

## Deletion Mutation Analysis of the *mutS* Gene in *Escherichia coli*\*

(Received for publication, September 17, 1998, and in revised form, November 21, 1998)

Te-Hui Wu and Martin G. Marinus‡

From the Department of Pharmacology and Molecular Toxicology, University of Massachusetts Medical School, Worcester, Massachusetts 01655

**The MutS protein is part of the *dam*-directed MutHLS mismatch repair pathway in *Escherichia coli*. We have constructed deletion derivatives in the *mutS* gene, which retain the P-loop coding region for ATP binding. The mutant proteins were assayed for ATP hydrolysis, heteroduplex DNA binding, heterodimer MutS formation, and the ability to interact with MutL. Dimerization was assayed by expressing His<sub>6</sub>-tagged wild-type and non-tagged deletion mutant proteins in the same cell and isolating the His<sub>6</sub>-tagged protein followed by MutS immunoblotting after SDS-polyacrylamide gel electrophoresis. MutS-MutL interaction was measured using the same technique except that the MutL protein carried the His<sub>6</sub> tag. Our results indicate that DNA binding ability resides in the N-terminal end of MutS, and dimerization and MutL interactions are located in the C-terminal end. Given the extensive amino acid homology in the MutS family our results with *E. coli* should be applicable to MutS homologues in other prokaryotes and eukaryotes.**

The dimeric MutS protein is part of the *Escherichia coli* MutSLH DNA repair system that corrects mismatches arising in DNA as biosynthetic errors (1). The mutator phenotype of strains with an inactivated *mutS* gene is consistent with this idea (2). In addition MutS also participates in homeologous recombination, transcription-coupled repair, very short patch repair, resolution of directly repeated DNA sequences, and sensitivity to *cis*-platin and alkylating agents (3–6). MutS binds specifically to mismatched base pairs and insertion/deletion mispairs of up to four nucleotides as well as adducted base mismatches (7, 8). The DNA-bound MutS protein binds ATP and in association with dimeric MutL can form  $\alpha$ -looped structures with concomitant hydrolysis of ATP, suggesting movement of the protein complex on DNA away from the mismatch (9). An alternative model suggests that ADP stabilizes DNA binding and ATP increases dissociation (10). After addition of MutH, the MutSLH complex cleaves DNA 5' to hemimethylated -GATC- sequences in the unmethylated strand. Subsequent excision repair from this nick can occur in either direction by one of several exonucleases, and directionality is imparted through interaction of the MutLS complex with helicase II, the *uvrD* gene product (11). Resynthesis of the gapped DNA is accomplished by DNA polymerase III holoenzyme and subsequent ligation by DNA ligase.

\* This work was supported by Grant GM33233 from the National Institute of General Medical Sciences. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Pharmacology and Molecular Toxicology, University of Massachusetts Medical School, 55 Lake Ave., Worcester, MA 01655. Tel.: 508-856-3330; Fax: 508-856-5080; Internet: Martin.Marinus@ummed.edu.

The basic elements of the scheme outlined above have been conserved in eukaryotes (3–5). This includes MutS and MutL homologues, each of which shows conservation with the *E. coli* protein at the amino acid level. Unlike bacteria, however, multiple MutS (MutS homologue) and MutL (MutL homologue, postmeiotic segregation) homologs are present as heterodimers in eukaryotes. Mutation in these genes results in microsatellite sequence instability and may dispose individuals to hereditary or sporadic cancer, especially colon carcinoma. The mechanism for strand discrimination during the repair process in eukaryotes is unknown.

At present, there is no structural information for any MutS homologue. The amino acids that are important for specific and nonspecific contact with DNA are not known. Similarly the residues important for dimerization and contacting MutL are unknown. To identify which regions of MutS are essential for these functions, we have made specific deletions in the *mutS* gene and have examined the properties of the truncated proteins. The results obtained with the *E. coli* protein should be applicable to other members of the MutS family given their overall amino acid conservation (4).

### EXPERIMENTAL PROCEDURES

**Bacterial Strains and Culture Conditions**—Strain *E. coli* K-12 GM5864 is GM4271 lysogenized with phage  $\lambda$ DE3. GM4271 is a *mutS458::mTn10Kan* derivative of CC106 (12). Bacteria were grown in L broth supplemented with 100  $\mu$ g/ml ampicillin and/or 10  $\mu$ g/ml chloramphenicol. The methods for determining mutation frequency to rifampicin resistance or Lac<sup>+</sup> papillation have been described previously (12).

**Construction of Plasmid-borne Mutations and Protein Expression**—Plasmid pMQ372 is a pET3d (Novagen) derivative that contains the wild-type *mutS* gene and lacks the *Ava*I site of pET3d. pMQ395 is derived from pMQ372 but contains the N-terminal His<sub>6</sub> tag of pET15b fused to the *mutS* coding sequence. Plasmids pMQ372 and pMQ395 fully complement the *mutS458* mutation in strain GM4271, as determined by monitoring for spontaneous mutation to rifampicin resistance, even in the absence of T7 RNA polymerase induction (see Table II). Plasmids pMQ382 and pMQ393 are derivatives of pACYC184 and carry His<sub>6</sub>-tagged *mutS* and *mutL*, respectively (12).

Deletions in the *E. coli mutS* gene were made in pMQ372 at naturally occurring restriction enzyme recognition sites, with appropriate endonucleases (New England Biolabs), to produce derivatives with promoter-proximal, promoter-distal, or midgene mutations (see Fig. 1). The fusion joint for each deletion end point is shown in Table I. The method of constructing the deletion at the *Ssp*I site resulted in the addition of 13 additional amino acids to the mutant protein. Mutations from the pMQ372 series were moved into pMQ395 by fragment swapping to generate proteins with an N-terminal His<sub>6</sub> tag. The His<sub>6</sub> tag wild-type and deleted mutant proteins were prepared as recommended by the manufacturer (Novagen) in strain GM5864. Although the specific conditions for culturing cells varied for each construct, in general GM5864 transformed with a pMQ395 mutant derivative was grown at 37 °C to an A<sub>600</sub> of 0.8, shifted to room temperature, and isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG)<sup>1</sup> was added to 50  $\mu$ M final concentration. Incubation was continued for 2–3 h at room temperature, and the cells were harvested and lysed in a French pressure cell (Aminco) in

<sup>1</sup> The abbreviations used are: IPTG, isopropyl-1-thio- $\beta$ -D-galactopyranoside; bp, base pair; PAGE, polyacrylamide gel electrophoresis.

TABLE I  
DNA sequences of fusion joints

The plasmid structures are shown in Fig. 1. The underlined sequences are the fusion joints using the restriction enzymes shown in Fig. 1. The H after the plasmid name indicates an N-terminal His<sub>6</sub>-tag.

Plasmid	Sequence	Codons
pΔ1-311H	AGC . <u>CAT</u> . <u>ATG</u> . CCA . GTG	312 start
pΔ1-260	AGATATACC . <u>ATG</u> . GAA	261 start
pΔ26-260H		
pΔ26-260	CAT . <u>CCC</u> . <u>GAG</u> . GAA . CGT	26-260 deleted
pΔ261-556H		
pΔ261-556	ATC . ACC . <u>ATG</u> . <u>AAC</u> . CTG	261-556 deleted
	GCC . <u>AAT</u> . <u>TTG</u> . ATC . GTG . GAC . CGG . AGA .	
	L    I    V    D    R    I	680-853 deleted
pΔ680-853H	GTC . TTG . ACG . TCC . TGC . GCC . TGC . TAA	13 additional codons
pΔ680-853	V    L    T    S    C    A    C	

lysis buffer (20 mM Tris-HCl, pH 7.9, 5 mM imidazole, 500 mM NaCl) followed by a brief sonication to reduce viscosity. The lysate was centrifuged in a Beckman Ti70 rotor at 39,000 rpm for 30 min at 4 °C, followed by filtration through a 0.45-μm syringe filter (Acrodisc). His<sub>6</sub> tag protein was bound to and eluted from nickel-affinity resin (His-Bind, Novagen) as recommended by the manufacturer. At least 80% of the wild-type and 10-30% of the mutant MutS were recovered as soluble protein. All proteins were at least 95% pure as judged by Coomassie Brilliant Blue staining of polyacrylamide gels and by Western blotting (e.g. see Fig. 4). Protein concentration was assayed using the Bradford reagent (Bio-Rad). ATPase activity was assayed by the method of Weinstock *et al.* (13), and percent [ $\alpha$ -<sup>32</sup>P]ATP hydrolysis was measured by scanning polyethyleneimine chromatography plates using a Molecular Dynamics PhosphorImager equipped with ImageQuant software.

**Heteroduplex DNA Construction**—A 154-bp DNA oligonucleotide with a centrally located G-T mismatch at bp 76 was constructed as follows. Oligonucleotides MM181 (86-mer, 5'-CGGCGATATTCTAGACACAGGCGATGGTTTTGATAGAGCATCTTGGACGATTTGTAACA-ACTCGGAGTTCATAGATCTCCCATTCG-3') and MM186 (92-mer, 5'-AGAGGATCCGCACCTTAACTTCCGTATGCCTATGGAAGTCAGAG-AGAAATTTAAATTCAGAGCGGAGGCGAATGGGAGGTCTATGAAC-TCCG-3') were synthesized by Dr. Kendall Knight (University of Massachusetts Medical School). The underlined bases are complementary, and the mismatched bases are in bold. The DNA sequence of these oligonucleotides is derived from the phage P22 *mnt* gene (14). The oligonucleotides were mixed, annealed in 20 mM Tris-HCl, pH 7.6, 10 mM EDTA, 5 mM MgCl<sub>2</sub> at 70 °C for 10 min, and then slowly cooled to room temperature. The single-stranded regions were converted to duplex DNA in the presence of dNTPs and [ $\alpha$ -<sup>32</sup>P]dATP (800 Ci/mmol) and DNA polymerase I Klenow fragment (New England Biolabs) in reaction buffer supplied by New England Biolabs. Following the reaction, labeled double-stranded DNA was recovered after passage through a Qiaquick (Qiagen) column. Binding of MutS to heteroduplex DNA was measured by band shifting in polyacrylamide gels as described previously (8).

**MutS Heterodimer Detection and MutS-MutL Protein Interaction**—To measure MutS heterodimer interaction, strain GM5864 was transformed with pMQ382, encoding wild-type His<sub>6</sub>-tagged MutS, and with a pMQ372 *mutS* deletion derivative. MutS-MutL interaction utilized strain GM5864 transformed with pMQ393, which encodes wild-type His<sub>6</sub>-tagged MutL, and with a pMQ372 *mutS* deletion derivative. For the His<sub>6</sub>-tagged derivatives listed in Fig. 1, the same procedure was used except that the wild-type MutS was not His<sub>6</sub>-tagged. The strains were grown to A<sub>600</sub> of 0.7 at 37 °C (no IPTG was added) and harvested, and His<sub>6</sub>-tagged proteins were isolated as described above. A portion of each fraction from the affinity column was subjected to SDS-PAGE. Immunoblotting on Immobilon-P membranes (Millipore) after semi-dry electroblotting (Owl Scientific) employed rabbit polyclonal antiserum to MutS and MutL (BAbCO) using chemiluminescence according to the manufacturer's (Tropix) instructions.

RESULTS

**Construction of Deletion Mutations**—The amino acids important for MutS dimerization, for binding to heteroduplex DNA, and for interaction with MutL are not known. We have constructed deletion derivatives in the *E. coli mutS* gene to determine whether these functions can be localized to specific regions of the MutS protein. Because ATP binding and/or

hydrolysis is necessary for heteroduplex DNA-induced MutS conformational change (15) as well as movement of MutS along the heteroduplex DNA (9) and for MutS-MutL interaction (16), we constructed deletion mutations in the *mutS* gene that retained the coding sequence of the P-loop motif for nucleoside triphosphate binding (between the *Hpa*I and *Ssp*I sites in Fig. 1). All mutant proteins derived from the deletion constructs had similar low ATPase activity (about 0.34 μM ATP hydrolyzed/μM MutS/min) as the wild type.

**Mutator Phenotype of Cells with Plasmid-borne *mutS* Deletions**—Plasmids containing the wild-type and mutant *mutS* deletion alleles were introduced into wild-type and *mutS E. coli* strains. The presence of the plasmids in the wild-type strain did not significantly alter the mutation frequency to rifampicin resistance (Table II) or the frequency of Lac<sup>+</sup> reversion as measured by papillation (less than 1% of colonies showing one or more papillae). Similarly the plasmids did not significantly alter the mutation frequency of the *mutS* strain to rifampicin resistance (Table II) or Lac<sup>+</sup> papillation (>90% of cells showing papillation). The deletion mutations, therefore, inactivate the protein for genetic complementation and do not show dominance in the wild type.

**Heteroduplex DNA Binding of Mutant Proteins**—MutS protein binds specifically to base mismatches and insertion/deletions in DNA (7, 8). The wild-type and deletion mutant proteins were tested for their ability to bind to a 154-bp heteroduplex containing a G-T mismatch at position 76 by monitoring band-shifting in polyacrylamide gels (Fig. 2). The wild-type protein bound the radioactive substrate oligonucleotide efficiently at low concentration, and apparent binding was decreased by 85% in the presence of a 50-fold excess of non-radioactive competitor DNA (Fig. 2). At a wild-type protein concentration that resulted in complete loss of the free oligonucleotide band, mutant proteins produced by pΔ680-853H, pΔ26-260H, pΔ261-556H, and pΔ1-311H showed 9-, 44-, 87-, and 100-fold reduction in specific binding, respectively (Fig. 2). These data indicate that the N-terminal end of the MutS protein is essential for substrate binding.

**MutS Heterodimer Detection**—MutS protein is a dimer in solution (7). We have used an *in vivo* assay to test for the ability of mutant proteins to dimerize with wild-type MutS. Compatible plasmids were used to express His<sub>6</sub>-tagged wild-type and native mutant proteins in the same cell. His<sub>6</sub>-tagged protein was purified from cell lysates and subjected to SDS-PAGE. The proteins were blotted to a membrane and probed with MutS antiserum. If mutant-wild-type heterodimers are formed, then two reactive bands, at different molecular weights, should be present (Fig. 3A). Expression of the wild-type His<sub>6</sub>-tagged MutS gene in the presence of the compatible plasmid vector produces predominantly a single protein band distribution at the predicted wild-type M<sub>r</sub> in eluates from the nickel column

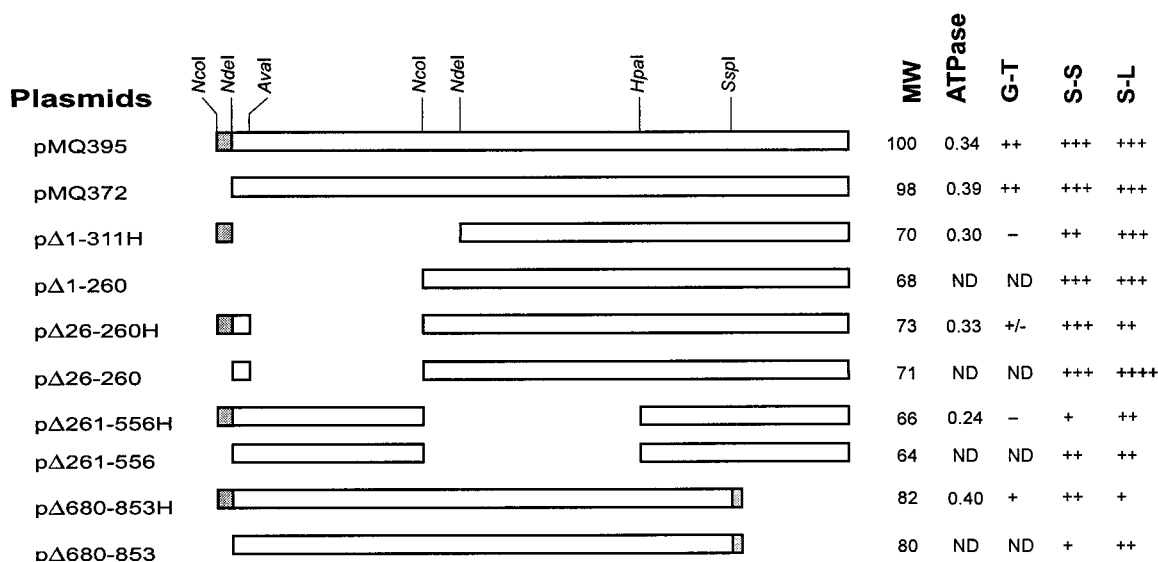


FIG. 1. Structure and properties of *mutS* deletion mutants. Plasmids pMQ372 and pMQ395 have the wild-type *mutS* sequence without (open rectangle) and with (gray box) an N-terminal His<sub>6</sub>-tag DNA, respectively. Pertinent restriction enzyme recognition sequences are shown. The DNA encoding the P-loop domain for ATP binding is between the *HpaI* and *SspI* sites. The sequence of fusion joints and extent of the deletions are given in Table I. The gray box at the 3'-end of pΔ680-853H and pΔ680-853 represents DNA encoding 13 additional amino acids. The ATPase activity of the proteins is given as  $\mu\text{M}$  ADP produced/min/ $\mu\text{M}$  MutS. MW, calculated molecular weight; G-T, ability to bind to G-T mismatched substrate DNA; S-S, extent of dimerization; S-L, extent of MutS-MutL interaction; ND, not determined.

TABLE II  
Mutation frequency to rifampicin resistance

Bacterial strains containing the plasmids listed above were grown to saturation in duplicate in L broth with ampicillin but without IPTG. Portions of the cultures were diluted, if necessary, and spread on plates containing ampicillin or rifampicin. The plates were scored after overnight incubation at 37 °C.

Plasmid	Mutation frequency ( $\times 10^{-8}$ ) in strain	
	GM4244 (wild)	GM4271 ( <i>mutS</i> )
pET3a	2.0	20
pMQ395	1.4	5
pMQ372	3.1	3
pΔ1-311H	0.2	110
pΔ1-311	0.3	37
pΔ26-260H	0.6	128
pΔ26-260	2.0	45
pΔ261-556H	0.9	52
pΔ261-556	2.7	83
pΔ680-853H	0.6	76
pΔ680-853	0.4	56

(Fig. 3B, vector). A similar result was obtained if both His<sub>6</sub>-tagged and non-tagged wild-type proteins were present in cell extracts (Fig. 3B, pMQ372). We assume the lower molecular weight band in these two figures is because of protein degradation. The distribution of wild-type and mutant proteins in eluates from the affinity column was similar if not identical for pΔ261-556 and pΔ1-260 encoded mutant proteins, indicating efficient heterodimer formation *in vivo*. Deletion of the N-terminal region of MutS encompassed by these two mutations (Fig. 1) therefore does not affect dimerization. For the pΔ680-853 and pΔ261-556 encoded proteins, however, the distributions of wild-type and mutant proteins were not the same (Fig. 3), indicating less efficient heterodimer formation. The basis for the altered distribution of the mutant proteins is unknown. These mutant proteins are as stable and soluble as those made from pΔ261-556 and pΔ1-260, suggesting that lack of heterodimer formation is not because of these causes. None of the mutant non-His<sub>6</sub>-tagged MutS proteins bind to the affinity column (data not shown). The results suggest that amino acid residues in the C-terminal end of the protein are responsible for efficient dimerization.

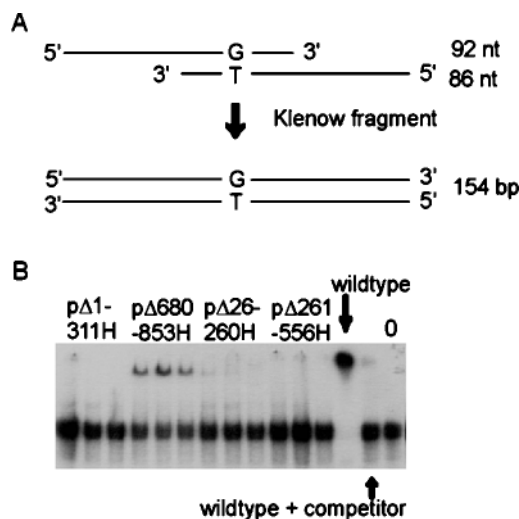
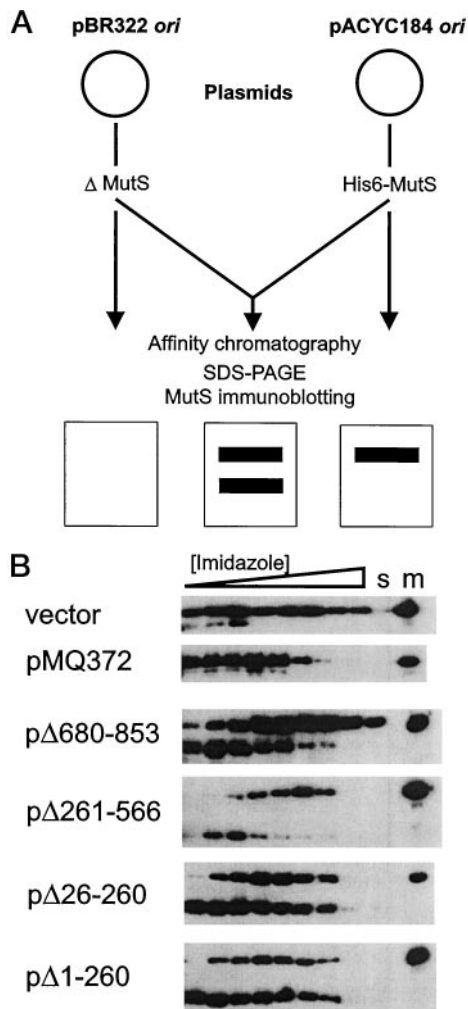


FIG. 2. MutS protein binding to a 154-bp G-T heteroduplex substrate. A, construction of the 154-bp DNA fragment substrate. B, the three lanes for each mutant protein indicate (from left to right) that fractions eluting at 30, 55, and 80 mM imidazole from the affinity column were tested. The 0 lane represents heteroduplex DNA only. The amount of wild-type protein was 0.6 pmol; for pΔ1-311H and pΔ261-556H, 1.2 pmol; and for pΔ680-853H and pΔ26-260H, 1.6 pmol. The amount of the heteroduplex DNA fragment was 200 fmol. A 50-fold excess of unlabeled competitor DNA was used. nt, nucleotide.

**MutS-MutL Interaction**—MutS protein bound to heteroduplex DNA is thought to recruit MutL protein into a complex (16). We have been unable to detect MutS-MutL complexes by co-immunoprecipitation.<sup>2</sup> We have used, instead, a variation of the technique to detect dimerization in which His<sub>6</sub>-tagged MutL was co-expressed *in vivo* with non-His<sub>6</sub>-tagged MutS. Affinity purification of MutL followed by SDS-PAGE and immunoblotting with MutS antibody should allow for detection of MutS-MutL complexes (Fig. 4A). The control experiments showed that a band at the expected  $M_r$  was detected in cells expressing MutS (Fig. 4B, pMQ372) but was not detected in its

<sup>2</sup> T-H. Wu and M. G. Marinus, unpublished data.



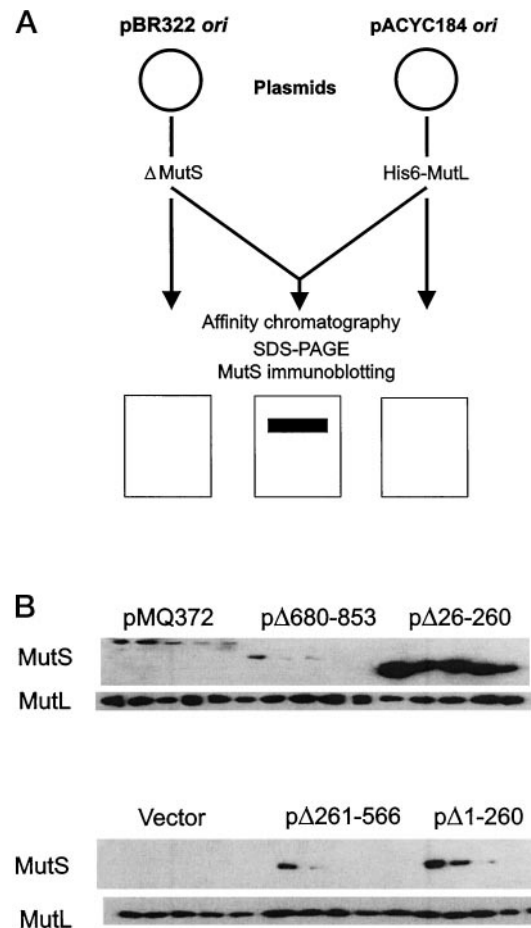
**FIG. 3. Analyzing MutS dimerization by affinity chromatography.** A, schematic representation of the experiment showing the result expected if heterodimerization occurs (2 bands) or not (1 band). B, for each plasmid construct, fractions eluting at 30, 55, 80, and 105 mM imidazole from the affinity column were tested in duplicate for MutS immunoblotting after SDS-PAGE. Lanes *m* and *s* are, respectively, marker wild-type MutS protein and the fraction obtained after stripping the column with EDTA.

absence (Fig. 4B, *vector*). Reduced amounts of mutant MutS were detected in cells containing the C-terminal and midgene *mutS* deletions (Fig. 4B, *pΔ680-853* and *pΔ261-566*). One mutation deleting the N-terminal end produced almost as much cross-reacting material as wild-type (Fig. 4B, *pΔ1-260*) whereas another resulted in a vast excess of it (Fig. 4B, *pΔ26-260*). We assume the latter result indicates a higher affinity of the mutant protein for MutL than wild type or binding of more MutS molecules to MutL than normal. The results above suggest that the MutS-MutL interaction region is at the C-terminal end of MutS, perhaps overlapping with the P-loop domain of the ATP binding site (Fig. 1).

DISCUSSION

The mutant proteins we have used all have approximately the same ATPase specific activity as the wild-type MutS protein, suggesting that gross structural alterations are not present. This is supported by the observation that the mutant proteins are able to dimerize and to interact with MutL unless specifically defective in one or both of these processes.

The *mutS* deletion mutations produce little, if any, residual mismatch repair *in vivo*, and they do not impart a dominant phenotype in a wild-type strain (Table II). The latter finding is



**FIG. 4. Analyzing MutS-MutL interaction by affinity chromatography.** A, schematic representation of the experiment showing the result expected if MutS-MutL interaction occurs (1 band) or not (no bands). B, for each plasmid construct, fractions eluting at 30, 50 (two), and 80 mM (two) from the affinity column were tested for MutS immunoblotting after SDS-PAGE. As a control, MutL immunoblotting was also carried out on each fraction and is shown below that for MutS.

surprising for those mutant proteins, such as *pΔ1-311*, that fail to bind DNA but still interact with downstream proteins. It should be noted that IPTG was not added to the cultures to induce the phage T7 RNA polymerase because the wild-type *mutS* plasmids complemented the mutator phenotype in its absence (Table II) and because the plasmid instability increased in its presence. Perhaps the ratio of mutant protein to wild type is low *in vivo* in uninduced cultures because of a lower than expected level of expression and/or poor solubility of the mutant proteins, thereby precluding sequestration of sufficient wild-type and mutant monomers into inactive dimers.

The results from Fig. 1 suggest that the N-terminal end of MutS is important for heteroduplex DNA binding. This is in agreement with the observation of Malkov *et al.* (17) that Phe-39 of the *Thermus aquaticus* (Phe-36 in *E. coli*) MutS cross-links to mismatched DNA. We also find, however, that specific DNA binding occurs at a low level in the mutant deleted for residues 26-260 but is not detectable if residues 261-556 are deleted (Fig. 2). In *Thermus thermophilus*, a proteolytic fragment containing residues 275-570 of MutS is able to bind double-stranded DNA (18), confirming the importance of this region for DNA binding.

The ability of MutS to dimerize appears to reside within the region containing residues 557-853 (Fig. 3). This agrees with the finding by Alani (19) that in yeast the C-terminal 114 amino acids of Msh2 are important for interaction with Msh6.

Taken together these findings suggest that the same region of MutS homologs is used for homo- and heterodimerization. Alani *et al.* (15) have also described a mutation (A859E) in yeast Msh2 located in the putative helix-turn-helix domain, which affected Msh2-Msh6 heterodimer formation. The equivalent residue in *E. coli* MutS is Ala-776 that is within the predicted interaction region (residues 557–853). Because none of the mutations we tested were completely defective in dimerization, it is highly probable that additional residues are involved.

The C-terminal region of MutS also seems to be important for MutS-MutL interaction, and the results in Fig. 4 are the only available mutational data addressing this question. Given that MutS-MutL interaction was detected in the presence of MutS, but not in its absence, and that the extent of the interaction could be varied by mutation (Fig. 4), we believe the assay detected bonafide interaction. However, given the high NaCl concentration (500 mM) used in these experiments, it is probable that the MutS-MutL complexes detected in these experiments are not bound to heteroduplex DNA. This suggests that a fraction of the total MutS protein *in vivo* may be loosely bound to MutL in the absence of DNA. Even when MutS is bound to DNA *in vitro*, its interaction with MutL is not readily detectable (20). This might explain why the MutS-MutL complexes are detected in column eluates only at low concentrations of imidazole (Fig. 4). None of the mutant proteins tested was totally deficient in MutS-MutL interaction, suggesting that amino acids 556–630, which include the P-loop motif, are required.

The methods we have used for protein interaction do not allow a quantitative measurement of the interaction affinities. We have, therefore, used a qualitative scale to express the results (Fig. 1).

The MutS family of proteins from prokaryotes and eu-

karyotes is highly conserved (3–5, 21). The results we have obtained with the *E. coli* MutS protein should be applicable to other members of the family. It should now be possible to target individual amino acid residues in the regions we have identified in MutS or its homologues to further define those important for DNA binding, dimerization, and MutL interaction.

*Acknowledgments*—We thank Dr. Kendall Knight for synthesis of oligonucleotides and for advice on the ATPase assays and Dr. R. Lahue for advice and suggestions in the early part of this project. We also thank Patricia Hess, who constructed some of the plasmids used here and helped devise protein expression conditions during a graduate student research rotation.

#### REFERENCES

1. Modrich, P., and Lahue, R. (1996) *Annu. Rev. Biochem.* **65**, 101–133
2. Horst, J. P., Wu, T. H., and Marinus, M. G. (1999) *Trends Microbiol.* **7**, 29–36
3. Modrich, P. (1991) *Annu. Rev. Genet.* **25**, 229–253
4. Kolodner, R. (1996) *Genes Dev.* **10**, 1433–1442
5. Umar, A., and Kunkel, T. A. (1996) *Eur. J. Biochem.* **238**, 297–307
6. Karran, P., and Bignami, M. (1994) *Bioessays* **16**, 833–839
7. Su, S. S., and Modrich, P. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 5057–5061
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. Gradia, S., Acharya, S., and Fishel, R. (1997) *Cell* **91**, 995–1005
11. Yamaguchi, M., Dao, V., and Modrich, P. (1998) *J. Biol. Chem.* **273**, 9197–9201
12. Wu, T. H., and Marinus, M. G. (1994) *J. Bacteriol.* **176**, 5393–5400
13. Weinstock, G. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 8829–8834
14. Wu, T. H., Clarke, C. H., and Marinus, M. G. (1990) *Gene (Amst.)* **87**, 1–5
15. Alani, E., Sokolsky, T., Studamire, B., Miret, J. J., and Lahue, R. S. (1997) *Mol. Cell. Biol.* **17**, 2436–2447
16. Grilley, M., Welsh, K. M., Su, S. S., and Modrich, P. (1989) *J. Biol. Chem.* **264**, 1000–1004
17. Malkov, V. A., Biswas, I., Camerini-Otero, R. D., and Hsieh, P. (1997) *J. Biol. Chem.* **272**, 23811–23817
18. Tachiki, H., Kato, R., Masui, R., Hasegawa, K., Itakura, H., Fukuyama, K., and Kuramitsu, S. (1998) *Nucleic Acids Res.* **26**, 4153–4159
19. Alani, E. (1996) *Mol. Cell. Biol.* **16**, 5604–5615
20. Drotschmann, K., Aronshtam, A., Fritz, H. J., and Marinus, M. G. (1998) *Nucleic Acids Res.* **26**, 948–953
21. Eisen, J. A. (1998) *Nucleic Acids Res.* **26**, 4291–4300