The Role of Mismatched Nucleotides in Activating the hMSH2-hMSH6 Molecular Switch*

(Received for publication, August 5, 1999, and in revised form, November 18, 1999)

Scott Gradia, Samir Acharya, and Richard Fishel‡

From the Genetics and Molecular Biology Program, Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Mismatch repair (MMR) is an important cellular pathway that facilitates genome stability by correcting mismatched nucleotides in DNA that arise from chemical and physical damage, replication errors, and recombination events between heteroallelic parental DNAs (for review see Ref. 6). Mutation of several of the human MMR genes have been shown to result in a mutator phenotype and are associated with a common cancer predisposition syndrome, hereditary nonpolyposis colorectal cancer (HNPPC), as well as a variety of sporadic tumors (7,8).

Initiation of MMR is fundamentally dependent on the proto-
typical Escherichia coli MutS or its eucaryotic homologs: a highly conserved family of proteins responsible for mismatch recognition (for review see Ref. 9). The bacterial MutS protein appears to recognize mispaired DNA as a homodimer, while the eucaryotic MSHs (MutS homologs) appear to function as heterodimers of MSH2 and MSH6 or MSH2 and MSH3 (10–12). Like their yeast counterpart, the human hMSH2-hMSH6 heterodimer primarily recognizes and participates in the repair of single-base and small insertion/deletion DNA mismatches, while the hMSH2-hMSH3 heterodimer is associated with the repair of small and large insertion/deletion DNA mismatches (12–14).

Homology between the MutS homologs is largely based upon a highly conserved Walker-A/B adenosine nucleotide and magnesium-binding domain (3,9). Although aspects of ATP binding/hydrolysis by the bacterial and yeast MutS homologs have been examined (15–17), more comprehensive studies of the human hMSH2-hMSH6 heterodimer have demonstrated coupled ATP and DNA binding properties as well as an intrinsic ATPase activity that is stimulated by mispaired DNA (1, 2, 5, 18, 19). A defining observation is that binding to mismatched DNA by MutS and hMSH2-hMSH6 is abolished in the presence of ATP (1, 10, 20). The ATP-induced release of E. coli MutS from mispaired DNA has been reported to occur by hydrolysis-driven translocation of the protein along the DNA backbone (4). This conclusion is based on the appearance of growing loop structures observed by electron microscopy that depend on MutS, MutL, and ATP. Moreover, the poorly hydrolyzable analog of ATP, ATPγS, appears to block the growth of these loops, which has been interpreted to suggest a requirement for ATP hydrolysis.

An alternative model for signaling MMR suggests that hMSH2-hMSH6 functions as a dinucleotide-regulated molecular switch (1–3). This conclusion is grounded on the biochemical properties of the hMSH2-hMSH6 ATPase and the observation that ADP and ATP have opposing effects on mispair binding. These studies have further demonstrated that: (i) the rate-limiting step for the intrinsic ATPase is mismatched nucleotide provoked ADP→ATP exchange; (ii) hMSH2-hMSH6 undergoes a conformational transition associated with ADP→ATP exchange similar to that demonstrated for G protein signaling molecules; (iii) the adenosine nucleotide conformational transition of hMSH2-hMSH6 results in the formation of an ATP-bound hydrolysis-independent sliding clamp (preincubation of hMSH2-hMSH6 with ATPγS, results in a conformation that is topologically refractory to mispair binding); and (iv) hydrolysis of ATP only occurs when hMSH2-hMSH6 dissociates or is dissociated from the DNA (thus recycling the mispair binding switch).

An argument against the Molecular Switch model was recently forwarded by Blackwell et al. (5,19) and is based on the following observations: (i) a similar dissociation constant (kₜ)}
between hMSH2-hMSH6 and mismatched DNA in the presence or absence of ADP; (ii) plasmion resonance spectroscopy demonstrating ADP-induced release of hMSH2-hMSH6, which was prebound to mismatched DNA in the absence of nucleotide (albeit >10-fold slower than ATP-induced release); (iii) an ATPase “salt profile” that appeared similar to MMR and mismatch-provoked excision reactions in vitro, and 4.) a “significant” DNA length-dependent increase in the $k_{cat\text{-DNA}}$ for the ATPase. It was concluded that hMSH2-hMSH6 movement along the DNA backbone occurred by a modified Hydrolysis-Driven Translocation model (5, 19).

Quantitative measure of bacterial MMR in vitro and in vivo have shown that repair efficiency is primarily determined by the type of mispaired base (21, 22) and can be influenced by the sequence context surrounding the mispair (23). Although genetic experiments have implicated hMSH2-hMSH6 in the repair of most single base mismatches, nearly all of the biochemical studies have focused on its interaction with G/T mismatched DNA. Here we have examined the affinity of hMSH2-hMSH6 for a variety mismatched DNA substrates as well as the extent to which these mismatched DNA substrates provoke ADP→ATP exchange and stimulate the intrinsic ATPase activity. We have additionally characterized the affinity of hMSH2-hMSH6 for ATP and examined the chain length dependence of homoduplex and mismatched DNA substrates on the hMSH2-hMSH6 ATPase. Our results suggest that the rate-limiting step in hMSH2-hMSH6 initiation of MMR is tied to the ability of individual mispaired nucleotides to provoke ADP→ATP exchange. Moreover, in contrast to previous reports (19), we observe a modest decrease in the $k_{cat\text{-DNA}}$ with increasing DNA chain length under physiologically significant conditions. These and other results reduce the likelihood of a Hydrolysis-Driven Translocation mechanism, while providing further support for the Molecular Switch model.

**MATERIALS AND METHODS**

The hMSH2-hMSH6 heterodimer was prepared and quantitated as described previously (1).

**Preparation of DNA Substrates—Oligonucleotides** were synthesized using a 3948 Nucleic Acid Synthesis and Purification System (Applied Biosystems). Unlabeled duplex DNA substrates were made by annealing equal molar amounts of an upper strand, 5′-GCT TAC CAT CGA GGA TCG TCA AGC ACA TGC GGA-3′, to a lower strand, 5′-CCG TTT AAT TGC ACC GAG CTT GAT CCT CGA TTA GC-3′. For example a G/T mismatch was constructed by positioning a G in place of X in the upper strand and a T in place of Y in the lower strand. All single base pair mismatched DNA substrates followed this position. Insertion/deletion mismatched DNA substrates were constructed by annealing an upper strand 5′-GCT TAC CAT CGA GGA TCG XAG TCT GGA AGT CAG CCG-3′, where $X = A$ for (+A), $X = C$ for (+C), and $X = CACACA$ for (+GA), to the lower strand, 5′-CGC TGT AAT TGC ACC GAG CTT GAT CCT CGA TTA GC-3′. Labeled 41-base pair DNA substrates were prepared by annealing 32P-end-labeled oligonucleotide to an equal molar amount of unlabeled complementary oligonucleotide. Duplex DNA was purified from an 8% acrylamide gel. Excised gel slices were crushed and incubated in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 100 mM NaCl for 12 h. Buffer containing the DNA was separated from the acrylamide using a Ultrafree-MC 0.22 μm Filter Unit (Millipore) and concentrated using a microconcentrator (Amicon).

The 25′-, 251′-, and 501-bp heteroduplex DNA substrates were made by amplifying a region of a modified pBSK vector containing a central G/C or A/T base pair using the Pfu polymerase in a standard PCR reaction (Stratagene). The sequence directly surrounding the G/C or A/T base pair is 5′-TGC AGC AGC TGC ATG CCT TCT-3′ where $X = A$ or A. PCR products were purified using the Qiaquick PCR purification kit. Equal molar amounts of G/C and A/T DNA were combined in buffer M (10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM MgCl2, 1 mM DTE) (Roche Molecular Biochemicals), denatured for 5 min at 95°C, and then reannealed by cooling to 37°C. DNA was then digested with BglII and PvuII, which cleaves only homoduplex DNA (leaving G/T or C/A heteroduplex DNA intact) (24). Full-length heteroduplex DNA was purified by high pressure liquid chromatography using a Waters Gen-Pak FAX Column (Millipore). DNA was loaded onto the column in buffer M at 0.5 ml/minute, washed 10 min with 25 μM Tris (pH 8.0), 500 mM NaCl, 1 mM EDTA, and then eluted with a 35 min gradient to 750 mM NaCl. Full-length heteroduplex DNA fraction typically separated 2–4 min earlier than the 32P-terminated DNA fraction, and ethidium bromide-stained substrate eluted in 10 mM Tris (pH 7.5), 100 mM NaCl, 2 mM MgCl2, and then quantitated by spectrophotometry.

**DNA and ATP-γ-S Filter Binding Assays—**DNA and ATP-γ-S filter binding assays were performed in 25 mM Hepes-HCl (pH 7.8), 110 mM NaCl, 2 mM MgCl2, 1 mM dithiothreitol, and 15% glycerol, unless otherwise indicated. The concentrations of hMSH2-hMSH6, DNA, and ATP-γ-S are indicated in the figure legends. DNA binding assays were performed by incubating hMSH2-hMSH6 with the indicated labeled DNA substrates in the presence of 25 μM ATP at 37°C for 15 min in a 20-μl reaction. Unlabeled competitor DNA or ATP was included in the reaction where indicated. Reactions were placed on ice, diluted with 4 ml of buffer A (which consisted of 25 mM Hepes-HCl (pH 7.8), 2 mM MgCl2, 15% glycerol, and NaCl concentration equivalent to that of the reaction), and then were immediately filtered through a prewet Millipore HAWP nitrocellulose membrane and washed with 8 ml of buffer A. Filters were incubated overnight in scintillation fluid and quantitated using a Beckman counter. Data from the DNA competition assays were fit to the equation: $Y = NS + (T - NS)(1 + 10^{log[IC50]} - [comp])$ as described by Motulsky (25) where, [comp] is the concentration of competitor, $T$ is the total binding in the absence of competitor, NS is the binding at saturating concentration of competitor, and $Y$ is the binding measured at various concentrations of competitor. $IC_{50}$ values represent the concentration of unlabeled competitor that reduced G/T DNA binding by 50%. $K_s$ values were determined by the equation $K_s = IC_{50}/(1 + (\text{radioligand}/K_{D}^{{\text{DNA}}}))$, where the concentration of radioligand (labeled G/T DNA) was 20 nM and the $K_s$ for hMSH2-hMSH6 binding G/T DNA was 10.5 nM. ATP-γ-S binding assays were performed by incubating hMSH2-hMSH6 with ATP-γ-S at 37°C for 5 min in the presence or absence of DNA; the reactions were then analyzed by filter binding as described above. $K_d$ and $B_{max}$ (equivalent to the moles of substrate bound at saturation) were determined by fitting the data to a square hyperbola (25).

**ATPase Assays—**ATPase assays were performed in 25 mM Hepes-HCl (pH 7.8), 110 mM NaCl, 1 mM dithiothreitol, and 15% glycerol, unless otherwise indicated. The concentrations of hMSH2-hMSH6 and ATP are indicated in the figure legends. For experiments shown in Fig. 3A and Table I, the concentration of hMSH2-hMSH6 was varied so that the amount of ATP hydrolyzed remained below 15%. Reactions were incubated at 37°C for 30 min, and the fraction of hydrolyzed [ATP-γ-S]ATP was determined by charcoal desorption as described previously (1).

**ADP Exchange Assays—**Assays were performed in 25 mM Hepes-HCl (pH 7.8), 100 mM NaCl, 1 mM dithiothreitol, 15% glycerol, and 2 mM MgCl2. hMSH2-hMSH6 (75 nM) was incubated with 2.3 μM [3H]ADP at 25°C for 25 min and then put on ice. DNA (93 nM) and 25 μM ATP was added to start the reaction, which was then stopped at the indicated time by the addition of 4 ml of ice-cold stop buffer (25 mM Hepes-HCl (pH 7.8), 100 mM NaCl, 2 mM MgCl2). The solution was immediately filtered on a Millipore HAWP nitrocellulose membrane and washed with 10 ml of cold stop buffer. Filters were air dried, added to 3 ml of scintillation fluid, and quantitated. The amount of ADP bound before the addition of DNA and ATP was used as the zero time point. We found that multiple filter washes (up to four) did not significantly alter (≤10%) the results.

**RESULTS**

**Mismatch Binding Specificity of hMSH2-hMSH6** The hMSH2-hMSH6 heterodimeric protein complexes has been purified from human cell extracts based on its ability to restore MMR to mutant cell lines (10) and to specifically bind DNA containing mismatched nucleotides (1). Biochemical studies of the hMSH2-hMSH6 protein have primarily focused on its interaction with G/T mismatch. We examined the affinity of hMSH2-hMSH6 for the eight possible single nucleotide mispairs by gel shift analysis and found a strong bias for the G/T mispair followed by a C/A mispair (data not shown). To further quantitate these interactions, a filter binding assay was developed, and the association of hMSH2-hMSH6 with several DNA substrates containing defined mis-
matched nucleotides in an otherwise identical sequence context was examined (Fig. 1A). We found that none of the mismatched DNA substrates reached a level of binding saturation comparable with that of a G/T mismatch. These results suggested that the hMSH2-hMSH6 interaction(s) with mismatched DNA was complex, and simple binding studies were unlikely to be sufficient for accurate interaction comparison.

Competition studies have been used to overcome complex binding activities and to gauge the relative affinity MutS homologs for mismatched DNA substrates (16, 26). We performed a similar competition analysis in which an unlabelled DNA was tested for its ability to compete with a labeled DNA substrate containing a G/T mismatch (Fig. 1B). IC_{50} and K_i values for these competition assays were calculated by fitting the data to a one-site competition equation (see “Materials and Methods”) and are shown in Table 1. We observe a hierarchy for mispair discrimination between homoduplex (G/C) and heteroduplex (G/T) DNA and the T/T mismatch binding, which was the next best competition substrate. Although there appeared to be little or no discrimination between a T/T mismatch and homoduplex (G/C) DNA. Data were fit to a one-site competition equation described under “Materials and Methods.” To assess the relative affinity for mismatched DNA, we calculated that G/T DNA has a 6.5-fold higher IC_{50} than C/A DNA (which is poorly but significantly repaired) (19). This agreement between the G/T repair efficiencies and our relative binding data further suggests that the hMSH2-hMSH6 interaction(s) with mismatched DNA is solely due to hMSH2-hMSH6 function (19), the wide range of salt concentrations where mispair discrimination occurs supports the idea that mismatch binding is unlikely to be the critical function for hMSH2-hMSH6 in MMR.

**Activation of the hMSH2-hMSH6 ATPase by Mismatched DNA**—Previous studies have demonstrated that hMSH2-hMSH6 ATPase activity requires MgCl_2 (1, 2). We have further examined the salt profile and DNA concentration dependence of the G/T mismatch-stimulated hMSH2-hMSH6 ATPase (Fig. 2). Homoduplex (G/C) stimulation of hMSH2-hMSH6 ATPase activity peaked at ~65 mM, while G/T mismatched DNA peaked at ~110 mM (Fig. 2A). Moreover, below 65 mM and above 300 mM NaCl there appeared to be no discrimination between homoduplex (G/C) and heteroduplex (G/T) stimulation of the hMSH2-hMSH6 ATPase. Interestingly, the NaCl concentrations that produce a discrimination between homoduplex (G/C) and heteroduplex (G/T) DNA for the hMSH2-hMSH6 ATPase appear to closely correlate with the salt profile observed for MMR in vitro (19).

At the salt concentration of peak ATPase activity (110 mM), a wide range of DNA concentrations (25–900 nM) appeared to provide continuous discrimination between homoduplex (G/C) and heteroduplex (G/T) DNA (Fig. 2B). We calculated that G/T mismatched DNA stimulated hMSH2-hMSH6 ATPase activity displayed a k_{cat} of ~10 min^{-1} and K_{cat,GT-DNA} of ~40 nM, whereas the k_{cat,G-C DNA} for homoduplex (G/C) was lower (~6.4}

**Table 1**

| DNA substrate | Log(IC_{50}) | IC_{50} | K_i |
|---------------|--------------|---------|-----|
| G/T           | -7.516 ± 0.069 | 30.5   | 10.5 |
| C/A           | -6.599 ± 0.058 | 251.8  | 86.6 |
| + (C/A)       | -6.507 ± 0.041 | 311.2  | 107.0 |
| T/T           | -6.167 ± 0.056 | 680.3  | 234.1 |
| G/C           | -6.146 ± 0.062 | 714.5  | 245.6 |

cells in vivo and in vitro in which G/T > C/A = G/G = +A > A/A > G/A > T/T > C/T = C/C (21, 26–33). There is a general agreement between the G/T repair efficiencies and our relative binding data. However, there appears to be a discordance between the relative binding and repair of the C/A mismatch as well as the complete lack of discrimination between the homoduplex (G/C) DNA and the T/T mispair (which is poorly but significantly repaired) (19, 34).

We have compared the ability of hMSH2-hMSH6 to bind a DNA substrate containing a G/T mismatch with an identical homoduplex (G/C) DNA substrate as a function of salt concentration (Fig. 1C). The binding preference for a G/T mismatch at low salt appears comparable with previous observations (19). We have additionally found that the binding of homoduplex (G/C) DNA by hMSH2-hMSH6 also increases at low salt concentrations. However, the preference for mismatched DNA compared with homoduplex (G/C) DNA remains fairly constant at NaCl concentrations below 150 mM. Interestingly, the peak of MMR activity appears to occur at a salt concentration of 110–130 mM (19). Although it would appear premature to suggest that peak salt activity of a multicomponent MMR system is solely due to hMSH2-hMSH6 function (19), the wide range of salt concentrations where mismatch discrimination occurs supports the idea that mismatch binding is unlikely to be the critical function for hMSH2-hMSH6 in MMR.

**Activation of the hMSH2-hMSH6 ATPase by Mismatched DNA**—Previous studies have demonstrated that hMSH2-hMSH6 ATPase activity requires MgCl_2, is significantly stimulated by DNA containing a G/T mismatch, and is controlled by mismatched DNA provoked ADP → ATP exchange (1, 2). We have further examined the salt profile and DNA concentration dependence of the G/T mismatch-stimulated hMSH2-hMSH6 ATPase (Fig. 2). Homoduplex (G/C) stimulation of hMSH2-hMSH6 ATPase activity peaked at ~65 mM, while G/T mismatched DNA peaked at ~110 mM (Fig. 2A). Moreover, below 65 mM and above 300 mM NaCl there appeared to be no discrimination between homoduplex (G/C) and heteroduplex (G/T) stimulation of the hMSH2-hMSH6 ATPase. Interestingly, the NaCl concentrations that produce a discrimination between homoduplex (G/C) and heteroduplex (G/T) DNA for the hMSH2-hMSH6 ATPase appear to closely correlate with the salt profile observed for MMR in vitro (19).

At the salt concentration of peak ATPase activity (110 mM), a wide range of DNA concentrations (25–900 nM) appeared to provide continuous discrimination between homoduplex (G/C) and heteroduplex (G/T) DNA (Fig. 2B). We calculated that G/T mismatched DNA stimulated hMSH2-hMSH6 ATPase activity displayed a k_{cat,G-T DNA} of ~10 min^{-1} and K_{cat,G-T DNA} of ~40 nM, whereas the k_{cat,G-C DNA} for homoduplex (G/C) was lower (~6.4
Fig. 2. Salt and DNA concentration affect hMSH2-hMSH6 ATPase activity. Velocity \((\text{mol ATP hydrolyzed})\) (mol hMSH2-hMSH6 \(\times 10^{-3}\) min\(^{-1}\)) of the DNA-stimulated ATPase in the presence of a 41-bp oligonucleotide containing a G/T mismatch (G/T) or homoduplex (G/C) at a central base pair (see "Materials and Methods"). Assays were performed in the presence of 10 mM MgCl\(_2\) and 500 \(\mu\)M ATP. A, salt profile (NaCl) of the hMSH2-hMSH6 (80 nM) ATPase activity. DNA concentration dependence (185 nM molecules). B, DNA concentration profile of the hMSH2-hMSH6 (80 nM) ATPase activity. DNA concentrations are expressed in nM molecules at 110 mM NaCl. Kinetic parameters were calculated by fitting the data to the Michaelis-Menten equation: for heteroduplex DNA, \(k_{cat\text{-G/T}} = 9.9 \text{ min}^{-1}\) and \(K_m\text{-G/T} = 40 \text{ nM}\); for homoduplex DNA, \(k_{cat\text{-G/C}} = 6.4 \text{ min}^{-1}\) and \(K_m\text{-G/C} = 260 \text{ nM}\).

We then examined the activation of the hMSH2-hMSH6 ATPase by a variety of DNA substrates (Fig. 3A). Estimations of the apparent \(K_m\) for ATP and \(k_{cat\text{-ATP}}\) was determined by fitting the data directly to the Michaelis-Menten equation (Table II). It is interesting to note that the rate of hMSH2-hMSH6 ATP hydrolysis \(k_{cat\text{-ATP}}\) induced by individual mismatched nucleotides largely correlated with the reported mismatch repair efficiencies (Refs. 29–33 and see above). The G/T mismatch, which is efficiently repaired in human cells, readily activated the ATPase activity, whereas the poorly repaired single nucleotide mismatches, such as the C/C mismatch, stimulate the ATPase activity poorly. A (+CA\(_4\)) insertion-deletion loop-type (insertion/deletion) mismatch, which would be predicted to be repaired largely by a hMSH2-hMSH3-mediated repair event, does not stimulate the ATPase above that of homoduplex DNA (11, 33, 35, 36). More importantly, a (+CA) insertion/deletion, which has been proposed to be repaired by both the hMSH2-hMSH6 and hMSH2-hMSH3 pathways, is capable of stimulating the hMSH2-hMSH6 ATPase activity. It should be noted that the ATPase assays were performed at a single DNA concentration (185 nM) from which \(k_{cat}/K_m\) was derived for comparison. These should be regarded as snapshots of catalytic efficiency because an accurate comparison would require an analysis of the DNA concentration dependence and calculation of the \(k_{cat\text{-DNA}}/K_m\text{-DNA}\) as determined for the G/T and G/C oligonucleotides (see above; see also Fig. 6 and Table III), which displayed an order of magnitude difference in \(k_{cat\text{-DNA}}/K_m\text{-DNA}\).

In addition, we found that the rate of ADP→ATP exchange \(k_{cat\text{-ATP}}\) correlated with the relative \(k_{cat}\) values observed for the mismatch-stimulated ATPase activity (G/T \(\sim +\text{(CA)} \sim \text{CA} > \text{T/T} > \text{G/C} \gg \text{no DNA}) (Fig. 3B). These results support the idea that the rate-limiting step for ATP hydrolysis is mismatch provoked ADP→ATP exchange. Previous studies have demonstrated that ADP→ATP exchange results in the formation of a

| DNA substrate | \(k_{cat}\) \(\times 10^{-3}\) min\(^{-1}\) | \(K_m\) \(\times 10^{-4}\) | \(k_{cat}/K_m\) \(\times 10^{-4}\) |
|--------------|-----------------|-----------------|-----------------|
| +CA\(_4\)    | 16.44 ± 0.63    | 93.8 ± 9.6      | 17.5            |
| G/T         | 15.04 ± 0.65    | 71.2 ± 8.7      | 21.1            |
| +CA        | 14.91 ± 0.65    | 110.7 ± 12.1    | 13.3            |
| G/C        | 12.71 ± 0.46    | 95.5 ± 9.1      | 13.3            |
| C/A        | 12.35 ± 0.49    | 90.1 ± 9.5      | 13.7            |
| A/A        | 10.16 ± 0.35    | 115.4 ± 9.7     | 8.8             |
| ssDNA      | 9.89 ± 0.53     | 85.1 ± 12.4     | 11.6            |
| T/T        | 8.57 ± 0.53     | 109.3 ± 17.1    | 7.8             |
| G/T        | 8.39 ± 0.27     | 95.7 ± 8.2      | 8.8             |
| G/A        | 8.22 ± 0.24     | 96.4 ± 7.5      | 8.5             |
| C/C        | 5.44 ± 0.18     | 81.5 ± 7.6      | 6.7             |
| +CA\(_4\)  | 4.37 ± 0.27     | 129.7 ± 20.3    | 3.4             |
| G/C        | 4.00 ± 0.12     | 53.2 ± 5.0      | 7.5             |
| no DNA     | 0.97 ± 0.08     | 45.8 ± 11.7     | 2.1             |
hydrolysis-independent sliding clamp and that ATP hydrolysis occurs when hMSH2-hMSH6 transits a free end (2). These observations, combined with the mismatch-dependent ATPase data presented here, strongly argue that it is the unique events associated with ADP—ATP exchange at the site of the mismatch that are important for the control of MMR.

ATP Binding Activity by hMSH2-hMSH6—Previous studies using plasmon resonance suggested dissociation of hMSH2-hMSH6 from mismatched DNA in the presence of ADP, which was taken as support for a Hydrolysis-Driven Translocation model and further implied that the protein complex initially recognized mismatched DNA in a nucleotide-free form (5, 19). This hypothesis depends critically on hMSH2-hMSH6 adenine nucleotide binding activities under physiological conditions. We have quantitatively determined the conditions of adenine nucleotide binding by hMSH2-hMSH6 (Fig. 4). In the absence of DNA, we have found that hMSH2-hMSH6 binds ADP (see Ref. 1), ATP (Kd \( \approx 0.38 \pm 0.10 \mu M \) in the absence of MgCl2 (data not shown), and ATP\( \gamma S \) (Kd \( \approx 0.75 \mu M \) in the presence of MgCl2 (Fig. 4A). Because it has been estimated that the concentration of ATP in a metabolizing cell approaches 1–3 mM (37, 38), it would appear likely that hMSH2-hMSH6 remains saturated with adenine nucleotide in vivo. Furthermore, in the absence of mismatched nucleotides (but only in the presence of MgCl2), we have shown that hMSH2-hMSH6 rapidly hydrolyzes bound ATP to ADP and remains in the ADP bound state unable to exchange ADP—ATP (Ref. 1 and Fig. 3B). Taken as a whole, these observations are consistent with the idea that under physiological conditions, hMSH2-hMSH6 should be largely bound by adenine nucleotide. We cannot rule out the possibility that mispair binding induces the release of ADP. These studies also suggest that quantitative binding studies between hMSH2-hMSH6 and ATP\( \gamma S \) are a reasonable measure of binding between hMSH2-hMSH6 and ATP.

We have additionally explored the interaction between ATP and hMSH2-hMSH6 in the presence of DNA containing a G/T mismatch (1). Using a filter binding assay (see “Materials and Methods”), we determined the ATP\( \gamma S \) binding activity of hMSH2-hMSH6 (100 nM) binding to ATP\( \gamma S \) in the presence of a 41-bp duplex DNA (185 nM) containing a central G/T mismatch. The Kd and B max (see “Materials and Methods”) were determined by fitting the data to a square hyperbola. In the absence of DNA, Kd = 0.74 \( \pm 0.10 \mu M \) ATP\( \gamma S \) and B max = 0.45 \( \pm 0.01 \) pmol ATP\( \gamma S \); in the presence of G/T DNA, Kd = 2.03 \( \pm 0.13 \mu M \) ATP\( \gamma S \) and B max = 0.46 \( \pm 0.01 \mu M \) ATP\( \gamma S \). B, salt dependence (NaCl) of ATP\( \gamma S \) (2 \( \mu M \)) binding to hMSH2-hMSH6 (80 nM) in the presence or absence of a 41-bp duplex DNA (185 nM) containing a central G/T mismatch.

**Fig. 4.** Binding of hMSH2-hMSH6 to ATP\( \gamma S \) in the presence or absence of DNA. A, filter binding activity of hMSH2-hMSH6 (100 nM) binding to ATP\( \gamma S \) in the absence or presence of a 41-bp duplex DNA (185 nM) containing a central G/T mismatch. The Kd and B max (see “Materials and Methods”) were determined by fitting the data to a square hyperbola. In the absence of DNA, Kd = 0.74 \( \pm 0.10 \mu M \) ATP\( \gamma S \) and B max = 0.45 \( \pm 0.01 \mu M \) ATP\( \gamma S \); in the presence of G/T DNA, Kd = 2.03 \( \pm 0.13 \mu M \) ATP\( \gamma S \) and B max = 0.46 \( \pm 0.01 \mu M \) ATP\( \gamma S \). B, salt dependence (NaCl) of ATP\( \gamma S \) (2 \( \mu M \)) binding to hMSH2-hMSH6 (80 nM) in the presence or absence of a 41-bp duplex DNA (185 nM) containing a central G/T mismatch.

**Fig. 5.** Comparison of ATP-induced dissociation of DNA from hMSH2-hMSH6 at low and high NaCl concentrations. G/T or G/C DNA substrates refers to a 41-bp duplex DNA containing a central G/T or G/C base pair as indicated. 100% DNA binding represents the amount of DNA bound at 6 or 110 mM NaCl in the absence of ATP. ATP-induced dissociation of hMSH2-hMSH6 (15 nM) and G/T mismatched DNA (20 nM) at 6 and 110 mM NaCl. B, ATP-induced dissociation of hMSH2-hMSH6 (15 nM) and G/C homoduplex DNA (20 nM) at 6 and 110 mM NaCl.

(\( ATP\gamma S \)) and hMSH2-hMSH6 in the presence of DNA containing a G/T mismatch (185 nM) and found the affinity of hMSH2-hMSH6 for ATP\( \gamma S \) decreased 2.5-fold (Kd \( \approx 2.03 \mu M \)) (Fig. 4A). These results are consistent with the pseudo-uncompetitive behavior of the mismatch-stimulated hMSH2-hMSH6 ATPase (see Ref. 1 and Fig. 3), as well as the single-step ATP hydrolysis results where the amount of prebound ADP was found to decrease in the presence of increasing concentrations of mismatched DNA (see Ref. 1).

Examination of the salt profile revealed that the DNA-dependent inhibition of ATP\( \gamma S \) binding by hMSH2-hMSH6 could be overcome at high salt concentrations (Fig. 4B), which also appears to correlate with a similar salt-induced inhibition of DNA binding (Fig. 1C). Although the DNA binding domain(s) of hMSH2 and hMSH6 have not been elucidated, it has been shown that mutations within Walker A/B consensus adenine nucleotide binding domain that eliminate ATPase activity do not affect mismatch DNA binding (18). These results support the conclusion that there are independent binding sites as well as compulsory ordered binding mechanisms for DNA and ATP as was suggested by Gradia et al. (1).

In addition, we found that both the DNA-dependent inhibition and overall affinity of hMSH2-hMSH6 for ATP\( \gamma S \) were reduced at low salt concentrations (Fig. 4B). These observations led us to test whether the IC50 for ATP-induced dissociation of hMSH2-hMSH6 from mismatched DNA would be altered at low NaCl concentrations (Fig. 5). Previous gel shift analysis identified an IC50 \( \approx 3 \mu M \) (at 100 mM NaCl) for ATP-induced dissociation of hMSH2-hMSH6 from DNA containing a G/T mismatch (1). Using a filter binding assay (see “Materials and Methods”), we determined a similar IC50 \( \approx 10 \mu M \) (at 110 mM NaCl) for ATP-induced dissociation of hMSH2-hMSH6 from mismatched DNA (Fig. 5A). Interestingly, the IC50 for ATP-induced dissociation of hMSH2-hMSH6 from mismatched DNA at low salt concentrations (Fig. 5B).
TABLE III
Stimulation of hMSH2-hMSH6 ATPase activity by heteroduplex and homoduplex DNA substrates of varying lengths

ATPase assays were performed by incubating hMSH2-hMSH6 with 100 μM ATP and varying concentrations of 41, 125, 251, and 501-base pair heteroduplex (het) or homoduplex (home) DNA substrates. Heteroduplex DNA consists of an equimolar mixture of molecules with a central G/T or C/A heteroduplex base pair (as described under “Materials and Methods”). The data generated (as shown in Fig. 6) was fit to the Michaelis-Menten equation. $K_{1/2}$ and $K_{16}$ (concentration of DNA corresponding to half-maximal velocity) values are presented with standard deviations. $K_{16}$ values are presented in terms of both DNA molecules and DNA base pairs.

| DNA substrate     | $k_{cat}$ | $K_{1/2}$ DNA molecules | $K_{1/2}$ base pairs |
|-------------------|-----------|-------------------------|----------------------|
| 41-bp het         | 9.92 ± 0.49 | 40.0 ± 6.6              | 1.64 ± 0.27          |
| 125-bp het        | 8.94 ± 0.21 | 17.3 ± 1.8              | 2.17 ± 0.23          |
| 251-bp het        | 8.32 ± 0.40 | 15.7 ± 2.5              | 3.95 ± 0.63          |
| 501-bp het        | 8.33 ± 1.34 | 11.5 ± 3.7              | 5.75 ± 1.84          |
| 41-bp homo        | 6.42 ± 0.04 | 253.2 ± 41.8            | 10.39 ± 1.71         |
| 125-bp homo       | 5.74 ± 1.34 | 259.4 ± 108.6           | 32.43 ± 15.57        |
| 251-bp homo       | 5.38 ± 0.69 | 46.2 ± 16.3             | 11.58 ± 4.08         |
| 501-bp homo       | 5.26 ± 1.38 | 45.7 ± 24.5             | 22.88 ± 12.31        |

DNA increased to ~50 μM at low salt (6 mM) (Fig. 5A). These results indicate an apparent preference for mismatched DNA over ATP at low salt concentrations and the possibility that the reduced ATPase was tied to the an inability of hMSH2-hMSH6 to appropriately process adenosine nucleotide and/or form a hydrolysis-independent sliding clamp. It is interesting to note that the addition of saturating amounts of ATP released 90% of the hMSH2-hMSH6 from the mismatched DNA at low salt (6 mM NaCl), thus further minimizing the role of ATP hydrolysis in hMSH2-hMSH6 dissociation.

Although hMSH2-hMSH6 binding to homoduplex DNA is substantially weaker than binding to DNA containing a G/T mismatch, we observed a significant ATP-induced dissociation of hMSH2-hMSH6 from homoduplex (G/C) DNA. This result is intriguing and suggests an adenosine nucleotide induced binding and release mechanism that is similar to that observed for mismatched DNA. Further analysis is necessary to determine whether this interaction contributes to mismatch recognition or whether it is nonspecific.

Length-dependent Stimulation of the hMSH2-hMSH6 ATPase—An extensive mathematical analysis of hydrolysis-dependent translocation by DNA helicases has suggested that the length of the DNA lattice on which translocation occurs could influence its enzyme concentration-dependent maximum velocity ($k_{cat}$-DNA) and/or its DNA dependence ($K_{DNA}$) (39; see the Addendum). We have examined the stimulation of the hMSH2-hMSH6 ATPase by DNAs ranging in length from 41 to 501 bp at a physiological relevant salt concentration (Fig. 6). These PCR-derived DNA substrates contain an equal molar mixture of G/T and C/A mismatched molecules within identical sequence contexts. Data were fit to the Michaelis-Menten equation, and the resulting $k_{cat}$-DNA and $K_{DNA}$ values are displayed in Table III. A small but reproducible (n = 3) decrease in the $k_{cat}$-DNA was observed in the presence of both mismatched and homoduplex DNA. This decrease in $k_{cat}$-DNA with increasing DNA length is opposite to that found with E. coli helicase II (40) and predicted by a theoretical analysis of ATP-dependent transloconce (39). Moreover, we observe a 3–4-fold decrease in the $K_{DNA}$ (in units of DNA molecules) and/or an equivalent increase in the $K_{base}$ pairs (in units of DNA base pairs) as the length of the mismatched DNA is increased from 41 to 501 bp. By comparison we found no consistent change in $K_{DNA}$ (DNA molecules or base pairs) with varying homoduplex (G/C) DNA lengths. The larger standard deviations for homoduplex (G/C) $K_{DNA}$ may reflect the uncertainty of curve fitting with these shallower binomial functions.

Our findings contrast those of Blackwell et al. (19) who have reported a “significant” increase (approximately one half the order of magnitude as shown here) in the $k_{cat}$-DNA of hMSH2-hMSH6 in the presence of increasing length homoduplex and mismatched DNAs. These differences could be the result of nonuniform substrates or biochemical conditions. For example,
the ATPase assays contained in Blackwell et al. (19) were performed at 50 mM KCl, a concentration at which hMSH2-hMSH6 shows no ATPase discrimination between mismatched and homoduplex (G/C) DNA (Fig. 2). The length-dependent ATPase studies described here were performed at the peak salt concentration (110 mM) for both the ATPase and MMR in vitro (Fig. 2 and Ref. 19). Moreover, the mismatch context varied significantly in Blackwell et al. (19).

The modest decrease in at least the $k_{cat, DNA}$ with increasing DNA length appears to support the Molecular Switch model (see the Addendum), although we recognize the limitations of such meager alterations. However, the decrease in $k_{cat, DNA}$ clearly suggests that further studies of the length dependence are warranted. Although we cannot rule out the possibility that hydrolysis associated with a translocation event may occur in steps greater than 250 bp (the arm length of the 501-bp substrate), it is worth noting that comparison of linear 2.9-kilobase heteroduplex DNA to an equal amount of the same length circular DNA yielded a decrease in the velocity of the hMSH2-hMSH6 ATPase (2). In this latter case the Hydrolysis-Driven Translocation model would predict an increase in ATPase velocity as the DNA length was increase from a linear 2.9-kilobase DNA to a circular molecule of infinite length (40).

**DISCUSSION**

Following our initial report (1), there now appears to be general agreement that mismatched DNA stimulates ATP hydrolysis by hMSH2-hMSH6 (19). However, there is substantial dissent regarding the role of ATP hydrolysis in the mechanism of MMR (2, 3, 5). The Hydrolysis-Driven Translocation model suggests that mismatched DNA activates an ATP hydrolysis translocation of hMSH2-hMSH6 along the DNA backbone (5). The Molecular Switch model suggests that mismatched DNA provoked ADP$\rightarrow$ATP exchange results in an ATP-bound sliding clamp that can diffuse along the DNA backbone with subsequent ATP hydrolysis upon release from DNA, which recycles the recognition complex (2, 3). The results presented here appear to provide accumulating support for the Molecular Switch model.

Although the competing MMR mechanisms accomplish the same goal of transducing a mismatch signal along the DNA backbone to downstream repair machinery, the concept of a “threshold signaling” mechanism via a Molecular Switch versus the “single-event signaling” of Hydrolysis-Driven Translocation may not be immediately obvious. In the Molecular Switch model, we have proposed and demonstrated that multiple molecules of hMSH2-hMSH6 may become associated with the mismatched DNA (2); once a mismatch provoked ADP$\rightarrow$ATP exchange event occurs, the complex forms a hydrolysis-independent sliding clamp that diffuses away from the mismatch, which then leaves it exposed for subsequent binding, adenosine nucleotide exchange, and further rounds of stochastic bidirectional clamp formation events. It would be the threshold number of molecules in the ATP-bound sliding clamp form that ultimately transduces the signal that a mismatch is present in the DNA and is similar to G protein-mediated signaling events. Moreover, the timing and/or assembly of the MMR machinery would be authorized by this threshold signal. Hydrolysis of ATP in this model merely recycles the switch in a continuous turnover process, also similar to G protein-mediated signaling events. In our in vitro system, this hydrolysis occurs when the molecules transit a free end (1). However, in vivo the hydrolysis may be intrinsic or driven by an ATPase accelerator protein similar to GTPase-activating proteins (GAPs) in the G protein signaling system. The Hydrolysis-Driven Translocation model suggests that a single-binding event results in MMR by controlled motoring to the downstream repair machinery. Because it is unclear how many ATP molecules would be required to propel either of these models, the “efficiency” of the process cannot be compared. However, it would appear unlikely that they are significantly different.

Although part of the disagreement between these models resides in the interpretation of similar observations, there is also some deviation in data that might be traced to biochemical conditions. One of the interpretation differences resides in binding of adenine nucleotide, which results in release of hMSH2-hMSH6 from the mismatched nucleotides. It has been proposed that mispair binding occurs in an adenosine nucleotide-free state of hMSH2-hMSH6 and is based on the observation that ATP is capable of promoting the release of hMSH2-hMSH6 from a mismatched DNA substrate (5). The $t_{1/2}$ of this release is approximately 10-fold slower than that observed for ATP and approximately 20-fold faster than in the absence of nucleotide. We have found that both hMSH2-hMSH6 and hMSH2-hMSH3 can adopt distinct conformations (as determined by partial proteolysis) dependent on whether they are bound by ADP, ATP, or in the absence of adenosine nucleotide (2, 34). A binding constant in the low $\mu M$ range (Fig. 4 and Ref. 1) appears to suggest that hMSH2-hMSH6 is associated with adenosine nucleotide at most times in vivo and that the physiologically significant mismatch recognition form would be ADP-bound. Thus, the release of hMSH2-hMSH6 by ADP, as measured by plasmon resonance methodology, might reflect a transition/reversion to a physiologically significant equilibrium binding form (which may dissociate in the continuous-flow Biacore plasmon resonance system). It is possible that separate comparison of the on rate and the off rate of the ADP-bound and adenosine nucleotide-free hMSH2-hMSH6 by plasmon resonance may be informative. These studies are in progress. With regard to the Molecular Switch model, we consider the possibility that mispair recognition promotes the release of ADP much as guanine nucleotide exchange factors enhance the release of GDP from signaling G proteins.

In the present form of the Hydrolysis-Driven Translocation model, the role of a mismatched nucleotide is merely to target the hMSH2-hMSH6 to the mismatched DNA (5). Importantly, there does not appear to be a role for mismatch provoked ADP$\rightarrow$ATP exchange. Here we have demonstrated that individual mismatch-provoked ADP$\rightarrow$ATP exchange is unique and rate-limiting as predicted by the Molecular Switch model. This conclusion is underlined by the observation that the hierarchy of mismatched DNA provoked ATPase by hMSH2-hMSH6 largely correlates with the hierarchy of MMR in vitro and in vivo.

One of the most important distinctions raised by Blackwell et al. (19) is that a Hydrolysis-Driven Translocation model should result in a DNA length-dependent increase in $k_{cat, DNA}$ similar to that observed with DNA helicase II (40). We have examined the DNA length dependence of both the $k_{cat, DNA}$ and $K_{M, DNA}$ using a series of DNA substrates that contain a single mismatch imbedded in an identical sequence context. In contrast to the predictions of the Hydrolysis-Driven Translocation model, we find no increase in the $k_{cat, DNA}$ for either heteroduplex (G/T) or homoduplex (G/C) DNA. We consider the possibility that the low salt conditions or the use of DNA substrates containing multiple mismatched nucleotides contributed to the different $k_{cat, DNA}$ observations (19). The modest decrease in the $k_{cat, DNA}$ as a function of DNA length appears to be predicted by a kinetic model for an ATPase that is controlled by structure-provoked ADP$\rightarrow$ATP exchange, hydrolysis-independent diffusion along the DNA backbone, and subsequent hydrolysis upon reaching an end and/or dissociating from the DNA (see the Addendum). This kinetic model also predicts a DNA length-de-
pendent decrease in the $K_{m}$, which can be interpreted to suggest that as the length of the mismatched DNA increases, the apparent affinity for the mismatched DNA increases. One could imagine that the increasing length of time in which a hydrolysis-independent sliding clamp may be associated with a DNA of increasing length might be translated to an apparent decrease in the $K_{m}$. However, another intriguing possibility is that hMSH2-hMSH6 associates with the DNA in a “search mode” prior to mismatch recognition and ADP→ATP exchange. Thus, the longer the DNA substrate, the more likely it may associate in this search mode prior to mismatch recognition and ADP→ATP exchange. It is important to note that the role of the MutL homologs is unknown and may modify the function of the MutS homologs.

**Acknowledgments**—We thank our laboratory colleagues for helpful discussions, M. Germann for advice DNA binding analysis, H. Adler and the Kimmel Nucleic Acid Facility for preparation of oligonucleotides, and P. von Hippel for helpful discussions and review of the kinetic derivations contained in the Addendum.

**Addendum**—A steady-state ATPase rate description for a hydrolysis-independent sliding clamp can be formulated based on partition analysis and the concept of net rate constants first described by Cleland (41). The method is similar to that described by Young et al. (39) for ATP hydrolysis-driven translocases. A description of the kinetic steps and net rate constants associated with hMSH2-hMSH6 mismatch recognition $(k_{1})$, adenine nucleotide exchange $(k_{2})$, hydrolysis-independent enzymatic transition $(k_{3})$, and ATP hydrolysis $(k_{4})$ is shown in Scheme III. In this scheme, it is clear that the rate of ATP hydrolysis depends on the formation of E*DNA, which is the terminal hydrolysis-independent translocation species of the protein.

$$
\frac{k_{\text{cat}}h_{\text{DNA}}}{[E_{o}]_{\text{DNA}}} = \frac{[E_{o}]\
\text{DNA}}{k_{d}(k_{1}+k_{3}) + [\text{DNA}]} 
$$

(Eq. 3)

Consideration of Scheme III and the concept of net rate constants (41) leads to an expression for the steady-state rate of ATP hydrolysis (Equation 3) where $[E_{o}]$ is the total enzyme concentration and [DNA] represents the concentration of DNA on a molecular basis. Such a steady-state function is similar to that derived by Young et al. (39) and contains terms for $V_{\text{max}}$ and $k_{\text{cat}}$. We have found that these terms are most conveniently expressed as $h_{\text{cat, DNA}}$ (the product of $V_{\text{max}}$ and the total enzyme concentration [E$_{o}$]) and $K_{d, \text{DNA}}$ ($K_{d}$). The expression for $h_{\text{cat, DNA}}$ is shown in Equation 4.

$$
K_{1/2, \text{DNA}} = \frac{k_{d}(k_{1}+k_{3})}{k_{1}(k_{1}+k_{3})} 
$$

(Eq. 7)

$$
K_{1/2, \text{DNA}} = \frac{k_{d}(k_{1}+k_{3})}{k_{1}(k_{1}+k_{3})} 
$$

(Eq. 8)

$$
K_{1/2, \text{DNA}} = \frac{k_{d}h_{\text{DNA}}(\lambda+1) + k_{1}2h_{\text{DNA}}}{k_{d}h_{\text{DNA}} + k_{1}2h_{\text{DNA}}} 
$$

(Eq. 9)

Because the term $h_{\text{DNA}}$ contains the lattice length dependence $(\lambda)$, we have solved Equation 4 using the derivations of length dependence shown in Equations 1 and 2 (see Equations 5 and 6). Assuming a unidirectional or biased diffusion mechanism, $h_{\text{cat, DNA}}$ appears inversely proportional to the DNA length (1/λ) (Equation 5), whereas $h_{\text{cat, DNA}}$ appears inversely proportional to the square of the length (1/λ$^{2}$), assuming a random walk diffusion mechanism (Equation 6). There do not appear to be enough data points in Table III to distinguish between unidirectional and random walk mechanisms. However, the $h_{\text{cat, DNA}}$ for both heteroduplex (G/T) and homoduplex (G/C) DNA appears inversely proportional to the DNA length, which is consistent with the molecular switch model (derived above), and appears inconsistent with an ATP Hydrolysis-Driven Translocation model (see Ref. 39).

$$
K_{1/2, \text{DNA}} = \frac{k_{d}(h_{\text{DNA}}+k_{3})}{k_{1}(h_{\text{DNA}}+k_{3})} 
$$

(Eq. 7)

$$
K_{1/2, \text{DNA}} = \frac{k_{d}(h_{\text{DNA}}+k_{3})}{k_{1}(h_{\text{DNA}}+k_{3})} 
$$

(Eq. 8)

$$
K_{1/2, \text{DNA}} = \frac{k_{d}h_{\text{DNA}}(\lambda+1) + k_{1}2h_{\text{DNA}}}{k_{d}h_{\text{DNA}} + k_{1}2h_{\text{DNA}}} 
$$

(Eq. 9)

We have additionally solved the $h_{\text{DNA}}$ term embedded in $K_{1/2, \text{DNA}}$ (Equation 7), assuming a unidirectional or biased walk diffusion mechanism (Equation 8) or a random walk diffusion mechanism (Equation 9). Inspection of Equations 8 and 9 reveals the length dependence term (λ) in both the numerator and denominator. However, the numerator term also contains the reversible kinetic constant $k_{-1}$, which may be considered to be inordinately low in the case of hMSH2-hMSH6 mismatch binding. This is because the forward kinetic rate constant, $k_{1}$, is coupled with ADP→ATP exchange and the formation of a hydrolysis-independent sliding clamp. Reversibility of this process would appear substantially reduced compared with the forward rate. A discounted influence of the numerator term containing the length dependence (λ) suggests that $K_{1/2, \text{DNA}}$ should be inversely proportional to the DNA length for both the unidirectional or biased (1/λ) and random walk mechanisms (1/λ$^{2}$). Although the number of data points is not sufficient to distinguish between the unidirectional or biased versus a random walk diffusion mechanism(s), these derivations are entirely consistent with the results.
presented in Table III. Moreover, the consistency in length dependence of both the $k_{\text{cat, DNA}}$ and $K_{1/2, DNA}$ appear to further support the validity of these kinetic derivations.

REFERENCES
1. Gradia, S., Acharya, S., and Fishel, R. (1997) Cell 91, 995–1005
2. Gradia, S., Subramanian, D., Wilson, T., Acharya, S., Makhor, A., Griffith, J., and Fishel, R. (1999) Mol. Cell 3, 253–261
3. Fishel, R. (1998) Genes Dev. 12, 2096–2101
4. Allen, D. J., Makhor, A., Grilley, M., Taylor, J., Thresher, R., Modrich, P., and Griffith, J. D. (1997) EMBO J. 16, 4467–4476
5. Blackwell, L. J., Martik, D., Bjornson, K. P., Bjornson, E. S., and Modrich, P. (1998) J. Biol. Chem. 273, 32055–32062
6. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) DNA Repair and Mutagenesis, American Society for Microbiology, Washington, D. C.
7. Fishel, R., Lescoe, M. K., Rao, M. R., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. (1994) Cell 75, 1027–1038
8. Bocker, T., Ruschoff, J., and Fishel, R. (1999) Biochim. Biophys. Acta 31, 1–10
9. Fishel, R., and Wilson, T. (1997) Curr. Opin. Genet. Dev. 7, 105–113
10. Drummond, J. T., Li, G.-M., Longley, M. J., and Modrich, P. (1995) Science 268, 1909–1912
11. Marsischky, G. T., Files, N., Kane, M. F., and Kolodner, R. (1996) Genes Dev. 10, 407–420
12. Acharya, S., Wilson, T., Gradia, S., Kane, M. F., Guerrette, S., Marsischky, G. T., Kolodner, R., and Fishel, R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13629–13634
13. Risinger, J. I., Umar, A., Boyd, J., Berchuck, A., Kunkel, T. A., and Barrett, J. C. (1996) Nat. Genet. 14, 102–105
14. Palombo, F., Iaccarino, I., Nakajima, E., Ikejima, M., Shimada, T., and Jiricny, J. (1996) Curr. Biol. 6, 1181–1184
15. Hafer, L. T., and Walker, G. C. (1991) EMBO J. 10, 2707–2715
16. Chi, N. W., and Kolodner, R. D. (1994) J. Biol. Chem. 269, 29993–29997
17. Alani, E., Sokolsky, T., Studamire, B., Miret, J. J., and Lahue, R. S. (1995) Mol. Cell. Biol. 17, 2436–2447
18. Iaccarino, I., Marra, G., Palombo, F., and Jiricny, J. (1998) EMBO J. 17, 2657–2667
19. Blackwell, L. J., Bjornson, K. P., and Modrich, P. (1998) J. Biol. Chem. 273, 32049–32054
20. Grilley, M., Welsh, K. M., Su, S. S., and Modrich, P. (1989) J. Biol. Chem. 264, 1000–1004
21. Su, S. S., Lahue, R. S., Su, K. G., and Modrich, P. (1988) J. Biol. Chem. 263, 6829–6835
22. Kramer, B., Kramer, W., and Fritz, H.-J. (1984) Cell 38, 879–887
23. Wernig, H., Steger, C., Riesner, D., and Fritz, H. J. (1986) Nucleic Acids Res. 14, 3773–3790
24. Fishel, R. A., and Kolodner, R. (1983) UCLA Symp. Mol. Cell. Biol. New Series 11, 309–324
25. Motulsky, H. J. (1999) Analyzing Data with Graphpad Prism, Graphpad Software Inc., San Diego, CA
26. Alani, E. (1996) Mol. Cell. Biol. 16, 5604–5615
27. Kramer, B., Kramer, W., and Fritz, H. J. (1984) Cell 38, 879–887
28. Bishop, D. K., Andersen, J., and Kolodner, R. D. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 3713–3717
29. Holmes, J. J., Clark, S., and Modrich, P. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5837–5841
30. Thomas, D. C., Roberts, J. D., and Kunkel, T. A. (1991) J. Biol. Chem. 266, 3744–3751
31. Parsons, R., Li, G.-M., Longley, M. J., Fang, W.-H., Papadopoulos, N., Jen, J., de la Chapelle, A., Kinzler, K. W., Vogelstein, B., and Modrich, P. (1993) Cell 75, 1227–1236
32. Fang, W. H., and Modrich, P. (1993) J. Biol. Chem. 268, 11838–11844
33. Genschel, J., Littman, S. J., Drummond, J. T., and Modrich, P. (1998) J. Biol. Chem. 273, 19895–19901
34. Wilson, T., Guerrette, S., and Fishel, R. (1999) J. Biol. Chem. 274, 21659–21664
35. Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1996) Curr. Biol. 6, 1186–1187
36. Sia, E. A., Jinks Robertson, S., and Petes, T. D. (1997) Mutat. Res. 383, 61–70
37. Arthur, P. G., and Hochachka, P. W. (1995) Anal. Biochem. 227, 281–284
38. Rostovtseva, T., and Colombini, M. (1997) Biophys. J. 72, 1954–1962
39. Young, M. C., Kuhl, S. B., and von Hippel, P. H. (1994) J. Mol. Biol. 235, 1436–1446
40. Matson, S. W., and George, J. W. (1987) J. Biol. Chem. 262, 2066–2076
41. Cleland, W. W. (1975) Biochemistry 14, 3220–3224