Functional Expression of hMYH, a Human Homolog of the *Escherichia coli* MutY Protein

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We have previously described the hMYH cDNA and genomic clones (M. M. Slupska et al., J. Bacteriol. 178:3885–3892, 1996). Here, we report that the enzyme expressed from an hMYH cDNA clone in *Escherichia coli* complements the mutator phenotype in a mutY mutant and can remove A from an A·8-hydroxydeoxyguanine mismatch and to a lesser extent can remove A from an A·G mismatch in vitro.

Reactive oxygen species generated either as a by-product of cellular respiration or by ionizing radiation and other oxidizing agents can cause damage to DNA. Cells have developed mechanisms to prevent mutations from different types of oxidative damage. Among oxidatively damaged DNA bases, 7,8-dihydro-8-oxodeoxyadenine (also termed 8-hydroxydeoxyguanine [8-oxodG]) is particularly mutagenic. It can easily mispair with adenine, and if it is not repaired before replication, such a mispair can result in a G·C→T·A transversion at the next round of replication (19). Cells evolved repair systems to prevent mutation from 8-oxodG. One of the enzymes involved in that process in *Escherichia coli* is the MutY protein (11), a simple glycosylase that removes A when paired with 8-oxodG or G (1, 10) and to a lesser extent removes A from a 8-oxodG mismatch (2). Previous work has shown that strains with defects in mutY have increased G·C-to-T·A transversions (16), so we used the *E. coli* CC104 strain carrying the F- lac plasmid with the lac gene engineered for detection of G·C-to-T·A transversions (2). Previous work has shown that strains with defects in mutY have increased G·C-to-T·A transversions (16), so we used the *E. coli* CC104 mutY::Tn10 (12) derivative to check whether hMYH complements the mutY mutator phenotype. The complementation experiment was performed as described previously (14). Cells of strain CC104 carrying both the pREP4 and pQE30/hMYH plasmids showed suppression of the mutator phenotype. The suppression was complete when cells were grown on lactose minimal medium and pREP4 was present.

**Table 1. Complementation of *E. coli* mutY defect with cloned hMYH**

<table>
<thead>
<tr>
<th>Expression plasmid</th>
<th>IPTG</th>
<th>Avg no. of Lac&lt;sup&gt;+&lt;/sup&gt; revertants/10&lt;sup&gt;8&lt;/sup&gt; cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>pQE30</td>
<td>−</td>
<td>98.3 ± 12.8</td>
</tr>
<tr>
<td>pQE30</td>
<td>+</td>
<td>127.9 ± 22.7</td>
</tr>
<tr>
<td>pQE30/hMYH</td>
<td>−</td>
<td>8.9 ± 8.8</td>
</tr>
<tr>
<td>pQE30/hMYH</td>
<td>+</td>
<td>1.2 ± 1.1</td>
</tr>
</tbody>
</table>

* Four or five single colonies of the CC104 mutY mutant carrying pREP and expression plasmids were inoculated into Luria-Bertani medium with 100 µg of ampicillin and 25 µg of kanamycin per ml, with or without 1 µM IPTG, and grown overnight at 37°C. Samples were plated on lactose minimal medium and titered on glucose minimal medium. The number of Lac<sup>+</sup> revertants (mean ± standard deviation) of strain CC104 resulting from G·C-to-T·A transversion is shown.

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The expressed hMYH was rapidly degraded, in both a E. coli strain that lacked the hMYH, lysate of the same strain with pQE30. (B) Western blot analysis of hMYH. Proteins were separated on a 10% polyacrylamide gel, transferred onto a Hybond enhanced chemiluminescence nitrocellulose membrane (Amer-sham Pharmacia Inc., Piscataway, N.J.), and reacted with antibodies against the histidine tag [anti-RGS(H); Qiagen, Inc.]. Western blotting was performed by enhanced chemiluminescence analysis (ECL; Amersham). Arrows point to the position of full-length hMYH. Lanes C, C+, C−, and E are as described for panel A.

Expression and purification of hMYH. The hexahistidine-tagged hMYH protein was purified by Ni2+-agarose affinity chromatography, followed by ssDNA-cellulose chromatography. Conditions for Ni2+-agarose purification were set according to the protocols of the manufacturer (Qiagen, Inc.), except that a pH of 6.5 and no NaCl were used for binding and the column was washed with buffer containing 100 mM NaCl and 30 mM imidazole at pH 6.5. Conditions for ssDNA-cellulose were as described for the E. coli MutY purification (6). The purified protein reacts with antibodies against the histidine tag [anti-RGS(H)]2; Qiagen, Inc.] (Fig. 1).

The expressed hMYH was rapidly degraded, in both a CC104 mutY mutant (12) (normally used for expression) and a strain that lacked the E. coli outer membrane protease (ompT mutant) (BL21; Novagen Inc., Madison, Wis.). The degrees of degradation were similar during growth at different temperatures (15 to 37°C). Purified fractions contained other proteins, some of which cross-reacted with anti-RGS(H)2 antibody (Fig. 1).

The cloned hMYH gene has seven AGA and AGG codons in the 5′ terminus of the mRNA. These are rare arginine codons in E. coli and can result in poor expression in E. coli (7), as was the case for hMYH. It has been shown that cloning the argU gene, encoding the arginine tRNA that reads AGA and AGG codons, can improve the expression of some eukaryotic genes with rare arginine codons (18). Therefore, we cloned the argU gene with its natural promoter into the same expression plasmid but did not observe any improvement in hMYH levels. We also cloned and expressed N-terminally truncated clones of hMYH. We expressed a 54-kDa clone (missing the first 41 amino acids), a 52-kDa clone (missing the first 62 amino acids), and a 45-kDa clone (missing the first 120 amino acids). We observed high-level expression only for the 45-kDa clone (data not shown). It turned out that the protein expressed by this clone was inactive (data not shown). Also, this was the only clone that did not have rare arginine codons in the 5′-terminal part of the gene.

hMYH activity. To determine the specificity of hMYH, we investigated its glycosylase activity on various mismatches placed in 96-mers. The sequence of 96-mers containing 8-oxoG or G was 5′AATTTGCTTCTCCCTCTTCTCTCGCC ACGTTGCAGAATTGGGTCTTCCCCCTCAAGCCTA AAATCGGGGCTCTCCCTTTAGGGTTCCGATCCGGCC3′ (the bold G marks the position of 8-oxoG). We tested A·G, A·G, G·C, A·A, A·T, G·T, C·G, C·T, C·C, G·8-oxoG, G·T, G·G, T·T, and T·8-oxoG mismatches. We detected activity for the A·8-oxoG and A·G mismatches but not for the A·C mismatch (Fig. 2). As for E. coli MutY, cleavage of the oligomer by hMYH was seen only for the strand containing A (data not shown). After prolonged incubation and strong overexposure of the film, we could also see a small degree of cleavage for the G·8-oxoG mismatch but not for the A·C mismatch (data not shown). The same substrates were processed by the mouse MutY homolog (data not shown). The only difference we observed between the hMYH and the E. coli MutY enzymes was the lack of glycosylase activity for the A·C mismatch. This activity was also undetectable in HeLa cell extracts (23) and very weak for the Schizosaccharomyces pombe MutY homolog (5), but this activity for a partially purified bovine MutY homolog has been reported (8).

We also tested hMYH activity towards A·8-oxoG and A·G mismatches placed in shorter oligomers. We could easily detect the activity for the A·8-oxoG mismatch, but surprisingly, we did not see any activity for the A·G mismatch placed in a 23-mer. We saw, however, the activity of hMYH with the A·G mismatch in 45-mer (Fig. 3) and 72- and 96-mer (data not shown) templates. To detect hMYH glycosylase activity on A·G mismatches we had to use more enzyme and longer reaction times (Fig. 3). The time and concentration courses for the A·8-oxoG mismatch in a 23-mer template and the A·G mismatch in...
a 42-mer template are shown in Fig. 3. The lack of activity of hMYH with an A·G mismatch in a 23-mer is an unexpected observation. An S. pombe MutY homolog (5), a partially purified calf MutY homolog (8), and a mouse MutY homolog from our lab (data not shown) all cleave a 20- or 23-bp DNA containing an A·G mismatch. The A·8-oxodG-containing oligomers are, however, much better substrates for both hMYH and mMYH (mouse MutY homolog), and this difference is particularly obvious when short reaction times and smaller amounts of enzyme are used (data not shown). One explanation for this phenomenon might be that both hMYH and mMYH have low affinity to the A·G mismatch placed in shorter templates but the affinity of the mouse MutY homolog to A·G-containing short oligomers is higher than that of hMYH. The differences between DNA glycosylase homologs of closely related species have already been described for methylpurine-DNA glycosylase from mice and humans (17). It was also observed that human methylpurine-DNA glycosylase has a 10-fold-lower binding capacity for shorter oligomers (<15 bp) than for longer ones (9). We also cannot exclude the possibility that the lack of hMYH glycosylase activity on A·G mismatches is due to the neighboring sequence context. The investigated mismatches were placed in a different context in the 23-mers than those of the 45-, 72-, and 96-mers used in this work, and the sensitivity to sequence context of the MutY glycosylase activity towards A·G mismatches has been noted for E. coli (22).

To determine whether hMYH has both glycosylase and AP-lyase activities, we repeated conditions for a similar experiment with E. coli MutY, described previously (25). In this experiment, the product of the same reaction was resolved on acrylamide gels under different, carefully controlled heating conditions. As can be seen in Fig. 4, for hMYH as well as E. coli MutY, under the “no-heat” conditions the product of DNA nicking is barely visible, whereas when the same reaction mixture was heated at 95°C for 5 min, the β-elimination product is obvious. Additional treatment with piperidine (known to cleave AP sites) resulted in a shift of the β-elimination product into a ß-elimination product (Fig. 4). These results indicate that, similar to the E. coli MutY protein, hMYH is a simple glycosylase with only residual, if any, AP-lyase activity.

In summary, the data presented in this paper show that hMYH, cloned in our laboratory on the basis of DNA sequence homology to the E. coli mutY gene, is the true homolog of the bacterial MutY glycosylase.

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REFERENCES