Differential subcellular localization of human MutY homolog (hMYH) and the functional activity of adenine:8-oxoguanine DNA glycosylase

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ABSTRACT

The post-replicative adenine:8-oxo-7,8-dihydroguanine (GO) mismatch is crucial for G:C to T:A transversion. This mismatch is corrected by Escherichia coli MutY which excises the adenine from A:GO. A candidate gene coding for the human counterpart of MutY has been cloned as hMYH. However, the function and enzyme activities of the gene product have not been identified. We previously demonstrated that an epitope-tagged hMYH protein behaves as a mitochondrial protein. In the present study, we have identified an alternative hMYH transcript, termed type 2, which differs in the exon 1 sequence of the known transcript (type 1). A nuclear localization for the type 2 protein was revealed by detection of epitope-tagged protein in COS-7 cells. Expression of both type 1 and type 2 transcripts was reduced in post-mitotic tissues. hMYH cDNA suppressed the mutator phenotype of E.coli mutY. In vitro expressed hMYH showed adenine DNA glycosylase activity toward the A:GO substrate. The protein can bind to A:GO, and to T:GO and G:GO without apparent catalysis. These results represent the first demonstration of the function of the hMYH gene product which is differentially transported into the nucleus or the mitochondria by alternative splicing.

INTRODUCTION

Continuous oxidative DNA damage is implicated in carcinogenesis and aging (1,2). 8-oxo-7,8-dihydroguanine (GO) is one of the most significant oxidative lesions in DNA because of its mutagenic potency (3,4). The primary repair enzyme for GO:C is 8-oxoguanine DNA glycosylase, known as Escherichia coli Fpg (5) and eukaryotic OGG1 (6–13). A post-replication repair system is needed, however, because DNA synthesis is not blocked at the GO lesion and dA is frequently incorporated by many DNA polymerases, including nuclear polymerases (14) and mitochondrial polymerase (15). Such a repair mechanism is present in E.coli, where the MutY DNA glycosylase removes the adenine base at an A:GO mismatch (16). A number of biochemical studies (17 and references therein) as well as genetic evidence that the E.coli strains lacking MutY is a mutator for G:C to T:A transversions (18,19) indicate that MutY is a post-replication safeguard against mutation. Recently, the catalytic activity of MutY toward G:GO and its preventive role against G:C to C:G transversion have been revealed (20). The MutY protein has a helix–hairpin–helix (HhH) motif characteristic of the base excision repair (BER) glycosylase superfamily (21) and a [4Fe-4S] cluster (22) conserved in the endonuclease III family. Proteolytic treatment of the MutY protein yields an N-terminal catalytic domain and a C-terminal fragment (23,24). The primary and tertiary structures (25) of the catalytic domain are strikingly homologous to E.coli endonuclease III (26) which catalyzes several oxidized pyrimidines (27).

A eukaryotic homolog of E.coli mutY has recently been characterized from Schizosaccharomyces pombe (28). The gene product (SpMYH) has adenine DNA glycosylase activity toward A:GO and A,G mismatches as does E.coli MutY. In humans, a gene exhibiting substantial homology to mutY has been cloned as hMYH (29). The ‘MutY’ activity of the gene product has been inferred from the sequence homology and from biochemical evidence that mammalian cells have an adenine DNA glycosylase cross-reactive to E.coli MutY antibodies (30). However, the enzyme activity and cellular function of hMYH remained to be demonstrated. A recent study using shuttle vectors containing an A:GO mismatch has shown slow mismatch repair and a consequent high level of mutation to A:T in human cells (31), raising questions about the functional role and regulation of human ‘MutY’ protein.

Eukaryotic cells have nuclear and mitochondrial genomes and thus the cells have necessarily had to develop either two distinct repair enzymes or a transport system that delivers the same enzymes into the separate organella. A DNA polymerase required for BER is an example of former case, pol γ is involved in mitochondrial BER (32) while repair synthesis in nuclear BER is performed by pol β or pol δ/ε with the aid of the aid of nuclear proteins (33). In contrast, several DNA glycosylases

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are transported into both the nucleus and mitochondria. The human uracil DNA glycosylase gene (UNG) generates two alternative transcripts designated for either the nuclear or mitochondrial transport (34). Alternative splicing of the hOGG1 gene also gives rise to nuclear and mitochondrial isoforms (35). The human endonuclease III homolog (hNTH1) is an example of dual localization achieved by having two transport signals in one protein (35).

In a previous study, we found that the hMYH gene product is localized in the mitochondria when a tagged construct is transiently expressed. Nuclear localization was detected either weakly or not at all in COS-7 cells or in HEK and CHO-9 cells, respectively (35). This localization is not consistent with the activity of the mammalian adenine DNA glycosylase which has been identified in nuclear extracts (30). To resolve this discrepancy, we consider that it is essential to characterize the function of the hMYH gene product and to identify its subcellular localization. In this report, we identify a nuclear isoform which is generated by alternative splicing of the hMYH gene. We further describe, for the first time, the hMYH function as evidenced by the ability to suppress the mutator phenotype of E. coli mutY and by the catalytic activity of adenine (A:GO) DNA glycosylase in vitro.

MATERIALS AND METHODS

Cloning of cDNA fragment

5′ cDNA fragments of hMYH were amplified from a placental cDNA pool by the 5′ RACE method (Marathon Amplification Kit, Clontech) with adapter primers from the kit and nested gene-specific primers designed from the sequence of exon 3 (TCC ATG GTA GGT CCC GTT TCT TT GT C and CAG GCT CCC TCG GAA GGC TGT GAC TTC A). The PCR products were cloned in pT7Blue (Novagen) and the randomly selected clones were sequenced. Two splicing isoforms (type 1 and type 2) with a 5′ sequence distinct from each other were identified. Additional clones for the type 2 cDNA were obtained by nested PCR with the inner adapter primer and a primer specific for the type 2 sequence (GAT GGC CCG CGG CTC ACG CTG ATG AAG). Sequencing was performed by Shimadzu DSQ-1000.

Immunofluorescence

The coding sequences for hMYH isoforms were C-terminally tagged with a FLAG sequence and introduced into a mammalian expression vector (pTARGET, Promega). Indirect immunofluorescence with anti-FLAG (Sigma) following transient expression vector (pTARGET, Promega). Indirect immunofluorescence with anti-FLAG (Sigma) following transient expression was estimated by incorporation of 35S-methionine in a transcription/translation coupled in vitro translation system based on a rabbit reticulocyte lysate (TNT for Sp6, Promega) at a concentration of 1 µg/25 µl reaction. The yield of the translation was estimated by incorporation of 35S-methionine in a separate reaction. For the DNA glycosylase assay, a 30mer oligonucleotide containing GO (oligo-GO) was synthesized: 5′-CTCGTCAGCATCT-GO-CATCATACAGTCAGTG-3′.

The complementary oligonucleotide containing A, G, C or T opposite GO was 5′-32P-labeled and annealed with the oligo-GO to generate dsDNA substrates: *A:GO, *G:GO, *C:GO or *T:G. The TNT reaction lysate (4 µl) was incubated with the substrate (40 fmol) in a 20 µl reaction buffer containing 10 mM Tris–HCl (pH 7.5), 0.5 mM dithiothreitol, 5 µM MnSO4, 30 mM NaCl and 5 mM MgCl2 at 37°C for 60 min unless otherwise mentioned. The reaction was stopped by adding phenol/ chloroform followed by DNA extraction and ethanol precipitation. The DNA was dissolved in water and treated with 0.1 M NaOH at 95°C for 5 min in order to convert the abasic DNA into nicked form. The sample was mixed with formamide-loading dye, heated at 75°C for 2 min, and loaded onto 20% polyacrylamide/8 M urea gel. The gels were visualized by autoradiography and the quantification was done using the BAS 2000 system (Fujix).

DNA binding assay

The hMYH cDNA (type 2) was C-terminally tagged with an HA sequence and in vitro-translated as described above. Binding of the hMYH protein to the 30mer DNA probe was performed under the same conditions as for the DNA glycosylase assay except for the incubation on ice for 30 min. For the preparation of probes, the oligo-GO or oligo-G was 5′-32P-labeled, divided into aliquots and annealed to the complementary strand. The binding reaction contained 80 ng plasmid DNA from the TNT samples were loaded on 1% agarose gel and densitometric analysis was made using the NIH image program.

Spontaneous mutation assays

The strains of E.coli CC104 [ara, A(gpt-lac)s, rpsL/F′ (lacI98, lacZ461, proA*B*)] and CC104mutY::Tn10 were used. The lacZ461 can only revert to wild-type via a GC TA transversion event (36). The hMYH (type 2) cDNA was subcloned in pMAL-c2 (NEB) to generate pMAL-cY2. CC104 and CC104mutY were transformed with pMAL-cY2 or the empty pMAL-c2 vector. Five to ten independent overnight cultures were analyzed for Lac+ and RifR mutations as previously described (20). Aliquots of the cultures were plated on LB agar containing ampicillin (50 µg/ml) and rifampicin (100 µg/ml), or on minimal glucose and minimal lactose plates containing ampicillin (50 µg/ml). Ampicillin was not added for the experiments with E.coli CC104 and CC104mutY without plasmid. The plates were then incubated at 37°C for ~20 and 48 h for determining the frequency of Rifr mutation and Lac+ reversion mutation, respectively.

DNA glycosylase assay

MutY protein was obtained from Trevigen. For hMYH expression, the hMYH cDNA was subcloned behind the SP6 promoter of pSPUTK (Stratagene). The plasmid was used as a substrate for the transcription/translation coupled in vitro translation system based on a rabbit reticulocyte lysate (TNT for Sp6, Promega) at a concentration of 1 µg/25 µl reaction. The yield of the translation was estimated by incorporation of 35S-methionine in a separate reaction. For the DNA glycosylase assay, a 30mer oligonucleotide containing GO (oligo-GO) was synthesized: 5′-CTCGTCAGCATCT-GO-CATCATACAGTCAGTG-3′.

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reaction and 80 ng of poly dIdC. The DNA–protein mixture was loaded onto 6% polyacrylamide gel in 0.4 TBE buffer (pH adjusted to 8.0).

RESULTS

Alternative transcript for a nuclear isoform

By analogy with the E. coli model that MutY and Fpg cooperate to prevent GO-induced mutation, we expected co-localizations of hMYH and hOGG1 in the nucleus and mitochondria. However, previous studies (35) had shown that hMYH is essentially a mitochondrial protein while hOGG1 is transported into both organella. Studies using a green fluorescent protein (GFP) fusion revealed that an N-terminal portion of hMYH acts as a mitochondrial targeting sequence (MTS) and that a nuclear localization signal (NLS) found near the C-terminus is functional. Since a deletion of the N-terminal 63 amino acids resulted in the nuclear transport of the truncated hMYH, we hypothesized that there might be an alternative nuclear isoform which lacks or is altered in the N-terminal MTS. Using 5' RACE from human placental cDNA we were able to find such RT–PCR products. Two RACE clones out of nine analyzed exhibited a 5' sequence distinct from the rest, i.e. the known hMYH sequence (29). The sequence in exon 1 of type 1 was entirely displaced in the newly isolated clones as shown in Figure 1. This indicates that alternative splicing skips the type 1 exon 1. Additional RACE clones were obtained using a nested primer specific for the new sequence. All six RACE clones for the alternative transcript are identical in sequence with the 5' ends being close to each other. We considered that some of these 5' ends could represent the intact transcription start(s). An open reading frame for the new cDNA (type 2) starts at the second ATG of the previous cDNA (type 1). This results in a deletion of 14 amino acid residues including two R and three S/Ts that would constitute the MTS elements (37) of the type 1 protein. A possible NLS, RKPR, appears in the type 2 N-terminus although it is not functional in the context of the mitochondrial targeting signal because the MTS–GFP fusion was transported only to the mitochondria (35). Thus, the type 2 protein having the N-terminal NLS and C-terminal NLS appears to favor a nuclear localization. To determine the actual intracellular localization, an epitope-tagged version of each type of cDNA was expressed in COS-7 cells. As shown in Figure 2, the type 2 protein was localized only in the nucleus (right panel) while type 1 protein was transported into the mitochondria (left panel).

Expression of hMYH transcripts in tissues

The presence of two mRNAs starting from different first exons implies that they utilize different promoters. We thus examined expression of the two transcripts in tissues by northern blot analysis. However, clear blots were not obtained with a respective oligo probe or even with a full-length cDNA probe, suggesting that the expression of hMYH is very low (not shown). We next used a semi-quantitative assay with an equalized human tissue cDNA panel. Although this approach does not reveal the relative quantities of type 1 and type 2 transcripts, we found that they had a similar expression pattern and a varied expression level depending on tissues. As shown in Figure 3A, a lower yield of the amplifications in post-mitotic tissues (i.e., in brain, heart and skeletal muscle) than in the other tissues was observed.
Adenine (A:GO) DNA glycosylase activity of hMYH protein

To characterize the catalytic activity of hMYH, we attempted to express the recombinant protein in E. coli. However, this proved to be difficult, probably because of its toxicity in the bacteria. We then used an in vitro transcription/translation (TNT) coupled system for protein expression. The TNT reaction produced a major polypeptide with the expected molecular size of hMYH (type 1) and hMYH (type 2) in an amount, 50–100 fmol/µl, comparable to the control protein expression (Fig. 4A). Although the in vitro translated type 1 (lane 1) protein showed a slightly larger size than the type 2 (lane 2) the former is evidently processed during mitochondrial transport in cells. Since the exact N-terminus of the mature type 1 protein is not known, we only show the data on the in vitro-translated type 2 protein. However, all the results for the ‘full-length’ type 1 protein were similar (not shown). Using the TNT reaction lysate as the enzyme source, we examined DNA glycosylase activity with a 5’-labeled, 30mer duplex DNA (5:A:GO). The rabbit TNT lysate itself did not contain significant DNA glycosylase activities toward substrates for MutY (5:A:GO, 5:G:GO, 5:A:G) and for OGG1 (5:A:GO). However, a mock reaction showed AP-nicking activity and weak exonuclease activity probably due to the rabbit AP endonuclease. Thus, we treated the DNA with NaOH at 95°C prior to denaturing gel analysis so that the nicked 17mer product represented the DNA glycosylase activity whether or not the enzyme is monofunctional or has AP-lyase activity. As shown in Figure 4B, hMYH (type 2)-expressing lysate showed DNA glycosylase activity toward the 5:A:GO substrate in the presence of Mg²⁺ (lane 5). The optimum concentration of Mg²⁺ is ~5 mM (Fig. 4C). The activity was, however, weak and complete incision did not occur even with a 5–10 molar excess of translated protein versus substrate. The

Table 1. Suppression of spontaneous mutations by expression of hMYH (type 2) cDNA in E. coli CC104mutY

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Rif⁹/₁₀⁹</th>
<th>Lac⁴/₁₀⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC104</td>
<td>–</td>
<td>3–10</td>
<td>5–12</td>
</tr>
<tr>
<td>CC104mutY</td>
<td>–</td>
<td>195–264</td>
<td>89–206</td>
</tr>
<tr>
<td>CC104mutY</td>
<td>pMAL-c2</td>
<td>145–245</td>
<td>ND</td>
</tr>
<tr>
<td>CC104mutY</td>
<td>pMAL-cY2</td>
<td>12–37</td>
<td>17–32</td>
</tr>
</tbody>
</table>

Figure 3. Semi-quantitative analysis of tissue-specific expression. An equalized tissue cDNA panel was employed to evaluate expression of the hMYH transcripts in tissues. The PCR product of type 1 or type 2 (440 or 275 bp, respectively) was amplified by a primer set specific for either transcript (A). As an example of low and high expression, PCR products from the heart (closed circles) and liver (open circles) at a given cycle number are compared in (B). G3PDH amplification is shown as a control for the cDNA panel.

Figure 4. DNA glycosylase activity of in vitro-expressed hMYH (type 2) protein. (A) SDS–PAGE of 35S-labeled proteins. hMYH (type 1), hMYH (type 2) and control luciferase proteins were expressed with the transcription/translation-coupled system in the presence of 35S-methionine (lanes 1–3, respectively). The indicated migration of the major product corresponds to the predicted size of type 1 (59 070.34 Da) or type 2 (57 445.34 Da). (B) DNA glycosylase assay. A double-stranded 30mer substrate (5:A:GO) was incubated with MutY protein (lane 1), MutY protein in mock lysate (lane 2), luciferase-expressing lysate (lane 3) or hMYH (type 2)-expressing lysate (lanes 4 and 5). The incidence product was analyzed in a denaturing polyacrylamide gel. Either 5 mM MgCl₂ or 5 mM EDTA was included in the reaction as indicated. (C) Enhanced incision by the addition of MgCl₂. The incision reaction was performed in the presence of MgCl₂ or EDTA. The bold broken line represents the background level of the assay, obtained from the reaction of the luciferase-expressing lysate.

The increase of the product from the heart and liver with PCR cycle number is depicted in Figure 3B as an example of the lower- versus the higher-expressing tissue. The band for type 1 cycle number is depicted in Figure 3B as an example of the increase of the product from the heart and liver with PCR amplification is shown as a control for the cDNA panel.
addition of the following cations did not enhance the activity: Zn\(^{2+}\) (0.1–1 mM), Fe\(^{2+}\) (0.01–1 mM), Co\(^{2+}\) (0.01–0.1 mM), Ca\(^{2+}\) (2 mM), NaCl (50–150 mM). Inclusion of ATP (0.1 mM) did not affect the activity and dithiothreitol (0.5 mM) seemed to be required. Since TNT lysate does not affect incision for bacterial MutY (Fig. 4B, lane 2), the weak activity on hMYH may be due to a small quantity of ‘active’ polypeptide produced in the in vitro translation reaction.

The reaction for the *A:GO substrate reached a plateau after 30 min (Fig. 5A and B). Nicks at T or C opposite GO were undetectable. The nick at G:GO was not obvious due to the high background in the assay. The incision at an unmodified A:G mismatch, if it occurred, was within the background level; this was also the case with G:G and T:G mismatches (Fig. 5C and D). Nicks were not observed on the GO-containing strand with any combination of the mismatches (not shown).

### Binding of hMYH to GO-containing mismatch DNA

We performed a band-shift assay in order to determine the affinity of the in vitro translated hMYH (type 2) protein for mismatch DNA probes (Fig. 6). The hMYH protein was C-terminally tagged by an HA sequence to discern the specific complex with anti-HA antibody. Although binding reactions of both luciferase-expressing control lysate and hMYH-expressing lysate yielded bands not specific to any of the DNA probes, a specific band appeared in the binding reaction of hMYH-expressing lysate with T:*GO, G:*GO and A:*GO (Fig. 6A). This band was supershifted by the antibody, thus indicating the hMYH–DNA complex. The hMYH–DNA complex was undetectable in the reaction with C:*GO and in that of any of the non-oxidative mismatch DNA probes (Fig. 6B) even on a longer exposure (not shown). The binding to A:*GO was weaker than those to T:*GO and G:*GO, which might be due to a reduced amount of substrate by the progress of DNA glycosylase reaction under the conditions. The results indicate that hMYH has an affinity for T and G opposite GO without apparent catalysis.

### DISCUSSION

We found in this work that the catalytic function of the *hMYH* gene product, unidentified until now, is adenine (A:GO) DNA glycosylase. Furthermore, the presence of nuclear isoform
complements the previous studies on the subcellular localizations of hMYH and hOGG1 (35), hOGG1 (types 1a, 1b, 1c and 2) and hMYH (type 1) are in the mitochondria, and hOGG1 (type 1a) and hMYH (type 2) are in the nucleus. Together with the cytosolic localization of hMTH1 and its mitochondrial localization for the unique nucleotide pool of the organelle (38), the results suggest that the metabolism of human nuclear and mitochondrial DNA is protected against GO-induced mutation by hOGG1, hMYH and hMTH1 as is the case in E.coli by Fpg, MutY and MutT (39,40).

The expression of both type 1 and type 2 transcripts at various levels was found in all tissues examined. Although the relative amount of the type 1/type 2 transcripts was not determined here, type 2 seemed to be expressed at a lower level than type 1 because we found the type 2 sequence in two out of nine RACE clones. Consistent with this view is the fact that the type 2 sequence has not been entered in the EST database, while there are two entries for the 5′ sequence of type 1. The major cause of the generation of A:GO is a DNA replication error at the GO site in the parent strand. MutY corrects the mismatch by way of post-replication DNA repair. In this regard, the mammalian homolog may be required less in post-mitotic than in dividing cells. The observed low expression of hMYH transcripts in post-mitotic tissues is, therefore, consistent with the presumed role of hMYH.

In our attempt to overexpress the recombinant hMYH protein in E.coli, we typically found that the bacteria would not grow further upon induction. Although a small quantity of FLAG-tagged, MBP-fused or intein-fused recombinant protein was detected in cell extracts by western blot analysis, positive DNA glycosylase activity of the polypeptide. From these observations, the expression of both type 1 and type 2 transcripts at various levels was found. Although the relative amount of the type 1/type 2 transcripts was not determined here, type 2 seemed to be expressed at a lower level than type 1 because we found the type 2 sequence in two out of nine RACE clones. Consistent with this view is the fact that the type 2 sequence has not been entered in the EST database, while there are two entries for the 5′ sequence of type 1. The major cause of the generation of A:GO is a DNA replication error at the GO site in the parent strand. MutY corrects the mismatch by way of post-replication DNA repair. In this regard, the mammalian homolog may be required less in post-mitotic than in dividing cells. The observed low expression of hMYH transcripts in post-mitotic tissues is, therefore, consistent with the presumed role of hMYH.

In our attempt to overexpress the recombinant hMYH protein in E.coli, we typically found that the bacteria would not grow further upon induction. Although a small quantity of FLAG-tagged, MBP-fused or intein-fused recombinant protein was detected in cell extracts by western blot analysis, positive DNA glycosylase activity was not recovered through the purification. Expression of a truncated MBP-protein (from Gln64 to Gly340) corresponding to the MutY catalytic core (24,25) allowed cell growth/induction but resulted in undetectable catalytic activity of the polypeptide. From these observations, the hMYH protein appears to be toxic in the bacteria and rather unstable in purification. Nevertheless, the suppression of the E.coli mutY mutator phenotype with hMYH cDNA suggests that the low level of expression with intact activity in vivo can partially complement the MutY function.

Our data on the DNA glycosylase activity and binding characteristics of hMYH cannot be compared in detail with those of MutY and the recently characterized S.pombe homolog, SpMYH, because we were only able to perform the assay in the presence of rabbit TNT lysate proteins. The lysate contained some background activities including relatively high AP-nicking activities and non-specific DNA binding activities. Nevertheless, the results contrast the unique characteristics of hMYH with MutY and SpMYH. (i) The A:G mismatch is not a good substrate for hMYH catalysis and binding while the substrate is efficiently catalyzed as A:GO by MutY and SpMYH (28). (ii) The DNA glycosylase activity of hMYH requires Mg\textsuperscript{2+} and the reaction is inhibited by the addition of EDTA. EDTA inhibition is not observed in MutY or SpMYH (17). These points are also incompatible with the characteristics of a calf protein co-purified with A:G mismatch DNA glycosylase activity from nuclear extracts, termed calf MYH protein (30). The calf MYH protein in the purified fraction catalyzes A:G substrate twice as efficiently as A:GO and Mg\textsuperscript{2+} is not required in the reaction buffer. We initially used the same buffer in the hMYH DNA glycosylase assay but failed to detect the activity. The calf MYH protein, if it is a counterpart of hMYH, might have slightly different properties because of post-translational modifications or by forming a complex with unknown proteins.

Data on the binding of hMYH to mismatch substrates support the view that A:GO is a preferred catalytic substrate. C:GO is not a substrate for either catalysis or binding. We found that the enzyme has the ability to bind to T:GO and G:GO without apparent catalytic action. The mechanism for the formation of G:GO in vivo is not clear. However, a replication error at a GO site may account for the G:GO mismatch since oxidative stress and ionizing radiation induce G to C transversion in E.coli (41,42). The T:GO mismatch could be generated as a result of a replication error and the deamination of 5-methylcytosine opposite GO analogous to the frequent occurrence of G:T mismatches. We also observed binding of hMYH to a probe containing a U:GO mismatch which could be generated by cytosine-deamination at a C:GO lesion in vivo (data not shown). Interestingly, these substrates bound to by hMYH coincide with the substrates for ‘unfavorable’ GO repair. The removal of GO in the parent strand from a GO:T or GO:G mismatch leads to G:C to A:T or G:C to C:G mutations, respectively. Indeed, several groups have reported that recombinant hOGG1 (6,8,10,12) and purified enzyme (43) can catalyze GO:T and GO:G mismatches. In particular, the activity toward the former substrate is rather high and it appears to occur in vivo. NER may also recognize a GO-containing strand for repair (44) and induce repair-mediated mutations (45). From this point of view, one might expect that the binding of hMYH to these mismatches would attenuate the ‘unfavorable’ GO repair. In our preliminary experiments, the hMYH-expressing lysate did not affect the catalysis and substrate specificity of hOGG1 (data not shown). Because only a small proportion of the in vitro-translated hMYH polypeptide seemed to be active in these conditions, it remains to be determined whether or not hMYH protein has a protective function against repair-mediated mutations. Recently, DNA glycosylase preferentially catalyzing GO-A and GO-G mismatches (OGG2) has been purified from HeLa cells (43). The activity of OGG2 rivals that of hMYH for the GO:A repair. Thus, coordination of repair proteins acting on the same GO lesion may occur to avoid mis-coding repair. The hMYH protein is ~1.5-fold larger than MutY. The sequence of hMYH is well-aligned to MutY within the catalytic core but has several gaps in the remainder and extended N-terminal and C-terminal segments (29). In this regard, it is of interest to determine if there is an interaction of hMYH with other DNA repair and replication components.

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