The DNA Binding Activity of MutL Is Required for Methyl-directed Mismatch Repair in Escherichia coli*

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The DNA binding properties of the mismatch repair protein MutL and their importance in the repair process have been controversial for nearly two decades. We have addressed this issue using a point mutant of MutL (MutL-R266E). The biochemical and genetic data suggest that DNA binding by MutL is required for dam methylation-directed mismatch repair. We demonstrate that purified MutL-R266E retains wild-type biochemical properties that do not depend on DNA binding, such as basal ATP hydrolysis and helicase II stimulation, are severely compromised. In addition, there is a modest effect on stimulation of MutH-catalyzed nicking. Finally, genetic assays show that MutL-R266E has a strong mutator phenotype, demonstrating that the mutant is unable to function in dam methylolation-directed mismatch repair in vivo.

Methyl-directed mismatch repair (MMR) is the primary pathway for correcting replication errors and unwanted recombination events in the Escherichia coli bacterial cell (1–3). Therefore, a functional MMR pathway is essential for ensuring the integrity of the chromosome as well as for maintaining an acceptable cellular mutation rate. The primary components of the bacterial MMR system are the mutator proteins (MutL, MutS, MutH, and UvrD), several exonucleases including Exo I and Exo X (3’ to 5’ polarity), Exo VII and Rec J (5’ to 3’ polarity) (4–6), DNA polymerase III, single-stranded DNA-binding protein, and DNA ligase. These proteins act in concert to correct base-base mismatches after passage of the replication fork (7).

A current model of MMR (for reviews, see Refs. 2 and 8–10) posits that the MutS protein recognizes and binds the mismatched base pair followed by the binding of MutL to form a ternary complex (11). In E. coli both of these proteins function as homodimers (12, 13). This complex forms an α-shaped loop in the DNA (14) that is dependent on ATP and thought to be the mode for communicating the signal from the mismatch to the nearest hemi-methylated d(GATC) site, where MutH binds to provide the strand discrimination essential in methyl-directed mismatch repair. Once the MutS-MutL complex locates a MutH-bound hemi-methylated d(GATC) site, MutH is stimulated, presumably by MutL (15, 16), to nick the unmethylated DNA strand, resulting in discrimination of the nascent and parental DNA strands (4, 17). The use of the nearest hemi-methylated d(GATC) as the site of MutH-directed incision provides MMR with a bidirectional capability since this site could be located on either side of the mismatch. Helicase II (uvrD gene product) is then loaded on the appropriate strand at the nick and translocates in a 3’ to 5’ direction (18), unwinding the DNA duplex until the mismatch has been removed (19). The signal indicating that sufficient DNA has been unwound, to complete the repair process, is not known. One of the several nucleases with an appropriate polarity degrades the nascent DNA strand, and single-stranded DNA-binding protein binds and stabilizes the single-stranded DNA (ssDNA) template until DNA polymerase III is recruited to fill the gap. The resulting nick is sealed by DNA ligase, completing the repair process and restoring the integrity of the DNA (13).

The role of MutL protein in MMR remains to be completely defined on a mechanistic level. The protein was originally purified using a biochemical complementation assay as an essential component for partially reconstituted mismatch repair in cell extracts lacking MutL (12). Subsequent experiments demonstrated its interaction with MutS at a mismatch (13, 20), and the solved crystal structure of the amino-terminus domain of MutL demonstrated an ATP binding/hydrolysis fold common to the GHKL group of ATP-hydrolyzing enzymes (21). Purified MutL catalyzes a slow ATP hydrolysis reaction that is stimulated by the presence of ssDNA and is essential for MMR (20–22). In addition, MutL has been shown to interact with and stimulate the hemi-methylated d(GATC)-directed nicking reaction catalyzed by MutH (15, 16) as well as stimulating the unwinding of duplex DNA catalyzed by UvrD (19). Thus, MutL has been characterized as the master regulator of MMR in light of its interaction with many of the proteins required for MMR.

The stimulation of MutL-catalyzed ATP hydrolysis by the addition of DNA has prompted an investigation of the DNA binding properties of MutL. Several groups have demonstrated that MutL binds to both ssDNA and double-stranded DNA (dsDNA) (20, 23), whereas others report that MutL does not bind DNA (24). Recently, the DNA binding activity of MutL has been suggested to be an artifact of in vitro assays designed to measure DNA binding (25). Indeed, a current model for MMR postulates that DNA binding by MutL is not required for the MMR process (26).

Using a combination of biochemical and genetic assays, we present evidence to suggest that DNA binding by MutL is a requirement for MMR. These studies take advantage of an mutL point mutant (MutL-R266E), in which the arginine at position 266 has been altered to a glutamic acid. Expression of this protein in a cell strain lacking functional MutL results in a mutator phenotype similar to that observed with a complete deletion of the mutL gene. The purified mutant protein exhibits a weak ssDNA binding affinity, no ssDNA-stimulated ATPase...
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activity (the basal ATPase activity in intact), and very poor stimulation of UvrD-catalyzed DNA unwinding. Taken as a whole, these data are consistent with a primary DNA binding defect since the purified protein is able to interact with the other MMR proteins as effectively as wild-type MutL protein. We conclude that the DNA binding activity of MutL is required for full functionality of the MMR process.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—E. coli BL21(DE3) (F- ompT [lon] hsdS4 rB mBgal λDE3) was from Novagen. A derivative of the strain, BL21(DE3)uvrD::Tn5 mutL::Tn10, has been described (27). The mutL gene was cloned into the pET15b expression vector (Novagen) by removing the mutL gene from pET3C-MutL (28) with BamHI and inserting the gene in pET15b cut with the same restriction enzyme. This plasmid is referred to as pET15b-MutL. The pET15b-MutL-R266E construct was a gift from Peggy Hsieh (National Institutes of Health). Constructs were sequenced to verify the coding sequence of the gene and to ensure the absence of unintended mutations.

Protein Purification—MutL and MutL-R266E were expressed in E. coli BL21(DE3) uvrD::Tn5 mutL::Tn10 cells containing the appropriate MutL expression vector. Two liters of cells were grown at 37 °C to an A600 of 0.8, and protein expression was induced by adding IPTG to 0.5 mM. After an additional 4-h incubation at 37 °C, the cells were harvested by centrifugation, washed with cold H2O, suspended in binding buffer (50 mM Na2PO4 (pH 7.0), 500 mM NaCl, 5 mM imidazole, 10% (v/v) glycerol), and frozen at −75 °C for future use. Cells were thawed on ice and lysed by the addition of lysozyme to 200 μg/ml followed by incubation at 0 °C for 60 min. The lystate was sonicated briefly to reduce the viscosity, applied to a TALON metal affinity resin (BD Biosciences; 1 ml of resin/L cells), and extensively washed with binding buffer. To decrease the NaCl concentration before elution, the column was washed with several column volumes of binding buffer containing 25 mM NaCl instead of 500 mM NaCl. The column was eluted using 200 mM imidazole in the low NaCl binding buffer. This step resulted in a substantial purification of MutL, but contaminants were still present. The protein that eluted from the TALON column was diluted 3-fold with Mono Q buffer (25 mM Tris-HCl (pH 7.0), 0.1 mM EDTA (pH 8.0), 1 mM dithiothreitol, and 10% (v/v) glycerol) containing no NaCl to achieve a final NaCl concentration of ~75 mM and applied to a Mono Q column equilibrated in Mono Q buffer containing 75 mM NaCl. The protein was eluted using a gradient from 75 mM NaCl to 500 mM NaCl in Mono Q buffer. MutL was detected by SDS-PAGE. The protein eluted at ~180 mM NaCl, and fractions containing MutL were pooled. To achieve greater than 95% purity, the proteins were further purified by gel filtration using a Superdex 200 column. The pooled fractions from the Mono Q column were concentrated using solid polyethylene glycol 20,000 and loaded onto a Superdex 200 column equilibrated with Superdex buffer (50 mM Na2PO4 (pH 7.0), 250 mM NaCl, 0.5 mM EDTA (pH 8.0), and 10% (v/v) glycerol). Fractions containing MutL were identified by SDS-PAGE, and MutL eluted at the position expected for a MutL dimer. Final purity was assessed by SDS-PAGE (see Fig. 1). UvrD was purified as described (27) from a strain that contained an insertion in mutL to ensure that purified UvrD was not contaminated with MutL. The protein was greater than 95% pure as determined by SDS-PAGE.

MutS and MutH were purified in a manner similar to the purification of MutL. Each protein was expressed in E. coli BL21(DE3) using pET15b-MutS or pET15b-MutH constructs. After the initial TALON metal affinity column, the proteins were further purified using heparin agarose and Superdex 200 as appropriate. The final protein preparations were judged to be >95% pure as determined by SDS-PAGE.

DNA Substrates—The various DNA substrates (ligands) used in DNA binding assays and DNA unwinding assays were prepared using synthetic DNA oligomers. The sequences and sources of the oligomers used in these studies were as follows: 50-mer, 5′-TTT TGG GGC GAA GTT TTA TGG TCT ACC TAG GCC GGC TAT TCA GGG TT-3′ (MWG Biotech); 91-mer, 5′-AGT AGC ACC ATT ACC ATT AGC AAG GCC GGA AAC GTC ACC AAC AAT GAA ACC ACC GTA ATC GAG ACA GAC AGA ATC AAG TTT G-3′ (Integrated DNA Technologies); complement to 50-mer, 5′-AAC CCT GAA TAG CCG GCC TAG GTA GGA GAC CAT AAA ACT TCG CCC CAA AA-3′ (MWG Biotech); 3′-overhang 70-mer, 5′-AAC CCT GAA TAG CCG GCC TAG GTA GGA GAC CAT AAA ACT TCG CCC CAA AAT CAA TAA TCA AAT CCA A-3′ (MWG Biotech). To construct DNA binding ligands, 2.3 pmol (molecules) of 50-mer were labeled on the 5′-end using 3.33 μM [γ-32P]ATP (Amer sham Biosciences) and T4 polynucleotide kinase (New England Biolabs) using reaction conditions suggested by the supplier. Free nucleotides were removed using a Qiagen nucleotide removal kit. The 50- and 50/70 3′ overhang duplex DNA were made by annealing 2.8 pmol of the 50-mer complement or the 3′ overhang 70-mer to the labeled 50-mer in annealing buffer (40 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 1 mM dithiothreitol, 50 mM NaCl). The DNA was mixed and heated to 100 °C for 5 min, cooled to 65 °C, incubated for 30 min, cooled to 25 °C, and incubated for 10 min. The substrate DNA was purified from the unannealed 50-mer on a 10% native polyacrylamide gel run in TBE buffer (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA (pH 8.3)) at 3.1 V/cm for 12 h. DNA was extracted from the gel slice by electroelution in 1× Tris borate-EDTA. An electric potential of 3.1 V/cm was passed across the gel slice for 8 h at 4 °C. The electroeluted DNA was then dialyzed against TEN buffer (40 mM Tris-HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl) buffer. The eluted DNA was concentrated using 2-butanol (29).

The 93-bp partial duplex DNA substrate was prepared as described (30). Briefly, 2 μg of M13mp7 ssDNA, 40 ng of 91-mer, annealing buffer, and water were added to a final volume of 50 μl. The mixture was heated to 100 °C for 5 min, cooled to 65 °C, incubated for 30 min, and finally incubated at room temperature for 10 min. [α-32P]dCTP (3.3 μM) and 5 units of DNA polymerase I (Klenow fragment, New England Biolabs) were added. The extension reaction was allowed to proceed for 30 min at 25 °C, chased by the addition of 20 nmol of dCTP, and allowed to incubate at 25 °C for an additional 10 min. The volume of the solution was increased to 100 μl with STE buffer (100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA), phenol/CHCl3 was extracted, and free nucleotides were removed using an A5M column (Bio-Rad). The partial duplex substrate eluted in the void volume and was used directly in helicase assays. The concentration of the DNA substrate was estimated at 20 μM DNA-Pi.

The 710-bp blunt duplex was prepared by digesting pLitmus28 with Scal and XbAl (New England Biolabs). The resulting 710-base pair fragment was labeled using DNA polymerase I (Klenow fragment, New England Biolabs). The reaction contained an 87.7 μM 710-bp DNA fragment (DNA molecules), 50 mM NaCl, 10 mM Tris-HCl (pH 7.9), 10 mM MgCl2, 1 mM dithiothreitol, 200 μM DATP, 200 μM dGTP, 200 μM dTTP, [α-32P]dCTP, and 5 units of DNA polymerase I (Klenow Fragment, New England Biolabs) (30 μl). The reaction was incubated at 25 °C for 20 min and chased by the addition of 20 nmol of dCTP at 25 °C for 15 min. Free nucleotides were removed using a nucleotide removal kit (Qiagen). Heteroduplex DNA containing a single G:T mismatch and the corresponding homoduplex DNA were prepared as previously described (15).
Mutator Assays—Nine independent cultures of GE1752, GE1752 mutL::Tn10, GE1752 mutL::pET15b, GE1752 mutL::pET15b-MutL, and GE1752 mutL::pET15b-MutL-R266E were grown to saturation at 37 °C in the presence of appropriate antibiotics. Appropriate dilutions of each cell strain were made, and cells were plated on LB plates containing appropriate antibiotics to determine a cell titer and on LB plates containing rifampicin (100 μg/ml) to measure the number of rifampicin resistant colonies. Plates were incubated at 37 °C overnight, and colonies were counted. Mutation frequencies and rates were calculated using the method of the mean as previously described (31–34).

Small Scale Mutator Assays—Two cultures each of BL21(DE3), BL21(DE3)::Tn10, BL21(DE3)::pETcoco-2, BL21(DE3) mutL::Tn10/pETcoco-2-MutL, BL21(DE3) mutL::Tn10/pETcoco-2-MutL-E29A were grown for 15 h and 30 min at 37 °C in the presence of the appropriate antibiotics (pETcoco-2 from Novagen). One of the two cultures was grown in the absence of IPTG, whereas the other culture was grown in the presence of 250 μM IPTG. Serial dilutions (0, 10−1, and 10−2) were plated on an LB plate containing rifampicin (100 μg/ml). The plate was incubated at 37 °C overnight and was analyzed qualitatively to determine the mutator phenotype of the particular mutant.

To confirm that the appropriate MutL protein was expressed, a Western blot of each of the plated samples was run. Cells (1 ml) were sedimented by centrifugation and suspended in TE buffer according to the following formula to ensure equal loading: resuspension volume of Tris-EDTA buffer = (A280 × 100)/0.5. Cells (12.5 μl) were then lysed in an equal volume of SDS gel loading buffer (32 mM Tris-HCl (pH 6.8), 3.2% (w/v) SDS, 1.2 M 2-mercaptoethanol, 20% (v/v) glycerol) and boiled for 5 min. The samples were resolved by SDS-PAGE, and proteins were transferred to nitrocellulose at 4 °C. The nitrocellulose filter was blocked with 2% (w/v) powdered milk (Carnation) in TTBS (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% (v/v) Tween 20) for 60 min while shaking. The nitrocellulose filter was then washed 4 times with TTBS for 15 min while shaking. The filters were subsequently incubated with rabbit anti-MutL antibodies in TTBS (1:25,000) for 2 h. The nitrocellulose filter was washed 4 times with TTBS and incubated with goat anti-rabbit antiserum conjugated to alkaline phosphatase in TTBS (1:10,000) for 15 min while shaking. The filters were subsequently incubated with rabbit anti-MutL antibodies in TTBS (1:25,000) for 2 h. The nitrocellulose filter was washed 4 times with TTBS and incubated with goat anti-rabbit antiserum conjugated to alkaline phosphatase in TTBS (1:10,000) for 30 min. The filter was washed 4 times with TTBS and then reacted with Western Blue alkaline phosphatase substrate (Promega) until a visible blue color reaction was observed (~5 min).

ATP Hydrolysis Assays—Reaction mixtures (120 μl) contained 25 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 20 mM NaCl, 50 μg/ml BSA, 5 mM 2-mercaptoethanol, 3 mM ATP ([γ-32P]ATP). For reaction mixtures containing DNA, the 50-mer was added at concentrations varying from 0 to 4 μM as indicated in the figure legends. Reactions were initiated by the addition of purified MutL or MutL-R266E to a final concentration of 1 μM followed by incubation at 37 °C. Aliquots (20 μl) were removed at 0, 15, 30, 45, and 60 min and added to 280-μl quench solution (20 mM H3PO4, 1 mM EDTA (pH 8.0), 5% (v/v) Norit activated charcoal USP). The quenched reactions were incubated on ice for 15 min, and the activated charcoal was sedimented in a microcentrifuge. A 200-μl aliquot of the supernatant was removed, added to 3 ml of scintillation mixture (Ecocint A, National Diagnostics), and counted in a liquid scintillation counter.

DNA Binding Assays—Double filter DNA binding assays were performed as described (35). Briefly, nitrocellulose (Millipore) and DEAE (DE81, Whatman) filters (2.5 cm) were prepared as previously described (35), with the exception that the filters were incubated at 4 °C with MutL reaction buffer (25 mM Tris-HCl (pH 7.5), 3 mM MgCl2, 20 mM NaCl, 5 mM 2-mercaptoethanol) before use. Reaction mixtures (20 μl) contained 25 mM Tris-HCl (pH 7.5), 3 mM MgCl2, 20 or 100 mM NaCl (as indicated), 5 μg/ml BSA, 5 mM 2-mercaptoethanol, 3 mM AMP-PNP, 1 mM oligonucleotide 50-mer, 1 mM homoduplex, 1 mM 70/50 3′-overhang DNA, or 4 μM 93-bp partial duplex DNA (DNA-P) and protein as indicated. The reactions were incubated at 37 °C for 10 min, diluted with 1 ml of MutL reaction buffer, then filtered across treated nitrocellulose and DEAE filters in a vacuum manifold. The filters were washed twice with reaction buffer, dried, and counted in a liquid scintillation counter. Data were analyzed as previously described (35).

Helicase Unwinding Assays—Reaction mixtures (20 μl) contained 25 mM Tris-HCl (pH 7.5), 3 mM MgCl2, 20 mM NaCl, 5 mM 2-mercaptoethanol, 50 μg/ml BSA, 3 mM ATP, 93 bp of partial duplex DNA (final concentration of 2 μM DNA-P) or 710 bp of blunt duplex DNA (final concentration of 1 nM DNA molecules), 1.25 or 16 nM UvrD, and a titration of MutL or MutL-R266E. Reactions were initiated by the addition of ATP after pre-warming 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 710-bp blunt duplex by the addition of 10 μl of stop solution (50% (w/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× Tris borate-EDTA and 0.1% (w/v) SDS. Polyacrylamide gels were imaged using a phosphor screen (Amersham Biosciences) and quantified using ImageQuant on a Storm PhosphorImager (Amersham Biosciences).

MutL-stimulated MutH Nicking Assays—Reaction mixtures (16 μl) contained 20 mM Tris-HCl (pH 7.6), 4 mM MgCl2, 20 mM NaCl, 50 μg/ml BSA, 3 mM ATP, 50 ng of heteroduplex DNA, 33.8 nM MutS, 1.9 nM MutH, and the indicated concentrations of MutL or MutL-R266E. 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. 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.

MutH Affinity Column Chromatography—MutH (560 μg) in MutH affinity chromatography buffer (100 mM Hepes-KOH (pH 7.5), 25 mM NaCl, 100 μM EDTA, 10% (v/v) glycerol) was bound to 1.5 ml Affi-Gel 10 (Bio-Rad) overnight at 4 °C. The resin was blocked with 100 μl of 1 M ethanolamine (pH 8.0) for 1 h at 4 °C. The blocked resin was added to a column and washed in MutH affinity chromatography buffer until the flow-through contained no detectable protein (determined by measuring the A280 of the flow-through). MutL (100 μg), MutL-R266E (80 μg), or BSA (100 μg) was loaded onto the column in binding buffer, and the column was washed with four column volumes of binding buffer and eluted with increasing concentrations of NaCl. Five fractions (300 μl each) were collected at each NaCl concentration (50 and 200 mM, 1 and 2 mM NaCl) in MutH affinity chromatography buffer and analyzed by a Bradford protein assay and SDS-PAGE. The gels were analyzed by densitometry, measuring the density of the protein on the gel relative to a marker protein.

Enzyme-linked Immunosorbent Assay—UvrD (62.5 fmol) or BSA (62.5 fmol) in 25 mM Hepes-KOH (pH 7.5), 50 mM NaCl, and 1.6 mM sulfo N-hydroxysuccinimide were added to each of 16 wells (100 μl/well) on a CovaLink plate (Nunc). To initiate the binding reactions of UvrD or BSA, 6.5 mM N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride was added to each of the wells (50 μl/well). The binding reaction was incubated for 2 h at 25 °C. The wells were washed 4
times with 350 μl of wash buffer (25 mM Hepes-KOH (pH 7.5), 50 mM NaCl, 0.1% (v/v) Tween 20) overnight at 4 °C. The wells were washed 4 times with 350 μl of wash buffer, and a titration of MutL or MutL-R266E in wash buffer was added to the wells (100 μl/well) as indicated and incubated for 2 h at 25 °C. The wells were rinsed 4 times with 350 μl of wash buffer. Goat anti-rabbit IgG conjugated to alkaline phosphatase (100 μl; 1:10,000) in wash buffer was added to the wells and incubated at 25 °C for 2 h. The wells were washed with 350 μl of wash buffer. Goat anti-rabbit IgG conjugated to alkaline phosphatase substrate (1 m diethanolamine, 1 mg/ml p-p-nitrophenyl phosphate disodium salt (Pierce)) were added to the wells for 30 min at 25 °C. The wells were washed with 350 μl of wash buffer, and 100 μl of p-nitrophenyl phosphate alkaline phosphatase substrate was added to the wells for 30 min at 25 °C. The color reaction was read at A405 using a MicroQuant plate reader. The data were analyzed by subtracting the background MutL or MutL-R266E bound to BSA from the MutL or MutL-R266E bound to UvrD.

RESULTS

Since its discovery several years ago, the DNA binding activity of MutL has been investigated in several laboratories with conflicting results (11, 23–25, 28, 36, 37, 47). Taken together, most investigations show that MutL binds ssDNA and probably duplex DNA, but the biological significance of this result remains to be demonstrated. Recently, the ssDNA binding activity of MutL has been suggested to be an artifact in vitro assays and not essential for the role of MutL in MMR (25). We (28) and others (19, 28) have shown that MutL stimulates the unwinding of duplex DNA by helicase II, presumably by facilitating the loading of helicase II at an appropriate site to initiate DNA unwinding. It has been presumed that an interaction between MutL and DNA is important in this reaction. However, the DNA binding activity of MutL has never been shown to have a direct role within the context of the bacterial MMR system in vivo.

We have used a MutL mutant that has a single point mutation at position 266, where the basic arginine residue has been changed to an acidic glutamic acid (MutL-R266E) to investigate the role of DNA binding by MutL in MMR. Previous studies (20, 36) have shown this mutant to exhibit a DNA binding defect. We have extended those studies here and suggest that DNA binding by MutL is required for MMR.

E. coli mutL-R266E Exhibits a Mutator Phenotype—The ability of the mutL-R266E allele to substitute for the wild-type mutL gene in MMR was evaluated in a genetic complementation assay (Table 1). This assay measures the ability of plasmid-borne mutL alleles to substitute for the wild-type gene in a strain containing an insertion in the chromosomal mutL gene. As expected, the mutation frequency was elevated ~100-fold in the absence of MutL as demonstrated by the increased frequency of RifR cells. The addition of the vector alone (pET15b) to the strain lacking MutL did not significantly alter either the mutation frequency or the mutation rate. The addition of wild-type mutL on the pET15b expression plasmid restored both the mutation rate and frequency to that observed in the wild-type strain, indicating that the plasmid-borne copy of mutL could effectively replace the chromosomal copy of the gene. It is important to note that expression of MutL was not induced in these experiments, and therefore, a basal level of MutL expression from this plasmid is sufficient to restore MMR to wild-type levels. The mutL-R266E mutant allele failed to complement the loss of the chromosomal copy of mutL, indicating that this protein was not functional in MMR. Because the expression of the mutL-R266E allele in these cells was confirmed by Western blot, we conclude that mutL-R266E encodes a mutant MutL protein that is not functional in MMR. Similar results have been obtained by us3 and others (22, 38) with regard to the role of the ATP hydrolysis reaction catalyzed by MutL.

Purification and Biochemical Characterization of MutL-R266E—Because MutL-R266E was defective in mismatch repair, the mutant protein was purified and characterized using a variety of biochemical assays. The purification schemes for MutL and MutL-R266E were identical. Both proteins were overexpressed using the pET15b vector in the BL21(DE3) uvrD::Tn5 mutL::Tn10 E. coli cell strain. This strain was chosen to ensure there was no contamination by either wild-type MutL or helicase II. The latter has been shown to interact with and, in some cases, copurify with MutL, and its presence in final preparations of MutL-R266E could confound the interpretation of biochemical results. Purification of MutL and MutL-R266E was facilitated by the presence of an NH2-terminal six histidine tag that was provided on the vector chosen for expression. It is important to note that the histidine-tagged protein was also used in the complementation assays described above, indicating that the purification tag did not interfere with the ability of the protein to function in MMR. The histidine-tagged protein was bound to TALON resin and eluted using imidazole. The protein was further purified using a Mono Q ion exchange column followed by a Superdex 200 gel filtration column to remove essentially all impurities (for details see “Materials and Methods”). The purified proteins are shown in Fig. 1. Importantly, the purified proteins are identified as MutL using anti-MutL polyclonal antibodies, and the protein preparations lack any detectable helicase II as demonstrated by Western blot (data not shown). It should also be noted that both MutL and MutL-R266E elute from the Superdex 200 column in the position expected for a dimer of MutL. Thus, the dimerization properties of MutL-R266E were not affected by the mutation.

ATP Hydrolysis by MutL-R266E Is Not Stimulated by DNA—DNA has been shown to increase the low basal rate of ATP hydrolysis catalyzed by MutL (20, 21). This finding implies there is an interaction between DNA and the MutL dimer since DNA-bound MutL hydrolyzes ATP at a faster rate. However, to date the available direct evidence for DNA binding is considered controversial. Purified MutL and MutL-R266E exhibited equivalent levels of basal ATP hydrolysis (MutL ATPase rateNo DNA = 3.0 ± 0.6 pmol/min, MutL-R266E ATPase rateNo DNA = 2.9 ± 1.4 pmol/min) that compare favorably with values for the rate of basal MutL hydrolysis that have been reported previously (21, 22) (Fig. 2). This finding indicates that the mutant protein is able to bind and subsequently hydrolyze ATP. Together with the purification
properties of the protein, this result suggests that MutL-R266E is properly folded and observed in vivo defects in MMR cannot be attributed to improper folding of the protein.

When ssDNA was added to the reaction containing wild-type MutL, the rate of ATP hydrolysis increased as the ssDNA concentration was increased (Fig. 2). The rate dependence on the presence of ssDNA was well described by a rectangular hyperbola, where the ssDNA-stimulated ATP hydrolysis reaction was maximal at a DNA concentration of 4 μM and a rate of MutL-catalyzed hydrolysis of 14.8 ± 0.26 pmol/min. In a similar experiment the rate of MutL-R266E-catalyzed ATP hydrolysis did not increase as a function of increasing ssDNA concentration. In fact, the MutL-R266E hydrolysis rate remained at the basal level. Although indirect, these data suggest that the mutant protein was unable to interact productively with ssDNA since the rate of ATP hydrolysis was not stimulated by the addition of DNA.

MutL-R266E Is Defective for ssDNA Binding—To directly test the possibility that MutL-R266E fails to bind ssDNA or binds ssDNA with a significantly lower affinity than wild-type MutL, we performed double filter DNA binding assays (35). Previously we have shown that MutL is able to bind a 93-bp partial duplex DNA with an apparent KD of 25 nM in the absence of nucleotide (28). The data shown in Fig. 3A demonstrate that MutL is able to bind to a 93-bp partial duplex with an apparent KD of 2.8 nM in the presence of 3 mM AMP-PNP, a nonhydrolyzable analogue of ATP. Enhanced DNA binding in the presence of a nucleotide cofactor has been reported previously (20, 28). MutL-R266E was also able to bind to this substrate in the presence of AMP-PNP but with a severely decreased affinity. We observed that at low concentrations of MutL-R266E, almost no DNA was bound; however, at elevated concentrations of MutL-R266E, a significant fraction of this substrate was bound in the presence of 3 mM AMP-PNP. We obtained very similar results when the NaCl concentration in the reaction mixture was raised to 100 mM (Fig. 3B). In this case the apparent KD for binding of MutL to the substrate was 6 nM in the presence of AMP-PNP. Again, MutL-R266E was able to bind to this substrate but with a significantly reduced affinity. These data suggest that MutL-R266E has a very low affinity for DNA, although it is not completely DNA binding-deficient.

A 93-bp partial duplex DNA ligand has the characteristics of ssDNA, dsDNA, and 3’ overhang ssDNA and perhaps other secondary structures. To further characterize the DNA substrate preference of MutL and MutL-R266E for these varied DNA secondary structures, three DNA ligands were generated using oligonucleotides; they are a 50-mer ssDNA, a 50-mer dsDNA, and a substrate with a 50-bp duplex region containing a 20 base 3’ overhang ssDNA region. The binding of MutL and MutL-R266E was tested using the double filter DNA binding assay described under “Materials and Methods” with each of these ligands in the presence and absence of AMP-PNP. MutL bound to the ssDNA 50-mer (Fig. 3C) with an apparent KD of 246.7 nM, whereas MutL-R266E did not appear to bind to this synthetic oligomer even at protein concentrations in excess of 800 nM. The binding of MutL to an ssDNA ligand in the presence of AMP-PNP was also tested using a gel mobility shift assay (28, data not shown). The results obtained with both proteins using this method were identical with the results obtained using the filter binding assay.

The 50-mer dsDNA and the DNA substrate with a 50-bp duplex region containing a 20-base 3’ overhang ssDNA region were also tested. The results of these experiments show that MutL exhibits a preference for binding the DNA ligands in the following order: ssDNA (KD > 246.7 nM) > 3’ overhang DNA (KD > 4.1 μM) > dsDNA (KD > 5000 μM), suggesting that ssDNA is the preferred ligand for DNA binding. Under the same conditions, MutL-R266E was unable to bind significantly to any of these DNA ligands (data not shown). The absence of DNA binding by the mutant protein is a direct demonstration that MutL-R266E cannot bind DNA or binds DNA very poorly.

MutL-R266E Is Able to Stimulate Helicase II-catalyzed Unwinding—To address the issue of MutL-R266E and its interaction with helicase II, we compared the ability of MutL and MutL-R266E to stimulate UvD in helicase unwinding assays using both a 93-bp partial duplex (PD) and 710-bp blunt duplex substrates (Fig. 4). As shown here and in previous reports (19), MutL stimulates the helicase II-catalyzed unwinding of these DNA substrates with a KD of 28.4 nM and a KD of 210 nM for 710-bp blunt of 208 nM (K1/2 is defined as the MutL concentration required for 1⁄2 maximal stimulation). Under the same conditions, MutL-R266E was also capable of stimulating the helicase II unwinding reaction on a 93-bp partial duplex substrate, albeit
very poorly \( (K_{1/2} = 305 \text{ nM}) \), and was unable to stimulate the helicase II unwinding reaction on a 710-bp blunt duplex substrate. Again, this result is indirect evidence of the poor DNA binding activity associated with the mutant protein. It is likely that MutL-R266E is able to stimulate the helicase II unwinding reaction due to the fact that a single point mutation in MutL does not abolish DNA binding and, therefore, MutL-stimulated unwinding but does diminish the DNA binding activity significantly.

MutL-R266E Partially Complements the mutL Deletion When Overexpressed in Vivo—The data presented above are consistent with the possibility that MutL-R266E is able to interact with ssDNA, albeit with duplex DNA \( (4 \mu M \text{ DNA-P}) \) in the presence of 100 mM NaCl (B), and ssDNA 50-mer \( (1 \mu M \text{ molecules}) \) (C) are shown. All reactions contained 3 mM AMP-PNP, and each point represents the average of at least three experiments. Error bars are the S.D. about the mean.
a much lower affinity than the wild-type protein. We reasoned that if this were the case, then overexpression of the mutant protein in vivo might rescue the defect observed in a strain that does not express MutL in genetic complementation assays. The notable ability of MutL-R266E to stimulate helicase II to nearly the same extent as wild-type MutL, but only at very high concentrations of protein, is consistent with this possibility. To test this idea, a single copy vector (pETcoco-2) was utilized to reduce basal expression from the T7 promoter in the absence of IPTG. The final IPTG concentration chosen to induce protein expression was 250 μM, and the cell cultures were grown for exactly 15 h and 30 min before plating. Fig. 5 shows the results of these experiments with E. coli BL21(DE3)mutL::Tn10 and the indicated plasmid. All the cell strains used in this experiment exhibit a mutator phenotype, as indicated by the growth of RifR cells, in the absence of IPTG, as expected, since MutL is not expressed. However, in the presence of IPTG, protein expressed from the pETcoco-2-MutL plasmid was able to fully complement the MutL deletion, and importantly, the plasmid-borne MutL-R266E was able to partially complement the deletion such that the mutator phenotype was not as severe. This result is consistent with the interpretation that MutL-R266E has a decreased DNA binding affinity that can be partially overcome by increased expression of the mutant protein and that MutL DNA binding is essential for MMR in vivo.

The R266E Mutation Does Not Abrogate Any Protein-Protein Interaction in the MMR Pathway—MutL is known to interact with several proteins involved in MMR (12, 15, 24, 36, 39, 40). Thus, the mutator phenotype associated with MutL-R266E might be due to an inability to interact with one or more of the MMR proteins. We directly tested the ability of MutL-R266E to interact with both helicase II and MutH as indicated below. Previous studies (36) have shown that MutL-R266E interacts with MutS.

The interaction between MutL-R266E and MutH was assessed by affinity chromatography and by using MutL-R266E in a MutL-stimulated heteroduplex DNA nicking assay. As shown in Fig. 6, MutL-R266E binds an affinity column constructed using purified MutH and elutes at a NaCl concentration of 200 mM. This result is identical with the elution pattern observed for wild-type MutL and strongly suggests that MutL-R266E is able to interact with MutH.

We also examined the ability of these two proteins to interact in a functional assay based on a partial reconstitution of the mismatch repair pathway (Fig. 7). In this assay the ability of MutL to stimulate the latent endonuclease reaction catalyzed by MutH is evaluated in the presence of a base pair mismatch and MutS. It is clear that MutL-R266E is able to stimulate the MutH endonuclease reaction, albeit not to the same extent or with the same efficiency as wild-type MutL. Control experiments using DNA lacking a mismatch, with MutL alone, MutS alone, and MutH alone indicate this reaction is dependent on all three proteins as well as the mismatch, as expected.
Taken together, these data suggest that MutL-R266E interacts with MutH, and it is unlikely that the mutator phenotype associated with the MutL-R266E allele is due to an inability to interact with MutH. However, the DNA binding activity of MutL is important, although not essential, for the MutL-stimulated nicking reaction catalyzed by MutH. This is similar to what was observed with UvrD (see Fig. 4), where MutL-R266E is able to stimulate the helicase reaction but not as efficiently as the wild-type protein.

The interaction between MutL-R266E and helicase II has been shown earlier in the helicase II stimulation assays (see Fig. 4). To directly assess the interaction between MutL and helicase II, we performed a double sandwich enzyme-linked immunosorbent assay (Fig. 8). In this experiment, 62.5 fmol of helicase II was bound to an enzyme-linked immunosorbent assay plate, and a titration of MutL or MutL-R266E was performed. Bound protein was detected using an antibody directed against MutL. The data indicate that the interaction between MutL-R266E and helicase II is similar to that of MutL and helicase II. Thus, the mutator phenotype observed in cells expressing MutL-R266E is not due to a defect in the interaction between MutL-R266E and helicase II.

**DISCUSSION**

The role of DNA binding by MutL in the process of mismatch repair has been debated in the literature for the last 14 years. Although there is considerable evidence suggesting that MutL binds DNA (11, 20, 23, 28, 47), there is also evidence to the contrary (24), and recent experiments have suggested that DNA binding may be an artifact of in vitro experiments (25). Here we have used biochemical and genetic assays to characterize a MutL point mutant in an effort to evaluate the biological importance of DNA binding by MutL. Taken together, the in vivo and in vitro results presented in this study strongly suggest that MutL must bind DNA as part of the MMR process.

The in vitro DNA binding results clearly show that, under the variety of conditions used here, MutL-R266E is unable to bind a ssDNA oligonucleotide to any significant extent and binds very poorly to a partial duplex DNA which contains multiple DNA secondary structures (see Fig. 3). Importantly, the mutant protein failed to bind the ssDNA and 3' overhang DNA for which wild-type MutL has a demonstrated affinity. However, these conditions are not physiological and, therefore, it remained possible that the binding of wild-type MutL to DNA, as measured in these experiments, was an artifact of the in vitro conditions.
Thus, a careful analysis of the other biochemical properties of MutL-R266E and its function \textit{in vivo} were undertaken.

Purified MutL-R266E retains the biochemical properties of wild-type MutL that do not involve DNA binding. These properties include: (i) basal ATP hydrolysis, (ii) an ability to interact with MutH, MutS, and helicase II, and (iii) the ability to dimerize. These results demonstrate that the mutant protein retains both its tertiary structure and its dimeric form. In addition, it is able to catalyze the hydrolysis of ATP. However, the properties dependent upon DNA binding were severely compromised. The basal ATPase activity exhibited by the mutant protein was not stimulated by the addition of ssDNA, whereas the basal ATPase of wild-type MutL was significantly stimulated by the addition of ssDNA (see Fig. 2). The mutant protein was also significantly reduced in its ability to stimulate the unwinding reaction catalyzed by helicase II, although at high concentrations it was able to stimulate helicase II but only on a partial duplex DNA substrate. This, most likely reflects the low affinity exhibited by MutL-R266E for binding a circular partial duplex DNA. The nature of the interaction between MutL and the circular DNA is unknown at present and under active investigation.

The crystal structure of dimeric MutL (20, 21) positions arginine 266 facing inward and previous reports (11, 20, 36, 47) have suggested it could interact with the negatively charged backbone of DNA. Thus, arginine 266 may be a primary amino acid involved in recognizing the DNA through electrostatic interactions. Recently, a second potential DNA binding site, located in the COOH-terminal domain of MutL, was identified based on mutation of a series of positively charged amino acid residues followed by \textit{in vivo} and \textit{in vitro} analysis (47). However, a new model of the COOH-terminal domain of MutL (48) suggests that the MMR defect observed with these mutants may be due to altered mobility of a critical region of the COOH-terminal domain as opposed to a DNA binding defect. Thus, the contribution of the COOH-terminal domain to DNA binding, if any, is not clear at present.

At least one report has suggested that the binding of MutL to DNA is independent of the presence of ATP (23). However, we have shown that MutL does not bind model DNA substrates in the absence of a non-hydrolyzable analogue of ATP (AMP-PNP) under our conditions and as previously reported (20, 28). This result strongly suggests that DNA binding is dependent upon ATP binding. We hypothesize that ATP-bound MutL interacts with DNA and remains bound to the DNA until one or both of the bound ATP molecules are hydrolyzed to ADP and P\textsubscript{i}. After an ATP hydrolysis fueled conformational change, MutL is released from the DNA. Thus, ATP binding and its hydrolysis may serve as a switch to modulate the DNA binding affinity of MutL in MMR.

It is known that MutL "activates" helicase II in MMR (11, 19, 47), and we have proposed that MutL loads helicase II onto the incised MMR substrate (20) in a reaction dependent on DNA binding by MutL. Due to the slow ATPase intrinsic to MutL, we further postulate that the MutL ATPase may act as a molecular timer to regulate the loading of helicase II molecules onto the DNA. The processivity of helicase II-catalyzed unwinding of duplex DNA has been estimated at 40–50 base pairs in one study (42) and ~250 base pairs in another study (43). However, repair tracts \textit{in vivo} can be up to 2 kilobases in length (44, 45). Moreover, we have demonstrated significant unwinding of a 710-bp duplex DNA substrate by helicase II in the presence of MutL (see Fig. 4). In a recent study by Fischer \textit{et al.} (46), the processivity of helicase II was determined to be 2400 ± 600 bases as the protein \textit{translocates} along ssDNA. We suggest that MutL binds to the DNA and continuously loads helicase II molecules until MutL hydrolyzes its ATP and is subsequently released from the DNA. MutL, no longer associated with the DNA, is not able to load helicase II onto the DNA. The \textit{multiple} helicase II molecules that are loaded onto the duplex are able to unwind up to 2–3 kilobases of duplex DNA even though the processivity of helicase II for unwinding duplex DNA is much less than 2 kilobases. As one molecule of helicase II dissociates from the unwinding fork due to its intrinsic low processivity, a second molecule of helicase II takes its place and unwinding continues uninterrupted, taking advantage of the processivity of this protein as a translocase. This helps to ensure that, no matter what the distance between the initiating nick and the mismatch, adequate DNA is unwound by helicase II to ensure repair of the match. This model does not, however, address the termination of the repair tract, which has been shown to extend ~100 base pairs past the mismatch (17). This question remains unresolved.

We also note that MutL-R266E stimulated the mismatch-directed nicking of a heteroduplex DNA substrate by MutH (see Fig. 7), consistent with the fact that MutL-R266E interacts with MutH as determined by affinity chromatography. However, the DNA binding-deficient MutL was able to stimulate MutH-directed nicking less efficiently and to a lower extent than wild-type MutL. This suggests that the DNA binding activity of MutL is important for this reaction as well. Thus, the \textit{in vitro} experiments with MutL-R266E reveal two steps in the MMR pathway in which DNA binding by MutL contributes to the overall efficiency of repair; that is, stimulation of the incision reaction catalyzed by MutH and activation (loading) of helicase II.

With the characteristics of the purified protein in mind, it is not surprising that cells expressing MutL-R266E exhibit a strong mutator phenotype that is essentially identical to that of a \textit{mutL} null mutant. Based on both the genetic and biochemical data, we conclude that DNA binding by MutL is critical for MMR. We note that a similar conclusion has been reached for representative eukaryotic MutL homologues. In yeast it has been shown that point mutations in PMS1 (Pms1-K328E) and MLH1 (Mlh1-R273E,R274E), located within a proposed DNA binding groove, increase the mutation frequencies and rates \textit{in vivo} (41). These proteins also fail to bind DNA \textit{in vitro}. Thus, in addition to catalyzing ATP hydrolysis, MutL must also bind DNA to fulfill its function in the MMR pathway.

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\textbf{REFERENCES}

1. Feinstein, S. L. and Low, K. B. (1986) \textit{Genetics} 113, 13–33
2. Modrich, P., and Lahue, R. (1996) \textit{Annu. Rev. Biochem.} 65, 101–133
3. Rayssiguier, C., Thaler, D. S., and Radman, M. (1989) \textit{Nature} 342, 396–401
4. Cooper, D. L., Lahue, R. S., and Modrich, P. (1993) \textit{J. Biol. Chem.} 268, 11823–11829
5. Viswanathan, M., Burdett, V., Baum, C., Modrich, P., and Lovett, S. T. (2001) \textit{J. Biol. Chem.} 276, 31053–31058
6. Burdett, V., Baatinger, C., Viswanathan, M., Lovett, S. T., and Modrich, P. (2001) \textit{Proc. Natl. Acad. Sci. U.S.A.} 98, 6765–6770
7. Lahue, R. S., Au, K. G., and Modrich, P. (1989) \textit{Science} 245, 160–164
8. Harfe, B. D., and Jinks-Robertson, S. (2000) \textit{Annu. Rev. Genet.} 34, 359–399
9. Schofield, M. J., and Hsieh, P. (2003) \textit{Annu. Rev. Microbiol.} 57, 579–608
10. Nicholoff, J. A., and Hoeskstra, M. F. (1998) \textit{DNA Damage and Repair}, pp. 205–228, Humana Press Inc., Totowa, NJ
11. Junop, M. S., Yang, W., Fuchan, P., Clendenen, W., and Miller, J. H. (2003) \textit{DNA Repair} 2, 387–405
12. Grilley, M., Welsh, K. M., Su, S. S., and Modrich, P. (1989) \textit{J. Biol. Chem.} 264, 1000–1004
13. Su, S. S., and Modrich, P. (1986) \textit{Proc. Natl. Acad. Sci. U.S.A.} 83, 5057–5061
14. Allen, D. J., Makhov, A., Grilley, M., Taylor, J., Thresher, R., Modrich, P., and Griffith, J. D. (1997) \textit{EMBO J.} 16, 4467–4476
15. Hall, M. C., and Matson, S. W. (1999) \textit{J. Biol. Chem.} 274, 1306–1312
16. Au, K. G., Welsh, K., and Modrich, P. (1992) \textit{J. Biol. Chem.} 267, 12142–12148
17. Grilley, M., Griffith, J., and Modrich, P. (1993) \textit{J. Biol. Chem.} 268, 11830–11837
18. Matson, S. W. (1986) \textit{J. Biol. Chem.} 261, 10169–10175
19. Yamaguchi, M., Mao, V., and Modrich, P. (1998) \textit{J. Biol. Chem.} 273, 9197–9201
20. Ban, C., Junop, M., and Yang, W. (1999) Cell 97, 85–97
21. Ban, C., and Yang, W. (1998) Cell 95, 541–552
22. Spampinato, C., and Modrich, P. (2000) J. Biol. Chem. 275, 9863–9869
23. Bende, S. M., and Graffstrom, R. H. (1991) Nucleic Acids Res. 19, 1549–1555
24. Drotschmann, K., Aronshtam, A., Fritz, H. J., and Marinus, M. G. (1998) Nucleic Acids Res. 26, 948–953
25. Acharya, S., Foster, P. L., Brooks, P., and Fishel, R. (2003) Mol. Cell 12, 233–246
26. Yang, W. (2000) Mutat. Res. 460, 245–256
27. Runyon, G. T., Wong, I., and Lohman, T. M. (1993) Biochemistry 32, 602–612
28. Mechanic, L. E., Frankel, B. A., and Matson, S. W. (2000) J. Biol. Chem. 275, 38337–38346
29. Ausubel, F. M. (1987) Current Protocols in Molecular Biology, pp. 2.1.4–2.1.5, Greene Publishing Associates and Wiley-Interscience, J. Wiley, Inc., New York
30. Brosh, R. M., Jr., and Matson, S. W. (1995) J. Bacteriol. 177, 5612–5621
31. Miller, J. H. (1992) A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria, pp. 21.1–21.3, Cold Spring Harbor Laboratory Press, Plainview, NY
32. Rosche, W. A., and Foster, P. L. (2000) Methods 20, 4–17
33. Luria, S. E., and Delbruck, M. (1943) Genetics 28, 491–511
34. Lea, D. E., and Coulson, C. A. (1949) J. Genet. 49, 264–285
35. Wong, I., and Lohman, T. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5428–5432
36. Selmane, T., Schofield, M. J., Nayak, S., Du, C., and Hsieh, P. (2003) J. Mol. Biol. 334, 949–965
37. Schofield, M. J., Nayak, S., Scott, T. H., Du, C., and Hsieh, P. (2001) J. Biol. Chem. 276, 28291–28299
38. Aronshtam, A., and Marinus, M. G. (1996) Nucleic Acids Res. 24, 2498–2504
39. Giron-Monzon, L., Manelyte, L., Ahrends, R., Kirsch, D., Spergler, B., and Friedhoff, P. (2004) J. Biol. Chem. 279, 49338–49345
40. Hall, M. C., Jordan, J. R., and Matson, S. W. (1998) EMBO J. 17, 1535–1541
41. Hall, M. C., Shcherbakova, P. V., Fortune, J. M., Borchers, C. H., Dial, J. M., Torner, K. B., and Kunkel, T. A. (2003) Nucleic Acids Res. 31, 2025–2034
42. Ali, J. A., and Lohman, T. M. (1997) Science 275, 377–380
43. Dessimies, M. N., Lionnet, T., Xi, X. G., Plessisnon, D., and Croquette, V. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 6439–6444
44. Bruni, R., Martin, D., and Jiricny, J. (1988) Nucleic Acids Res. 16, 4875–4890
45. Lahue, R. S., Su, S. S., and Modrich, P. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1482–1486
46. Fischer, C. J., Maluf, N. K., and Lohman, T. M. (2004) J. Mol. Biol. 344, 1287–1309
47. Guarme, A., Ramon-Maiques, S., Wolff, E. M., Ghirlanda, R., Hu, X., Miller, J. H., and Yang, W. (2004) EMBO J. 23, 4134–4145
48. Kosinski, J., Steindorf, I., Bajnicki, J. M., Giron-Monzon, L., and Friedhoff, P. (2005) J. Mol. Biol. 351, 495–909