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Functional analysis of human MutSα and MutSβ complexes in yeast

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ABSTRACT

Mismatch repair (MMR) is initiated when a heterodimer of hMSH2•hMSH6 or hMSH2•hMSH3 binds to mismatches. Here we perform functional analyses of these human protein complexes in yeast. We use a sensitive genetic system wherein the rate of single-base deletions in a homopolymeric run in the *LYS2* gene is 10 000-fold higher in an *msh2* mutant than in a wild-type strain. Expression of the human proteins alone or in combination does not reduce the mutation rate of the *msh2* strain, and expression of the individual human proteins does not increase the low mutation rate of a wild-type strain. However, co-expression of hMSH2 and hMSH6 in wild-type yeast increases the mutation rate 4000-fold, while co-expression of hMSH2 and hMSH3 elevates the rate 5-fold. Analysis of cell extracts indicates that the proteins are expressed and bind to mismatched DNA. The results suggest that hMutSα and hMutSβ complexes form, bind to and prevent correction of replication slippage errors in yeast. Expression of hMSH6 with hMSH2 containing a proline substituted for a conserved Arg^{524} eliminates the mutator effect and reduces mismatch binding. The analogous mutation in humans is associated with microsatellite instability, defective MMR and cancer, illustrating the utility of the yeast system for studying human disease alleles.

INTRODUCTION

DNA mismatch repair (MMR) increases the stability of cellular genomes by correcting DNA replication errors that escape proofreading. This process is initiated when bacterial MutS protein or its eukaryotic homologs bind to mismatches. In humans, the hMSH2 protein can bind to mismatched DNA either alone (1,2) or when complexed with hMSH6 or hMSH3 to form hMutSα and hMutSβ heterodimers, respectively. hMutSα binds to and participates in the repair of single base•base mismatches as well as those containing 1–8 unpaired nucleotides (3–7). Although hMutSβ has little binding affinity for single base mismatches, it participates in the repair of substrates containing 1–8 unpaired nucleotides (5,6).

In addition to binding to their MSH partner and to mismatched DNA, the hMSH proteins work in concert with other proteins to conduct ATP-dependent reactions that excise replication errors and replace them with correct nucleotides (reviewed in 8–10). Perturbation of these multiple interactions and enzymatic reactions can result in inactivation of MMR, a defect that has been linked to both hereditary and sporadic cancers in several tissues (reviewed in 11,12). Loss of MMR activity leads to a mutator phenotype *in vivo*, whose magnitude depends on the nature of the defect and the reporter gene used to detect it. In yeast, complete loss of MMR elevates the rate of forward mutation to canavanine resistance ~40-fold (e.g. 13), while greater differences are seen with mutant alleles of reporter genes that can revert by deletions or additions in repetitive sequences (e.g. 14). The most sensitive microsatellite sequences for detecting loss of MMR are long homopolymeric runs having a high probability of misalignment and a low probability of proofreading during replication (15). In such sequences, inactivation of MMR leads to a very strong increase in mutation rate. For example, the rate of loss of a single A•T base pair from an $(A•T)_{14}$ run in the *LYS2* gene is 10 000-fold higher in an *msh2* mutant yeast strain than in a wild-type yeast cell strain (16). This highly sensitive reporter gene can be used to examine even partial loss of MMR capacity. Here we use it for functional analysis of the human MutSα and MutSβ complexes produced in yeast. We show that although the hMSH proteins are unable to complement the mutator phenotype of a yeast *msh2* mutant, concomitant expression of hMSH2 and hMSH6 or hMSH2 and hMSH3 in a wild-type yeast strain produces a mutator phenotype. We then use the system to demonstrate the functional consequence of an hMSH2 missense mutation found in a cancer patient.

MATERIALS AND METHODS

Yeast strains

Yeast strains E134 (wild-type) and DAG60 (*msh2::kanMX*) were constructed from the original strain CG379 (S1 in our collection): *Mat*α *ade5-1 his7-2 leu2-3,112 trp1-289 ura3-52* (17). Both E134 and DAG60 carry the *lys2::InsE-A$_{14}$* mutant allele which

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reverts via single base deletions in the A_{14} homonucleotide run (16). The *msh2::kanMX* mutation in DAG60 was created by replacing a major portion of the *MSH2* coding region (leaving 41 nt on the 5′ side and 96 nt on the 3′ side) using the PCR disruption technique by the kanMX module (18). The kanMX cassette was amplified by PCR using the following pair of primers (underlined are sequences of primers homologous to the kanMX cassette): msh2-kanMX-5′: d(ATGTCCTCCACTAGGCCAGAGCTAA-AATTCTCTGATGTATCCGTACGCTGCAGGTCGAC) and MSH2-kanMX-3′: d(CTGGTTCATTTGCTATAGCACGCAATA-GCTCTTGTATTTTATGCTGATCGATGAATTCGAGCTCG). Strain E134 was transformed to G418-resistance and *msh2* mutant transformants were verified by an allelism test. The yeast strain E203 (*msh6::kanMX*), containing a deletion of the entire open reading frame of *MSH6*, was constructed using the PCR disruption technique by the kanMX module (18,19). The strain E209 (*msh3::hisG*) was prepared from E134 by transformation with *Eco*RI digested pEN33 (20). All transformants for strain construction were verified by allelism tests.

Vector construction

Expression plasmids are derived from previously described yeast–*Escherichia coli* shuttle vectors (21). YEp195SPGAL, a gift from E. Perkins, was derived from the YEplac195 2μ origin based plasmid with a *URA3* selectable marker by replacing the YEplac195 *Pvu*II fragment with the *Pvu*II fragment from the pSPORT cloning vector (Life Technologies). An *Eco*RI/*Sal*I fragment containing *GAL1-10* promoter was ligated into the pSPORT multicloning sequence at the *Eco*RI and *Sal*I restriction sites providing multicloning sites on both sides of the divergent *GAL1-10* promoter. Both the YEp181SPGAL (*LEU2* marker) and the YEp112SPGAL (*TRP1* marker) expression plasmids were derived from YEplac181 and YEplac112 by replacing the original pUC *lacZ Pvu*II fragment with the *Pvu*II fragment containing the *GAL1-10* promoter with the pSPORT multicloning sites from YEp195SPGAL. The yeast *MSH2* expression plasmid pAC12, containing the yeast coding sequence for *MSH2* fused to the *GAL* promoter, was constructed by ligating the yeast gene isolated from pEN11 (a gift from R. Kolodner) as a blunt-ended *Xho*I/*Kpn*I fragment into the blunt-ended *Eco*RI/*Kpn*I digested YEp195SPGAL expression vector. This construction places the start codon of yeast *MSH2* 18 nt further downstream than the start codon of the *GAL10* gene (22). The human *MSH2* expression plasmid pAC20.2, containing the human coding sequence for *MSH2* fused to the *GAL* promoter, was constructed by ligating the human *MSH2* cDNA isolated from pBS-hMSH2 (a gift from Burt Vogelstein) as a blunt-ended *Xba*I/*Kpn*I fragment into the blunt-ended *Eco*RI/*Kpn*I digested YEp195SPGAL expression vector. This positioning of the human *MSH2* coding sequence places the start codon 18 nt further downstream than the start site of the *GAL10* gene (22) and includes 289 nt 3′ of the termination codon. The expression plasmid pAC31.3, containing the human *MSH3* coding sequence fused to the *GAL* promoter, was constructed by creating a *Hin*dIII and *Mlu*I fragment of *hMSH3* cDNA (a gift from T. Shimada) by PCR using deep vent DNA polymerase (New England Biolabs). The PCR fragment was digested 5′ and 3′ of the *hMSH3* coding sequence with *Hin*dIII and *Mlu*I, respectively, and ligated into the *Hin*dIII and *Mlu*I sites in YEp181SPGAL in which the *Bam*HI fragment within the multicloning sequence had been deleted. This construction places the start codon of *hMSH3* 18 nt further downstream than the start codon of the *GAL1* gene (22) and includes 46 nt 3′ of the termination codon. The expression plasmid pAC61.2, containing the human *MSH6* cDNA sequence, was isolated as a *Bam*HI/blunt-ended *Xho*I fragment from pBS-hGTBP (a gift from J. Jiricny) and ligated into the *Bam*HI/blunted *Mlu*I site of YEp181SPGAL, placing the *hMSH6* cDNA start codon 6 nt further downstream than the start codon of the *GAL1* gene. The sequence of the *hGTBP* overexpression construct and all of the above MutS homolog constructions were determined to be correct by DNA sequencing.

Overexpression of mismatch recognition proteins in yeast

Yeast strains were transformed with expression plasmids according to the modified lithium acetate procedure (21). Transformants were selected on synthetic media without uracil and leucine (23). For induction of the gene or cDNA sequences fused to the *GAL1-10* promoter, transformants were streaked onto synthetic media without uracil and leucine, but containing lysine and 2% galactose (Pfanstiehl Laboratories, Inc., Waukegan, IL) substituted for glucose. Quantitative measurements of the ability of the expressed sequences to either complement an *msh2* null mutant or exhibit a dominant negative mutator phenotype were determined on galactose containing media lacking lysine by fluctuation analysis as previously described (24).

Yeast extract preparation

Yeast cultures overexpressing human mismatch recognition proteins were grown in liquid synthetic media containing 2% galactose substituted for glucose and lacking uracil and leucine. Cells were harvested at a density of $\sim 5 \times 10^7$ cells/ml, resuspended in 1 ml of 10% sucrose, 50 mM Tris pH 7.5, 10 mM EDTA/g of cells, and frozen in liquid nitrogen. Upon thawing cells, zymolyase 100T (ICN, Costa Mesa, CA) was added to 1 mg/ml and the cells were incubated on ice for 30 min. An equal volume of lysis buffer 0.5 M NaCl, 50 mM Tris pH 7.5, 10 mM EDTA, 10% glycerol, 1 mM 2-mercaptoethanol, 0.1 M PMSF was added and the cells were incubated on ice for 30 min. Lysates were centrifuged at 25 000 *g* for 10 min and the supernatants were collected.

Western blot analysis of yeast extracts

SDS–PAGE gels containing 30 μg of yeast extract per lane were transferred to PVDF membrane (Millipore, Burlington, MA) overnight in 25 mM Tris, 192 mM glycine buffer at 0.1 mA. Blots were blocked with 3% gelatin (BioRad, Hercules, CA) before probing with antibodies. Purified rabbit polyclonal antibody against hMSH2 (Ab-3) was purchased from Oncogene Research Products (Cambridge, MA). A multiple antigen peptide of amino acids 136–156 of human MSH3, purchased from Research Genetics (Huntsville, AL), was used for making a polyclonal rabbit antiserum. Goat affinity purified polyclonal antibody against an hGTBP peptide containing amino acids 69–88 was purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). Alkaline phosphatase conjugated goat-anti-rabbit antibody was purchased from Promega (Madison, WI). Alkaline phosphatase conjugated rabbit–anti-goat antibody was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

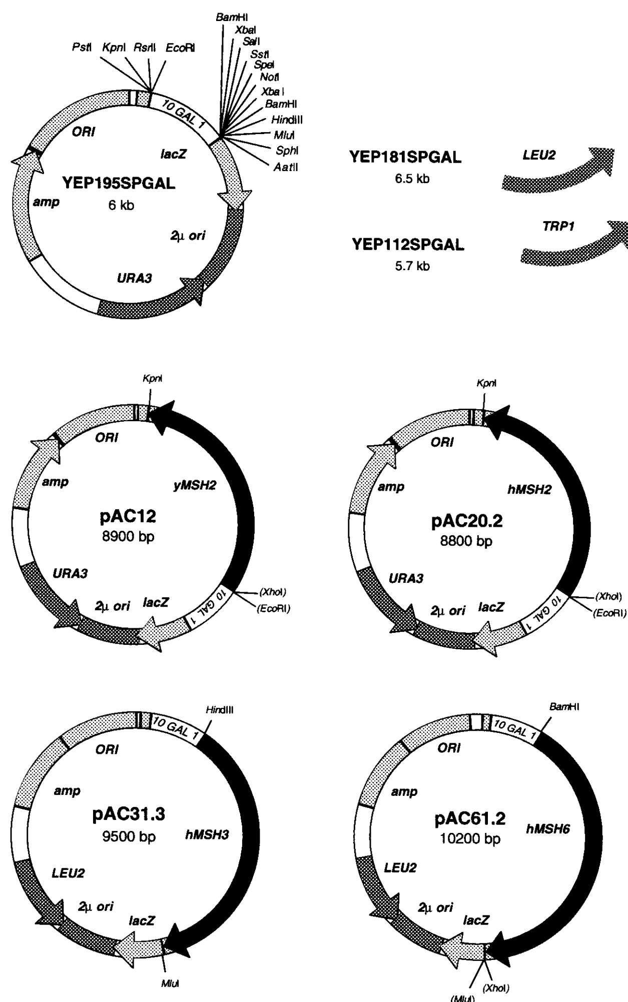

Figure 1. Vectors used in this study. Multicopy galactose expression vectors were constructed that contain the 2µ origin, the *GAL1-10* inducible promoter with multiple cloning sites, and either *LEU2*, *URA3* or *TRP1* for selection. For the vectors containing the MutS homolog genes used in this study, only the restriction sites used for cloning are shown. The sites in parentheses were rendered blunt-ended for ligation.

Mismatch binding assay

An oligo heteroduplex containing a G•T mispair was formed by annealing radiolabeled d(GAATTCACGGCGGC**G**GCGGCG-GGACCGG) with unlabeled d(CCGGTCCCGCCGC**T**GCCGC-CGTGAATTC) (bold type represent mispairs bases). Homoduplex and two base loop heteroduplex were formed by annealing radiolabeled d(ATATGACATGTGGAATTCTATTGTGGC) with d(GCCACAATAGAATTCCACATGTCATAT), and d(GCCAC-AATAGAA**CC**TTCCACATGTCATAT), respectively. A single T insertion simulating one of the two possible substrates occurring within *lys2::InsE-A*$_{14}$ was prepared from radiolabeled d(CTTTCC-CGTTTTTTTTTTTTTTAAGGATCT) containing a T_{14} run and unlabeled d(AGATCCTTAAAAAAAAAAAAACGGAAAG) with an A_{13} run. A heteroduplex oligonucleotide with a single A insertion was prepared by annealing radiolabeled d(AGATCCAAA-AAAAAAAAAAACGGAAAG) with an A_{14} run and unlabeled d(CTTTCCCGTTTTTTTTTTTTTAAGGATCT) having a T_{13} run. Homoduplexes were made by annealing radiolabeled oligonucleotides to unlabeled complementary oligonucleotides. Mismatch binding assays were performed with 15 µg of yeast extract and 100 fmol of duplex substrate in 20 µl reactions containing 20 mM Tris pH 7.5, 5 mM $MgCl_2$, 0.1 mM DTT, 0.01 mM EDTA, 2 µg of non-specific competitor. Mixtures were incubated on ice for 30 min, then mixed with 5 µl of loading buffer (20 mM Tris pH 7.5, 5 mM $MgCl_2$, 0.1 mM DTT, 0.01 mM EDTA, 50% glycerol, 0.015% Bromophenol Blue) and subjected to electrophoresis (100 V) at 4°C through 6% polyacrylamide gels in 0.5× TBE buffer. Gels were dried and exposed to phosphor screens for analysis by a PhosphorImager (Molecular Dynamics). Extent of binding was calculated as the ratio of pixels in the shifted band to the total number of pixels in the gel lane.

RESULTS

Multicopy, galactose-inducible expression vectors were constructed for expression of yMSH2, hMSH2, hMSH3 and hMSH6 in yeast (Fig. 1). They each have a 2µ origin, multiple unique restriction sites and the inducible *GAL1-10* promoter, and differ only in the selectable marker used for maintenance. The *GAL1-10* promoter was chosen because it is expected to promote a high level of expression of the MSH proteins (22). The effects of these vectors on mutation rate were assessed in strains carrying the *lys2::InsE-A*$_{14}$ allele, a highly sensitive reporter gene with a run of 14 A•T base pairs in the +1 reading frame within the *LYS2* coding sequence. Reversion to LYS+ occurs by deletion of a single A•T base pair within the homonucleotide run (16).

Table 1. Effect of expressing human MutS homologs on reversion rate Lys+ reversion rate in yeast *msh2*$^-$, *lys2::InsE-A*$_{14}$ strain

Strain/plasmid	Mutation rate ($\times 10^6$)	95% Confidence limits ($\times 10^6$)
MSH2$^+$/vectors	0.13	0.07–0.19
msh2$^-$/vectors	1900	980–4000
msh2$^-$/yMSH2	0.07	0.03–0.82
msh2$^-$/hMSH2	1400	1000–1800
msh2$^-$/hMSH3	1000	800–1200
msh2$^-$/hMSH6	2600	1400–3600
msh2$^-$/hMSH2 + hMSH3	1100	820–2400
msh2$^-$/hMSH2 + hMSH6	1600	1300–2200

Lack of complementation by human MutS homologs

The difference in LYS+ reversion rate between a wild-type and an *msh2* mutant yeast strain is 10 000-fold (16). The high reversion rate in an *msh2* mutant strain is unaffected by introducing control vectors that do not contain MMR genes (Table 1, line 2). However, it is fully complemented by introducing a yeast *MSH2* expression vector, consistent with Alani *et al.* (25; Drotschman *et al.*, submitted) (Table 1, line 3). In contrast, introduction of human *MSH2*, *MSH3* or *MSH6*, either individually or in combination (Table 1, lines 4–8), does not reduce the high reversion rate of the *msh2* mutant. That this lack of complementation is not due to lack of expression of the human proteins is suggested by western blot analysis of extracts of

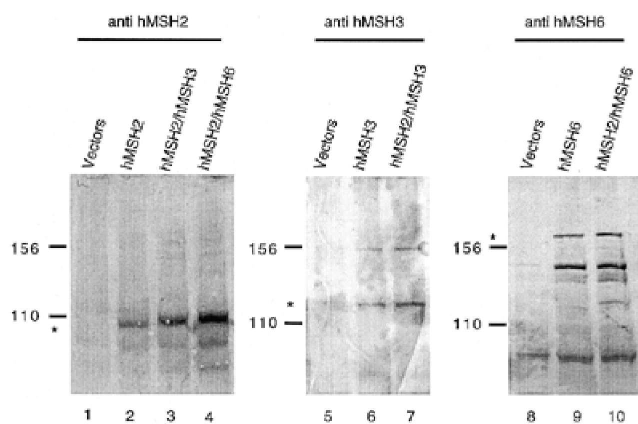

Figure 2. Immunoblots of yeast strains expressing MutS homologs. Each lane contains 30 µg of yeast extract with human proteins expressed individually or together, as indicated. The bands migrating with mobilities consistent with deduced mobilities for human MutS homologs are indicated by asterisks. Lanes 9 and 10 contain a higher mobility species stained with hMSH6 antibody. The smaller band is likely the result of proteolysis similar to that observed after purification of hMSH6 from both HeLa cells and baculovirus (5,37). The slower mobility species present in lanes 6 and 7 is a galactose-induced protein of unknown identity that cross reacts with hMSH3 antiserum.

galactose-induced strains harboring the *hMSH2*, *hMSH3* and *hMSH6* expression vectors alone or in combination (Fig. 2). Polypeptides with mobilities of 105, 123 and 167 kDa (indicated with asterisks) were detected with antibodies against hMSH2, hMSH3 and hMSH6, respectively. These bands are consistent with the deduced molecular weights of hMSH2 (105 kDa), hMSH3 (123 kDa) and hMSH6 (153 kDa), and the apparent molecular weight of 167 kDa observed for hMSH6 (5). These bands were not seen in uninduced yeast strains (data not shown) or in strains harboring vectors without the genes (Fig. 2).

Dominant negative mutator effects by human MutS homologs

We next examined whether expression of the human proteins interfered with yeast MMR as detected by an increase in reversion rate. Expression of *hMSH2*, *hMSH3* or *hMSH6* alone did not affect the reversion rate of a wild-type yeast strain (Table 2, compare line 1 with lines 5–7), indicating that these proteins individually do not influence MMR *in vivo* in yeast. However, co-expression of *hMSH2* with *hMSH6* yielded a reversion rate of 510×10^{-6}, a value 4000-fold higher than that of the wild-type strain (Table 2, line 9) and close to that of the yeast *msh2* null mutant strain. Co-expression of *hMSH2* and *hMSH3* yielded a reversion rate of 0.66×10^{-6}, a value 5-fold higher than that for the wild-type strain. For comparison, an *msh3*⁻ strain has a mutation rate that is 18-fold higher than the wild-type strain (Table 2, line 2). Although small, the 5-fold effect is reproducible and significant, since the lower 95% confidence limit for co-expression of *hMSH2* and *hMSH3* is greater than the upper 95% confidence limit for the wild-type strain. Moreover, both the 4000- and 5-fold mutator phenotypes are absent when the strains are grown on glucose containing media (i.e., without inducing expression), and they are not observed in strains in which either the *hMSH2*, *hMSH3* or *hMSH6* expression vector is lost when selection is not maintained (data not shown).

Table 2. Effect of expressing human MutS homologs on Lys+ reversion rate in yeast MSH2$^+$, *lys2::InsE-A$_{14}$* strain

Strain/plasmid	Mutation rate ($\times 10^6$)	95% Confidence limits ($\times 10^6$)	Fold increase over *MSH2*$^+$
MSH2$^+$/vectors	0.13	0.07–0.19	1
msh3$^-$	2.4	1.9–4.3	18
msh6$^-$	22	12–39	170
msh2$^-$/vectors	1900	1000–4000	14 000
MSH2$^+$/hMSH2	0.08	0.04–0.17	1
MSH2$^+$/hMSH3	0.13	0.08–0.25	1
MSH2$^+$/hMSH6	0.15	0.08–0.29	1
MSH2$^+$/hMSH2 + hMSH3	0.66	0.47–2.1	5
MSH2$^+$/hMSH2 + hMSH6	510	160–940	4000
MSH2$^+$/hMSH2$_{R542P}$ + hMSH6	0.20	0.10–0.57	1

Analysis of heteroduplex binding

The dominant negative mutator effects suggest that concomitant expression of *hMSH2* and *hMSH6* and of *hMSH2* and *hMSH3* may result in formation of hMutSα and hMutSβ complexes that inhibit yeast MMR. To determine if these human complexes form and bind to mismatched substrates, DNA mobility shift assays were performed with extracts made from both *msh2* and *MSH2* yeast strains overexpressing *hMSH2*, *hMSH3* or *hMSH6* individually, or from strains overexpressing both partners of hMutSα or hMutSβ. None of the extracts prepared from strains containing vectors lacking hMutS homologs or from strains expressing individual hMutS homologs displayed evidence of specific binding to heteroduplexes containing a 2 nt insertion/deletion mismatch (Fig. 3A, lanes 2–5) or a G•T mismatch (Fig. 3A, lanes 9–12). However, extracts of cells expressing either *hMSH2* and *hMSH3* or *hMSH2* and *hMSH6* bound to a 2 nt insertion/deletion mismatch, as indicated by a strong, unique band in lanes 6 and 7 of Figure 3A. Moreover, an extract prepared from cells expressing *hMSH2* and *hMSH6* yielded a strong, unique band with a G•T mismatch (Fig. 3A, lane 14), whereas cells expressing *hMSH2* and *hMSH3* yielded a much weaker band with a G•T mismatch (Fig. 3A, lane 13). These band-shift data are consistent with formation of hMutSα and hMutSβ complexes having heteroduplex binding specificities similar to those reported for these isolated human complexes (3–7).

DNA mobility shift assays were also performed with single-base deletion mismatches using the same A$_{14}$ run sequence that was used to score reversion rates *in vivo*. An extract of cells expressing *hMSH2* and *hMSH6* yielded a strong, unique band with substrates containing an unpaired A or T nucleotide (Fig. 3B, lanes 6 and 9, respectively). This binding was reduced upon addition of unlabeled heteroduplex competitor (data not shown), and, as expected, the intensity of the shifted band was much greater with heteroduplex substrate than with homoduplex substrate (Fig. 3B, lane 3). An extract of cells expressing *hMSH2*

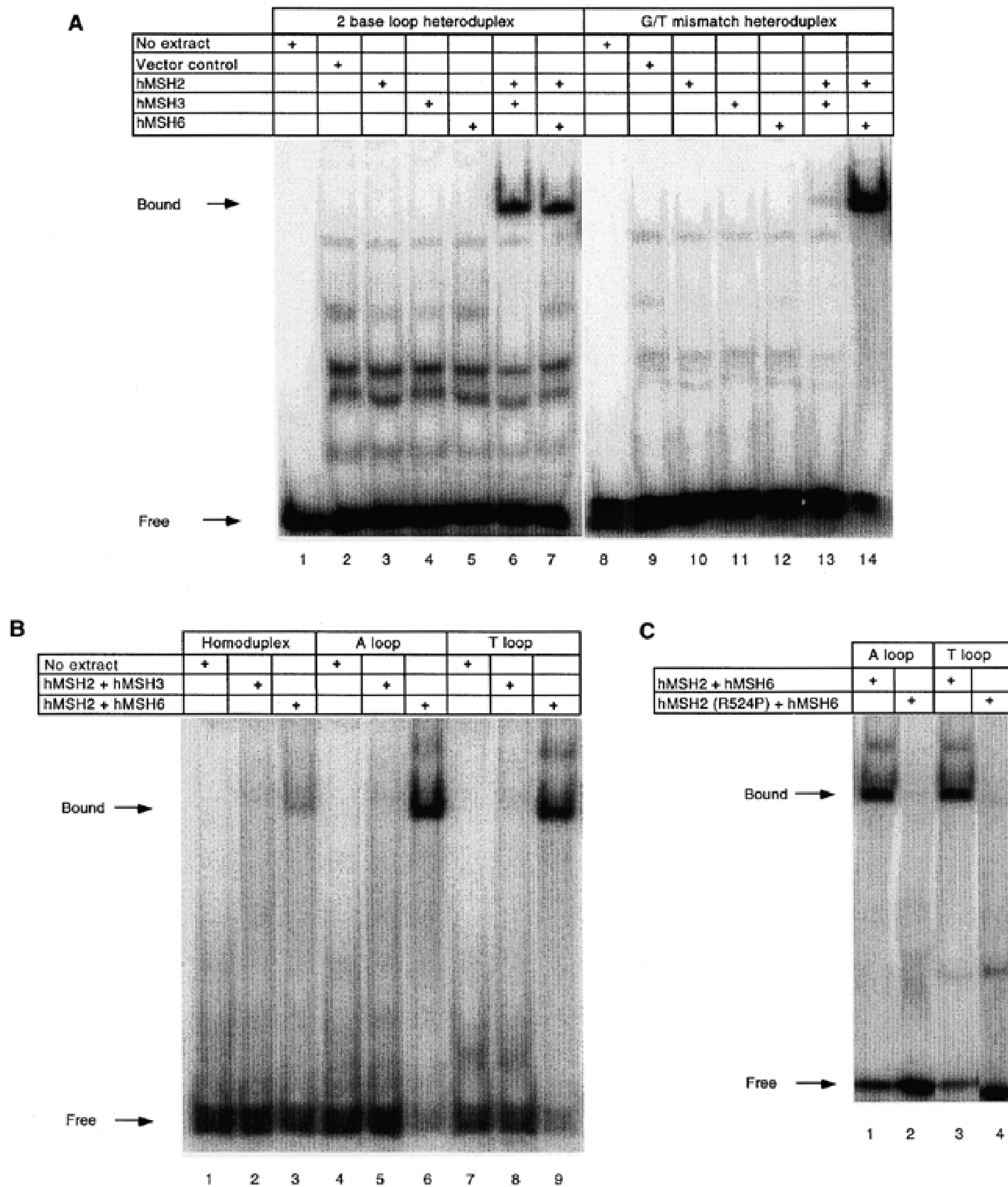

Figure 3. Band-shift analysis. Extracts were prepared from an *msh2* mutant yeast strain expressing the human protein(s) in order to abolish substrate recognition by the yeast MMR system. Similar results (not shown) were obtained with a wild-type yeast strain. Extracts are indicated above each lane. The positions of the free probe and the bound complex are indicated by arrows. (**A**) Binding by extracts expressing individual human MutS homologs or co-expressing the partners of human MutSα and human MutSβ complexes. (**B**) Binding by co-expressed hMSH2•hMSH3 and hMSH2•hMSH6. The homoduplex or heteroduplex substrates used are indicated above the lanes. (**C**) A comparison of binding by wild-type or mutant hMutSα to duplex oligonucleotides containing an extra A or T in one strand.

and *hMSH3* failed to produce a detectable band shift with these same two substrates (Fig. 3B, lanes 5 and 8). This result is consistent with the 800-fold smaller dominant negative effect seen when *hMSH2* and *hMSH3* are co-expressed in yeast (Table 2).

In addition to the results shown in Figure 3, we observed that extracts expressing individual MutS homologs did not detectably bind to homoduplex DNA. Extracts expressing both hMSH2 and hMSH6 did bind to homoduplex DNA; however, the proportion of homoduplex substrate present in the shifted band was 2.5% of the proportion observed for the binding of the G•T heteroduplex substrate. Likewise, extracts expressing hMSH2 and hMSH3 also bound to homoduplex DNA, but the proportion of bound substrate was 5% of that formed with a mismatch containing two unpaired nucleotides. The proportion of bound G•T heteroduplex by an extract expressing hMSH2 and hMSH3 was only 1.5-fold greater than that observed with the homoduplex.

Analysis of the effect of an hMSH2 missense mutation

We examined the ability of a missense mutation in *hMSH2* to modulate the 4000-fold mutator effect of the wild-type hMutSα complex. We chose to investigate the R524P missense

substitution that has been described as an HNPCC mutation (26). A human ovarian tumor cell line harboring this mutation exhibits microsatellite instability (26) and an extract of this cell line is defective in repair of base•base and insertion/deletion mismatches (27). We introduced the R524P mutation into *hMSH2* in the yeast expression vector and then co-expressed it with the wild-type *hMSH6* gene in a wild-type yeast strain. Immunoblots of a cell extract (not shown) revealed that the hmsh2-R524P and hMSH6 protein expression levels were similar to those of the wild-type proteins shown in Figure 2. However, the 4000-fold mutator phenotype characteristic of the wild-type hMutSα complex was eliminated (Table 2, compare lines 9 and 10). When an extract was used for band-shift analysis with either of the two single-base deletion mismatches within the *LYS2* A_{14} run sequence, the signal was greatly reduced relative to results obtained with wild-type *hMSH2* and *hMSH6* (Fig. 3C, compare lanes 2 and 4 with lanes 1 and 3).

DISCUSSION

The results presented here are relevant to several aspects of DNA MMR in eukaryotes. Although considerable evidence indicates that hMSH2, hMSH3 and hMSH6 participate in repair of single-base deletion mismatches in human cells (4,6,27–31), our data suggest that these proteins cannot fulfil this same role in yeast, since they fail to complement the strong single-base deletion mutator phenotype of an *msh2* mutant strain. This may reflect the inability of the human proteins to productively interact with yeast proteins downstream in the MMR pathway, such as Mlh1p, Pms1p or PCNA. Nonetheless, the western blot analysis demonstrates that the human genes are expressed in yeast, and the band-shift results indicate that they bind mismatched DNA. An extract of yeast cells co-expressing *hMSH2* and *hMSH6*, but neither protein alone, yields a band shift with DNA containing a G•T mismatch or two extra nucleotides. This is consistent with the mismatch binding specificity of natural and recombinant hMutSα (3,5,31) and with its participation in strand-specific repair of these mismatches *in vitro* (4,6). An extract of yeast cells co-expressing *hMSH2* and *hMSH3* also yields a strong band shift with a substrate containing two extra nucleotides, but not with DNA containing a G•T mismatch. Again, this is consistent with the reported mismatch binding specificity of hMutSβ (5), its role in correcting frameshift heteroduplexes, and the fact that it has a minor (29,32) or undetectable role (4,6,7) in correcting base•base mismatches. Collectively, the data indicate that human protein complexes with mismatch binding capacity are present in these yeast extracts.

The mutator phenotypes in the wild-type yeast strain are observed only upon galactose-induced expression of both partners of the hMutSα and hMutSβ heterodimers, demonstrating that the complexes are also present *in vivo*. The data also suggest that hMutSα and hMutSβ alone are sufficient for the recognition of mismatches *in vivo*. The increase in mutation rate conferred by hMSH2•hMSH6 (4000-fold) and by hMSH2•hMSH3 (5-fold) correlates with their respective strong versus weak binding to deletion mismatches in the *lys2::InsE-A*$_{14}$ target DNA sequence. These differential effects are consistent with a recent *in vitro* study indicating that hMutSα is much more active in repairing single nucleotide insertion/deletion mismatches than is hMutSβ (6). The results may be explained in several ways, including a model wherein suppression of MMR *in vivo* results from mismatch binding by the hMutS heterodimer, with subsequent inability to interact with heterologous yeast proteins required for MMR. The human and yeast complexes may be sufficiently diverged such that the proteins cannot be interchanged. Regardless of the explanation for the increased mutation rate, the results demonstrate that, in addition to many functional studies of single mammalian repair genes in yeast (33–36), yeast can also be used for functional analysis of human multiprotein complexes.

The 4000-fold mutator effect and strong binding activity of co-expressed *hMSH2* and *hMSH6* are completely eliminated by the R524P missense mutation in *hMSH2* found in an HNPCC patient (26). These strong effects in yeast cells are consistent with the microsatellite instability (26) and MMR defects (27) observed for a human ovarian tumor cell line that is hemizygous for this mutation. Arg^{524} is conserved among eukaryotic MSH2 proteins, and the non-conservative change to proline may effect function by eliminating heterodimer formation or nucleic acid binding. The yeast model system described here will be useful for functional studies of the human MutSα complex, including examination of the functional consequences of missense mutations found in hereditary and sporadic cancers. Modification of the reporter gene to monitor instability in different repetitive sequences should also provide a more sensitive assay for hMutSβ function.

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