Role of MutS ATPase Activity in MutS,L-dependent Block of in Vitro Strand Transfer*

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In addition to mismatch recognition, *Escherichia coli* MutS has an associated ATPase activity that is fundamental to repair. Hence, we have characterized two MutS mutant gene products to define the role of ATP hydrolysis in homologous recombination. These mutants, denoted MutS501 and MutS506, have single point mutations within the Walker A motif, and rate constants for ATP hydrolysis are down 60–100-fold as compared with wild type. Both MutS501 and MutS506 retain mismatch binding and, unlike wild type, fail to relinquish this specificity in the presence of ATP, adenosine 5’-O-(thiotriphosphate), and adenosine 5’-(β,γ-imino)triphosphate.

Both MutS501 and MutS506 blocked the level of strand transfer between M13 and fd DNAs. The level of inhibition varied between the mutants and corresponded with the relative affinities to a G/T mispair. Neither MutS501 nor MutS506, however, would afford complete block of full-length heteroduplex in the presence of MutL. DNase I footprinting data are consistent with these results, as the region of protection by MutS501 and MutS506 was unchanged in the presence of ATP and MutL. Taken together, these studies suggest that 1) MutS impedes RecA-mediated homologous exchange as a distinct mismatch-provoked event and 2) the role of MutL is coupled to MutS-dependent ATP hydrolysis. These observations are in good agreement with the present model for *E. coli* methyl-directed mismatch repair.

In addition to its role in replication fidelity, mismatch repair contributes to genome stability by controlling the level of recombination between closely related sequences (1–9). In *Escherichia coli*, MutS and MutL act to block recombinant yield when phenotypic selection arises in the vicinity of heteroallelic markers (10). Recent work from Zahrt and Maloy (11) provided more evidence implicating mismatch repair in recombination. Specifically, they showed that the efficiency of chromosomal gene transfer between *Salmonella typhimurium* and the closely related *Salmonella typhi* is low due to mismatch repair. Zahrt and Maloy (11) attributed this barrier of genetic exchange to DNA divergence and the ability of MMR to correct this event in favor of the recipient strand. Abdulkarim and Hughes (12) also showed that the mutHLSU genes increased the fidelity of exchange 1000-fold between the TfuA and TfuB translation factors, whose sequences share 99% sequence identity. Mismatch repair in this case ensures that the integrity of the genome is preserved by allowing these genes to evolve in concert.

The first biochemical evidence to implicate mismatch repair in homologous recombination was provided by the *in vitro* RecA-catalyzed three-strand transfer reaction. *E. coli* MutS was shown to inhibit exchange between M13 and fd DNAs whose sequence heterology is ~3% at the nucleotide level (13). Since MutS was without effect on M13-M13 or fd-fd exchange, we attribute the block to the occurrence of mismatched base pairs in newly formed heteroduplex.

MutL enhanced the MutS-dependent block of M13-fd exchange. Indeed, these proteins completely abolished full-length heteroduplex between these DNAs and is reminiscent of the proposed role of these activities in MMR (14–19). MutL is believed to add to the MutS mismatch complex in an ATP-dependent fashion. Evidence for this stems from work by Grilley et al. (20), who showed the region of DNase I protection by MutS and ATP is extended by MutL. In the experiments described here, the role of ATP hydrolysis by MutS is examined on RecA-strand transfer. Previous work by Wu and Marinus (21) described dominant negative mutants of MutS from *MNN* treatment and showed a large fraction of the isolates were missense mutations within the nucleotide binding domain (21). It is here that we have characterized two of these mutants, MutS501 and MutS506, and show that 1) mismatch binding is retained, 2) ATP hydrolysis is reduced, and 3) mismatch correction with MutSS~ extracts is not restored.

These mutants also blocked RecA-catalyzed strand transfer between M13 and fd DNAs. That MutS501, and to a lesser extent MutS506, inhibited M13-fd and not M13-M13 exchange, suggests that mismatch recognition precludes RecA-dependent branch migration in regions of nonhomology. Moreover, that both mutants failed to completely block strand transfer in the presence of MutL suggests that the barrier to homologous recombination is dependent upon ATP hydrolysis.

EXPERIMENTAL PROCEDURES

*E. coli* proteins RecA, SSB, and MutL were purified as described previously (13). Restriction endonucleases *Sna*BI and *Msp*I and S1 nuclease were purchased from U.S. Biochemical Corp./Amersham Pharmacia Biotech. DNase I from bovine pancreas was from Worthington.

**Other Materials—**Proteinase K, phosphocreatine kinase, and phosphocreatine were purchased from Sigma. Oligonucleotides were from Oligos Etc. Replicative form and single-stranded M13 and fd bacteriophage DNAs were prepared as described (13). The replicative forms of M13 and fd DNAs were linearized with *Sna*BI restriction endonuclease. Linear DNA (50 pmol of 5’-ends) was 5’-end-labeled with [γ-32P]ATP using T4 polynucleotide kinase and purified as described previously (13).

**DNA Substrates—**Oligodeoxyribonucleotides were purchased from Oligos Etc. Purified oligomers were annealed and isolated as described previously (22). Covalently closed circular G/T heteroduplex used in the

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§ The abbreviations used are: SSB, single-stranded DNA-binding protein; MMR, methyl-directed mismatch repair.
MMR assays was prepared as described previously (23–26). Repair was scored by determining the fraction of molecules rendered sensitive to XhoI cleavage due to T → C correction on the unmethylated strand.

**Construction of Expression Vectors—**Plasmids harboring structural genes mutS501 and mutS506 (21) were amplified using the following primers: upstream primer 5′-GGGACCTTGGACCCGCAAGCTGTTAGGATCC-3′ and downstream 5′-TTACGAGCAGCGCTGCAGAAGTAAGATTACAAATTCGACG-3′. The resulting fragments (~3 kb) were inserted into the BamH1–HindIII sites of pQE10 expression vector (Qiagen) encoding an N-terminal His6-tag to yield pLW11 (mutS501) and pLW12 (mutS506). Recombinant wild-type MutS (pLW10) was constructed in a similar manner from plasmid pMS312.

**Cell Growth and MutS Purification—**All His6-tagged proteins were purified as described by Qiagen expressionist from Qiagen with the following modifications. M15pREP4 cells, transformed with pLW11 or pLW12, or pLW12, were grown in 4 × 11 L to an A600 of 0.7, chilled to 20–25 °C and induced with 0.05 mM isopropyl-1-thio-β-D- galactopyranoside (27). Cells were recovered in a sonication buffer (100 ml; 50 mM sodium phosphate buffer, pH 7.5, 0.1 mM dithiothreitol, 0.1 mM EDTA, 80 mM KCl, 1.2 mM phenylmethylsulfonyl fluoride at 17 μg/ml; and benzamidine- HCl, 20 μg/ml; 90 μg/ml of lysozyme, sonicated, and batch-eluted with nickel affinity resin. MutS was dialyzed against 11 of 50 mM HEPES, pH 7.5, 500 mM KCl, 1 mM dithiothreitol, 1 mM EDTA, and 13.3% glycerol for 3 h, followed by overnight dialysis using the same buffer containing 200 mM KCl. MutS, at 2.5–3 mg/ml and >95% purity, was stored at ~70 °C.

**Gel Mobility Shift Assay—**All gel shift assays were carried out at 4 °C for 30 min in the following buffer: 20 mM HEPES, pH 7.5, 5 mM MgCl2, 0.1 mM dithiothreitol, 0.1 mM EDTA, 100 μg/ml bovine serum albumin, 0.02 μM γ-32P-labeled G/T oligonucleotide (T lower 5′-CCACGTTAGCCGTAATGCGG-3′ and the underlined nucleotides indicate position of mismatch or G:C base pair). Incubations were resolved on a 6% polyacrylamide gel in 40 mM Tris-HCl, pH 8.5, and 2 mM EDTA at 4 °C for 1.5 h at 110 V using 10% glycerol and 0.1% bromophenol blue. Analysis was done with ImageQuanNT from the Molecular Dynamics STORM 860 PhosphorImager.

**Determining Kinetic Parameters Km and kcat for MutS—**MutS ATPase activity was carried out as described by Hughes and Jiricny (28). Reactions (24 μl) were performed in 50 mM HEPES-KOH, containing 0.75 μM of [γ-32P]ATP and 2, 4, 8, 16, 25, 37.5, 50, or 75 μM ATP. MutS (0.35 μM; mutS501 and MutS506, 2 μM) was added to initiate the reactions at 27 °C, and aliquots (2 μl) were quenched (4 μg/ml EDTA, pH 8.0; 2 μl formamide) and resolved for 0.5 h at 25-μA constant current as described in the figure legends. Rates for ATP hydrolysis were determined by quantitating the amount of labeled Pi constant current as described in the figure legends. Rates for ATP hydrolysis were determined by quantitating the amount of labeled Pi constant current as described in the figure legends.

**Results**

**Purification of wild-type Histidine Reconstitute MutS—** Both MutS mutants (501 and 506) and wild-type were overexpressed and purified using a N-terminal hexameric histidine tag. Induction with 0.4 μM isopropyl-1-thio-β-D-galactopyranoside resulted in high levels of expression, since ~30% of the total E. coli lysate (Fig. 1.) was MutS. However, a vast majority (~80%) of the recombinant protein precipitated as inclusion bodies (30). This was corrected by reducing the isopropyl-1-thio-β-D-galactopyranoside to 0.05–0.1 mM and lowering the growth temperature to 25 °C. Recombinant Hist6-tagged proteins were purified to greater than 95% purity using nickel affinity chromatography (Fig. 1). The yield from 4-liter preparations was 40–50 mg of protein at 2.5–3.0 mg/ml.

**Gel Retardation Assay/NheI Endonuclease Assays—** Both MutS501 and MutS506 were examined for mismatch recogni-
tion by the ability to retard a 51-mer G/T-containing oligonucleotide. Titration of His$_6$-tagged MutS revealed a $K_D$ for a G/T mispair at 40 nM (Fig. 2a) (15). MutS$_{501}$ mismatch was marginally affected with a $K_D$ of 55 nM. Mismatch recognition by MutS$_{506}$, however, was down almost 3-fold with a $K_D$ of 125 nM. The gel retardation pattern was identical in nature to both tagged and nontagged wild-type MutS (data not shown). Discrimination for G/T heteroduplex over homoduplex DNA was 10-fold for both wild-type and MutS$_{501}$ (Fig. 2b).

Previous studies have shown *E. coli* MutS leaves the mismatch in the presence of ATP (31, 35). This nucleotide-driven process was tested for wild-type, His$_6$-tagged wild-type, MutS$_{501}$, and MutS$_{506}$ in the presence of 2 mM ATP. Like nontagged wild-type, His$_6$-tagged MutS failed to bind the mismatch site in the presence of ATP (Fig. 2b). In contrast, MutS$_{501}$ and MutS$_{506}$ retained mismatch binding. Both mutants essentially bound a G/T mismatch with the same affinity as that when ATP was not present. Nonspecific binding (G:C homoduplex) by these proteins was slightly affected by ATP. Further studies with nonhydrolyzable ATP analogs ATP$_g$ and AMP-PNP revealed similar results.

In a related experiment, we tested binding specificity of these mutants by examining the relative accessibility of a restriction site adjacent to the mismatch (31). As detailed in Fig. 3a, G/T-containing duplex DNA was protected against digestion by *NheI* at increasing concentrations of His$_6$-tagged MutS and abolished by 2 mM ATP. G:C homoduplex was sensitive to cleavage by *NheI* at the highest level of MutS, demonstrating the mismatch-specific nature of protection.

MutS$_{501}$ protected against endonucleolytic attack in a similar fashion as wild-type. MutS$_{506}$ was less efficient in its ability to protect against cleavage. As illustrated in Fig. 3b, a 2-fold increase in sensitivity to *NheI* digestion was seen for MutS$_{506}$ as compared with MutS$_{501}$. Even at substoichiometric amounts of protein, both MutS$_{501}$ and MutS$_{506}$ remained bound to the mismatch site in the presence of ATP. These results further reinforce the gel retardation data in illustrating...
the mismatch specificity of these mutants in the presence of ATP.

**Kinetics of ATP Binding/Hydrolysis**—ATPase activity was examined for MutS501 and MutS506 to further characterize the dominant negative phenotype (21). Kinetic parameters, $K_m$ and $k_{cat}$, were determined for wild type, MutS501, and MutS506 to verify their role in MMR. As shown in Fig. 4, E. coli MutS hydrolyzes ATP to ADP + P, and thus corroborates the early findings of Grilley et al. (20). From three independent experiments, $k_{cat}$ and $K_m$ values were determined, and the data were analyzed by both Lineweaver-Burk and Eadie-Hofstee double reciprocal plots. As a comparison, values for MutS from S. typhimurium are presented (29). In terms of $k_{cat}$, E. coli wild-type MutS is approximately 30 times faster. This rate of hydrolysis does not seem to be dependent upon better binding, since the $K_m$ values are virtually identical (Table I). Moreover, it is unlikely that this difference in $k_{cat}$ is due to an ATPase contaminant in the E. coli preparations, because both MutS501 and MutS506 possessed different rates of ATP hydrolysis.

Based upon the mutations (29) defining MutS506 and MutS501, we observed a dramatic drop in ATPase activity (Fig. 5) as compared with wild type. A turnover number of 7.4 min$^{-1}$ for wild type was down 60–100-fold for MutS501 and MutS506 with values of 0.11 and 0.08, respectively (Table I). $K_m$ values were slightly affected with a 2–3-fold decrease in binding as compared with wild-type. It is important to note that the ATPase activities presented here were measured in the absence of DNA. We observed no difference in ATP hydrolysis in the presence of duplex DNA (data not shown), but effects of a mismatch were not tested.

**Mismatch Correction**—Mismatch repair was examined to compare the repair efficiency of MutS501 and MutS506 with His$^6$-tagged wild-type by complementing mutS$(\text{MutS201::Tn5})$ extracts. Maximum repair of closed circular G/T heteroduplex by His$^6$-tagged MutS occurred at 0.2 pmol of protein (Table II). This corresponded well with wild-type MutS as optimum repair occurred between 0.2 and 0.8 pmol of MutS. Unlike wild type, His$^6$-tagged MutS started to inhibit repair above 0.3 pmol.$^2$

Neither MutS501 nor MutS506 was able to complement MutS$^+$ extracts (Table II). Increasing the protein concentration an additional 5-fold resulted only in minimal repair ($<7$ fmol). These proteins also failed to block repair of wild-type MutS when added in a 1:1 ratio at 0.4 pmol of total protein (data not shown).

**MutS501 and MutS506 Inhibit M13-fd RecA-catalyzed Strand Exchange**—To better define the mechanism behind the MutS,L block of RecA-mediated strand transfer, we examined the role of MutS ATPase activity in this process. Functional His$^6$-tagged MutS was therefore tested against the ATPase-

defective mutants in the ability to block M13-fd exchange (13). As illustrated in Fig. 5, His$^6$-tagged wild-type limits full-length heteroduplex formation at increasing MutS concentrations. This concentration-dependent block is in good agreement with previous studies on nontagged MutS and again illustrates no unusual perturbations due to the N-terminal histidines (34). Maximal inhibition was observed at a MutS to mismatch ratio of 1:2.5. MutS501 shows a similar ability to inhibit strand transfer as wild-type MutS. The extent of inhibition by MutS506, however, was down approximately 2–3-fold.

**Effects of MutS,L on Homologous Exchange**—A concentration of MutS to mismatch of 1:4 yielded a 2-fold decrease in full-length heteroduplex. Consistent with previous studies (13), the rate of strand transfer between M13 and M13 was about 3 times faster than M13-fd (Fig. 6, a and b), thus illustrating that homology search by RecA is sensitive to non-Watson-Crick base pairing. M13 duplex was taken up by the RecA filamented single-stranded circular M13 within the first 10 min of incubation. The population of branched intermediates for M13-M13 exchange appeared more dispersed, indicating no barrier to branch migration. MutS failed to change this distribution in the homologous reaction (Fig. 6a), an observation consistent with previous studies (13). Values for E. coli MutS were the average of three independent experiments (S.D. of ±3.4) involving initial velocities from single ATP concentrations (see “Experimental Procedures”).

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**TABLE I**

| Protein            | $K_m$ (μM ATP) | $V_{max}$ (μM P$_i$/min) | $k_{cat}$ (μM P$_i$/μM MutS) | $K_m$ (μM ATP) | $V_{max}$ (μM P$_i$/min) | $k_{cat}$ (μM P$_i$/μM MutS) |
|--------------------|----------------|--------------------------|-------------------------------|----------------|--------------------------|-------------------------------|
| S. Typhimurium MutS| 5.9            | 0.30                     | 0.23                          | 6.7            | 0.34                     | 0.26                          |
| MutS622            | 198.0          | 0.050                    | 0.038                         | 250            | 0.064                    | 0.049                         |
| E. coli MutS       | 14.8           | 2.34                     | 7.20                          | 17.1           | 2.56                     | 7.56                          |
| MutS501            | 32.5           | 0.228                    | 0.114                         | 35.0           | 0.244                    | 0.122                         |
| MutS506            | 57.6           | 0.148                    | 0.074                         | 66.0           | 0.169                    | 0.084                         |

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**TABLE II**

| Repair                  | With 200 fmol of MutS | With 800 fmol of MutS |
|-------------------------|-----------------------|-----------------------|
| Wild type               | 8.4                   | 14.2                  |
| His$^6$-MutS            | 15.7                  | 5.4                   |
| His$^6$-MutS501         | 0.7                   | 0.5                   |
| His$^6$-MutS506         | 0.6                   | 0.6                   |

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$^2$ Repair efficiency varied among MutS preparations and inhibited at higher concentrations when purified in HEPES versus phosphate buffer.
with the formation of normal canonical base pairing. Conversely, branched intermediates between M13 duplex and fd single-strands accumulated as a defined species that appeared to stabilize when MutS was present (Fig. 6b).

His6-tagged MutS and MutL revealed a similar kinetics profile as that observed for nontagged wild type (13). These combined repair activities completely blocked formation of full-length heteroduplex DNA between M13 and fd (Fig. 7). This barrier to RecA-catalyzed strand transfer was MutS-dependent, since MutL alone had no effect on either M13-M13 or M13-fd exchange. As illustrated in Fig. 6b, it appears that MutL participates in this block by destabilizing branched intermediates that have accumulated in the presence of MutS (13).

**Effects of MutL on RecA-catalyzed Strand Transfer in the Presence of Mutants MutS501 and MutS506**—In examining the role of mismatch repair proteins in homologous recombination, we evaluated the effects of MutS501 and MutS506 on both M13-M13 and M13-fd exchange in the presence of MutL. As illustrated in Fig. 8 and 9a, we observed that MutS501 inhibits M13-fd exchange similar to that observed for wild type. In contrast, the level of inhibition by MutS506 was down 2-fold (Fig. 9b). This inhibition of M13-fd exchange was dependent upon heteroduplex formation as both mutants contribute little to the block of M13-M13 strand transfer (data not shown). MutS501 did not completely block full-length heteroduplex in the presence of MutL during M13-fd exchange. The extent of product formation was the same whether MutL was present or not (Figs. 8 and 9a). It appears from these results that the effect of MutL requires MutS and ATP hydrolysis. In addition, it suggests the block by MutL is mismatch-dependent. A similar loss of MutL function was observed with MutS506. The amount of heteroduplex DNA, however, was 2–3 times higher for MutS506 than MutS501 (Figs. 8 and 9b).

**Footprinting of Wild-type MutS, MutS501, and MutS506 at a Mismatch**—We further characterized the DNA binding properties of MutS501 and MutS506 using DNase I footprinting. Grilley et al. (20) showed that MutS will extend the MutS footprint at a G/T mispair in the presence of ATP. MutS501 protein retains its mismatch binding specificity and protects a region of ~20 base pairs spanning the G/T mismatch (Fig. 10a). This footprint is identical to that observed for wild type. Consistent with previous studies with gel retardation, MutS501 remained at the mismatch site in the presence of ATP. His6-tagged wild-type MutS, however, does not protect against DNase I digestion in the presence of ATP (Fig. 10b). This observation is different from Grilley et al. (20) with nontagged MutS. We do, however, point out that in the studies of Grilley et al., they did observe a decrease in protection due to ATP. Evidence here and elsewhere suggests MutS leaves the mismatch in the presence of ATP (31).
MutS506 bound specifically at the G/T mismatch and produced a different protection pattern than MutS501 or wild-type (Fig. 10a). A region of approximately 8–10 bp 5’ to the mismatch is more sensitive to DNA cleavage with MutS506 than MutS501 or wild-type (Figs. 8 and 10a). To help clarify MutL function in recombination, we examined this activity with MutS501 and MutS506 in the presence of ATP. Consistent with studies of Grilley et al. (20) we see that His$_6$-tagged wild-type MutS protection from DNase I increases upon the addition of MutL and ATP (Fig. 10b, right). Neither MutS501 nor MutS506 extended the region of protection in the presence of MutL and ATP. Both mutants remained in the vicinity of the G/T mispair. Studies with ATP alone revealed a similar pattern of protection (Fig. 10b, middle). This is in good agreement with the results from the gel retardation assays, where the presence of ATP failed to disrupt mismatch binding.

**DISCUSSION**

Here, we present data that define the enzymatic properties of MutS mutants MutS501 and MutS506 in MMR and recom-
bination. Gel retardation analysis shows both MutSS01 and 506 retain the ability to bind mismatches. Indeed, MutSS01 was as efficient as wild-type in binding a G/T mispair. MutSS06 binding specificity, however, was reduced almost 3-fold. This difference in mismatch binding is consistent with the in vivo studies of Wu and Marinus (21), who showed repair of a 2-base deletion was more efficient in mutSS01 than mutSS06 mutant strains. That both mutSS01 and mutSS06 are dominant over wild type suggests mismatch binding is not the only criterion defining this phenotype. We do believe the results presented here offer a plausible explanation for the relative difference in repair efficiencies by these mutants (21).

The initial goal of these studies was to define the role of MutSSL during RecA-catalyzed strand transfer between closely related DNAs. Previous work has shown that the formation of mismatched base pairs in heteroduplex is modulated by MutSSL (13). In E. coli, MutS initiates repair by binding to the mismatch site. In subsequent steps, incision at a hemimethylated(dGATC) site is dependent upon MutS, MutL, MutH, and ATP hydrolysis. It is known that a persistent and strand-specific nick will direct repair and thus bypass the MutH requirement (15, 36, 38, 39). That MutSL and ATP hydrolysis are still required for excision reflects an intimate interaction between these proteins in governing a repair event. In this study, we examined how these repair activities interact during strand transfer by examining the role of ATP hydrolysis by MutS in this process. We demonstrate that the extent of exchange between homologous DNAs is affected by the ability of MutS to bind newly formed mismatches. More precisely, strand exchange between M13 and fd DNAs is hampered by both MutSS01 and MutSS06. Since both mutants have reduced ATPase activity (60–100-fold) and failed to leave the mismatch in the presence of ATP, we attribute this block of strand exchange to mismatch binding.

Based on studies from Cox (40, 41), RecA promotes the exchange of homologous DNAs at a rate of 350 bp min⁻¹. Given the size of M13 phage DNA, RecA should drive the reaction to completion in approximately 18 min. Indeed, we observed 100% conversion of linear duplex to nicked circles between M13 DNAs in 20 min. The rate of branch migration for M13-fd exchange was slower at ~105 bp min⁻¹. Because of this difference in rates, as manifested by regions of nonhomology, we believe MutS has time to test for the presence of non-Watson-Crick base pairing. Indeed, it would be fair to assume that mismatch binding interferes with normal RecA reassociation in regions where the density of mispairs is energetically unfavorable (13).

The effect of MutL on homologous strand transfer could result from MutS-dependent translocation along the DNA via a shaped loop structures, a process that would help stabilize MutSSL on duplex DNA after mismatch binding. Recent studies from Allen et al. (31) are consistent with this idea. The rate of loop formation catalyzed by MutS and ATP increased 2-fold in the presence of MutL. Moreover, the ATPase-defective mutants MutSS01 and MutSS06 failed to show the enhanced block of full-length heteroduplex between M13 and fd DNAs in the presence of MutL. It appears the role of MutL is confined to a step following mismatch recognition and is probably coupled to MutS ATPase activity. DNAse I footprinting studies presented here are consistent with this line of reasoning. Unlike wild-type MutS, both mutants failed to expand the region of nuclease protection in the presence of ATP and MutL. However, it is not immediately obvious whether binding or hydrolysis is required for MutL recruitment to the complex. MutS could in principle undergo a conformational change once ATP is bound. Mismatch binding would then be lost, and the hydrolytic step would ensure that MutS remains in this state, thus facilitating DNA translocation over mismatch recognition. It is also likely that MutL somehow contributes to this form of MutS by stabilizing the complex on duplex DNA (28). Taken together, these results suggest MMR exerts a similar strategy in testing for the occurrence of mismatched base pairs during RecA-catalyzed strand transfer.

Certainly, further studies are needed to help define other roles of mismatch repair in recombination. Based on studies in yeast S. cerevisiae two MutS homologues, MSH4 and MSH5 are implicated in meiotic recombination (42–44). Just recently, the human homologue of yMSH4 was identified (45). This, along with studies illustrating yMSH2-specific binding to Holliday junctions (46), could implicate further roles for mismatch repair in genome stability.

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