process of branch migration but have little, if any, effect on preformed heteroduplex.

DISCUSSION

The results described here show that MutS and MutL proteins inhibit the branch-migration stage of RecA-catalyzed strand transfer when mismatched base pairs occur during the course of heteroduplex formation. When viewed in the context of the RecA mechanism, these observations are surprising. The active species in strand transfer is a nucleoprotein filament formed by directional polymerization of the protein onto a single strand (27). Subsequent to synapsis with a homologous sequence, strand exchange commences from a free end, with the heteroduplex product retained in the RecA filament (28). We therefore assume that MutS and MutL access mispairs that occur within this structure. Since MutS exists in solution as dimers and tetramers of a 97-kDa subunit and MutL is a dimer of 70-kDa subunits (17, 18), the mode of access could be complex and remains to be clarified, as does the mechanism by which heteroduplex binding by MutS and MutL leads to termination of branch migration.

Although we have not examined the effect of MutS and MutL on strand transfer between two DNAs that differ by only one base pair, the experiment shown in Fig. 4 suggests that a more significant degree of divergence is necessary for the proteins to effectively block branch migration. While the rate and extent of homeologous heteroduplex formation is reduced in the presence of the Mut proteins, branch migration proceeds to about 1 kb in the 70-min period examined to yield heteroduplex containing about 25 single base–base mispairs. These findings are in accord with genetic experiments that have documented the influence of *mutS* and *mutL* gene products on recombinant yield. Large effects have been observed only in those cases where homology differences ranged from 1% to 20% at the nucleotide level (6–8).

Smaller effects of *mutS* and *mutL* mutations on recombinant yield have been demonstrated in intragenic crosses involving near-perfect homology (4), but in this case *mutH* and *mutU* have also been implicated, suggesting that reduction in recombinant yield might involve heteroduplex processing by conventional methyl-directed excision (3). The limited effect of *mutH* and *mutU* mutations on recombination between diverged sequences (6, 8) may indicate that heteroduplex regions containing a high density of mispairs, and MutS–MutL complexes within such regions, have an alternative fate. Since MutS and MutL block branch migration but do not destabilize a previously formed homeologous heteroduplex, it seems likely that branch-migration intermediates that have been trapped by MutS and MutL are subject to some sort of disassembly process. Although we have not addressed the fate of this species, the genetic experiments alluded to above suggest that MutH and DNA helicase II (*mutU* product) do not have a major role in processing such structures.

Several laboratories have recently identified activities that enhance branch migration in heteroduplexes initiated by the action of RecA. RuvA and RuvB enhance the rate of strand exchange mediated by RecA (29), whereas RecG inhibits this reaction (30). Inhibition of exchange in the latter case has been attributed to RecG-facilitated directional branch migration that occurs with a polarity opposite to that promoted by RecA (30). This mechanism for resolution of recombination intermediates could provide a simple means for disassembly of homeologous strand-exchange intermediates that have been trapped by MutS and MutL. We have not tested the effects of MutS and MutL on homeologous strand exchange mediated by RecA in the presence of RuvA and RuvB proteins. RuvA and RuvB facilitate branch migration in strand-exchange intermediates initiated by RecA (29, 30), although it is not clear how much RecA-dependent heteroduplex formation occurs prior to initiation of Ruv-mediated branch migration.

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