Escherichia coli MutS,L Modulate RuvAB-dependent Branch Migration between Diverged DNA*

This study examines the interaction between Escherichia coli MutS,L and E. coli RuvAB during E. coli RecA-promoted strand exchange. RuvAB is a branch migration complex that stimulates heterologous strand exchange. Previous studies indicate that RuvAB increases the rate at which heteroduplex products are formed by RecA, that RuvA and RuvB are required for this stimulation, and that RuvAB does not stimulate homologous strand exchange. This study indicates that MutS,L inhibit the formation of full-length heteroduplex DNA between M13-fd DNA in the presence of RuvAB, such that less than 2% of the linear substrate is converted to product. Inhibition depends on the time at which MutS,L are added to the reaction and is strongest when MutS,L are added during initiation. The kinetics of the strand exchange reaction suggest that MutS,L directly inhibit RuvAB-dependent branch migration in the absence of RecA. The inhibition requires the formation of base-base mismatches and ATP utilization; no effect on RuvAB-promoted strand exchange is seen if an ATP-deficient mutant of MutS (MutS501) is included in the reaction instead of wild-type MutS. These results are consistent with a role for MutS,L in maintaining genomic stability and replication fidelity.

DNA mismatch repair (MR) contributes to genetic stability by recognizing and correcting errors made during DNA synthesis and by controlling recombination between closely related sequences (1–6). In Escherichia coli, the methyl-directed or “long patch” repair system has been characterized extensively (7, 8), and the models developed for the E. coli MR pathway provide a paradigm for examining and understanding MR in higher organisms. The role of MR in recombination, however, remains unclear. Studies in bacteria (1, 5, 6, 9–11) Saccharomyces cerevisiae (3, 12–18), and higher eukaryotes (19–21) have shown that sequence divergence acts as a potential barrier to recombination and that this barrier is enhanced by the action of MR proteins. Specifically, in conjugational crosses between E. coli and Salmonella typhimurium, whose sequences are 80% identical, mutations in the E. coli MR genes mutH, mutL, mutS, and mutU increase recombination rates 50- to 3000-fold (1). In E. coli, the antirecombinational activity of MutS and MutL modulates recombination between diverged sequences (9) and between large chromosomal duplications that differ by as little as 1% at the nucleotide level (1, 22, 23).

The antirecombinational activity associated with key components of MR presumably stems from their ability to recognize and bind mismatched base pairs in newly formed heteroduplex DNA. However, the precise mechanism by which MutS and MutL modulate homologous recombination has not been defined. In principle, the following potential roles for MR are envisioned in this process. (1) Functioning as a mismatch-sensing system, MutS,L modulate the extent of exchange by testing for the presence of mismatched base pairs during DNA exchange. In addition, or alternatively, (2) mimicking replication fidelity, MR signals downstream components and ultimately resolves/aborts newly formed heteroduplex DNA in response to the presence of mismatches. Worth et al. (24) have shown that MutS inhibits the RecA-catalyzed in vitro three-strand transfer reaction between M13 and fd DNA, whose sequences are diverged by 3%. MutL enhances the MutS-dependent inhibition of strand exchange. That MutL was without effect in the absence of MutS suggests that the role of MutL in homologous recombination is similar in nature to its role in replication error repair. MutS and MutL have no effect on homologous strand exchange using M13-M13 (or fd-fd) DNA, indicating that MutS,L-dependent inhibition of strand exchange requires the presence of base-base mismpairs in newly formed heteroduplex DNA. In support of a mechanism in which recombination is regulated by MR, it has been demonstrated that the enhanced block by MutL is coupled to MutS and ATP (25).

Escherichia coli RuvA and RuvB proteins are involved in repair of UV damage and form a two-component multimer that facilitates branch migration through regions of heterology during later stages of recombination (26, 27). Indeed, genetic studies suggest that the ruvA and ruvB gene products are largely responsible for bypass of DNA heterology during conjugative recombination or interspecies DNA exchange (28, 29). Therefore, it is highly probable that the role of RecA is somewhat diminished during this type of recombination, especially when sequence divergence is encountered.

Biochemical studies have shown that RuvAB binds specifically to three- and four-stranded junctions, promotes DNA exchange, bypasses regions of DNA heterology, and when divergence is high, actually reverses the polarity of branch migration in vitro (27, 30). This latter observation leads to the idea that RuvAB could act as an anti-recombinase when DNA heterology is encountered.

Studies by Bianchi and Radding (31) show that RecA promotes branch migration through regions of imperfect homology (Fig. 1); however, as little as 3% heterology between DNA substrates interferes with the efficiency of strand exchange in vitro (24, 31). Larger heterologies (i.e. 200 bp and up) are not

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1 The abbreviations used are: MR, mismatch repair; bp, base pairs; SSB, single-strand binding protein.

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Mismatch Repair and RuvAB Complex

**Fig. 1.** Strand exchange reaction between M13 and fd DNA promoted by RecA. A three-strand exchange reaction between circular single-stranded fd DNA and linear duplex M13 DNA produces open circular heteroduplex DNA containing mismatches and a displaced single-stranded fd DNA and linear duplex M13 DNA produces open circular heteroduplex DNA containing mismatches and a displaced single-strand. Two forms of branched intermediates are shown.

bypassed by RecA alone but require RuvAB in addition to RecA. This result is consistent with the suggestion that RuvAB plays a role in recombination, specifically after strand invasion (26). The goal of this work is to examine the influence of RuvAB on M13-fd strand exchange initiated by RecA in the absence and presence of MutS,L. The results show that RuvAB enhances the rate of formation of open circular heteroduplex DNA between M13-fd DNA by 2- to 3-fold. Addition of MutS,L to the reaction abrogates the ability of RuvAB to enhance the rate of RecA-catalyzed product formation. Because M13-M13 exchange is not affected by the presence of RuvAB, it is likely that this block is a mismatch-dependent event. Sequence divergence inhibits branch migration in the presence of MutS,L, suggesting that the MR proteins recognize heterology and destabilize the RecA-DNA and/or RuvAB-DNA complexes on heterologous DNA.

**Experimental Procedures**

**Materials**

*E. coli* proteins MutL and 6x-His-tagged wild-type MutS and MutS501 were purified as described previously (24, 25). Recombinant nontagged wild-type MutS was constructed by inserting the *BanHI-HindIII* fragment from pLW10 into the *BanHI-HindIII* site of pQE51 expression vector (Qiagen) to yield pLW40. M15pREP4 cells transformed with pLW40 were grown in 6° Luria Broth to an *A*$_{600}$ of 0.6, chilled, and induced as described previously (26). MutS protein was purified as previously described (24). RecA and RuvB proteins were purified as described (26, 33) from *E. coli* strain BL21 (DE3) bearing plasmids that overexpress RecA (pEA106) and RuvB (pEA112; a gift from L. V. Lane). Protein concentrations were determined by the method of Bradford (34) with bovine serum albumin as standard (Bio-Rad assay kit). Protein concentrations are expressed in moles of monomer. SSB protein was purchased from Amersham Pharmacia Biotech. Restriction endonuclease SstBI and T4 polynucleotide kinase were from New England Biolabs, Inc. RecA protein, proteinase K, phosphocreatine kinase, and phosphocreatine were purchased from Sigma.

**Other Materials**

Single-stranded circular M13 and fd bacteriophage DNA was prepared as described (26). Replicative forms of M13 and fd bacteriophage DNA were linearized with *SnaBI* restriction endonuclease. For experiments with branched intermediates, linear DNA (50 pmol of 5'-ends) was 5'-end-labeled with γ-[^32]P-ATP using T4 polynucleotide kinase and purified as previously described (24).

**Methods**

**Isolation and Purification of E. coli RuvA, RuvB, and Wild-type MutS—**RuvA, RuvB, and MutS were purified to greater than 95% homogeneity (Fig. 2) and characterized (26, 32, 33). The DNA binding activity was examined for all purified proteins. The ATPase activities of RuvA and MutS were also characterized as described (26, 25).

**Standard Strand Exchange Reaction—**A standard reaction was carried out in a total volume of 60 μl containing 50 mM HEPES-KOH (pH 7.5), 12 mM MgCl$_2$, 0.5 mM dithiothreitol, 3 mM ATP, 6 mM phosphocreatine, 140 units/ml creatine phosphokinase, 10.6 μM single-stranded circular fd DNA (M13), 3.5 μM RecA, 8.4 μM linear double-stranded M13 DNA (fd), 0.4 μM SSB protein, and 100 μg/ml bovine serum albumin. Preincubation of RecA and single-stranded circular DNA was for 10 min at 37 °C followed by linear duplex DNA and an additional 10 min at this same temperature. Reactions were initiated by the addition of SSB and ATP. Aliquots (14.5 μl) were removed at indicated times, quenched with a final concentration of 0.1% SDS, 25 mM EDTA (pH 8.0), and 153 μg/ml proteinase K, and incubated for 15 min at 37 °C. After deproteinization, 2 μl of load buffer (20% Ficoll 400, 0.1 mM EDTA, 1.0% SDS, 0.25% bromphenol blue, and 0.25% xylene cyanol) was added, and samples were subjected to electrophoresis on a 8% agarose gel for 18 h at 2 V/cm. DNA was visualized with ethidium bromide, photographed using the FOTO/Analyst documentation system from Fotodyne, and quantified by NIH Image 1.61.

**Strand Exchange with RuvAB—**Reactions in the presence of RuvA (60 nm) and RuvB (200 nm) were performed in a similar manner. Initially, a time course of 0, 15, 30, and 45 min was performed to examine the effect of RuvAB on both M13-M13 and M13-fd incubations. RuvAB inhibited the reaction when added before branched intermediates could form (data not shown); therefore, RuvAB was added at 10–15 min (M13-M13) or 35–40 min (M13-fd) after ATP was added to initiate the reaction. Branched intermediate formation was relatively steady during these times. Controls were supplemented with RuvA and RuvB diluted buffer (20 mM Tris-HCl (pH 7.6), 1 mM EDTA, 0.5 mM dithiothreitol, 150 mM NaCl, 100 μg/ml bovine serum albumin, 10% glycerol).

**Strand Exchange with MutSL—**Reactions were carried out as described previously, except that MutS (90 nm) and MutL (90 nm) were added just prior to SSB and ATP. Control reactions were supplemented with MutS and MutL dilution buffers (20 mM potassium phosphate (pH 7.5)) and 50 mM HEPES, 12 mM MgCl$_2$, 100 mM NaCl, 0.5 mM dithiothreitol, and 150 units/ml creatine phosphokinase.

**Fig. 2.** Expression and purification of MutS, RuvA, and RuvB proteins. 10% SDS-polyacrylamide gel stain with Coomassie Brilliant Blue. Lanes 1, 3, and 5, lysates after induction from RuvA, RuvB, and wild-type MutS, respectively. Lanes 2, 4, and 6 are purified RuvA, RuvB, and MutS (2 μg) proteins, respectively. *M*, molecular mass standards.
subject to the energetic barrier associated with heterologous strand exchange between M13 and fd DNA (24, 31). Thus, it is expected that RuvAB does not affect M13-M13 exchange (Fig. 4). The rate at which RecA forms open circular homoduplex DNA is essentially unchanged by addition of RuvAB (Fig. 4A, compare left and right top panels). Approximately 50% of the linear DNA was converted to branched intermediates, and ~95% of the intermediates were converted to product after 60 min, indicating that there is little energetic barrier to DNA exchange (Fig. 4A). This corroborates the findings of Lype et al. (26); however, in those experiments the ratio of RuvAB to RecA and ssDNA was ~2.5-fold higher than in the experiments described here.

Fig. 4B shows that E. coli MutS,L do not block RecA-promoted strand exchange between homologous DNA, and the kinetic profile for formation of branched intermediates and open circular homoduplex DNA is similar in both the presence and absence of MutS,L (Fig. 4B and 5B). The addition of RuvAB to RecA reactions with MutS,L had no effect on strand exchange between M13-M13 DNA (Fig. 4B, lower panels).

Strand Exchange between Heterologous DNA in the Presence of RuvAB—RecA-promoted heterologous strand exchange between M13-fd was also carried out in the presence and absence of RuvAB (Fig. 5A). Two reaction intermediates (intermediate I and intermediate II) were detected ~15–40 min after initiation of the reaction. A third, less abundant intermediate was not detected until after 40 min (intermediate III). The kinetic profile of intermediates I and II is shown in Fig. 5A (left lower panel). The amount of intermediates I and II is similar until the reaction reaches ~42 min. Intermediate I is constant from 42–55 min and only begins to decline during the last 10 min of the reaction. During this same time intermediate II accumulates rapidly and remains constant over the final 10 min. Intermediate III also accumulates late in the strand exchange process (50–65 min). However, less intermediate III forms than does intermediate II, and the levels of intermediate III coincide closely with the formation of full-length open circular product.

The kinetics of strand exchange differ during homologous and heterologous strand exchange with M13-M13 or M13-fd DNA substrates. During homologous strand exchange, a small amount of intermediate III is observed after formation of open circular homoduplex DNA (i.e., later than 30 min). Most of intermediate I is resolved prior to the formation of product. With heterologous M13-fd substrates, product begins to accumulate before the level of intermediate I begins to decline. This result is consistent with the idea that sequence divergence acts as a barrier to strand exchange. Thus, the accumulation of intermediates I and II may be due to stalling of the branch point (11, 24, 25), and intermediate III might form during heterologous strand exchange due to secondary loading of RecA onto a linear duplex.

As indicated above (Fig. 3), the greatest fraction of branched intermediates between M13-fd occur after 40 min in strand exchange reactions with RecA. Consequently, RuvaB was added after 42 min, and incubations were allowed to continue an additional 23 min. As shown in Fig. 5, open circular heteroduplex product is detected ~2 min after addition of RuvaB, and a similar amount or product forms after 10 min in the absence of RuvaB. Thus, as shown previously, RuvaB stimulates the rate of heterologous strand exchange by RecA. In addition, Fig. 5 shows that the distribution of reaction intermediates is altered in the reaction to which RuvaB is added after 42 min. Intermediate II is depleted within 20 min after RuvaB is added to the reaction (Fig. 5A). During this same time intermediate I
remains at a constant level. Thus, in the presence of RuvAB, the relative amount of intermediate I increases, the relative amount of intermediate II decreases ~3-fold, and the rate of product formation is accelerated. However, RuvAB does not increase the amount of product formed by RecA during M13-fd strand exchange. In fact, RuvAB limits uptake of substrate, because more than 50% of the linear duplex remains after incubation for 65 min.

MutS,L Modulate the Ability of RuvAB to Promote Branch
FIG. 6. Addition of MutS,L to M13-fd strand exchange at different time points. Reaction conditions were as in Fig. 5 except that MutS,L were added at different times. The RuvAB complex was added after 42 min. Aliquots were removed at the indicated times and quenched as described. Kinetic profiles of open circular heteroduplex formation are shown in the absence of MutS,L (—- —) or MutS,L and RuvAB (—- —- ) or in the presence of MutS,L after 0, 40, 47, or 55 min (—- —) as illustrated. The second 40-min profile (—- —) is an incubation with twice the amount of MutS (180 nM) and MutL (180 nM).

Migration between M13 and fd DNA—MutS,L inhibit RecA-promoted formation of open circular heteroduplex DNA between M13 and fd DNA, but MutS,L do not inhibit homologous strand exchange (Fig. 4B and 5B) (25). The majority of the branched molecules are intermediate I, which accumulates slightly between 40–65 min; a small amount of intermediate II is produced after 65 min.

In the presence of RuvAB and MutS,L, formation of open circular heteroduplex DNA is almost completely abolished; <2% product is generated after 65 min, and the reaction kinetics are similar to a reaction with RecA and MutS,L. The relatively low level of open circular heteroduplex DNA probably reflects the small amount of intermediate II present when RuvAB was added to the reaction. Intermediate I was the only species present when RuvAB was added and is the only substrate for RuvAB. It is likely that intermediate I forms when RecA encounters DNA heterology in the presence of MutS,L, and intermediate I may persist because of an energetic barrier to strand exchange. Thus, the amount of intermediate II is reduced in the presence of MutS,L, and RuvAB has to compete with MutS,L to bind the branched molecule.

Addition of MutS,L to RecA and RuvAB-dependent Strand Exchange Reactions at Different Time Points—To better understand the interaction between MutS,L, RecA, and RuvAB, the order and time of addition of these activities to heterologous strand exchange reactions were varied. Fig. 6 shows the kinetics of strand exchange when MutS,L were added at different time points. The level of inhibition by MutS,L is reduced almost 4-fold when these activities are added 40 min after initiation, as compared with reactions when MutS,L are present throughout the incubation. This is consistent with previous studies (24) and is consistent with the notion that the number of mismatched base pairs increases as heterologous strand exchange proceeds, such that the capacity of MutS,L to bind mismatches could be saturated as heteroduplex product accumulates. If this explanation is true, then it is expected that a higher amount of MutS,L could block strand exchange when added in the later phase of the reaction. As shown in Fig. 6, a 2-fold higher concentration of MutS,L blocks strand exchange when added after a 40-min reaction.

FIG. 7. Effect of MutS,L on RuvAB-promoted branch migration. Deproteinated recombination intermediates were used as substrates under standard reaction conditions, except that RecA was omitted (see “Experimental Procedures”). Upper panel, aliquots (15 μl) were incubated with either RuvAB only (60 nM RuvA and 200 nM RuvB) (lanes 2 and 5) or RuvAB and MutS,L (20 nM MutS and 20 nM MutL) (lanes 3 and 6). Control incubations without the RuvAB complex were also included (lanes 1 and 4). The final DNA concentration was 2 μM. All reactions were incubated for 20 min at 37 °C, quenched, deproteinized, and analyzed as described. Lower panel, relative comparison of the distribution of open circular DNA generated by RuvAB between M13-M13 DNA (unshaded; lanes 1–3) or M13-fd DNA (shaded; lanes 4–6).

MutS,L Inhibit the Action of RuvAB on Branched Intermediates Generated by RecA between M13-fd DNA—The strand exchange reactions described above were carried out in the presence of RecA. Because RecA might influence the way in which RuvAB and MutS,L interact with strand exchange intermediates and each other, it was of interest to carry out reactions with RuvAB and/or MutS,L using purified strand exchange intermediates. M13-fd and M13-M13 branched intermediates were deproteinized, isolated from strand exchange reactions (36), and used as substrates for RuvAB in the presence and absence of MutS,L. As shown in Fig. 7, RuvAB promotes branch migration of protein-free intermediates between M13-fd and M13-M13 DNA. Branched intermediates I and II are rapidly converted to open circular DNA, and most of the product forms after 10 min (data not shown).

MutS,L were added to reactions with RuvAB and purified branched intermediates, and the results are also shown in Fig. 7. As expected, MutS,L did not inhibit M13-M13 exchange. However, RuvAB-dependent branch migration between M13-fd DNA was inhibited 3–4-fold by MutS,L. Upon closer inspection, it appears that intermediate I, unlike intermediates II and III, was resolved less efficiently in the presence of MutS,L. Indeed, intermediates I–III were completely consumed in the control. In the presence of MutS,L, RuvAB processed all of intermediates II and III, with an increase in product yield of 1.5-fold over background. This relatively low level bypass corroborated the results obtained with RecA, establishing low but consistently detectable open circular heteroduplex DNA in the presence of MR. Nonetheless, that MutS,L still limited RuvAB action on intermediates of M13-fd exchange supports the notion that MutS,L inhibit strand exchange when these enzymes recognize mismatched bases in the growing heteroduplex.

MutS501,L Do Not Inhibit Strand Exchange between M13 and fd DNA in the Presence of RuvAB—The effects of ATP and
the MutS ATPase on RuvAB-promoted strand exchange were also examined. Previously, it was shown that ATPase-defective MutS mutants have altered in vitro function. One such mutant is \textit{mutS}501, which is a dominant negative \textit{mutS} allele with a changed amino acid in the p-loop motif (25, 39). Studies of these mutants show that mismatch recognition and ATP binding and/or hydrolysis contribute to the ability of MutS to block RecA-catalyzed strand exchange between M13-fd DNA. MutS501 has an altered ATPase but binds mismatches with specificity and remains bound to a mismatch in the presence of ATP (25). As shown in Fig. 8 and in previous studies (25), MutS501,L blocks RecA-catalyzed M13-fd exchange, such that no open circular heteroduplex DNA is detected after 65 min. This block by MutS501 does not require the presence of MutL (data not shown) (25), which suggests that mismatch recognition alone contributed to this effect. It is noted that in this work, MutS501,L completely blocks RecA-mediated heterologous exchange; however, this was not reported previously (25). This discrepancy is attributed to a lower RecA to ssDNA ratio, which in the experiments described here was down 2-fold.

When MutS501 or wild-type MutS is added to RecA-promoted heterologous strand exchange, intermediates I and II are generated to a similar extent. However, addition of RuvAB to a reaction with MutS501,L stimulates the reaction, and open circular heteroduplex DNA appears within 4 min after RuvAB is added (Fig. 8). The kinetics and amount of product (\approx 2.5 fmol) were similar to the reaction with only RuvAB. Interestingly, only intermediate I was detected during this period. The amount of product is almost 10-fold higher in the presence of MutS501,L than in the presence of MutS,L. Thus, whereas MutS501,L blocks RecA-mediated strand exchange between M13-fd DNA, RuvAB-dependent branch migration can abrogate this effect. This result suggests that ATP binding and/or hydrolysis by MutS is essential in this block of RuvAB-promoted branch migration through regions of heterology.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig8.png}
\caption{Strand exchange between M13-fd in the presence of MutS501. Reaction conditions were identical to those in Fig. 5 except with MutS501. Aliquots were removed at the indicated times and quenched as described. Reactions in the presence of (left) RuvAB and (right) MutS,L and RuvAB are shown. Upper panels, protein additions were as in Fig. 4. Lower panels, kinetics of M13-fd strand exchange in the absence (left) or presence (right) of RuvAB. Open circular heteroduplex DNA (\textbullet{}), branched intermediates I (\circ{}), II (\bigcirc{}), and double-stranded linear duplexes (\textcircled{}), were quantitated as described.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig9.png}
\caption{Model for the role of mismatch repair in homeologous strand pairing. Bold lines illustrate regions of sequence divergence (>3%). Open triangles are newly formed mismatches in heteroduplex DNA. Two modes are shown for heterologous block by MR: 1) destabilization of RecA monomers due to mismatch recognition by MutS and 2) ATP-dependent DNA translocation by the MutS,L complex and destabilization of the RuvAB complex at the branch point.}
\end{figure}

\section*{DISCUSSION}

This report examines the interaction between \textit{E. coli} MutS,L and RuvAB during in vitro strand exchange. Previous studies have shown that MutS,L inhibit RecA-catalyzed strand exchange between slightly diverged DNA (24, 25). RuvAB acts in late stages of homologous recombination and will facilitate bypass of DNA heterology in the presence of RecA (30, 40). This study demonstrates for the first time that MutS,L prevent RuvAB from stimulating RecA-promoted branch migration through regions of sequence heterology (Fig. 5, A and B). The results suggest that MutS,L may act at an earlier step in strand exchange than RuvAB. This study also shows that MutS501,L cannot prevent RuvAB from stimulating RecA-promoted heterologous strand exchange (Fig. 8), suggesting that ATP binding and/or hydrolysis plays an intricate role in how wild-type MutS,L control homologous exchange.

MR contributes to genome stability, in part by regulating the amount of sequence divergence that can be tolerated during homologous strand exchange. Thus, components of MR may play a vital role during early stages of strand pairing and recombination (24). A model for the role of MR during homologous exchange is presented in Fig. 9. This model is supported by studies on the three-strand exchange reaction catalyzed by RecA that is inhibited by MutS,L when heterologous substrates are used. In addition, as shown here, MutS,L also prevent RuvAB-dependent stimulation of branch migration. Unlike RecA, however, this block was dynamic and required
that MutS utilizes ATP. Thus, if MutS501, an ATPase-defective mutant, is substituted for wild-type MutS, RuvAB can still stimulate heterologous strand exchange. This suggests that nucleotide binding and/or hydrolysis is important to the mechanism that inhibits branch migration by RuvAB. However, because MutS501,L does inhibit branch migration by RecA (in the absence of RuvAB), it is likely that mismatch recognition per se is inhibitory to RecA and that MutS senses subtle perturbations within the RecA filament caused by mismatched base pairs.

Previous studies have shown that MutS501 binds mismatches but does not activate MutH-dependent incision at a hemimethylated GATC site (25). MutS is thought to undergo an ATP-dependent conformational change after mismatch recognition, leading to its dissociation from the mispair (41, 42). ATP hydrolysis by MutS may facilitate communication between the mismatch and a hemimethylated GATC site up to 1 kilobase away (32, 43–45). Several models have been proposed for this communication process. One model, initially proposed by Modrich and Lahue (7), involves bidirectional DNA translocation that forms α-shaped loops. Allen et al. (32) showed that loop formation is rapid and measured translational rates from 160 bp/s to ~300 bp/s, when MutL is present (43–45). Another model proposes ATP-dependent DNA sliding and a conformational switch that allows MutS to travel along the contour of the DNA helix away from the mismatch. These models differ in how the signal is communicated, but they are similar in suggesting that MutS assumes a nonmismatch-specific DNA binding mode that allows transmission of the signal from the mismatch to a distal site on the DNA. RecA and RuvAB promote branch migration at a slower rate than MutS (∼2–10 bp/s to 10–20 bp/s (46, 47)); thus, translational choice by the MutS,L complex might interfere with branch migration by destabilizing the RecA-DNA or RuvAB-DNA complex. The exact mechanism of the MutS,L block of DNA exchange is still poorly defined and requires further study.

Two models have been proposed to define the mechanism by which RecA utilizes ATP to drive branch migration through regions of nonhomology. Kowalczykowski and colleagues (49) suggest that RecA-mediated strand exchange is less efficient when there are discontinuities in the interior of the filament. They propose that the energy from ATP hydrolysis promotes redistribution of RecA monomers so that gaps and discontinuities in the filament are removed. A second model suggests that ATP hydrolysis is coupled to facilitated rotation of the DNA substrate (48). In this case monomer exchange is not necessary. It is possible to propose a mechanism for the MutS,L inhibition of heterologous strand exchange that would be consistent with either of these two models. The simplest concept is that MutS,L block strand exchange because MutS recognizes and binds mismatches in regions of sequence heterology. This mismatch recognition process would occur (a) during RecA monomer redistribution (i.e., facilitated by ATP hydrolysis) or (b) at branch points in the three-strand reaction, where MutS,L might compete with RuvAB (Fig. 9). It is proposed that MutS,L are recruited by RecA through protein-protein interactions, or this process is dynamic in nature, and MutS,L compete for mismatch binding when heterology is encountered. It has been reported that RuvAB directly displaces RecA monomers from supercoiled DNA (35). Similarly, MutS could recognize mismatches at or near the branch point in the three-strand reaction and thereby destabilize the filament or prevent RuvAB from loading.

The results presented here are not completely in agreement with studies by Stambuk and Radman (29), which demonstrate that MR has no effect on RuvAB-dependent recombination.

This difference could be attributed to the low level of sequence divergence (3%) used in our study compared with that between S. typhimurium and E. coli (~16%). In addition, RuvAB acts during later stages of recombination, and thus MR may have been saturated in the experiments described by Stambuk and Radman (29). This possibility is consistent with the result that MutS,L were less effective in blocking strand exchange when they were added later during an in vitro strand exchange reaction (Fig. 6). In addition, if the SOS response is activated, the RuvAB level increases, and a high level of RuvAB is expected to overcome any MutS,L-dependent barrier to heterologous strand transfer by mass action.

In summary, this work contributes to our understanding of the role(s) played by MutS,L in recombination. The action of MutS,L raises the energetic barrier for heteroduplex formation and destabilizes the RecA-DNA or RuvAB-DNA complexes when heterology is present. The models discussed here are based primarily on in vitro experiments, and the influence of MutS,L on RuvAB-dependent branch migration in vivo remains to be established. Future studies will be needed to better define the interactions between MutS,L and RuvAB. In addition, it will be useful to study the effects of base-base mismatches on RuvAB binding to three- and four-stranded junctions.

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