Mismatch Recognition and DNA-dependent Stimulation of the ATPase Activity of hMutSα Is Abolished by a Single Mutation in the hMSH6 Subunit*

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The most abundant mismatch binding factor in human cells, hMutSα, is a heterodimer of hMSH2 and hMSH6, two homologues of the bacterial MutS protein. The C-terminal portions of all MutS homologues contain an ATP binding motif and are highly conserved throughout evolution. Although the N termini are generally divergent, they too contain short conserved sequence elements. A phenylalanine → alanine substitution within one such motif, GXYF(X)₃X₃DA, has been shown to abolish the mismatch binding activity of the MutS protein of Thermus aquaticus (Malkov, V., A., Biswas, I., Camerini-Otero, R. D., and Hsieh, P. (1997) J. Biol. Chem. 272, 23811–23817). We introduced an identical mutation into one or both subunits of hMutSα. The Phe → Ala substitution in hMSH2 had no effect on the biological activity of the heterodimer. In contrast, the in vitro mismatch binding and mismatch repair functions of hMutSα were severely attenuated when the hMSH6 subunit was mutated. Moreover, this variant heterodimer also displayed a general DNA binding defect. Correspondingly, its ATPase activity could not be stimulated by either heteroduplex or homoduplex DNA. Thus the N-terminal portion of hMSH6 appears to impart on hMutSα not only the specificity for recognition and binding of mismatched substrates but also the ability to bind to homoduplex DNA.

Postreplicative mismatch repair (MMR) plays a key role in the maintenance of genomic integrity by eliminating base-base mismatches and insertion-deletion loops (IDLs) that arise in DNA during replication or recombination (1, 2). The repair process consists of four principal steps: (i) mismatch recognition, (ii) repairosome assembly, (iii) degradation of the newly synthesized strand from the strand discrimination signal to and past the mismatch, and (iv) resynthesis of the excised DNA tract (1). The mismatch recognition step is of particular interest; numerous in vitro and in vivo experiments, carried out over the past decade, have shown that the MMR system is capable of addressing a large number of substrates ranging from purine-pyrimidine, purine-purine, and pyrimidine-pyrimidine mismatches to IDLs of one to more than 10 extrahelical nucleotides (3–9). How are these various substrates recognized? In Escherichia coli, the role of the mismatch recognition factor is played by a homodimer of the MutS protein, which can initiate the repair of base-base mismatches (5, 10) and IDLs up to four nucleotides (7). In human cells, this function is fulfilled by one of two heterodimers consisting of MutS homologues (MSH): hMutSα, a heterodimer of hMSH2 and hMSH6 (11–13), which acts in the correction of base-base mismatches and small IDLs (8, 12, 14), and hMutSβ, a heterodimer of hMSH2 and hMSH3, which addresses principally IDLs (8, 14, 15). The situation in Saccharomyces cerevisiae is similar (16–19).

The highly conserved C termini of MutS homologue proteins contain the ATP binding sites as well as helix-turn-helix motifs (20–22). As nucleotide binding alters the conformation of the DNA-bound factors, such that they dissociate from the mismatch (22–24), and as helix-turn-helix motifs are frequently involved in DNA binding, it had been assumed that the mismatch recognition capability of these proteins resides in the C terminus. However, Malkov et al. (25) have shown that a short, highly conserved N-terminal motif with the consensus sequence GXYF(X)₃X₃DA (see Fig. 1) plays a crucial role in mismatch binding. They demonstrated that the MutS protein of Thermus aquaticus could be cross-linked to the heteroduplex substrate via the phenylalanine residue within this motif and that substitution of this residue with alanine effectively abolished mismatch binding. The overall structure of the protein, its ability to dimerize, and its ATPase activity were not impaired by this mutation (25). As both subunits of the human mismatch binding factor hMutSα possess the above consensus sequence (Fig. 1), it might be expected that both polypeptides contact DNA. However, our earlier experiments showed that only hMSH6 could be cross-linked to mismatch-containing DNA (20, 26, 27). These latter results invoke the asymmetric nature of the heterodimer and imply that the two subunits play distinct roles during mismatch recognition. Therefore, we introduced the Phe → Ala mutations into hMSH2 and hMSH6 and tested the effects of these changes on mismatch binding and mismatch repair. We showed that the F432A mutation in hMSH6 abolished not only mismatch recognition but also the binding to DNA in general, whereas the substitution of Phe-42 with alanine in hMSH2 was without effect. Our results are in general agreement with those of Alani and co-workers (28) who described a similar study with the MutSα heterodimer of S. cerevisiae. However, although the ATPase activity of the human factor was insensitive to the presence of both homo- and heteroduplex DNA, the S. cerevisiae MSH2-msh6 F337A heterodimer could be stimulated by a mismatch-containing oligonucleotide substrate at low salt concentrations. The possible reasons underlying this difference are discussed.
EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis and Production of the Recombinant Baculovirus Vectors—The conserved phenylalanine residues within hMSH2 and hMSH6 were changed to alanine by PCR-based site-directed mutagenesis as described previously (20, 29).

hMSH2F2A (hMSH2FA) was obtained using the following pairs of primers (the site of the Phe → Ala codon change is underlined): mr-34 (5'-GATTATCTACGTCCACCCAC-3') and PDU-2 (5'-GTTGCCGCTGATGCTGGCCCGG-3') and PDU-1 (5'-CCGGGCGAGCGATATTCCGGGAC-3') and PDU-3 (5'-CCAGGGAGAAGCACTTATGCCC-3'). In two separate PCR reactions, the two fragments complementary to each other near the site of the mutation were generated. After purifica-
tion and annealing, they then served as templates together with the outer primers mr-34 and PDU-3 in a second PCR reaction. The resulting product was digested at the ends with BamH1 and SmaI and substituted for the corresponding wild type fragment in pFastBac human hMSH2 (20).

hMSH6F32A (hMSH6FA) was obtained in a similar way, using the following pairs of primers: PDU-4 (5'-GAGGAGAGATGAGCACAGGAGCAGCAGGAG-3') and PDU-6 (5'-GATACGCTATACTTCTTTCCCCCCATTCCTTGT-3'), and PDU-5 (5'-CAAGGAGAAGGAAATGATGCGCTTAC-3') and PDU-7 (5'-GATCTCATGAAACTCGCTACTTGT-3'). As no convenient restriction enzymes could be found to clone the final PCR product directly into pFastBac1 human hMSH6, it had to be cloned first between the BamHI and MscI sites of pBluescriptKS− and transferred into pFastBac1 human hMSH6 (30). The entire hMSH6FA from BamHI to XhoI was then transferred into pFastBac1 human hMSH6.

Both plasmids were sequenced to ensure the integrity of the cDNA inserts and to confirm the presence of the mutations. The recombinant baculoviruses were produced using the Bac-to-Bac Baculovirus Expression System (Life Technologies, Inc.) according to the manufacturer’s recommendations.

Production and Purification of the Recombinant Proteins—Infection of Spodoptera frugiperda 9 (Sf9) cells with different pairs of recombinant baculoviruses gave rise to the four possible heterodimers: hMSH2/hMSH6, hMSH2F2A/hMSH6, hMSH2/hMSH6FA, and hMSH2F2A/hMSH6FA (29, 30). After 72 h of incubation, the cells were harvested and the total protein extracts were prepared as described (26). The proteins were purified by fast protein liquid chromatography (FPLC) essentially as described (24) except that the heparin-Sepharose fractions containing hMutSo were first loaded on a Resource-S FPLC column. Even though the desired protein was in the flow-through, the latter matrix bound many contaminating polypeptides. The Resource-S flow-through was then loaded onto a Resource-Q column (all FPLC columns were from Amersham Pharmacia Biotech). The protein preparations were judged by 7.5% SDS-PAGE to be >95% pure (Fig. 2). Protein concent-
trations were estimated in triplicate by the Bradford assay as directed by the manufacturer (Bio-Rad) using bovine serum albumin as a standard.

Electrophoretic Mobility Shift Assays (EMSA)—All EMSAs, including the determinations of the dissociation constants, were carried out as described previously (24). 100 ng of purified protein were incubated with 40 fmol of radioactively labeled DNA substrate (G/C, G/T, +2) for 20 min at room temperature. The oligonucleotide duplexes were ob-
tained by annealing the 32P-labeled, 34-mer upper strand 34GU (5'- AATTCCGGGGTACCTCGAGCTCGGACAAGCT-3') with one of

the three different lower strands: 34CL (5'-AGCTTGCTGAGGTCTTGACGGATCCCCGGGAATT-3'), 34TL (5'-AGCTTGCTGAGGTCTTGAGGTCTTGACGGATCCCCGGGAATT-3'), and 34W (5'-AGCTTGCTGAGGTCTTGAGGTCTTGAGGTCTTGACGGATCCCCGGGAATT-3'). 

The protein concentration was reduced to 0.1 μg/μl (5'-32P]ATP. When the assays were carried
out in the presence of DNA, the substrates (2 μM homo- or heteroduplex DNA G/T) were added at the beginning of the incubation period, and the protein concentration was reduced to 0.1 μg (0.52 μg, 2 pmol) so as to make the stimulation effect more apparent. At selected time points, 2-μl aliquots were removed, the reaction was stopped by the addition of 5 μl of 80% formamide dye, and 2 μl of this mixture were loaded on a 20% sequencing gel as described previously (27). The results were visualized on Biomax™ MR films (Kodak) and quantified using the ImageQuant v1.2 software (Molecular Dynamics). The determination of the dissociation constants was performed as described (20).

ATPase Assays—The ATPase assays were carried out at 37 °C in a 20-μl mixture containing 20 m mM Tris-HCl (pH 7.5), 5 mM MgCl2, 120 mM KCl, 1 mM dithiothreitol, 0.5 μM (12.6 μg, 10 pmol) purified recombinant protein, 4 μM ATP, and 33 radioactivity units of [γ-32P]ATP. When the assays were carried out in the presence of DNA, the substrates (2 μM homo- or heteroduplex DNA G/T) were added at the beginning of the incubation period, and the protein concentration was reduced to 0.1 μg (0.52 μg, 2 pmol) so as to make the stimulation effect more apparent. At selected time points, 2-μl aliquots were removed, the reaction was stopped by the addition of 5 μl of 80% formamide dye, and 2 μl of this mixture were loaded on a 20% sequencing gel as described previously (27). The results were visualized on Biomax™ MR films (Kodak) and quantified using the ImageQuant v1.2 software (Molecular Dynamics). The hMutSo2.6KR variant, which is defective in ATP binding and which was used in these assays as a negative control, was prepared as described previously (20). All assays were carried out in triplicate.

In Vivo Mismatch Repair Assays—M13mp2 heteroduplexes containing either a single G/T mispair (G/T) or a loop of two extrahelical nucleotides (+2) were incubated with the cytoplasmic cell extracts as described (20). The DNA was then purified, electrophorated into a mutant strain of E. coli, and plated along with the α-complementation strain CSH50, isopropyl-1-thio-β-D-galactopyranoside, and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal). If no repair occurred, mixed plaques were observed containing both blue and colorless progeny. Reduction in the percentage of mixed plaques and a concomitant increase in single-color plaques was indicative of repair. The recombinant hMutSo variants (0.18 μg) were used to complement the cytoplasmic cell extracts and to confirm the presence of the mismatch repair-deficient cell lines HCT15 (hMSH6−) and LoVo (hMSH2−). The repair reactions were allowed to proceed at 37 °C for 18 min. Repair efficiency (%) = 100 × (1 − %) of the.
The Phe → Ala Mutations in hMSH2 and hMSH6 Do Not Affect the Stability of hMutSa—The conserved phenylalanine residues 42 and 432 in hMSH2 and hMSH6, respectively, were substituted with alanine by site-directed mutagenesis of the respective cDNAs. Baculovirus vectors expressing the mutated and wild type proteins were constructed and used to infect Sf9 cells. By co-infecting with all the possible combinations, four heterodimers were obtained: wild type hMutSo, hMutSo2FA, hMutSo6FA, and hMutSo2,6FA. Examination of the total protein extracts of the infected Sf9 cells by SDS-PAGE showed that all polypeptides were expressed with similar efficiency (Fig. 2, lanes Sf9 TE). During subsequent purification by FPLC, all four hMutSo variants behaved identically and were obtained in similar yields; this suggests that the mutations did not bring about any significant alterations in the structural properties of the polypeptides. As shown in Fig. 2 (lanes purified hMutSo), all the mutated polypeptides migrated through SDS-PAGE gels with velocities identical to the respective wild type proteins, and the hMSH2/hMSH6 stoichiometry of the four hMutSo variants was also similar to the wild type heterodimer. Because the stability of hMSH6 depends on its interaction with hMSH2 (14, 30), these results indicate that the mutant polypeptides were not significantly altered structurally and were able to interact with their cognate partners to form stable heterodimeric factors.

Mutation of Phenylalanine 432 of hMSH6 to Alanine Abolishes Mismatch Recognition—To test whether the Phe → Ala mutations in hMSH2 and hMSH6 affected the specific binding of the purified recombinant hMutSo variants to base-base mismatches and small IDLs, EMSAs were carried out using radioactively labeled, double-stranded 34-mer homo- or heteroduplex oligonucleotides. As shown in Fig. 3A, wild type hMutSo and the hMutSo2FA variant formed specific protein-DNA complexes with substrates carrying either a G/T mismatch or a loop of two extrahelical thymines. In contrast, the hMutSo6FA and hMutSo2,6FA factors failed to form stable complexes with these substrates under identical conditions. Similar results were obtained when a substrate carrying a 1-nucleotide loop was used (data not shown). The binding affinities of the wild type hMutSo and the hMutSo2FA variant for the G/T-containing substrate were comparable, with both apparent dissociation constants being ~4 nM (data not shown, for details see “Experimental Procedures”). This value agrees well with that reported previously (20). The data showed that substitution of alanine for phenylalanine in the putative mismatch binding motif of hMSH6 abolished the mismatch recognition ability of hMutSo, although an identical mutation in hMSH2 was without effect in the mismatch binding assays. This finding helps explain the results of our earlier experiments where only hMSH6 could be cross-linked to mismatched substrates (20, 26, 27). The fact that identical substitutions in the MSH2 and MSH6 subunits of MutSo of S. cerevisiae were described to have similar phenotypes (28) further underscores the conservation of function of eukaryotic MutS homologues (see also “Discussion”).

The F432A Mutation in hMSH6 Abolishes the Binding of hMutSo6FA and hMutSo2,6FA to Homoduplex DNA—Wild type human and yeast MutSo is also able to bind homoduplex DNA albeit about one order of magnitude less efficiently than heteroduplex substrates (9, 20, 28, 31). As the Phe → Ala mutation in hMSH6 abolished mismatch recognition, we were interested to see if this mutation affected also the nonspecific binding to homoduplex DNA. To this end, we modified the conditions of our standard EMSA so as to augment binding to the latter substrate. By reducing the amount of nonspecific competitor DNA 10-fold, we were able to detect the formation of protein-DNA complexes between the 34-mer homoduplex G/C and hMutSo or hMutSo2FA. In contrast, no such complexes were noticeable when hMutSo6FA or hMutSo2,6FA were incubated with this substrate (Fig. 3B). This indicates that the F432A mutation in hMSH6 affects the binding of hMutSo not only to mismatch-containing substrates but also to DNA in general.

The Phe → Ala Mutations in hMSH2 and hMSH6 Do Not Affect the ATPase Activities of the Variant Heterodimers—In previous studies, the specific DNA-protein complex formed by the mismatched substrate and hMutSo could be shown to be sensitive to ATP (20, 22, 23). The binding of the nucleotide to the so-called Walker type A motifs at the C termini of the MutS homologues was shown to induce distinct conformational changes, which lead to the dissociation of the heterodimer from the substrate (22, 24). To ensure that the F432A mutation had not affected the ability of hMutSo2FA to dissociate from mismatch-containing DNA substrates in the presence of the nucleotide, we added ATP to the EMSA mixtures after a 10-min incubation. As shown in Fig. 4, both factors, the wild type hMutSo and the hMutSo2FA mutant, dissociated from the

**FIG. 3.** DNA binding activities of the recombinant hMutSo variants. In these EMSA experiments, 32P-labeled 34-mer oligonucleotide probes (perfectly matched (G/C) containing a single mismatch (G/T), or an IDL of two extrahelical thymidine residues (2+2), were incubated with the purified hMutSo variants indicated above the lanes (see “Experimental Procedures” for details). **A:** wt, wild type hMutSo; 2FA, hMutSo2FA; 6FA, hMutSo6FA; 2,6FA, hMutSo2,6FA; comp. (ng), amount in nanograms of nonspecific competitor poly[d(C)·d(G)] added to the sample (B). The figure is an autoradiograph of a native 6% polyacrylamide gel.
substrates to a similar extent upon nucleotide addition. Therefore, we conclude that the constitution of the hMutSα2FA-DNA complex, as well as its sensitivity to ATP, are unaffected by the F42A substitution in hMSH2.

Because the hMutSα6FA and hMutSα2,6FA variants failed to bind mismatch-containing DNA, we could not examine their ATP binding and/or hydrolysis properties by EMSA. To test the unlikely possibility that the F432A mutation in hMSH6 altered the DNA binding properties of this variant through a structural change in the ATP binding domain, we carried out ATPase assays. As shown in Fig. 5, the kinetics of ATP hydrolysis by hMutSα and hMutSα6FA were comparable and clearly distinguishable from those of the hMutSα2,6KR variant defective in ATP binding (20). This suggests that the Phe → Ala mutation in hMSH6 affected only mismatch and DNA binding but not the ATPase activity of the hMutSα6FA heterodimer.

The ATPase Activity of the hMutSα6FA and hMutSα2,6FA Variants Is Insensitive to DNA—Though weak, the ATPase activity of MutSα is essential for its function in MMR as shown by the phenotype of hMutSα variants in which ATP binding was diminished through Lys → Arg mutations in the Walker type A ATP binding motifs (20, 21). Previous studies showed that the ATPase activity of MutS homologues could be stimulated by homoduplex and heteroduplex DNA albeit to different extents (28, 33, 34). We wanted to test whether this stimulatory effect was absent in hMutSα variants bearing mutations that affect heteroduplex and homoduplex recognition.

The purified heterodimers were incubated with [γ-32P]ATP either in the presence or in the absence of DNA. In these experiments, five times lower protein concentrations (0.1 μM as opposed to 0.5 μM used in the experiments described in the paragraph above) were used to make the stimulation effect more apparent. As shown in Fig. 6, the ATPase activities of all four hMutSα variants were comparable in the absence of DNA. The Phe → Ala substitutions thus did not affect the DNA-independent ATPase activity. Addition of homoduplex DNA to the reactions resulted in a 3-fold stimulation of ATP hydrolysis by hMutSα or hMutSα2FA, whereas heteroduplex DNA stimulated the ATP hydrolysis by these factors approximately 7-fold (Fig. 6, A and B, respectively). In contrast, the ATPase activity of hMutSα6FA or hMutSα2,6FA was unaffected by both homoduplex and heteroduplex DNA (Fig. 6, C and D, respectively). These results differ somewhat from those of Alani and co-workers (28) who reported that the ATPase activity of the yeast heterodimer MSH2-msh6 F337A (equivalent of hMutSα6FA) was stimulated by heteroduplex DNA. This effect is hard to explain as the yeast heterodimer, similarly to its human counterpart studied here, demonstrated no mismatch binding activity. However, as the stimulation of the yeast factor was seen only at low salt concentrations, this effect may be of questionable relevance (see also “Discussion”). The human factor differs from its yeast homologue in that no stimulation of the hMutSα6FA ATPase was observed under a range of different salt concentrations.2

The F432A Mutation in hMSH6 Abolishes Mismatch Repair in Vitro—In an attempt to test the functionality of the four hMutSα variants, we used the recombinant proteins in in vitro MMR assays. To this end, we used extracts of MMR-deficient lines that lack hMutSα: HCT15 that carries truncating mutations in both alleles of hMSH6 and LoVo, carrying a homozygous deletion in hMSH2. As anticipated, purified recombinant hMutSα or hMutSα2FA fully complemented the MMR deficiency of the LoVo extracts on circular heteroduplex substrates with either a single G/T mismatch or a 2-nucleotide IDL. Similarly, the G/T repair efficiency was restored in HCT15 extracts by these recombinant proteins; the +2 substrate was not tested in HCT15 as these extracts contain a full complement of hMutSβ and are thus proficient in loop repair. In contrast, addition of hMutSα6FA or hMutSα2,6FA had no significant effect on the MMR efficiency of these extracts (Fig. 7).

DISCUSSION

Although the eukaryotic mismatch binding factor MutSα is a heterodimer of two highly conserved polypeptides, it binds to mismatch-containing substrates in an asymmetric fashion. Our experiments with whole cell extracts (26) and later with the purified factor (27) have shown that only a single polypeptide of about 160-kDa molecular mass could be cross-linked to the DNA. We were subsequently able to show by immunoprecipitation of the cross-linked protein-DNA complexes that the polypeptide covalently bound to the mismatch-containing substrate was hMSH6 (20). When Malkov et al. (25) showed that the MutS protein of T. aquaticus was cross-linked to DNA via a phenylalanine residue in a highly conserved N-terminal motif (Fig. 1), we decided to examine this region in hMutSα. As our

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hMSH6 Point Mutation Abolishes Mismatch Recognition

Fig. 6. Stimulation of ATPase activity of the recombinant hMutSo variants by homoduplex G/C (squares) and heteroduplex G/T (filled triangles) 34-mer oligonucleotides. A, wild type hMutSo; B, hMutSo2FA; C, hMutSo6FA; D, hMutSo2,6FA. The assays were performed as described under “Experimental Procedures.” Note that in these experiments five times less protein (0.1 μM) was used than in the experiment shown in Fig. 5 to make the stimulation effect more apparent. The 10-min time point of reactions in which the ATP binding-deficient variant hMutSo2,6KR (open triangles) was incubated with the heteroduplex substrate G/T was used as the negative control. Diamonds designate reactions carried out in the absence of DNA. The experiment was carried out in triplicate. Error bars represent the standard deviations from the mean.

Fig. 7. Complementation of protein extracts from mismatch repair-deficient cell lines with recombinant hMutSo variants in in vitro mismatch repair assays. Circular substrates containing a single mismatch (G/T) or a 2-nucleotide IDL (+2) were incubated with extracts of either HCT15 (hMSH6−−) or LoVo (hMSH2−−) cells, respectively. After an 18-min incubation at 37 °C, the proportion of repaired substrate was determined as described previously (20) and depicted as % repair efficiency. no compl., no recombinant protein added to the extracts; wt, complementation with the wild type hMutSo; 2FA, complementation with hMutSo2FA; 6FA, complementation with hMutSo6FA; 2,6FA, complementation with hMutSo2,6FA. The figure shows the results of two independent experiments (deviations are shown by error bars).

The present data show, mutation of this conserved phenylalanine to alanine in hMSH6 appears to have severely attenuated binding of the heterodimer to mismatch-containing substrates (Fig. 3A) as well as to homoduplex DNA (Fig. 3B). The fact that an identical mutation in hMSH2 was entirely without effect in our assays suggests that this latter polypeptide does not participate in mismatch recognition. It is interesting to note that the Phe → Ala mutation in hMSH6 did not affect the basal level of ATPase activity of the heterodimer, but that it abolished the ability of DNA, both homo- and heteroduplex, to stimulate ATP hydrolysis (Fig. 6, C and D). These results differ somewhat from those reported by Alani and co-workers (28) for the S. cerevisiae factor who reported that, at low salt concentrations, the ATPase activity of the yeast MSH2-msh6, F337A variant could be stimulated by heteroduplex DNA even though its binding to heteroduplex DNA was affected as severely as in the case of the human factor. As noted above, the ATPase activity of the human hMutSo variant hMSH2/hMSH6F432A could not be stimulated by DNA substrates, not even at low salt concentrations (data not shown). The partial proteolytic pattern of the F39A mutant of T. aquaticus MutS (Fig. 1) in the presence of ATP was recently described to be unaltered by the presence of heteroduplex DNA (35), which further strengthens the suggestion that this mutation abolishes mismatch binding. The anomalous behavior of the yeast mismatch binding factor cannot be easily explained. However, the latter factor does appear to differ somewhat from the human hMutSo. In cross-linking experiments, both MSH2 and MSH6 could be seen to form a covalent complex with the heteroduplex DNA (28), whereas no such reaction was observed with the human protein (20). The yeast factor appears to bind mismatches containing 8-oxoguanine and act in their correction (36), whereas no recognition of the 8-oxoguanine/C or 8-oxoguanine/A-containing substrates was observed with the human heterodimer.3 In addition, the mismatch binding motif of MSH6 contains a second phenylalanine residue at position 338 (Fig. 1), which might conceivably compensate, at least to some extent, for the loss of Phe-337 in MSH2. However, neither experiment helped us to understand the role of this conserved site in binding of hMutSo to DNA. This

3 G. Crouse and J. Jiricny, unpublished data.
situation has changed with the resolution of the crystal structure of the bacterial MutS proteins from E. coli (37) and T. aquaticus (32). The structure of co-crystals of these proteins with DNA reveals that the phenylalanine residue in question is located in an α-helix that contacts the DNA in the vicinity of the structural distortion and that the phenylalanine residue partially intercalates into the DNA at the site of the mismatch. This explains its key role in mismatch recognition. However, the most exciting finding concerns the fact that the two subunits of the homodimeric MutS factor are not equivalent inasmuch as only one subunit contacts the substrate DNA via its N-terminal α-helix, whereas the equivalent helical motif of the second subunit is turned away from the DNA. Thus, the mismatch binding factor is a functional heterodimer already in the initial stages of this project and Claudia Perrera for numerous discussions. The expert technical assistance of Darinka Bohrer is also gratefully acknowledged.

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