The MutL ATPase Is Required for Mismatch Repair*

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Members of the MutL family contain a novel nucleotide binding motif near their amino terminus, and the Escherichia coli protein has been found to be a weak ATPase (Ban, C., and Yang, W. (1998) Cell 95, 541–552). Genetic analysis has indicated that substitution of Lys for Glu-32 within this motif of bacterial MutL results in a strong dominant negative phenotype (Aronshtam, A., and Marinus, M. G. (1996) Nucleic Acids Res. 24, 2498–2504). By in vitro comparison of MutL-E32K with the wild type protein, we show the mutant protein to be defective in DNA-activated ATP hydrolysis, as well as MutS- and MutL-dependent activation of the MutH d(GATC) endonuclease and the mismatch repair excision system. MutL-E32K also acts in dominant negative manner in the presence of wild type MutL in vitro, inhibiting the overall mismatch repair reaction, as well as MutH activation. As judged by protein affinity chromatography, MutL and MutL-E32K both support formation of ternary complexes that also contain MutS and MutH or MutS and DNA helicase II. These findings imply that the MutL nucleotide binding center is required for mismatch repair and suggest that the dominant negative behavior of the MutL-E32K mutation is due to the formation of dead-end complexes in which the MutL-E32K protein is unable to transduce a signal from MutS that otherwise results in mismatch-dependent activation of the MutH d(GATC) endonuclease or the unwinding activity of helicase II.

Mismatch repair stabilizes the bacterial genome by correcting DNA biosynthetic errors and by ensuring the fidelity of homologous genetic recombination (1–4). The pathway displays a broad specificity for different mispairs, with repair of DNA biosynthetic errors targeted to the daughter strand by virtue of the transient absence of d(GATC) methylation on newly synthesized sequences (5). Repair is initiated by the binding of MutS to the mismatch, a reaction that can occur with the dimeric form of the protein (6–9). MutL, which also exists as a dimer in solution, binds to heteroduplex DNA in a MutS-dependent manner (9–11). Assembly of this ternary complex is sufficient to activate the MutH d(GATC) endonuclease activity of MutL, which cleaves the newly synthesized, unmethylated strand (12), and to activate unwinding by DNA helicase II, which enters the helix at the incised d(GATC) sequence and unwinds toward the mismatch (13). That portion of the incised strand unwound in this manner is subject to degradation by one of several single strand exouclease (14, 15). Repair synthesis of the ensuing gap is mediated by DNA polymerase III holoenzyme in the presence of single strand DNA-binding protein, and DNA ligase restores covalent continuity to the repaired strand (16).

MutL plays a critical role in mismatch repair, but the molecular functions of the protein in the reaction are only partially understood. It has been suggested that MutL serves to interface mismatch recognition by MutS to other activities involved in repair (17, 18), and evidence supporting this idea is available. As noted above, MutL binds to heteroduplex DNA in the presence of MutS (9–11). MutL also activates unwinding by DNA helicase II on conventional helicase substrates (19) and activates the d(GATC) endonuclease of MutH in the absence of MutS under certain conditions (20). Both of these effects are attributable at least in part to physical interaction of MutL with the latter two activities (19, 21, 22).

MutL homologs have been identified in yeast, mouse, and human cells (3, 4, 23). In contrast to the homodimeric structure of bacterial MutL, eukaryotic MutL function is provided by heterodimeric complexes of homologs of the bacterial protein, e.g. the MLH1-PMS1 complex in yeast and the MLH1-PMS2 heterodimer in mammalian cells (24, 25). These bacterial and eukaryotic polypeptides display sequence homology within their amino-terminal regions, which contains a novel nucleotide binding motif that was originally identified in type II topoisomerases, HSP90, and histidine kinase families (26, 27). A number of dominant negative mutations have been localized to this motif in the bacterial protein (28), and about 50% of missense mutations found in human MLH1 in nonpolyposis colon cancers are also within the conserved region (29). The structure of a 40-kDa amino-terminal segment of Escherichia coli MutL has been determined as the apoprotein (20) and as a complex with AMPPNP or ADP, work that has demonstrated a large conformational transition associated with nucleotide occupancy (30). In addition, the bacterial protein has been reported to be a weak ATPase (20, 30). However, others have failed to detect this activity (19, 22), perhaps because of the high MutL Km for ATP and the fact that purified MutL preparations are commonly contaminated by trace levels of DNA helicase II, which also hydrolyzes this nucleotide.

To further clarify the role of the MutL nucleotide binding site in mismatch repair, we have studied the biochemical properties of a mutant form of the protein that harbors a Glu to Lys substitution at position 32 within motif N of the nucleotide binding center. The E32K amino acid substitution results in a strong dominant negative mutL phenotype, and unlike other dominant negative mutL mutations that have been characterized genetically, the phenotype of the E32K substitution mutation is not suppressed by increased gene dosage of the wild type allele (28).
MutL ATPase Is Required for Mismatch Repair

MATERIALS AND METHODS

Bacterial Strains—E. coli BL21(DE3) mutL103::Tn5 uvrD::tet was constructed by P1 transduction of mutL103::Tn5 from NK7510 (a gift from Nancy Kleckner, Harvard University) into BL21(DE3) uvrD::tet (provided by Stephen Matson, University of North Carolina), with selection for resistance to tetracycline and kanamycin. Deficiency of both MutL and helicase II in the double mutant was confirmed by Western blotting and by in vitro complementation to restore mismatch repair (not shown).

MutL-E32K mutation was constructed by converting the Glu-32 GAA codon to a Lys AAA codon using the megaprimer polymerase chain reaction method (31). The mutant gene was inserted into pET3a after digestion with BamHI and Ndel as described previously (19) to yield plasmid pCS1. The sequence of the MutL-E32K gene was determined in its entirety to confirm the presence of the desired single mutation.

Protein Expression and Purification—Wild type MutL was purified using the overproducing plasmid L1-pET3a as described previously (19) and was isolated after expression in a BL21(DE3) uvrD::tet host to eliminate trace contamination by DNA helicase II that was observed previously (19). MutL-E32K mutant protein was expressed from plasmid pCS1 in E. coli BL21(DE3) mutL103::Tn5 uvrD::tet. Cells were grown in Luria broth to an optical density (600 nm) of 0.8 at 37 °C, induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 2 h at 37 °C, and harvested by centrifugation. The mutant protein was isolated by the procedure of Griley et al. (10) during which it fractionated like wild type MutL. Preparations had a purity greater than 98% as judged by electrophoresis in the presence of SDS. MutS, MutH, and DNA helicase II were isolated by published methods (6, 32, 33). Protein concentrations were determined by Bradford assay using bovine serum albumin (BSA) as standard (34). When cited in molar terms, protein concentrations are expressed as monomer equivalents.

Determination of Native Aggregation State—The aggregation state of MutL-E32K was compared with that of the wild type protein by gel filtration through a Superose 6 HR 10/30 (Amersham Pharmacia Biotech) fast protein liquid chromatography column equilibrated with 25 mM Mops (pH 7.4), 150 mM KCl, 8 mM NaCl, 0.5 mM dithiothreitol, 10% glycerol, and pH 8.0 at 37 °C. Fractions were analyzed using blue dextran.

ATPase and ATP Binding Assays—ATP hydrolysis was determined at 37 °C in reactions (20 μl) containing 20 mM Tris-HCl, pH 8.0, 90 mM KC1, 1 mM dithiothreitol, and 0.8 μM MutL or MutL-E32K. The effect of single-stranded DNA (30) on ATP hydrolytic activity was determined by inclusion of a 58-mer synthetic oligonucleotide (2 μM molecules unless noted otherwise). Hydrolysis was initiated by the addition of [γ-32P]ATP (5 μCi/ml) to prewarmed reactions to a final concentration of 1 mM. At 10 min intervals 2-μl samples were taken and quenched with 50 μl of 0.5 M EDTA, pH 8.0. The extent of ATP hydrolysis was determined by chromatography of 1 μl of quenched samples on polyethyleneimine-cellulose plates (EM Science, Gibbstown NJ) that were developed in 0.3 M KPO4, pH 7.0. Dried plates were phosophorimaged overnight and optically scanned using a STORM imager system, and data were analyzed using Imagequant software (Molecular Dynamics, Sunnyvale CA). Initial steady-state rates of ATP hydrolysis were determined by least squares analysis of the linear portion of the progress curve.

ATP binding was determined in reactions (20 μl) containing 20 mM Tris-HCl, pH 7.6, 25 mM KC1, 75 μg/ml BSA, 1 mM dithiothreitol, and 3.7–18.4 μM MutL or 8 μM MutL-E32K. Binding was initiated by the addition of indicated concentrations of [γ-32P]ATP (5 μCi/ml) or [α-32P]ATP (5 μCi/ml) (0.2 Ci/mmol). After incubation at 0 °C as indicated, 1 ml of reaction buffer was mixed with each sample, and the entire volume was passed through a 0.45-μm nitrocellulose membrane pre-wetted with reaction buffer. Filters were washed three times with 1 ml of reaction buffer lacking BSA and dried, and radioactivity was determined by liquid scintillation counting. ATP binding to membranes in the absence of MutL was typically less than 3% of that observed in the presence of MutL. ATP binding in the presence of heat denatured MutL was less than 6%. The latter values were used as blanks and were subtracted from the data shown.

The MutL-bound nucleotide was quantitatively released from filters by soaking for 2 h at room temperature in 0.4 ml of 20 mM Tris-HCl, pH 8.0, containing 0.2% SDS or 0.3 μg/ml proteinase K. The two methods yielded identical results. Samples were spotted onto polyethyleneimine-cellulose plates that were visualized and analyzed as described above.

Mismatch Repair and Partial Reaction Assays—MutL activity in mismatch repair was determined by complementation of extracts prepared from E. coli MG102 (mutL::Tn10) as described previously (10) using an θ heteroduplex containing a G-M mismatch at position 5632 and a single d(GATC) site at position 216 above 100 base pairs from the mismatch (7). d(GATC) cleavage was activated by MutH endonuclease was determined by a modification of the method of Au et al. (12) using a hemi-methylated 32P-end-labeled θ-G heteroduplex. This DNA was prepared by linearization of the circular, hemimethylated θ heteroduplex mentioned above with Bsp106 endonuclease, 3′ end-labeling of the product with exonuclease-free Klenow DNA polymerase I (Amersham Pharmacia Biotech) in the presence of dATP, dGTP, dTTP, and [α-32P]dCTP (NEN Life Science Products, 3000 Ci/mmol), termination of the reaction by heating to 75 °C for 20 min, and removal of unincorporated nucleotide by Sephadex G-50 chromatography in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. MutH endonuclease reactions (10 μl) contained 20 mM Tris-HCl, pH 8.0, 20 mM KC1, 5 mM MgCl2, 50 μg/ml BSA, 1 mM dithiothreitol, 10% glycerol, and 32P-end-labeled θ heteroduplex, and were incubated at 37 °C as indicated. After preincubation for 5 min at 37 °C, reactions were initiated by the addition of ATP to 3 mM. Reactions were terminated as indicated by the addition of one-third volume of 0.2 N NaOH, 0.04 mM EDTA, 10% Ficoll 400, 0.1% Brom cresol green, and samples electrophoresed through 1% agarose gels in 0.3% NaOAc, 1 mM EDTA. Gels were dried, quantitated using a Molecular Dynamics PhosphorImager and autoradiographed.

Mismatches-Induced gap formation was determined by purified proteins as described (15, 16). Reactions (10 μl) contained 35 ng of MutS, 30 ng of MutL, 10 ng of DNA helicase II, 200 ng of single strand-binding protein, 3 ng exonuclease I, and 24 fmol of nicked circular θ-T heteroduplex containing a single strand break at the gII cleavage site. This viral strand nick is located 181 base pairs 3′ to the mismatch as measured along the shorter path between the two sites in the circular molecule (35). Presence of a single-stranded gap in the DNA was determined by virtue of resistance of the gap intermediate to Nhel endonuclease, the recognition site for which is located 5 base pairs 5′ to the mismatch as viewed on the viral strand (35).

MutS Affinity Columns—Purified MutS (850 μg) was coupled to 1, carbonyldimidazole activated agarose (1 ml) according to the supplier (Pierce) in 0.1 M NaHCO3, pH 8.9. Residual active groups on the resin were then blocked by incubating the gel for an additional 2 h at room temperature with 1 mM ethanolamine, pH 9.0. A 1-ml column was transferred to a chemiluminescence assay buffer (Molecular Dynamics, Sunnyvale CA). Initial steady-state rates of ATP hydrolysis were determined by least squares analysis of the linear portion of the progress curve.
MutL ATPase Is Required for Mismatch Repair

Aronshtam and Marinus (28) have identified 72 mutations within the E. coli mutL gene that behave in a dominant negative fashion when expressed from a multicopy plasmid in the presence of a single wild type copy of the gene on the bacterial chromosome. With one exception, the dominant negative phenotype of these mutations was reversed when the wild type protein was also expressed from a multicopy plasmid, suggesting that dominant negative behavior in most cases was because of production of mixed mutant wild type MutL heterodimers or sequestration of other repair activities by the mutant protein (28). The latter possibility is consistent with the finding that dominant negative behavior in most cases was because of overproduction of MutS protein and MutL-E32K in this regard. MutL hydrolyzed ATP in a slow reaction that is enhanced substantially by single-stranded DNA (20, 30). We have confirmed this observation and have compared the wild type protein in essentially homogeneous form and have compared its biochemical properties with those of the wild type protein. Because MutL is known to activate and interact with DNA helicase II (19, 21), the mutant protein was isolated from a strain with insertion mutations in chromosomal mutL and uvrD loci (“Materials and Methods”).

ATP Binding and Hydrolysis—MutL has been reported to catalyze the hydrolysis of ATP in a slow reaction that is enhanced substantially by single-stranded DNA (20, 30). We have confirmed this observation and have compared the wild type protein and MutL-E32K in this regard. MutL hydrolyzed ATP with a $K_m$ of 0.41 ± 0.11 mM and a $k_{cat}$ of 0.86 ± 0.08 min$^{-1}$/monomer equivalent (not shown). This $k_{cat}$ value is similar to that reported by Ban and Yang (20), although the $K_m$ we have determined is somewhat higher than that of the previous study, perhaps reflecting their use of His-tagged MutL. MutL-E32K also hydrolyzed ATP with a similar $K_m$ of 0.35 ± 0.08 mM and a slightly reduced $k_{cat}$ of 0.38 ± 0.03 min$^{-1}$. The hydrolytic activity of the wild type protein was increased 10-fold in the presence of a 58-residue single strand oligonucleotide, which increased the $k_{cat}$ for hydrolysis to 8.7 ± 0.7 min$^{-1}$ without altering the $K_m$ for ATP, which remained at 0.40 ± 0.10 mM. However, as shown in Fig. 1 (upper panel), the presence of the single-stranded DNA had a minimal effect on the hydrolytic activity of the E32K mutant protein.

Binding of the magnesium chelate of [α-32P]ATP to wild type MutL was demonstrable by nitrocellulose membrane assay (Fig. 1, lower panel). Binding was hyperbolic with a dissociation constant of 0.48 ± 0.04 mM at 0 °C. Apparent stoichiometry
with $[\alpha^{32}\text{P}]\text{ATP}$ was 0.54 ± 0.05 mol nucleotide/mol MutL monomer, and a similar value was obtained with $[\gamma^{32}\text{P}]\text{ATP}$ (0.52 ± 0.03 mol/monomer). Because MutL is a dimer in solution (10), this may indicate that only one of the two nucleotide binding sites is active in the native oligomer. However, we cannot rule out the possibility that the reduced stoichiometry is because of a filter retention efficiency of less than unity. Despite the similar hydrolytic activities of wild type MutL and the E32K mutant protein in the absence of DNA, ATP binding by the mutant protein was reduced by more than an order of magnitude at a nucleotide concentration that is near saturating for the wild type protein (Fig. 1). Because the membranes used to trap MutL:ATP complexes were subjected to buffer wash prior to quantitation, this difference may be indicative of an increased rate of dissociation of the nucleotide from the mutant protein.

The similar binding stoichiometries observed for wild type MutL with $[\alpha^{32}\text{P}]\text{ATP}$ and $[\gamma^{32}\text{P}]\text{ATP}$ suggested that the bound nucleotide observed in the experiments above is predominantly the triphosphate. This was confirmed by elution of MutL-bound nucleotide from filtered complexes prepared with $[\alpha^{32}\text{P}]\text{ATP}$ (“Materials and Methods”). Analysis by thin layer chromatography showed 80% of the MutL-associated nucleotide to be ATP (not shown), suggesting that the rate-limiting step for hydrolysis occurs at or prior to the chemical step.

**Aggregation State of MutL-E32K—Sedimentation and gel filtration analysis** has previously shown that native MutL exists in solution as a homodimer with a significant degree of nonspherical asymmetry (10). Comparison of wild type and E32K proteins demonstrated identical behavior during gel filtration (Fig. 2). Furthermore, glutaraldehyde treatment yielded identical cross-linking patterns for both proteins. As judged by these criteria, the E32K amino acid substitution does not alter the oligomeric state of MutL.

**MutL-E32K Behaves as a Dominant Negative Inhibitor of Mismatch Repair in Vitro**—As shown previously, extracts of *E. coli* MG102 (mutL::Tn10) are defective in methyl-directed repair, but mismatch correction can be restored to normal levels by the addition of MutL (Fig. 3, lanes a–c). MutL-E32K not only failed to complement extracts of the MutL-deficient strain (lane d), but inhibited repair when present together with the wild type protein (lanes e–h). Quantitation of the repair products (“Materials and Methods”) from several experiments like those shown in Fig. 3 demonstrated that repair was reduced by 28–38% ($n = 2$) at a ratio of mutant:wild type protein 1:2 and by 71 ± 16% ($\pm 1$ standard deviation, $n = 11$) when the two proteins were present at equimolar concentration. These observations are in accord with the biological behavior of the E32K mutation (28).

**MutL-E32K Is Defective in MutH Activation and Mismatch-provoked Excision Reactions**—In a previous study, we showed that extracts of *E. coli* MG102 (mutL::Tn10) were defective in mismatch repair (20). As observed previously (12), efficient activation of the MutL endonuclease does occur with heteroduplex DNA in the absence of MutS, MutL, and ATP (Fig. 4, upper panel). By contrast, the MutL-E32K protein does not support this reaction, and in fact inhibits MutH activation that occurs in the presence of wild type MutL (Fig. 4, lower panel).
Reactions contained 24 fmol of a circular G-T heteroduplex with triangles contained wild type (circles) or E32K (circles) MutL. Gap formation was scored by conversion of the DNA to a form resistant to cleavage by NheI endonuclease. Because the recognition site for this endonuclease is located 5 base pairs from the DNA to a form resistant to cleavage by MutL or MutL-E32K. Gap formation was scored by conversion of the heteroduplex resistant to cleavage by this enzyme (35, 38). Reactions contained wild type (triangles) or E32K (circles) MutL.

The second step in the bacterial mismatch repair reaction is excision of that portion of the unmethylated strand spanning the incised d(GATC) sequence and the mispair (15). This reaction involves mismatch-, MutS-, and MutL-dependent activation of DNA helicase II (19), with unwinding initiating at the strand break and proceeding in an orientation-dependent manner toward the mispair (13). The single strand displaced in this manner is subject to degradation by a single strand-specific exonuclease (14). Because MutL activates and interacts with DNA helicase II (19, 21), we have compared the ability of wild type and E32K MutL with respect to their ability to support mismatch-provoked excision on a G-T heteroduplex containing a site-specific, strand-specific nick located 3′ to the mismatch as viewed along the shorter path joining the two sites in the circular substrate. As shown in Fig. 5, wild type MutL supported efficient excision in a purified system that also contained MutS, DNA helicase II, single strand-binding protein, and exonuclease I (46% of the heteroduplex converted to the gapped form in 15 min). However, MutL-E32K failed to support the excision reaction. The mutant protein is therefore defective with respect to activation of both the MutH endonuclease and the mismatch repair excision system.

MutL-E32K Retains Its Ability to Interact with MutS—The in vitro inhibitory properties of MutL-E32K described above are consistent with the dominant negative phenotype associated with this amino acid substitution in vivo (28). Whereas such effects might be due to the formation of mixed dimers of wild type and mutant polypeptides, we regard this possibility as unlikely on biological and biochemical grounds. In contrast to other characterized, dominant mutL mutations, the dominant negative behavior of the mutL705 (E32K) allele was not suppressed by a gene dosage increase in the wild type allele (28). Furthermore, experiments described above demonstrate that MutL-E32K forms a stable homodimer and inhibits the overall mismatch repair reaction in cell extracts at low molar ratios relative to the wild type protein.

An alternate explanation for MutL-E32K dominant negative effects invokes interaction of the E32K homodimer with other repair activities, leading to dead-end complexes. Because a MutS-MutL interaction has been demonstrated by protein affinity chromatography (36), we have used this method to assess potential interactions between MutL-E32K and other components of the methyl-directed system. MutL and MutL-E32K were retained to a similar degree on a MutS column (Fig. 6A). Serum albumin was not retained by the MutS column, nor did MutL bind to a serum albumin column (Fig. 6, B and C), confirming the specificity of this method.

Formation of MutS-MutL-MutH and MutS-MutL-Helicase II Ternary Complexes on MutS Affinity Supports—MutL has been postulated to function as an interface that couples mismatch recognition by MutS to other activities involved in mismatch repair (17, 18). In support of this view, MutL has been shown to bind to heteroduplex DNA in a MutS-dependent manner (9–11, 28).
and is required for mismatch- and MutS-dependent activation of MutH and helicase II on heteroduplex substrates (12, 13). MutL also activates DNA helicase II and MutH on model substrates and interacts with both proteins (19–22). As shown in Fig. 7 (A and C) neither MutH nor DNA helicase II (the uvrD product) bind detectably to the MutS affinity support. However, both proteins were bound by the column, when loaded in the presence of either MutL or MutL-E32K, and the presence of ATP did not significantly alter the outcome observed in the presence of wild type MutL (Fig. 7, B and D). Thus, MutS can form ternary complexes with MutL and MutH or MutL and DNA helicase II in the absence of DNA, and the MutL-E32K mutation does not interfere with formation of these assemblies as judged by protein affinity chromatography. It therefore seems likely that the in vivo and in vitro dominant negative effects associated with this mutation are because of the formation of dead-end complexes in which the MutL-E32K protein is unable to transduce a signal from MutS that otherwise results in mismatch-dependent activation of the MutH d(GATC) endonuclease or the unwinding activity of helicase II.

**DISCUSSION**

MutL function has been previously implicated in the initiation and excision steps of methyl-directed mismatch repair. MutL is required along with MutS for the mismatch-dependent activation of the MutH d(GATC) endonuclease (12), and both proteins are necessary for the activation of DNA helicase II that occurs at a strand break in an incised heteroduplex (13, 19). These observations have led to the suggestion that MutL serves to interface mismatch recognition by MutS to activation of downstream repair functions (17, 18). Indeed, MutL dramatically enhances the activity of DNA helicase II on conventional helicase substrates in a MutS-independent manner, an effect that is evident when the two proteins are present at comparable concentrations (19), and also activates MutH in the absence of MutS under some conditions (20, 22). The finding that the latter effect is supported by nonhydrolyzable ATP analogues implies that nucleotide hydrolysis is not necessary for MutH activation scored by this assay.

The analysis of the dominant negative MutL-E32K mutant protein described here confirms and extends these observations. The finding that this nucleotide binding site mutation renders the protein nonfunctional in the MutS- and MutL-dependent activation of MutH and the mismatch repair excision system implicates function of the MutL ATP hydrolytic function in multiple steps of the reaction. Although it could be argued that these effects are a consequence of secondary effects of the mutation on the structure of the protein, we regard this possibility as unlikely for several reasons. The mutant protein maintains the native dimeric structure characteristic of MutL in solution (10), and as in the case of wild type MutL, the mutant protein is able to form ternary complexes with MutS and MutH and with MutS and DNA helicase II. In fact, the finding that MutL-E32K behaves as a dominant negative inhibitor of MutH activation and the overall mismatch repair reaction strongly argues for retention of structural integrity. The simplest explanation for the inhibitory effects of the mutant protein, in the context of the other observations above, is that it supports repair complex assembly up to a point but is unable to couple ATP binding or hydrolysis to activation of required downstream functions.

Although effects of other repair proteins on the weak MutL ATPase have not been addressed, the hydrolytic activity of the protein is enhanced substantially by single-stranded DNA (Ref. 30 and Fig. 1). Whereas the $k_{cat}$ for ATP hydrolysis by MutL-E32K is reduced somewhat relative to that observed with wild type protein, the most obvious defect associated with the mutant protein is its failure to respond significantly to a single strand DNA cofactor. Although significance of this effect is uncertain at present, this finding may bear on the mechanism by which MutL activates DNA helicase II. Although it is clear that MutL functions with MutS to activate helicase II initiation at a strand break (13), it has also been suggested that MutL serves as a helicase activator during the subsequent course of unwinding by increasing the processive behavior of the protein (19). Because MutL and helicase II interact physically (19, 21), the MutL DNA binding site may function in this respect via interaction with a single strand product of helicase action.
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REFERENCES
1. Modrich, P. (1989) J. Biol. Chem. 264, 6597–6600
2. Radman, M., Matic, I., Halliday, J. A., and Taddei, F. (1995) Philos. Trans. R. Soc. Lond-Biol. Sci. 347, 97–103
3. Modrich, P., and Lahue, R. (1996) Annu. Rev. Biochem. 65, 101–133
4. Jiricny, J. (1998) EMBO J. 17, 6427–6436
5. Meselson, M. (1988) in Recombination of the Genetic Material (Low, K. B., ed) pp. 91–113, Academic Press, San Diego
6. Su, S.-S., and Modrich, P. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5057–5061
7. Su, S.-S., Lahue, R. S., Au, K. G., and Modrich, P. (1988) J. Biol. Chem. 263, 6829–6835
8. Parker, B. O., and Marinus, M. G. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1730–1734
9. Allen, D. J., Makhov, A., Grilley, M., Taylor, J., Thresher, R., Modrich, P., and Griffith, J. D. (1997) EMBO J. 16, 4467–4476
10. Grilley, M., Welsh, K. M., Su, S.-S., and Modrich, P. (1989) J. Biol. Chem. 264, 1000–1004
11. Gallo, L., Bouquet, C., and Brooks, P. (1999) Nucleic Acids Res. 27, 2325–2331
12. Au, K. G., Welsh, K., and Modrich, P. (1992) J. Biol. Chem. 267, 12142–12148
13. Das, V., and Modrich, P. (1998) J. Biol. Chem. 273, 9202–9207
14. Cooper, D. L., Lahue, R. S., and Modrich, P. (1993) J. Biol. Chem. 268, 11823–11829
15. Grilley, M., Griffith, J., and Modrich, P. (1993) J. Biol. Chem. 268, 11830–11837
16. Lahue, R. S., Au, K. G., and Modrich, P. (1989) Science 245, 160–164
17. Modrich, P. (1991) Annu. Rev. Genet. 25, 229–253
18. Sancar, A., and Heard, J. E. (1993) Science 259, 1415–1420
19. Yamaguchi, M., Mao, V., and Modrich, P. (1998) J. Biol. Chem. 273, 9197–9201
20. Ban, C., and Yang, W. (1998) Cell 95, 541–552
21. Hall, M. C., Jordan, J. R., and Matsen, S. W. (1998) EMBO J. 17, 1535–1541
22. Hall, M. C., and Matsen, S. W. (1999) J. Biol. Chem. 274, 1306–1312
23. Kolodner, R. (1996) Genes Dev. 10, 1433–1442
24. Proia, T. A., Pang, Q., Alani, E., Kolodner, R. D., and Liskay, R. M. (1994) Science 265, 1091–1093
25. Li, G.-M., and Modrich, P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1950–1954
26. Bergerat, A., de Massy, B., Gadal, D., Varoutas, P. C., Nicolas, A., and Forrester, P. (1997) Nature 386, 414–417
27. Mushegian, A. R., Bassett, D. E., Jr., Boguski, M. S., Bork, P., and Koonin, E. V. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5831–5836
28. Aronstam, A., and Marinus, M. G. (1996) Nucleic Acids Res. 24, 2498–2504
29. Peltomaki, P., and Vasen, H. F. (1997) Gastroenterology 113, 1146–1158
30. Ban, C., Junop, M., and Yang. W. (1999) Cell 97, 85–97
31. Picard, V., and Bock, S. C. (1997) Methods Mol. Biol. 67, 183–188
32. Walsh, K. M., Lu, A.-L., Clark, S., and Modrich, P. (1987) J. Biol. Chem. 262, 15624–15629
33. Runyon, G. T., Wang, L., and Lohman, T. M. (1993) Biochemistry 32, 602–612
34. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
35. Fang, W.-H., and Modrich, P. (1993) J. Biol. Chem. 268, 11838–11844
36. Wu, T.-H., and Marinus, M. G. (1999) J. Biol. Chem. 274, 5948–5952
37. Marquardt, D. W. (1963) J. Soc. Indust. Appl. Math. 11, 431–441
38. Blackwell, L. J., Bjornson, K. P., and Modrich, P. (1998) J. Biol. Chem. 273, 32049–32054
