MutL-catalyzed ATP Hydrolysis Is Required at a Post-UvrD Loading Step in Methyl-directed Mismatch Repair*

Adam B. Robertson[‡], Steven R. Pattishall[‡], Erin A. Gibbons[‡], and Steven W. Matson[‡§¶1]

From the [‡]Department of Biology, the [§]Curriculum in Genetics & Molecular Biology, and the [¶]Program in Molecular & Cellular Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Methyl-directed mismatch repair is a coordinated process that ensures replication fidelity and genome integrity by resolving base pair mismatches and insertion/deletion loops. This post-replicative event involves the activities of several proteins, many of which appear to be regulated by MutL. MutL interacts with and modulates the activities of MutS, MutH, UvrD, and perhaps other proteins. The purified protein catalyzes a slow ATP hydrolysis reaction that is essential for its role in mismatch repair. However, the role of the ATP hydrolysis reaction is not understood. We have begun to address this issue using two point mutants: MutL-E29A, which binds nucleotide but does not catalyze ATP hydrolysis, and MutL-D58A, which does not bind nucleotide. As expected, both mutants failed to complement the loss of MutL in genetic assays. Purified MutL-E29A protein interacted with MutS and stimulated the MutH-catalyzed nicking reaction in a mismatch-dependent manner. Importantly, MutL-E29A stimulated the loading of UvrD on model substrates. In fact, stimulation of UvrD-catalyzed unwinding was more robust with MutL-E29A than the wild-type protein. MutL-D58A, on the other hand, did not interact with MutS, stimulate MutH-catalyzed nicking, or stimulate the loading of UvrD. We conclude that ATP-bound MutL is required for the incision steps associated with mismatch repair and that ATP hydrolysis by MutL is required for a step in the mismatch repair pathway subsequent to the loading of UvrD and may serve to regulate helicase loading.

DNA mismatch repair is the primary mechanism for correcting DNA replication errors (base substitution mismatches and insertion-deletion loops) (1–5) and is also involved in preventing recombination between divergent DNA sequences (6, 7). Thus, an active mismatch repair system helps to ensure the fidelity of chromosomal replication and functions to maintain genomic stability in all organisms (5). Consistent with this idea, defects in mismatch repair genes in human cells have been linked to genomic instability and hereditary colon cancer, underscoring the importance of this repair pathway (8–14).

The methyl-directed mismatch repair (MMR)[2] pathway in *Escherichia coli* has been reconstituted *in vitro* (15), and the sequence of events in the mismatch repair process has been well described using this purified system (for reviews see Refs. 1, 16, and 17). In the first step, MutS recognizes the base pair mismatch (18, 19), and then MutL binds to the MutS-DNA complex (20). Both proteins are functional as homodimers, and the $MutS_2$-$MutL_2$ complex has been shown to loop out the DNA in what is presumed to be an active search for the nearest d(GATC) methylation site either 5′ or 3′ to the mismatch (20, 21). Then the $MutS_2$-$MutL_2$ complex stimulates MutH, bound to a hemi-methylated d(GATC) site, to nick the nascent, unmethylated DNA strand (22–24). Once the nascent DNA strand has been nicked, DNA helicase II (UvrD) and the appropriate exonuclease excise the error-containing DNA strand (25, 26). UvrD-catalyzed unwinding commences at the nick and continues past the mismatch, providing ssDNA for cleavage by an exonuclease (5). The resulting gap is filled by DNA polymerase III, and DNA ligase seals the nick (15).

Importantly, the MMR pathway has bi-directional capability (23, 26, 27). A nick is generated at the hemi-methylated d(GATC) located closest to the mismatch and therefore could exist on either side of the mismatch (25). However, UvrD unwinds DNA with a specific 3′ to 5′ polarity (28). As a result, UvrD must be loaded on the appropriate DNA strand to unwind toward the mismatch. We and others (29–31) have demonstrated a physical interaction between UvrD and MutL that results in a significant stimulation of the unwinding reaction catalyzed by UvrD. Notably, MutL fails to stimulate DNA unwinding catalyzed by other superfamily I DNA helicases,[3] and the unwinding activity of Rep protein (37% identical to UvrD), although slightly enhanced by MutL in helicase assays, is not detectably stimulated on a nicked heteroduplex circular DNA substrate in the presence of MutL and MutS (31). It seems likely that MutL serves to load UvrD onto the nicked DNA substrate with the appropriate polarity to ensure correction of the mismatch, although this has not been shown directly.

The mechanism by which the UvrD-catalyzed unwinding reaction is stimulated by MutL is not fully understood, although several important details of the reaction have been described. For example, on a nicked, circular molecule containing a mismatch, MutS, MutL, and UvrD initiate unwinding at the nick site and begin helix opening in the direction toward the mis-

---

[2] The abbreviations used are: MMR, methyl-directed mismatch repair; ssDNA, single-stranded DNA; AMP-PNP, adenosine 5′-(β,γ-imino)triphosphate.
[3] M. Hall, R. Jordan, and S. W. Matson, unpublished results.

match. This reaction requires all three protein components and the presence of a mismatch (30, 31). Moreover, although MutL dramatically stimulates UvrD-catalyzed DNA unwinding, it does not increase the rate of ATP hydrolysis (29). Recent studies using model DNA substrates are consistent with the notion that MutL acts to load UvrD onto a DNA substrate. MutL appears to be capable of loading multiple molecules of UvrD onto the DNA to allow the unwinding of long duplex regions. At present, there is no direct evidence for an increase in the processivity of the unwinding reaction catalyzed by UvrD in the presence of MutL (32), and we have suggested that the presence of multiple molecules of UvrD on the DNA allows for the unwinding of long duplex regions.

The biochemical activity associated with MutL protein has been a matter of debate for several years. Several laboratories have demonstrated that MutL binds to both ssDNA and double-stranded DNA (32–34), whereas others report that MutL does not bind DNA (35). It is now clear that MutL catalyzes a weak ATPase reaction, and this activity is required for mismatch repair (32, 34, 36, 37). In this report, we investigate the role of MutL-catalyzed ATP hydrolysis in the stimulation of the unwinding reaction catalyzed by UvrD. Using two MutL point mutants, MutL-E29A and MutL-D58A, we demonstrate that ATP-bound MutL is required for interaction with MutS, stimulation of MutH-catalyzed nicking of a hemi-methylated DNA mismatch-containing substrate, and loading of UvrD. MutL-catalyzed ATP hydrolysis is *not* required for either the stimulation of MutH-catalyzed incision at the hemi-methylated d(GATC) site or the stimulation of UvrD. In addition, we show that MutL-E29A stimulates UvrD-catalyzed unwinding with greater efficiency than wild-type MutL, and the ATP-bound form of MutL is responsible for stimulation of UvrD. Together, these results demonstrate that although the ATP-bound form of MutL is required for all steps prior to and including incision, the MutL-catalyzed ATPase activity is required in a step of mismatch repair subsequent to the loading of UvrD.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—*E. coli* BL21(DE3) (F⁻ *ompT* [lon] $hsdS_B r_B^- m_B^-$ *gal* λDE3) was from Novagen, Inc. BL21(DE3)Δ*uvrD* (38), BL21(DE3) *uvrD*::Tn5 *mutL*::Tn10 and GE1752Δ*mutL*::Tn10 (32) were constructed previously in this laboratory.

pET11d, pLysS, and pET3c were from Novagen, Inc. pLitmus28 was from New England Biolabs. M13mp18 ssDNA was purified as previously described (39). The UvrD expression plasmid has been described (38).

Oligonucleotides and Enzymes—Restriction endonucleases, DNA polymerase I (large fragment), and polynucleotide T4 kinase were from New England Biolabs and used as recommended by the supplier. Oligonucleotides were from IDT and were purified on a 20% polyacrylamide, 8 M urea denaturing gel prior to use.

Protein Purification—UvrD was purified as described previously (40). MutL and its various mutant forms were overexpressed prior to purification in BL21(DE3)*uvrD*::Tn5*mutL*::Tn10 containing pET15b-*mutL* in LB media at 37 °C and puri-

fied as described (41). MutH and MutS were expressed and purified as previously described (41).

The concentration of UvrD was determined using the published extinction coefficient of 1.29 ml mg⁻¹ cm⁻¹ (42). The concentration of MutL, MutL point mutants, MutS, and MutH was determined using a Bradford protein assay (Bio-Rad) with bovine serum albumin as a standard.

DNA Substrates—The 750-bp blunt duplex DNA fragment was prepared by digestion of pLitmus28 with DraI followed by treatment with calf intestinal phosphatase (Roche Applied Science) to produce a 5′-OH group on each end. The fragment was isolated from an agarose gel and was subsequently labeled at the 5′-ends using [γ-³²P]ATP and T4 polynucleotide kinase. The ³²P-labeled DNA fragment was separated from unincorporated [γ-³²P]ATP using an A-5M sizing column (Bio-Rad) equilibrated in 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA. The substrate concentration was estimated assuming an 85% recovery of the DNA, and the DNA was used directly in helicase assays.

The 93-bp partial duplex substrate used in standard helicase and DNA binding assays was prepared as described previously (43). The heteroduplex DNA substrate was prepared as described (41).

Mutator Assays—Seven independent cultures of GE1752, GE1752*mutL*::Tn10, GE1752*mutL*::Tn10/pET15b-MutL, GE1752*mutL*::Tn10/pET15b-MutL-E29A, and GE1752*mutL*::Tn10/pET15b-MutL-D58A were grown overnight to saturation at 37 °C in the presence of appropriate antibiotics. Serial dilutions of each cell strain were made, and appropriate dilutions were plated on LB plates containing antibiotics to determine a cell titer and on LB plates containing rifampicin (100 µg/ml) to measure the number of rifampicin-resistant colonies. The plates were incubated at 37 °C overnight, and the colonies were counted.

DNA Binding Assays—DNA binding reaction mixtures (20 µl) contained 25 mM Tris-HCl (pH 7.5), 3 mM MgCl₂, 20 mM NaCl, 5 mM β-mercaptoethanol, 0.5 nM ³²P-labeled DNA (93 base pair partial duplex helicase substrate), 2.9 mM AMP-PNP, and the indicated amount of either MutL or MutL-E29A. The reactions were incubated for 10 min at 37 °C, diluted to a final volume of 1 ml with reaction buffer containing 50 µg/ml bovine serum albumin, and filtered onto nitrocellulose and DEAE filters as described (44). The background values representing DNA retention on the nitrocellulose filter in the absence of protein were typically less than 3% and were subtracted from the binding values reported.

Gel mobility shift reaction mixtures (20 µl) contained 25 mM Tris-HCl (pH 7.5), 3 mM MgCl₂, 20 mM NaCl, 5 mM β-mercaptoethanol, and 1.0 nM ³²P-labeled DNA (5′-end-labeled 50-base oligonucleotide) (32) and 1 mM AMP-PNP. The reaction tubes were incubated for 20 min on ice followed by the addition of 5 µl of 75% (v/v) glycerol; glycerol and loading dyes were added to the control tube that contained only the oligonucleotide. The proteins were diluted in UvrD storage buffer (42).

The samples were immediately loaded onto an 8% polyacrylamide gel (67:1 cross-linking ratio) containing a Tris borate buffer (50 mM Tris, 50 mM borate, and 2.5 mM EDTA). The samples were electrophoresed at a constant voltage of 8 V/cm at 4 °C until the bromphenol blue marker had

migrated to ~1 inch from the bottom of the gel. The results were visualized using Storm 840 phosphorimaging device (Molecular Dynamics).

ATPase Assays—Standard ATPase reaction mixtures (30 μl) contained 25 mM Tris-HCl (pH 7.5), 3 mM MgCl$_2$, 20 mM NaCl, 5 mM β-mercaptoethanol, 50 μg/ml bovine serum albumin, 30 μM (DNA-Pi) M13 ssDNA, 2 mM [α-^{32}P]ATP, and the indicated amount of MutL or one of its mutant derivatives. Reaction mixtures lacking protein were assembled on ice and incubated for 2 min at 37 °C prior to the addition of protein to initiate the reaction. Aliquots (5 μl) were removed at the indicated times; quenched with an equal volume of 33 mM EDTA, 6 mM ATP, 6 mM ADP; and spotted on polyethyleneimine thin layer plates. The plates were developed in 1 M HCOOH, 0.8 M LiCl; air-dried; and analyzed using a phosphorimaging device.

Helicase Assays—Reaction mixtures (20 μl) contained 25 mM Tris-HCl (pH 7.5), 3 mM MgCl$_2$, 20 mM NaCl, 5 mM β-mercaptoethanol, 50 μg/ml bovine serum albumin, 3 mM ATP, a 93-bp partial duplex DNA (final concentration, 2 μM DNA-Pi) or a 750-bp blunt duplex DNA (final concentration, 1 nM DNA molecules), 1.25 or 16 nM UvrD, and a titration of MutL, MutL-E29A or MutL-D58A. The reactions were initiated by the addition of ATP after prewarming the reaction solution to 37 °C for 5 min. The reactions were terminated after 10 min for the 93-bp partial duplex or 20 min for the 750-bp blunt duplex by the addition of 10 μl of stop solution (50% (v/v) glycerol, 68 mM EDTA (pH 8.0), 0.022% (w/v) xylene cyanol, 0.022% (w/v) bromphenol blue, 0.3% (w/v) SDS, 44.5 mM Tris base, and 44.5 mM boric acid). The reaction products were resolved on an 8% native polyacrylamide gel run in 0.5× TBE and 0.1% (w/v) SDS. Polyacrylamide gels were imaged using a phosphor screen (Molecular Dynamics, GE Healthcare) and quantified using ImageQuant on a Storm phosphorimaging device (Molecular Dynamics, GE Healthcare).

MutL-stimulated MutH Nicking Assays—Reaction mixtures (16 μl) contained 20 mM Tris-HCl (pH 7.6), 4 mM MgCl$_2$, 20 mM NaCl, 50 μg/ml bovine serum albumin, 3 mM ATP, 50 ng of covalently closed heteroduplex DNA, 33.8 nM MutS, 1.9 nM MutH, and the indicated concentrations of MutL, MutL-E29A, or MutL-D58A. The reactions were initiated by the addition of MutH, incubated at 37 °C for 15 min, and terminated by the addition of EDTA to a final concentration of 12.5 mM. The samples were resolved on a 0.8% agarose gel run in the presence of ethidium bromide (0.5 μg/ml). To obtain quantitative results the gel was irradiated with UV light for 30 min, stained with ethidium bromide, and destained. Density in the nicked DNA species and the supercoiled DNA species was determined using an Alpha imager.

DNase I Footprinting Assays—Two complementary 91-base pair oligonucleotides (with the exception of a G:T mismatch at position 59 relative to the 5′-end label) were annealed to form a duplex with a specific mismatch. One oligonucleotide (the same oligonucleotide used in the preparation of the 93-bp partial duplex DNA substrate) was phosphorylated using T4 polynucleotide kinase and [γ-^{32}P]ATP, and the two strands were annealed. The annealed DNA substrate was isolated on an 8% native polyacrylamide gel. The appropriate labeled fragment was cut out of the gel and electroeluted at 100 V for 5 h at 4 °C in a TBE buffer. The substrate was then dialyzed overnight at 4 °C against 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 100 mM NaCl.

DNase I footprinting reaction mixtures (10 μl) contained 1 nM ^{32}P-labeled DNA, 25 mM Tris-HCl (pH 7.5), 20 mM NaCl, 100 μg/ml bovine serum albumin, 1 mM dithiothreitol, 5 mM MgCl$_2$, 1 mM ATP, 1 μM MutS, and either 1.3 μM, 650 nM, or 325 nM MutL or MutL-D58A. The reactions were incubated at 30 °C for 15 min to allow protein binding, and then 1 μl of CaCl$_2$ and 1 μl of DNase I were added to final concentrations of 1 mM and 0.5 μg/ml respectively. The reaction was incubated at 30 °C for an additional 4 min. The reactions were stopped using 12 μl of a stop solution containing 80% (v/v) formamide, 1 mM EDTA (pH 8.0), 10 mM NaOH, and 0.05% (w/v) xylene cyanol and bromphenol blue. The samples were boiled for 3 min, loaded onto an 8% denaturing polyacrylamide gel, and electrophoresed at 30 watts for 1.5 h. The gels were soaked in a drying solution of 40% methanol, 10% acetic acid, and 3% glycerol and then dried for 2 h under heat and vacuum. The gels were imaged using a Storm phosphorimaging device (Amersham Biosciences) and quantified using ImageQuant software.

Isothermal Titration Calorimetry—The experiments were performed in a Microcal VP-ITC microcalorimeter (Northampton, MA). The reaction mixture (2.1 ml) contained 50 mM Na-P$_i$ (pH 7.0), 37 μM MutL-E29A or 26.3 μM MutL-D58A, 200 mM NaCl, and 3 mM MgCl$_2$. ATP (1.43 mM for MutL-E29A or 526 μM for MutL-D58A) in reaction buffer was injected using a 600-μl syringe rotating at 300 rpm. The injection volumes were 2.5 μl added over 5 s for MutL-E29A or 5.0 μl added over 10 s for MutL-D58A. The time between injections was 180 s. The experiments were performed at 25 °C.

Circular Dichroism—Circular dichroism measurements were made in an Applied Photosystems Pistar-180 CD spectrometer. MutL and MutL-D58A protein concentrations were 3.8 and 3.9 μM, respectively, in CD buffer (150 mM NaF and 50 mM NaPi, pH 7.0). Circular dichroism measurements were taken from 185 to 260 nm in 0.2-nm increments. The data were analyzed using CDpro (lamar.colostate.edu/~sreeram/CDPro/main.html) software using three algorithms (selcon3, CONTINLL, and ProtSS).

RESULTS

Previous studies have shown that MutL catalyzes ATP hydrolysis (36) and that MutL-catalyzed ATP hydrolysis is required for MMR (37, 45). However, the rate of MutL-catalyzed ATP hydrolysis is slow in the presence of ssDNA (~9 min^{-1}) (37), and it seems unlikely this fuels active translocation along the DNA lattice, suggesting that ATP hydrolysis may play another role (46, 47). We and others (29–31) have shown that MutL interacts with and dramatically stimulates the duplex DNA unwinding activity of UvrD. An analysis of this reaction suggested that MutL loads UvrD onto the DNA substrate without significantly increasing the processivity of UvrD-catalyzed unwinding (32). We were interested in determining whether stimulation of the UvrD-catalyzed

unwinding reaction required the hydrolysis of ATP by MutL. If this were the case, then the MutL-catalyzed ATP hydrolysis requirement in MMR might be explained by the requirement for ATP hydrolysis in loading UvrD to begin resection of the damaged DNA strand. Such a requirement would be consistent with the characterization of MutL as a molecular matchmaker (48).

MutL-E29A Lacks Detectable ATPase Activity—To test this hypothesis two MutL point mutants, an ATP hydrolysis-defective mutant and an ATP binding-defective mutant, were constructed using site-directed mutagenesis. Based on the available three-dimensional structure of the amino-terminal domain of MutL (36) and previous studies (45, 50), several mutants were constructed, and the mutant proteins were purified, including MutL-E29A, MutL-E32K, MutL-N33A, MutL-R95F, MutL-D58N, and MutL-D58A. Each of the mutant proteins was purified and judged to be greater than 95% pure as determined by SDS-PAGE (Fig. 1). In each case, the purification properties were the same as those of the wild-type protein. The final step in the purification procedure involved gel filtration, and all of the MutL proteins used in this study eluted from this column at the position expected for a MutL dimer. We note that MutL-D58A migrates slightly slower than MutL and the other point mutants shown in SDS-PAGE. The reason for this is not clear. Sequencing of the mutant gene indicates the absence of any unintended mutations, and CD spectra indicate that the native protein is properly folded.
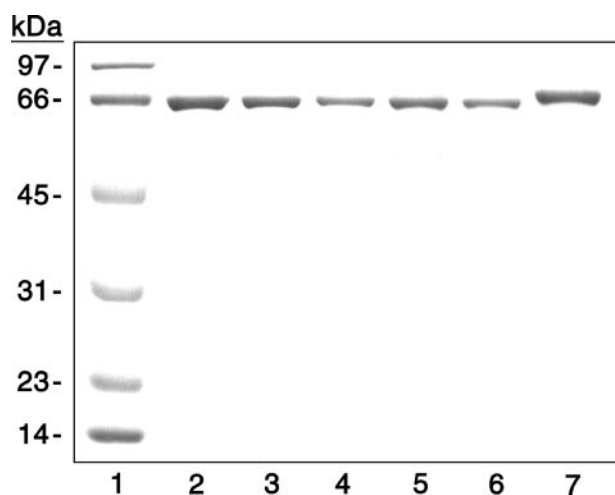
A ssDNA-stimulated ATPase assay was used to evaluate the ATP hydrolysis reaction catalyzed by each mutant as compared with the wild-type protein (Fig. 2 and data not shown). Wild-type MutL catalyzes a weak, but detectable, DNA-stimulated ATP hydrolysis reaction with a k_{cat} of ~9 min^{-1}. This value is in good agreement with a previously reported value for this protein (37) but higher than a recently reported value (45). The reason for this discrepancy is not clear but may be related to the ssDNA effector used in the studies reported here. We have used circular M13 ssDNA to stimulate the MutL ATPase reaction because several of the substrates used in DNA helicase activity assays are based on circular DNA molecules.

The MutL-N33A mutant protein exhibited a reduced rate of ATP hydrolysis, but ATPase activity was still clearly detectable (Fig. 2). MutL-E32K and MutL-R95F also exhibited slow rates of ATP hydrolysis (data not shown). A reduced, relative to wild type, ATPase activity associated with the MutL-R95F mutant has been reported previously (45). The ATPase activity of MutL-E29A, on the other hand, was essentially undetectable (Fig. 2). Even at high protein concentration a reproducible rate of ATP hydrolysis could not be measured (data not shown). Therefore, MutL-E29A was chosen to analyze the requirement for MutL-catalyzed ATP hydrolysis on stimulation of the unwinding reaction catalyzed by UvrD.

As expected, MutL-D58A, which does not bind ATP (see Fig. 5), failed to catalyze the hydrolysis of ATP (data not
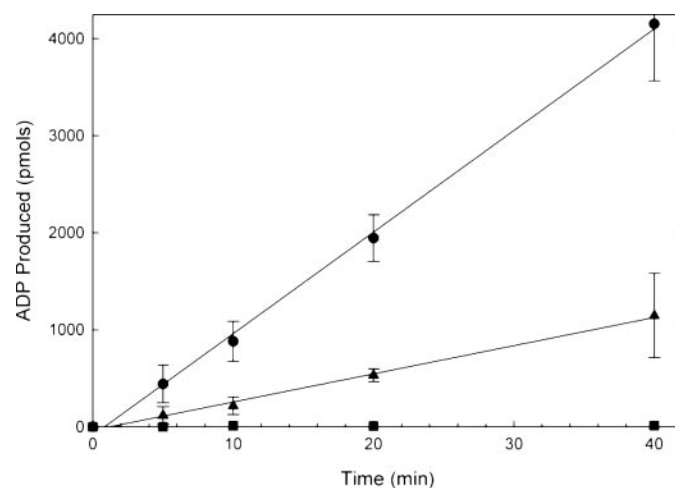

FIGURE 1. **SDS-PAGE analysis of MutL and MutL mutants.** The proteins (2 μg) were resolved on a 9.6% polyacrylamide gel run in the presence of SDS and stained with Coomassie Blue. *Lane 1*, molecular mass standards (size in kDa indicated on the *left*); *lane 2*, MutL; *lane 3*, MutL-E29A; *lane 4*, MutL-E32K; *lane 5*, MutL-N33A; *lane 6*, MutL-R95F; *lane 7*, MutL-D58A.

FIGURE 2. **ATP hydrolysis by MutL, MutL-N33A, and MutL-E29A.** DNA-dependent ATPase reactions were as described under "Materials and Methods" using 0.38 μM MutL (●), 0.37 μM MutL-N33A (▲), and 0.5 μM MutL-E29A (■). The reaction mixtures were preincubated at 37 °C prior to the addition of MutL. The aliquots were removed at the indicated times. The data represent the averages of at least three experiments; the *error bars* are the standard deviations about the mean.

TABLE 1

MutL-E29A and MutL-D58A exhibit a mutator phenotype

Strain	Relevant genotype	Mutation frequency[a]	Range[b]
GE1752	*mutL*[+]	3.3×10^{-8}	1.2–8.2×10^{-8}
GE1752*mutL*::Tn10	*mutL*[−]	7.0×10^{-6}	2.7–15.0×10^{-6}
GEΔ*mutL*/pET15b-MutL	*mutL*[+]	0.8×10^{-8}	0.3–2.0×10^{-8}
GEΔ*mutL*/pET15b-MutL-D58A	*mutL-D58A*	3.9×10^{-6}	2.0–6.7×10^{-6}
GEΔ*mutL*/pET15b-MutL-E29A	*mutL-E29A*	4.2×10^{-6}	3.3–9.2×10^{-6}

[a] The median mutation frequency is reported.
[b] The range of mutation frequencies obtained in the experiment is reported.
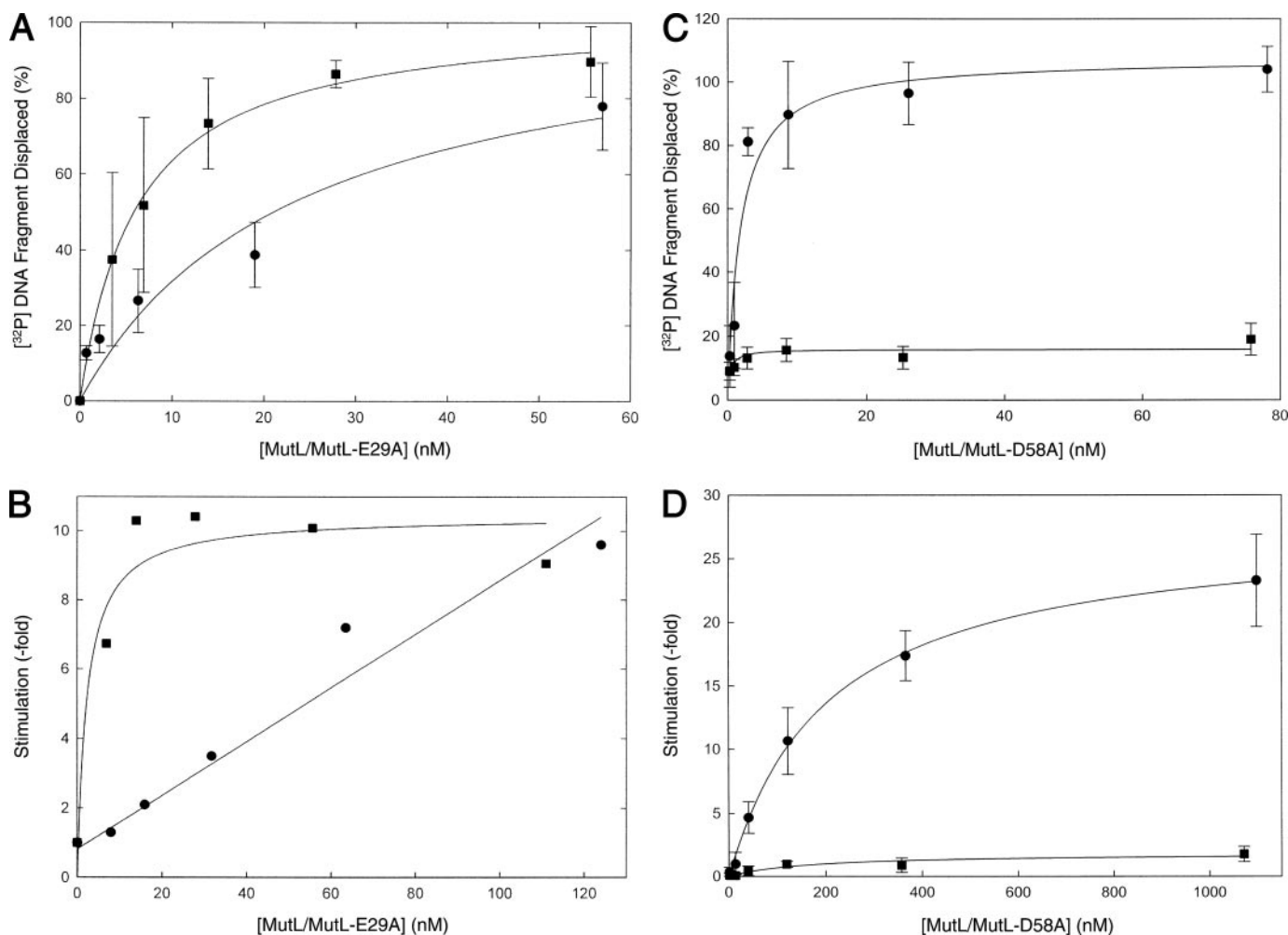
FIGURE 3. **MutL-E29A stimulates the helicase reaction catalyzed by UvrD.** DNA helicase activity assays were as described under "Materials and Methods." *A* and *C*, a 93-bp partial duplex DNA was incubated with UvrD (1.3 nM) and the indicated concentrations of MutL (●), MutL-E29A (■, *A*), or MutL-D58A (■, *C*) for 10 min at 37 °C. The products of the reaction were resolved on an 8% native polyacrylamide gel and quantified as indicated under "Materials and Methods." The data represent the averages of at least three experiments with *error bars* indicating the standard deviations about the mean. *B* and *D*, a 750-bp blunt duplex DNA was incubated with UvrD (16 nM) and the indicated concentrations of MutL (●), MutL-E29A (■, *B*), or MutL-D58A (■, *D*) for 20 min at 37 °C. The fraction of the substrate unwound in the presence of MutL was determined and compared with the fraction of the substrate unwound by UvrD alone to determine the level of stimulation. All of the data presented represent the averages of at least three experiments. Please note the different titrations of MutL used in the various experiments.

shown). It is important to note that MutL-E29A binds ATP and MutL-D58A fails to bind ATP as will be shown below (see Fig. 5). Therefore, MutL-E29A is ATP hydrolysis-defective but ATP binding-proficient, whereas MutL-D58A fails to bind nucleotide.

MutL-E29A and MutL-D58A Fail to Complement the Loss of MutL—Previous studies (37, 45) have shown that MutL-catalyzed ATP hydrolysis is required for MMR. Therefore, neither *mutL-E29A* nor *mutL-D58A* was expected to complement a strain containing a deletion of the *mutL* gene in a genetic complementation assay measuring mutation frequency. In this genetic test the wild-type and mutant proteins were expressed from the expression plasmid that was used for protein purification. It is important to note that neither wild-type nor mutant proteins were overexpressed in these experiments. Rather, expression was dependent on the adventitious use of an RNA polymerase promoter on the plasmid. The *mutL* deletion strain used in these experiments (GE1752*mutL*::Tn10) does not contain the gene encoding T7 RNA polymerase, and, therefore,

basal level expression of T7 RNA polymerase cannot explain the expression of MutL observed in these cells. Western blots using polyclonal antisera directed against MutL have shown the *mutL* deletion strain to be deficient in detectable MutL protein and have indicated that plasmid-based expression of both wild-type and mutant MutL is less than 5-fold higher than normal chromosomal levels (data not shown). Thus, the results obtained in the complementation assays do not reflect an artifact of high level expression of MutL.

Using a standard fluctuation test (51, 52), the frequency of mutation from Rif[S] to Rif[R] was measured using a wild-type strain containing a fully intact MMR system, a strain containing a deletion of the *mutL* gene, a strain in which the *mutL* gene was deleted and complemented using an expression plasmid harboring the wild-type *mutL* gene (pET15b-MutL), and strains in which the *mutL* gene was deleted and complementation was tested using an expression plasmid containing either *mutL-E29A* or *mutL-D58A* (Table 1). The mutation frequency increased by ~100-fold when the *mutL* gene was deleted, con-

sistent with previous reports (45, 53). The *mutL* deletion was effectively complemented by the wild-type protein expressed from pET15b-MutL. Thus, the histidine tag on the amino-terminal end of the protein does not interfere with biological activity as previously shown (53). The MutL-E29A protein and the MutL-D58A proteins did not complement the *mutL* deletion consistent with the previously described requirement for MutL-catalyzed ATP hydrolysis for MMR (37, 45). As indicated above, Western blots have shown that the mutant proteins are expressed (data not shown), and therefore, a lack of protein cannot explain the failure to complement MMR as measured by the increase in mutation rate.

MutL-E29A Stimulated UvrD-catalyzed DNA Unwinding— Purified MutL-E29A and MutL-D58A were compared with wild-type MutL in helicase activity assays that measure the ability of MutL to stimulate the unwinding reaction catalyzed by UvrD (Fig. 3). In these experiments two different DNA substrates were utilized. The first was a partial duplex DNA substrate containing 93 bp of duplex DNA on circular M13 ssDNA. The second substrate was a fully duplex 750-bp DNA fragment. As shown previously (32), MutL dramatically stimulated unwinding of the 93-bp partial duplex substrate by UvrD (Fig. 3*A*). At a concentration of 1.3 nM UvrD, less than 5% of the substrate was unwound in a 10-min incubation. The addition of increasing concentrations of MutL stimulated the unwinding reaction, and at a concentration of 57 nM MutL, nearly 80% of the DNA substrate was unwound. This represents a 20-fold stimulation of the unwinding reaction.

Our previous studies suggest that MutL binds the DNA and loads UvrD onto the substrate (32). Perhaps each loading event is associated with the ATP hydrolysis cycle of MutL. To test this possibility MutL-E29A was used in place of wild-type MutL in these reactions. Remarkably, the hydrolysis-defective mutant protein stimulated UvrD-catalyzed unwinding to essentially the same extent as wild-type MutL (Fig. 3*A*). However, the maximal unwinding reaction was achieved at a lower concentration of the mutant protein. This indicates that MutL-catalyzed ATP hydrolysis is not required for stimulation of the UvrD helicase reaction. Moreover, the ATP hydrolysis-defective mutant stimulated unwinding better than the wild-type protein. The ATP binding-defective MutL mutant, MutL-D58A, failed to stimulate the unwinding reaction catalyzed by UvrD (Fig. 3*C*).

UvrD also unwinds both nicked and fully duplex DNA molecules but at higher protein concentrations (42, 62). Presumably, thermal denaturation of the DNA at a nick or blunt end allows UvrD to bind and initiate an unwinding reaction. The requirement for an increased UvrD concentration in reactions using these two substrates is similar, and the physiologically relevant substrate in MMR is a nicked DNA molecule. In the following experiments we used a blunt duplex DNA substrate with a duplex region of significant length to approximate the conditions that might be encountered *in vivo*, making the assumption that loading at a blunt duplex end will be similar to loading at a nick. As shown previously (32) and in Fig. 3*B*, wild-type MutL stimulated the unwinding of duplex DNA catalyzed by UvrD. At a concentration of 16 nM UvrD, ~4–5% of the 750-bp blunt duplex DNA was unwound
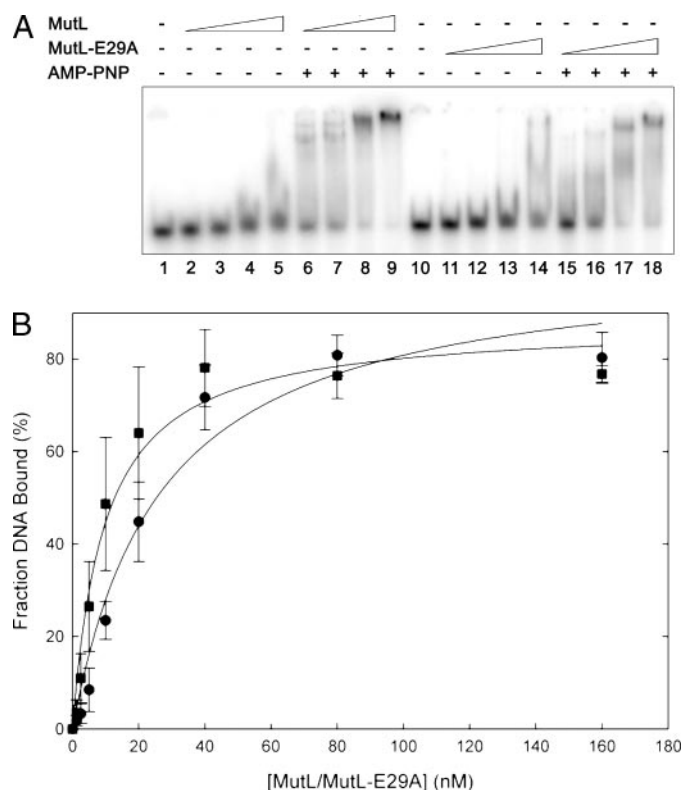

FIGURE 4. **MutL and MutL-E29A bind DNA in the presence of AMP-PNP.** *A*, electrophoretic gel mobility shift assays were as described under "Materials and Methods" using a 5′-end-labeled ssDNA 50-mer and either MutL or MutL-E29A. The MutL and MutL-E29A concentrations used were 22, 44, 88, and 175 nM. AMP-PNP at a final concentration of 3 mM was included in *lanes 6–9* and *lanes 15–18*. *B*, nitrocellulose filter binding assays using a 93-bp ^{32}P-labeled DNA partial duplex ligand were as described under "Materials and Methods" using the indicated concentrations of MutL (●) or MutL-E29A (■). All of the reaction mixtures contained AMP-PNP at a final concentration of 3 mM. The data represent the average of three or more experiments with *error bars* that represent the standard deviations about the mean.

in the absence of MutL. The addition of increasing concentrations of MutL increased the fraction of substrate unwound to greater than 50% representing a 10-fold stimulation. When wild-type MutL was substituted with the ATP hydrolysis-defective MutL-E29A, a similar extent of unwinding was achieved but at significantly lower MutL concentrations (Fig. 3*B*). This is essentially the same result observed using the partial duplex DNA substrate, although the effect is more pronounced at low MutL concentrations on this blunt-ended DNA substrate. Consistent with the results presented above using a partial duplex substrate, MutL-D58A failed to stimulate the UvrD-catalyzed unwinding reaction (Fig. 3*D*).

We conclude that ATP hydrolysis catalyzed by MutL is not essential to load UvrD on either a partial duplex substrate or a substrate with a blunt duplex end. Furthermore, an ATPase-deficient MutL mutant is significantly more efficient than the wild-type protein in promoting this reaction. In addition, these data show that the ATP-bound form of MutL participates in the loading of UvrD, as demonstrated using the MutL-D58A mutant, which failed to stimulate the unwinding reaction using either DNA substrate.

MutL-E29A Binds ATP—The data presented above suggest that although ATP hydrolysis by MutL is not required to load

UvrD onto a DNA substrate, it plays some role in regulating this loading event. That is, loading of UvrD is more efficient in the absence of ATP hydrolysis than it is in the presence of ATP hydrolysis. To gain a better understanding of this reaction, it was important to show that an ATP-bound form of MutL was participating in this reaction. The structure of the amino-terminal ATPase domain of MutL suggests that a mutation in glutamic acid 29 is likely to abrogate ATP hydrolysis but not ATP binding, and a previous study has indicated that MutL-E29A binds ATP (45). Importantly, an understanding of the role of ATP binding and hydrolysis by MutL in MMR requires an understanding of the role of the nucleotide in each partial reaction. Therefore, the ability of MutL-E29A to bind ATP was tested.

Previous studies (34, 35, 41, 45) have indicated that MutL binds ssDNA, and this binding is dependent on the presence of a nucleotide. Thus, we determined the ability of MutL-E29A to bind DNA in the presence of a nonhydrolyzable ATP analog (AMP-PNP), which has been shown to bind MutL at the ATP-binding site (36), and compared this with the ssDNA binding of wild-type MutL (Fig. 4). The data in Fig. 4 (*upper panel*) show the results of gel mobility shift assays using MutL, MutL-E29A, and a ssDNA oligonucleotide ligand. Both proteins bind the oligonucleotide in the presence of AMP-PNP (Fig. 4, *upper panel, lanes 6–9* and *15–18*). Importantly, neither protein bound the DNA to any significant extent in the absence of AMP-PNP (Fig. 4, *upper panel, lanes 1–4* and *10–14*). This is consistent with our previous studies (32) demonstrating the ability of MutL to bind ssDNA in the presence of AMP-PNP and provides indirect evidence that MutL-E29A binds AMP-PNP. If this were not the case then MutL-E29A would not bind the ssDNA oligonucleotide, even in the presence of AMP-PNP.

The binding of MutL and MutL-E29A to the partial duplex DNA substrate used in unwinding assays was also tested using a nitrocellulose filter binding assay (Fig. 4, *lower panel*). This ligand contains a variety of secondary structures including both ssDNA and duplex DNA. Again, both MutL and MutL-E29A bound this DNA molecule in the presence of AMP-PNP. At low protein concentrations MutL-E29A bound this ligand slightly better than wild-type MutL. We conclude, on the basis of DNA binding assays, that MutL-E29A binds ATP as represented by its ability to bind DNA in the presence of AMP-PNP. However, these tests of ATP binding are indirect, and we sought a more direct method to test the binding of ATP by MutL-E29A.

The binding of ATP to MutL-E29A was directly measured using isothermal microcalorimetry, which measures heat change caused by the binding of a ligand to a protein. These data are shown in Fig. 5*A* and clearly indicate the binding of
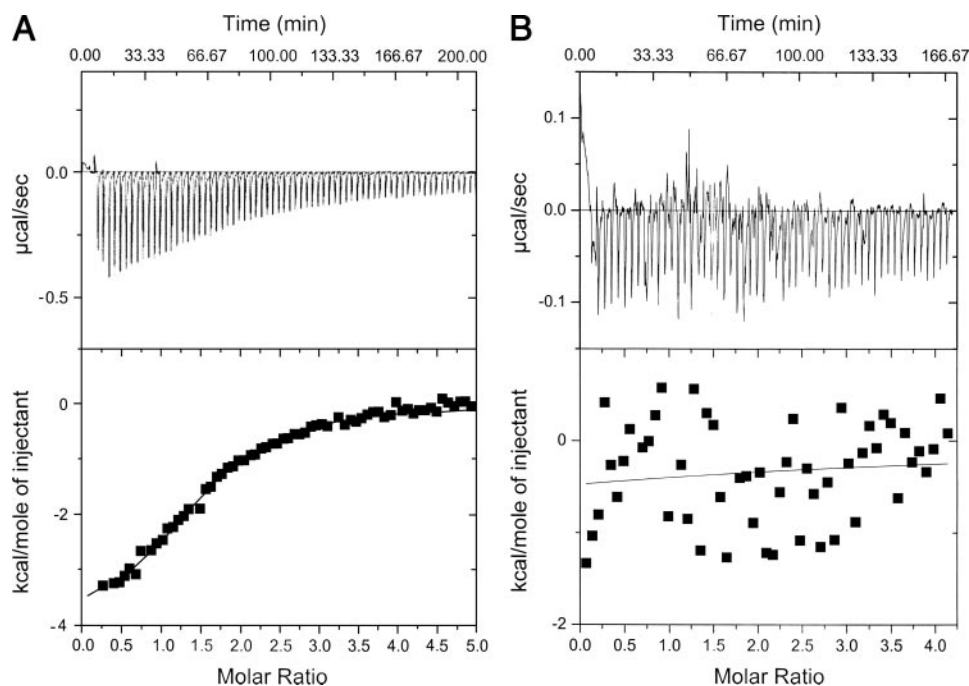

FIGURE 5. **MutL-E29A binds ATP.** Isothermal titration calorimetry was as described under "Materials and Methods" using MutL-E29A (*A*) and MutL-D58A (*B*).

ATP. In this experiment the MutL-E29A concentration was held constant at 37 μM, and the ATP concentration was increased. It is important to note that ATP is not hydrolyzed during the course of this experiment because MutL-E29A does not hydrolyze ATP. We conclude that MutL-E29A binds ATP, and under the conditions used in the experiments shown in Fig. 3, the ATP-bound form of MutL is responsible for stimulating the unwinding reaction catalyzed by UvrD.

Similar experiments were performed with MutL-D58A, which was predicted to have no ATP binding activity (Fig. 5*B*). The data indicate no binding of nucleotide to the protein. This is consistent with the lack of ATP hydrolysis activity and provides evidence supporting our conclusion that the ATP-bound form of MutL stimulates the UvrD-catalyzed unwinding reaction, whereas the ATP-free form of MutL does not.

MutL-E29A Stimulates MutH-catalyzed Nicking, Whereas MutL-D58A Fails to Simulate Nicking—In addition to interacting with UvrD, MutL also interacts with MutS (20) and stimulates the latent endonuclease reaction catalyzed by MutH (20–22). Efficient stimulation of MutH-catalyzed nicking at a hemi-methylated d(GATC) is dependent on the presence of a mismatch base pair and the presence of MutS. However, we have shown that MutL interacts with MutH and is capable of stimulating the MutH-catalyzed nicking reaction on model substrates in the absence of MutS (23). Importantly, this reaction is dependent on the presence of ATP but does not require ATP hydrolysis.

To ensure that MutL-E29A could interact with MutS and MutH and to evaluate the role of ATP hydrolysis in the MutL-stimulated nicking reaction catalyzed by MutH, we examined the ability of these proteins to interact in a functional assay based on a partial reconstitution of the mismatch repair pathway (Fig. 6). In this assay the ability of MutL to stimulate the latent endonuclease reaction catalyzed by MutH at a hemi-
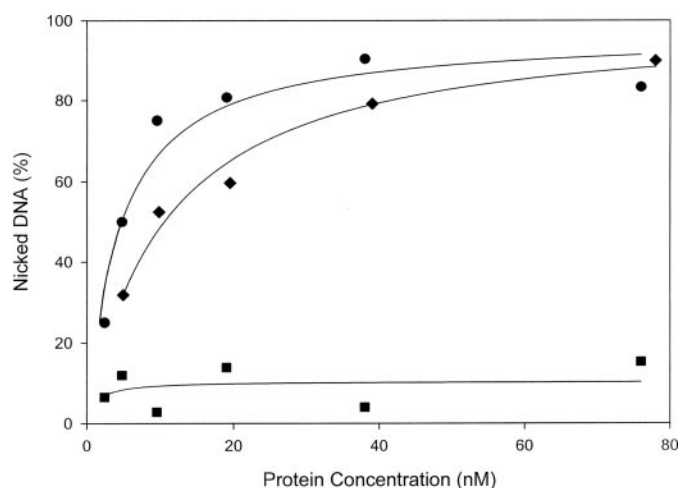
MutL ATPase Activity

FIGURE 6. **MutL-E29A stimulates MutH-catalyzed nicking of heteroduplex DNA.** Reaction mixtures containing either MutL (●), MutL-E29A (♦), or MutL-D58A (■) at the indicated concentrations were as described under "Materials and Methods." The reactions were incubated for 15 min at 37 °C and terminated by the addition of EDTA. The reaction products were resolved on a 0.8% agarose gel run in the presence of 0.5 μg/ml EtBr. The gels were quantified as described under "Materials and Methods." The data represent the averages of at least two determinations.

FIGURE 7. **MutL-D58A does not interact with MutS.** DNase I protection assays were as described under "Materials and Methods" using 1 μM MutS protein, 1 mM ATP, and 1.3 μM, 650 nM, or 325 nM of MutL or MutL-D58A as indicated. The reaction products were resolved on an 8% denaturing polyacrylamide gel. *Lane 1*, heteroduplex DNA cleaved with ClaI (50 bases) and HaeIII (25 bases); *lane 2*, marker ladder prepared using the ^{32}P-labeled 91-mer as previously described (63). The position of the G-T mismatch (position 59 relative to the 5'-end) is indicated by an *arrow* on the *left*.

methylated d(GATC) site was evaluated in the presence of a G-T mismatch and MutS. It is clear that MutL-E29A was able to stimulate the MutH endonuclease reaction with essentially the same efficiency as wild-type MutL. Control experiments (data not shown) using DNA lacking a mismatch, with MutL alone, MutS alone, and MutH alone indicated this reaction was dependent on all three proteins as well as the mismatch. We conclude that MutL-E29A interacts with both MutS and MutH. In addition, these data indicate that ATP hydrolysis catalyzed by MutL is not required at either the recognition or incision steps in the MMR pathway.

We also evaluated the ability of MutL-D58A to function in this partially reconstituted repair reaction. Because previous results suggested that ATP was required for MutL-stimulated nicking catalyzed by MutH, we expected that MutL-D58A would not substitute for MutL in this reaction. This was indeed the case (Fig. 6). In the presence of MutS, MutH, hemi-methylated DNA, and a G-T mismatch, there was no observable nicking of the heteroduplex DNA molecule when MutL-D58A was added to the reaction. Thus, ATP binding by MutL is essential for the incision step in MMR.

To further investigate the failure of MutL-D58A to function in this partial reconstitution of MMR, we investigated the interaction of MutL-D58A with MutS using footprinting experiments (Fig. 7). It has been demonstrated that MutS specifically binds a base pair mismatch, and the footprint formed upon MutS binding is extended in the presence of MutL (20). This can be clearly seen in Fig. 7 (*lanes 5–7*). When MutL-D58A is substituted for MutL in this reaction, the footprint is essentially identical to that of MutS alone, indicating that MutL-D58A does not interact with MutS (Fig. 7, *lanes 8–10*). We also investigated the interaction of MutL-D58A with MutH using affinity chromatography (data not shown). MutL-D58A does not interact with MutH, which is consistent with the fact that it does not stimulate MutH-catalyzed nicking of a heteroduplex DNA sub-
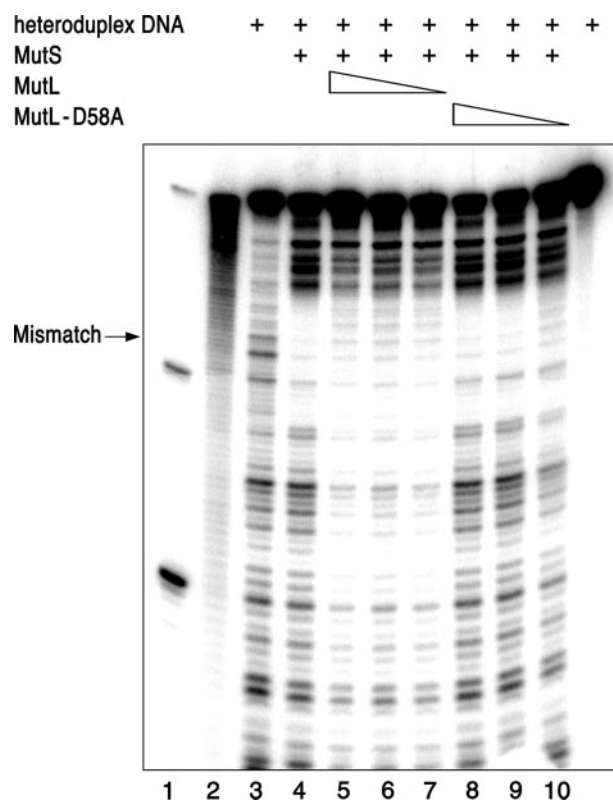
strate. Thus, the ATP-bound form of MutL participates in the initial steps of MMR, and nucleotide binding by MutL is essential for these steps in the pathway.

DISCUSSION

Methyl-directed mismatch repair in *E. coli* is a carefully orchestrated process with remarkable properties that is essential to the fidelity of DNA replication and the maintenance of the genome (1–5). Although the activities of more than 10 proteins are essential for MMR, this report focuses on two of those proteins: MutL and UvrD. Both are essential components of the *E. coli* MMR machinery (15). MutL binds DNA (32–35, 41); has been shown to interact with MutS, MutH, and UvrD (20, 23, 24, 29); and plays a key role in managing the repair process. In addition, the protein has a DNA-stimulated ATP hydrolysis activity (36) that is essential for MMR (37) but whose molecular role in the process is unknown. UvrD is a 3' to 5' DNA helicase that is essential for displacing the strand to be removed and ultimately replaced by the repair process. No other helicase in *E. coli* is able to substitute for UvrD in this role in the cell. Earlier work demonstrated a physical interaction between MutL and UvrD (29), and it has been shown that MutL dramatically stimulates the unwinding activity of UvrD (30–32, 45). We have proposed that MutL loads UvrD productively onto the DNA but does not clamp UvrD on the DNA during the unwind-
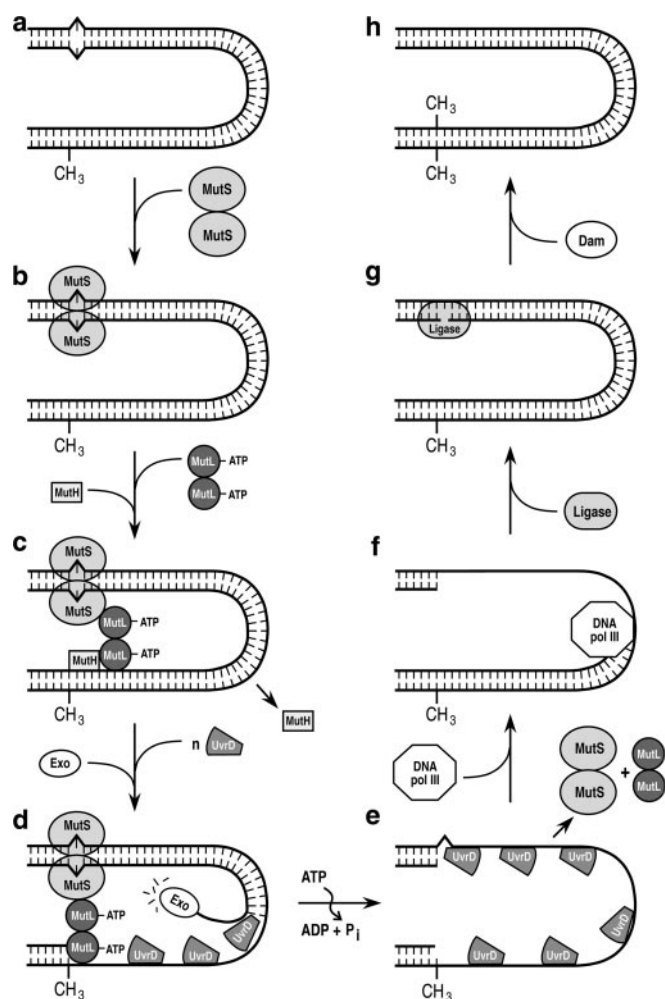
FIGURE 8. **A model for MMR.** The base pair mismatch shown in *panel a* is recognized and bound by the homodimer MutS protein (*panel b*). *Panel c*, the dimeric MutL protein, with the ATP bound, mediates communication between the MutS-bound mismatch and MutH bound at the nearest hemimethylated d(GATC) site. It should be noted that mismatch correction is bidirectional (26), and only a single direction is shown in this model. *Panel d*, MutL loads multiple molecules of UvrD onto the nicked intermediate to unwind the damaged DNA strand, which is degraded by one of four exonucleases with an appropriate polarity (61). MutL-catalyzed ATP hydrolysis is required for some step in the reaction pathway subsequent to the loading of UvrD. *Panel e*, resection of the damaged DNA strand extends past the mismatch, and presumably, MutL and MutS are displaced by an unknown mechanism. *Panel f*, the 3′-OH is then extended by DNA polymerase III, the resulting nick is sealed by DNA ligase (*panel g*) to restore the integrity of the DNA strand, and Dam methylase methylates the d(GATC) on the repaired DNA strand (*panel h*).

ing reaction (32). Therefore, the processivity of the unwinding reaction catalyzed by UvrD is not increased. We have also suggested that loading of UvrD by MutL is likely to be a continuous process with multiple molecules of UvrD loaded onto a single DNA substrate. This accounts for the unwinding of long duplex molecules that contain duplex regions far in excess of the processivity of UvrD. We suggest that the additional molecules of UvrD translocate from the initial loading site to the duplex region and thus contribute to the unwinding of longer duplex DNA molecules (32). The processivity of UvrD as a *translocase* is much higher than as a helicase (57), which is consistent with this proposed mechanism. In this report we have examined the role of the MutL-catalyzed ATP hydrolysis reaction in the loading of UvrD.

The data presented here clearly show that MutL-catalyzed ATP hydrolysis is not essential for stimulation of UvrD-catalyzed unwinding of duplex DNA (Fig. 3). Using a MutL point mutant (MutL-E29A) that does not catalyze ATP hydrolysis, the stimulation of UvrD-catalyzed duplex DNA unwinding has been directly measured using two DNA substrates. One DNA substrate contained a relatively short region of duplex DNA (93 bp) on a circular DNA molecule. The other substrate contained a much longer region of duplex DNA (750 bp) that may be more characteristic of the length of many of the repair tracks in MMR. In addition, this DNA substrate contained fully duplex ends and thus lacked a 3′-ssDNA tail to facilitate loading of UvrD. Remarkably, stimulation of the unwinding reaction catalyzed by UvrD was more robust with MutL-E29A than with the wild-type protein. In other words, the stimulation of UvrD-catalyzed unwinding was better in the *absence* of MutL-catalyzed ATP hydrolysis, particularly on longer duplex regions, than in the presence of MutL-catalyzed hydrolysis. Therefore, the ATPase reaction associated with MutL is not required to load UvrD onto the mismatch repair intermediate to be unwound by UvrD.

In addition, we have shown that the ATP-bound form of MutL is responsible for stimulating UvrD, and we suggest that the ATP-bound form of the protein loads UvrD onto the DNA substrate. The ability of MutL-E29A to bind ATP was directly demonstrated by isothermal titration calorimetry (Fig. 5), and because the protein fails to hydrolyze ATP, we assume that the ATP-bound form of the protein is predominant under the conditions used in the experiments reported here.

To provide additional support for this conclusion, MutL-D58A, a mutant that does not bind ATP, was constructed. The purified protein was properly folded, as evidenced by circular dichroism spectroscopy, and was able to bind a partial duplex DNA substrate that does not require binding of ATP by MutL (32). We directly demonstrated that the protein did not bind ATP, and it did not stimulate the unwinding reaction catalyzed by UvrD using either a partial duplex substrate or a blunt duplex substrate. Thus, the ATP free form of MutL does not stimulate UvrD-catalyzed unwinding.

The model for the action of MutL presented by Ban and Yang (36) posits that binding of ATP promotes the dimerization of the amino-terminal ATPase domain of MutL. The protein is a dimer in the absence of ATP binding as the protein dimerization interface is in the carboxyl-terminal region on the protein (58, 59). We suggest that the ATP-induced dimerization of MutL amino-terminal domain allows the protein to clamp onto the DNA substrate (41) at the nick generated by MutH. This provides a loading platform for UvrD, and helicase molecules are continuously loaded onto the DNA substrate as long as MutL remains clamped on the DNA. In the absence of ATP hydrolysis (*i.e.* with MutL-E29A), MutL remains bound and loads multiple molecules of UvrD. This results in a very dramatic stimulation of UvrD-catalyzed unwinding at low concentrations of MutL. In this case the most dramatic effect of MutL-E29A on the unwinding reaction would be expected when long duplex substrates are used as is seen here. Because the processivity of UvrD as a helicase has been reported to be ~50 bp (56), perhaps as few as two UvrD molecules must be loaded to

unwind the 93-bp partial duplex DNA, whereas many more molecules of UvrD must be loaded onto the 750-bp blunt duplex DNA to effect complete unwinding. This nicely explains the increased stimulation of UvrD-catalyzed unwinding in the presence of MutL-E29A. Importantly, in the absence of ATP-induced dimerization (*i.e.* with MutL-D58A), the protein does not clamp onto the DNA and does not form a loading platform for UvrD, explaining the lack of MutL-D58A-stimulated unwinding.

Furthermore, we suggest that the interaction between MutL and UvrD that results in stimulation of unwinding is abrogated upon ATP hydrolysis by MutL, perhaps because of the release of MutL from the DNA. The result is cessation of loading of UvrD onto the DNA substrate and, ultimately, cessation of unwinding. In the case of MutL-E29A the bound ATP is not hydrolyzed and MutL continues to load UvrD onto the DNA substrate with a resulting increase in the amount of DNA unwound as compared with wild-type MutL. A model that incorporates these findings is shown in Fig. 8.

These results have important implications for our understanding of the process of MMR. First, they serve to further refine our understanding of the role of ATP binding and hydrolysis catalyzed by MutL in MMR. It has been established previously that ATP binding but not hydrolysis by MutL is required for interaction with MutS (60). The requirement for ATP binding by MutL is confirmed in the footprinting assays using MutL-D58A and MutS (Fig. 7). MutL-D58A does not interact with MutS because it does not bind ATP. In addition, we have shown here (Fig. 6) and previously (23) that the ATP-bound form of MutL interacts with and stimulates the latent endonuclease reaction associated with MutH. In this report we demonstrate that MutL loads UvrD onto a DNA substrate in the absence of ATP hydrolysis but in a reaction that requires the ATP-bound form of MutL. Thus, the ATP-bound MutL is essential for the initial steps of MMR, and MutL-catalyzed ATP hydrolysis is required after strand incision and the beginning stage of strand resection. Second, the data presented here offer the possibility that ATP hydrolysis catalyzed by MutL regulates the amount of UvrD loaded onto the DNA substrate. In the absence of ATP hydrolysis the unwinding step may be uncoupled from the rescission step such that the exonuclease responsible for removing the unwound damage containing nascent strand is unable to keep up with the advancing helicase. If this were the case then repair events might not be properly completed because of the uncoupling, and there would be an increase in mutation rate as was observed when *mutL-E29A* was substituted for *mutL*. This might result in the genome becoming fragmented, resulting in genomic instability. This has not been measured.

Alternatively, MutL-catalyzed ATP hydrolysis may be required for a subsequent loading event in the MMR pathway. For example, MutL might recruit and load the appropriate exonuclease to digest the damaged DNA strand, or it might recruit and load the beta clamp as a prelude to loading DNA polymerase onto the available 3′-OH to complete strand resynthesis. In either case, the data presented here are consistent with the view that MutL and its associated ATPase serve as some kind of switch (47) regulating the overall process of MMR. Although we have

suggested that the MutL ATPase reaction might serve to regulate the loading of UvrD, this is not the only possibility. Additional data will be required to fully understand the role of the MutL ATPase activity.

REFERENCES

1. Modrich, P., and Lahue, R. (1996) *Annu. Rev. Biochem.* **65,** 101–133
2. Buermeyer, A. B., Deschenes, S. M., Baker, S. M., and Liskay, R. M. (1999) *Annu. Rev. Genet.* **33,** 533–564
3. Harfe, B. D., and Jinks-Robertson, S. (2000) *Annu. Rev. Genet.* **34,** 359–399
4. Schofield, M. J., and Hsieh, P. (2003) *Ann. Rev. Microbiol.* **57,** 579–608
5. Kunkel, T. A., and Erie, D. A. (2005) *Annu. Rev. Biochem.* **74,** 681–710
6. Rayssiguier, C., Thaler, D. S., and Radman, M. (1989) *Nature* **342,** 396–401
7. Worth, L., Clark, S., Radman, M., and Modrich, P. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 3238–3241
8. Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. (1993) *Cell* **75,** 1027–1038
9. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, S., and Perucho, M. (1993) *Nature* **363,** 558–561
10. Karran, P., and Bignami, M. (1994) *Bioessays* **16,** 833–839
11. Eshleman, J. R., and Markowitz, S. D. (1995) *Curr. Opin. Oncol.* **7,** 83–89
12. Jiricny, J. (1996) *Cancer Surv.* **28,** 47–68
13. Umar, A., and Kunkel, T. A. (1996) *Eur. J. Biochem.* **238,** 297–307
14. Loeb, L. A., Loeb, K. R., and Anderson, J. P. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 776–781
15. Lahue, R. S., Au, K. G., and Modrich, P. (1989) *Science* **245,** 160–164
16. Modrich, P. (1991) *Annu. Rev. Genet.* **25,** 229–253
17. Modrich, P. (1994) *Science* **266,** 1959–1960
18. Su, S. S., and Modrich, P. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 5057–5061
19. Su, S. S., Lahue, R. S., Au, K. G., and Modrich, P. (1988) *J. Biol. Chem.* **263,** 6829–6835
20. Grilley, M., Welsh, K. M., Su, S. S., and Modrich, P. (1989) *J. Biol. Chem.* **264,** 1000–1004
21. Allen, D. J., Makhov, A., Grilley, M., Taylor, J., Thresher, R., Modrich, P., and Griffith, J. D. (1997) *EMBO J.,* **16,** 4467–4476
22. Welsh, K. M., Lu, A. L., Clark, S., and Modrich, P. (1987) *J. Biol. Chem.* **262,** 15624–15629
23. Au, K. G., Welsh, K., and Modrich, P. (1992) *J. Biol. Chem.* **267,** 12142–12148
24. Hall, M. C., and Matson, S. W. (1999) *J. Biol. Chem.* **274,** 1306–1312
25. Grilley, M., Holmes, J., Yashar, B., and Modrich, P. (1990) *Mutat. Res.* **236,** 253–267
26. Cooper, D. L., Lahue, R. S., and Modrich, P. (1993) *J. Biol. Chem.* **268,** 11823–11829
27. Grilley, M., Griffith, J. D., and Modrich, P. (1993) *J. Biol. Chem.* **268,** 11830–11837
28. Matson, S. W. (1986) *J. Biol. Chem.* **261,** 10169–10175
29. Hall, M. C., Jordan, J. R., and Matson, S. W. (1998) *EMBO J.* **17,** 1535–1541
30. Dao, V., and Modrich, P. (1998) *J. Biol. Chem.* **273,** 9202–9207
31. Yamaguchi, M., Dao, V., and Modrich, P. (1998) *J. Biol. Chem.* **273,** 9197–9201
32. Mechanic, L. E., Frankel, B. A., and Matson, S. W. (2000) *J. Biol. Chem.* **275,** 38337–38346
33. Bende, S. M., and Grafstrom, R. H. (1991) *Nucleic Acids Res.* **19,** 1549–1555
34. Ban, C., Junop, M., and Yang, W. (1999) *Cell* **97,** 85–97
35. Drotschmann, K., Aronshtam, A., Fritz, H. J., and Marinus, M. G. (1998) *Nucleic Acids Res.* **26,** 948–953
36. Ban, C., and Yang, W. (1998) *Cell* **95,** 541–552
37. Spampinato, C., and Modrich, P. (2000) *J. Biol. Chem.* **275,** 9863–9869
38. George, J. W., Brosh, R. M. Jr., and Matson, S. W. (1994) *J. Mol. Biol.* **235,** 424–435
39. Lechner, R. L., and Richardson, C. C. (1983) *J. Biol. Chem.* **258,** 11185–11196
40. Mechanic, L. E., Hall, M. C., and Matson, S. W. (1999) *J. Biol. Chem.* **274,**

12488–12498

41. Robertson, A., Pattishall, S. R., and Matson, S. W. (2006) *J. Biol. Chem.* **281,** 8399–8408

42. Runyon, G. T., and Lohman, T. M. (1989) *J. Biol. Chem.* **264,** 17502–17512

43. Brosh, R.M., Jr., and Matson, S.W. (1995) *J. Bacteriol.* **177,** 5612–5621

44. Wong, I., and Lohman, T. M. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 5428–5432

45. Junop, M. S., Yang, W., Funchain, P., Clendenin, W., and Miller, J. H. (2003) *DNA Repair* **2,** 387–405

46. Schofield, M. J., Nayak, S., Scott, T. H., Du, C., and Hsieh, P. (2001) *J. Biol. Chem.* **276,** 28291–28299

47. Acharya. S., Foster, P. L., Brooks, P., and Fishel R. (2003) *Mol Cell* **12,** 233–246

48. Sancar, A., and Hearst, J. E. (1993) *Science* **259,** 1415–1420

49. Deleted in proof

50. Hall, M. C., Shcherbakova, P. V., and Kunkel, T. A. (2002) *J. Biol. Chem.* **277,** 3673–3679

51. Luria, S. E., and Delbruck, M. (1943) *Genetics* **28,** 491–511

52. Lea, D. E., and Coulson, C. A. (1949) *Journal of Genetics* **49,** 264–285

53. Aronshtam, A., and Marinus, M. G. (1996) *Nuc. Acids. Res.* **24,** 2498–2504

54. Deleted in proof

55. Deleted in proof

56. Ali, J. A., and Lohman, T. M. (1997) *Science* **275,** 377–380

57. Fischer, C. J., Maluf, N. K., and Lohman, T. M. (2004) *J. Mol. Biol.* **344,** 1287–1309

58. Guarne, A., Ramon-Maiques, S., Wolff, E. M., Ghirlanda, R., Hu, X., Miller, J. H., and Yang, W. (2004) *EMBO J.* **23,** 4134–4145

59. Kosinski, J., Steindorf, I., Bujnicki, J. M., Giron-Monzon, L., and Friedhoff, P. (2005) *J. Mol. Biol.* **351,** 895–909

60. Selmane, T., Schofield, M. J., Nayak, S., Du, C., and Hsieh, P. (2003) *J. Mol. Biol.* **334,** 949–965

61. Burdett, V., Baitinger, C., Viswanathan, M., Lovett, S. T., and Modrich, P. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 6765–6770

62. Runyon, G. T., Wong, I., and Lohman, T. M. (1993) *Biochemistry* **32,** 602–612

63. Sherman, J. A., and Matson, S. W. (1994) *J. Biol. Chem.* **269,** 26220–26226