MutY and MutY homologs (MYH) in genome maintenance

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1. ABSTRACT

The base excision repair carried out by bacterial MutY DNA glycosylase and eukaryotic MutY homolog (MYH) is responsible for removing adenines misincorporated into DNA opposite G and 7,8-dihydro-8oxo-guanines (8-oxoG); thereby preventing G:C to T:A mutations. Escherichia coli MutY (EcMutY) can also remove adenines from A/C and A/5-hydroxyuracil and can remove guanines from G/8-oxoG mismatches at reduced rates. Thus, MutY has a minor role in reducing the mutagenic effects on G:C to A:T transitions and G:C to C:G transversions. The eukaryotic MYH can excise adenines misincorporated opposite GO, G, or C; remove 2hydroxyadenines mispaired with A,G, and GO; excise G from G/GO mismatch weakly, thereby preventing G:C to T:A transversions. The in vitro and in vivo activities of

MYH can be modulated by several proteins including apurinic/apyrimidinic endonuclease (APE1), proliferating cell nuclear antigen (PCNA), and mismatch recognition enzymes MSH2/MSH6. Recently, MYH has been shown to associate with the checkpoint proteins, Rad9, Rad1, and Hus1 (referred as the 9-1-1 complex). Thus MYH-mediated base excision repair is coordinated with mismatch repair, DNA replication, cell-cycle progression, and DNA-damage checkpoints. Biallelic germ-line mutations in the human MYH gene are associated with recessive inheritance of multiple colorectal adenomas and carcinoma. MYH mutations can cause G:C to T:A mutations of the adenomatous polyposis coli (APC), K-ras, and other genes that control cellular proliferation in the colon.

2. INTRODUCTION

The human genome is vulnerable to an array of DNA-damaging agents of both endogenous and environmental origins. Thus, a variety of oxidation, alkylation, deamination, and radiation events produce thousands of cytotoxic and mutagenic base lesions per cell per day (1-3), which can lead to genome instability and degenerative conditions including aging and cancer (4). Constant scanning and repair of damaged DNA is essential to reduce mutagenic and cytotoxic accidents. Multiple repair pathways with different mispair specificities are utilized by all organisms to reduce replicative errors and to protect their genomes from various types of damage and maintain their genome stability (1,3,5,6). Sometimes, one lesion can be repaired by more than one pathway. Remarkably, the basic processes of DNA repair are highly conserved among diverse organisms. In mammalian cells, detection and correction of DNA damage by repair enzymes occurs in a coordinated fashion with DNA replication, DNA methylation, transcription, cell cycle control, and apoptosis (7).

Ionizing radiation, various chemical oxidants, and internal metabolism can cause damage to nuclear and mitochondrial DNA. 7,8-Dihydro-8-oxo-guanine (8-oxoG or GO) is one of the most stable products of oxidative DNA damage and has the significant deleterious effects. In Escherichia coli, MutT, MutM, MutY, MutS, and Nei (endonuclease VIII) are involved in defending against the mutagenic effects of 8-oxoG lesions (Figure 1) [reviewed protein has (9)]. The MutT in (8) and pyrophosphohydrolase activity which eliminates 8-oxodGTP from the nucleotide pool (Figure 1, reaction 1). MutM glycosylase (Fpg protein) removes both mutagenic GO adducts and ring-opened purine lesions paired with cytosines (Figure 1, reaction 2). MutS and MutY increase removing the fidelity replication by adenine misincorporated opposite GO or G (10,11) (Figure 1, reaction 3), and thus reduce G:C to T:A transversions. The MutS-dependent mismatch repair removes the mismatched A on the daughter DNA strand [reviewed in (12)]. MutY glycosylase excises A efficiently from A/GO mispairs when A is on the daughter strand to preserve genetic integrity (Figure 1, reaction 3). However, MutY reaction on A/GO mispairs when A is on the parental strand needs to be modulated (Figure 1, reaction 5). MutY can also remove adenine on A/G, A/C, and A/5-hydroxyuracil and can excise G from G/GO mismatches. Nei can excise GO when GO is opposite a cytosine or adenine during DNA replication (Figure 1, reactions 2 and 6) and can serve as a backup pathway to repair 8-oxoG in the absence of MutM and MutY (8,13).

The mechanism to defend against the mutagenic effects of 8-oxoG lesions is conserved among organisms (Table 1). Interestingly, *Saccaromyces cerevisiae* and *Schizosaccharomyces pombe* have no Nei homologs. Moreover, the MutY homolog (MYH or MUTYH) and the functional MutM/Fpg homolog (OGG) are not present in *S. cerevisiae* and *S. pombe*, respectively. Human cells possess functional homologs of MutT, MutM, MutY, MutS, and

Nei. The eukaryotic MYH DNA glycosylase can excise adenines misincorporated opposite GO, G, or C; remove 2-hydroxyadenines misincorporated with template A,G, and GO; excise G from G/GO mismatch weakly (9,14-19), thereby preventing G:C to T:A transversions (18-21). Germline mutations in the *hMYH* gene cause autosomal recessive colorectal adenomatous polyposis, which is characterized by multiple adenomas, some of which progress to cancer (22-26). Tumors from affected patients contain somatic G:C to T:A mutations in the adenomatous polyposis coli (*APC*), K-*ras*, and other genes (22,27,28).

The MutY and MYH interact with several proteins, some of which modulate their activities. The Endo VI. Exo III. and Endo VIII have been shown to enhance the turnover of MutY with A/G but not A/GO substrates (29,30). We have shown that MYH is directly associated with proliferating cell nuclear antigen (PCNA), replication protein A (RPA), apurinic/apyrimidinic (AP) endonuclease (APE1), and hMutS-alpha (MSH2/MSH6) via hMSH6 (31-33). The glycosylase and DNA binding activities of MYH can be stimulated by APE1 and MutS-alpha (32,34). Recently, we showed that S. pombe MYH (SpMYH) and hMYH are associated with the checkpoint proteins, Rad9, Rad1, and Hus1 (referred as the 9-1-1 complex) [(35) and unpublished results]. The 9-1-1 complex has predicted structural homology to the PCNA sliding clamp (36.37) and is involved in signaling the DNA damage response of cell cycle arrest or apoptosis [Reviewed in (38)]. Thus, MYH-mediated base excision repair (BER) is coordinated with mismatch repair, DNA replication, cell-cycle progression, and DNA-damage checkpoints.

3. MECHANISM OF BASE EXCISION REPAIR

The BER pathway recognizes a large variety of spontaneous and induced DNA lesions including base modification (3,5,6). The first step of BER is carried out by a lesion-specific DNA glycosylase. These enzymes find specific lesions in the vast genomic DNA, flip the target base out of the DNA helix, and excise the target base to generate potentially mutagenic apurinic/apyrimidinic (AP) sites (39).

3.1. Two types of DNA glycosylases

DNA glycosylases can be divided into two groups (40,41). Monofunctional glycosylases, such as AlkA, excise the target base (Figure 2, reaction I) but lack the conserved lysine and AP lyase activity (41-43). Bifunctional DNA glycosylases, such as E. coli Endo III, possess strong AP lyase activity (44) (Figure 2, reaction II). The associated AP lyase cleaves the phosphodiester bond 3' to the AP sites by the reaction of betaelimination. In general, a bifunctional glycosylase uses the epsilon-NH2 group of the conserved lysine to form a Schiff base intermediate. The nucleophile Lys residue is activated by a conserved Asp as a general base. Some bifunctional glycosylases such as MutM have a third activity that cleaves the DNA fragment containing an unsaturated aldehyde at 5'-phosphodiester bonds by delta-elimination (45,46) (Figure 2, reaction III).



Figure 1. 8-oxoG repair in *E. coli* and human cells. *E. coli* MutT, MutM, MutS, MutY, and Nei (Endo VIII) are involved in defending against the mutagenic effects of 8-oxoG lesions (structure is shown in the inset). The human functional homologs of MutT, MutM, MutY, MutS, and Nei are hMTH, hOGG1, hMYH, hMutS-alpha, and hNEIL1, respectively. The MutT/MTH protein hydrolyzes 8-oxo-dGTP (dG°TP) to 8-oxo-dGMP (dG°MP) and pyrophosphate (reaction 1). GO (G°) in DNA can be derived from oxidation of guanine or misincorporation of dG°TP during replication. The MutM/OGG1 glycosylase removes GO adducts while it is paired with cytosine (reactions 2, 4, and 7). Nei/NEIL1 can function as a backup for MutM/OGG1 to removes GO from GO/C. When C/GO is not repaired by MutM/OGG1, adenines are frequently incorporated opposite GO bases by DNA polymerase III or POLdelta/epsilon during DNA replication. A/GO mismatches are repaired to C/GO by the MutY/MYH-dependent or MutS/MutS-alpha-dependent pathway (reaction 3). When dG°TP is incorporated opposite adenine during DNA replication, MutY/MYH repair on GO/A can cause more mutation (reaction 5) while GO/A repair by MutS/MutS-alpha and Nei/NEIL1 can reduce mutation (reaction 6). This figure is adapted from Lu et al. (9) with permission from Humana Press Inc.

Table 1. 8-oxoG Repair Enzymes in Different	Organisms
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E. coli	Human	S. cerevisiae	S. pombe
Bacteria	Mammal	Baking yeast	Fission yeast
MutY	hMYH	No	SpMYH
MutT	hMTH1	ScMTH1	?
MutM	hOGG1	ScOGG1	No
	hOGG2	ScOGG2	
MutS	hMSH2/MSH6	ScMSH2/hMSH6	SpMSH2/MSH6
	hMSH2/MSH3	ScMSH2/MSH3	SpMSH2/MSH3
	Others	Others	Others
Nei (EndoVIII)	hNEIL1	No	No
	hNEIL2		
	hNEIL3		

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Although MutY can form a covalent Schiff base intermediate with its DNA substrates (47-52), it is controversial whether MutY is a bifunctional or monofunctional glycosylase (50,52). While several groups failed to detect AP lyase activity in their MutY preparations (41,50,53-56), a weak AP lyase activity of MutY has been reported (47-49,51,57-59). Lys142 of MutY has been identified as the residue to form the Schiff base intermediate (51,52,60,61). However, the formation of the Schiff base intermediate is not required for adenine



Figure 2. Reactions carried out by monofunctional and bifunctional DNA glycosylases. Step I, DNA glycosylase removes target base (circled). For example, MutY removes A which is paired with GO, G, C or 5-hydroxyluracil on the other strand (not shown). The product contains an AP site. Step II, bifunctional glycosylase containing AP lyase activity carries out the beta-elimination activity. The DNA backbone is cleaved to generate two fragments, one of which contains an unsaturated sugar moiety. Step III, some bifunctional glycosylases have the delta-elimination activity which removes the unsaturated sugar to produce a DNA fragment with 3' phosphate group. This figure is modified from Lu *et al.* (29) with permission from Biochemistry Society.

glycosylase activity by MutY (51,60,61). Thus, the biological significance of the Schiff base formation of MutY is not clear. A putative nucleophilic water is observed in the X-ray crystal structure of MutY complex with DNA (62), suggesting MutY is a monofunctional glycosylase. It is possible that the epsilon-amine of Lys142 may happen to lie closely to the active site and experience a chance encounter with the AP site. Asp138 of MutY is essential for its glycosylase and trapping activities (51,63) but the DNA binding activity of the D138N MutY protein is similar to that of the wild-type enzyme (51).

3.2. Enzymes involved in base excision repair

To complete the base excision repair after glycosylase action, the cytotoxic and mutagenic AP site is further processed by an incision step, DNA synthesis, an excision step, and DNA ligation. In *E. coli*, the AP-DNA can be processed by an AP endonuclease such as Exo III and Endo IV (Xth and Nfo proteins, respectively) to generate a 3' OH end for DNA synthesis. Exo III is the major constitutive AP endonuclease and Endo IV is inducible by reactive oxygen species (ROS). The BER repair is then completed by DNA polymerase I and DNA ligase (64). The *E. coli* MutY repair pathway is dependent on DNA polymerase I. The repair patch is 5-12 and 9-27 nucleotides as measured in cell extracts and *in vivo*, respectively (65,66).

In eukaryotes, there are two major sub-pathways for BER: a single-nucleotide short patch and a 2-10 nucleotide long patch pathway (2,67-69) (Figure 3). The short patch BER pathway requires four proteins: APE1, DNA polymerase beta (POLbeta) and DNA ligase III/XRCC1 heterodimer (70). The long patch BER pathway can be reconstituted with six proteins: APE1, replication factor C (RFC), proliferating cell nuclear antigen (PCNA), flap endonuclease 1 (FEN1), DNA polymerases delta/epsilon (POLdelta/epsilon), and DNA ligase I (69,71). However, the long-patch BER reaction is stimulated by POLbeta and trimeric replication protein A (RPA, a single-stranded DNA binding protein) (33,72-74). Repair by hOGG1 and hMYH are carried out via short and long patch pathways, respectively (33,75).

4. BACTERIAL MutY

Several bacterial MutY homologs have been characterized including ones from *E. coli, Salmonella typhimurium (76), Deinococcus radiodurans (77),* and *Bacillus stearothermophilus* (62). This review focuses on the functions of *E. coli* MutY (EcMutY) and structure of *B. stearothermophilus* MutY (BsMutY).

4.1. Substrate specificity of MutY

MutY is initially identified as an adenine glycosylase that can repair A/G, A/GO, and A/C mismatches (53,55,59,78,79). MutY is then shown to process weak guanine glycosylase repairing G/GOcontaining DNA (80,81). Recently, we showed that MutY has a weak adenine glycosylase activity on A paired with 5hydroxyuracil (hoU), a deaminated and oxidized form of cytosine (29). A/8-oxoG mismatches are particularly important biological substrates for MutY adenine glycosylase. MutY is unique because it removes an undamaged base mispaired with the damaged base GO or 5-hydroxyuracil.



Figure 3. Alterative base excision repair (BER) pathways in eukaryotes. A damaged base (solid square) is cleaved by a DNA glycosylase. For example, hMYH excises A which is paired with GO, G, or C. A bifunctional glycosylase with AP lyase activity cleaves the phosphodiester bond 3' to the AP site (represented by a wavy line) and the resulting DNA with 3' unsaturated aldehyde can be processed by AP endonuclease (APE1) to generate an 3' OH end for DNA synthesis. For monofunctional glycosylases, the phosphodiester bond 5' to the AP site is cleaved by APE1 and the resulting DNA with 5'-deoxyribose-5-phosphate (5'dRP) is processed by the DNA deoxyribophosphodiesterase (dRPase) of POLbeta. The DNA with one-nucleotide gap is filled-in by POLbeta and the nick is sealed by DNA ligase III/XRCC1 heterodimer. The long-patch BER pathway with a repair patch of 2-6 nucleotides, can be carried out by DNA polymerases delta/epsilon (POLdelta/epsilon) or POLbeta. The RFC and PCNA facilitate the polymerase activities of POLdelta/epsilon while PCNA and RPA stimulate the activity of flap endonuclease 1 (FEN1) which cleaves the flapped oligonucleotide. The newly synthesized DNA segments are represented by dotted lines. The nick is sealed by DNA ligase I whose activity is enhanced by PCNA. Repair by hMYH is thought to be carried out via long patch pathways (33,120). The 9-1-1 complex (dark red circle) has been shown to interact with MYH (35), POLbeta (146), FEN1 (147,148), RPA (141), and LIG1 (149) (shown in Red). This figure is adapted from Lu *et al.* (9) with permission from Humana Press Inc.

The DNA binding activity of MutY does not completely parallel its catalytic activity. The binding affinity of MutY with G/GO is slightly lower than that with A/GO-containing DNA, but higher than that with A/Gcontaining DNA (Table 2). However, the catalytic activity of MutY to G/GO-containing DNA is much lower than that to A/G- and A/GO-containing substrates (80). In addition, MutY binds tightly to T/GO and moderately to C/GO, both of which are not its catalytic substrates (80). Because MutM is able to remove GO from T/GO, G/GO, and C/GO efficiently *in vitro* (82), MutY may modulate MutM activity on these substrates. The inhibition of MutM activity is especially important if T/GO and G/GO mismatches arise from misincorporation of T and G opposite oxidized

DNA duplex	<i>Kd</i> (nM)
A/G-20	5.3 ± 0.5^b
A/C-20	15 ± 3^b
A/GO-20	0.07 ± 0.01^b
AP/G-20	2.2 ± 0.3^b
AP/GO-20	0.18 ± 0.11^{c}
C/GO-20	11.5 ± 3.8^{c}
T/GO-20	0.11 ± 0.01^{c}
G/GO-20	0.11 ± 0.01^{b}
A/G-44	1.8 ± 0.3^{b}
A/GO-44	0.14 ± 0.01^d
C:G-20	375 ± 80^b
C:G-44	315 ± 49^{d}
A/hoU	124 ± 14^{e}

 Table 2.
 Apparent Dissociation Constants (Kd) of E. coli

 MutY

^{*a*} The mismatched duplex DNA substrates are represented by a base-base mismatch followed by the chain length (number of base pairs). C:G represents a homoduplex DNA substrate. Dissociation constants for *E. coli* MutY are derived from references 57^b , 80^c , 114^d , and 29^e . This table is adapted from Lu (169) with permission from Elsevier.

template guanines as well as if T/GO mismatches are derived from deamination of 5-methylcytosine opposite GO. It has been suggested by Bridges *et al.* (83) that MutY may regulate MutM activity in resting cells.

MutY has high affinity to its DNA product containing an AP site (80,84,85). The purpose of the tight binding of MutY to its product (80,85) is to prevent the release of the toxic effect of AP site (Table 2) until other components are recruited to carry out the next repair step. Another biological significance of MutY binding to AP/GO mismatches after its glycosylase action may be to prevent removal of GO or cleavage at the AP site by MutM, and thus, to avoid the formation of double-strand breaks.

4.2. Function of the MutY domains

MutY is a 39 kDa protein with an iron-sulfur cluster [4Fe-4S] (59,86). The N-terminal domain of MutY retains the catalytic activity (47,49,58,80,87) while the C-terminal domain of MutY plays an important role in the recognition of GO lesions (47,80,84,87). The X-ray crystal structures of the catalytic domain of *E. coli* MutYwith bound adenine (63) and of *Bacillus stearothermophilus* intact MutY(D144N) bound to DNA (62) show that MutY distorts the bound DNA substrate and the mismatched A is flipped out of the helix.

The N-terminal domain of MutY contains two sub-domains: an iron-sulfur module and a 6-helix barrel module containing the conserved helix-hairpin-helix (HhH) motif (62,63). The N-terminal domain of MutY shares structural similarity with endonuclease III (endo III), AlkA, and hOGG1 (59,63,86,88-90). A deep cleft is formed between these two sub-domains that interact extensively with the strand containing the mismatched adenine. The active-site pocket that binds the flipped-out adenine consists of E37, L40, D138, N140, M185, Q182, and D186 (63). The iron-sulfur cluster of MutY is ligated by four

conserved cysteines at positions 192, 199, 202, and 208 that are spaced as $C-X_6-C-X_2-C-X_5-C$. The residues spacing the conserved cysteines are dominated by positively charged amino acids and are important for DNA recognition (91). The region of I191-C199 forms a surface exposed loop, referred to as the iron-sulfur cluster loop (FCL) motif (63) which is important in substrate recognition and MutY stability (92-94). The six-helix barrel module with the helix-hairpin-helix (HhH) motif has been shown to directly contact the backbone of the GO strand and has substantial interaction with the C-terminal domain (62). The HhH motif is involved in binding to the phosphate backbone (51,62,63). A MutY mutant (delete26-134) lacking the 6helix barrel domain can still bind DNA and has very weak catalytic activity, but it has no in vivo activity (95). The mutant protein is a dimer in solution and binds DNA substrates in highly cooperative fashion. These data support a model whereby MutY scans the DNA cooperatively as a dimer or a multimeric complex to locate base-base mismatches (95).

The C-terminal domain of MutY has been shown to play an important role in the recognition of GO lesions (47,80,84,87). The binding affinity and reaction rate of a truncated MutY (residues 1-226) against A/GO-containing DNA are reduced when compared to those of the intact MutY (80,87). Moreover, deletion of the C-terminal domain of MutY confers a mutator phenotype in vivo (80). Li and Lu (96) have shown that a region of the C-terminal domain of MutY corresponding to the cleft of MutT is involved in substrate recognition. F294A. R249A. and P262A MutY mutants have reduced binding and catalytic activities not only with A/GO but also with A/G mismatches, and are partially defective in in vivo complementation activity when they are expressed at low levels. These findings suggest that the C-terminal domain of MutY may modulate the N-terminal domain of MutY in DNA binding and catalytic activities. Sequence comparison (87,97), NMR data (98), and X-ray structure of MutY (62) indicate that the C-terminal domain of MutY is very similar to MutT (99,100). However, the C-terminal domain of MutY recognizes GO differently from MutT (62).

4.3. Mutator phenotype of the *mutY* mutants

As mentioned above, MutY can remove adenine from A/GO, A/G, A/C and A/hoU mismatches and guanine from G/GO mismatches (55,78-81). The adenine specificity to A/G and A/GO is consistent with the mutation phenotype of *mutY* mutants for G:C to T:A transversions (55,101,102). Because MutY activity on A/C, A/hoU, and G/GO is very weak, the most frequent mutations observed in *mutY* mutants are G:C to T:A transversions. The slightly increased G:C to C:G transversions observed in mutY mutant is likely contributed to the MutY activity on G/GO mismatches (81). It is unclear whether the minor role of MutY in reducing the mutagenic effects on G:C to A:T transitions (101,102) is due to its activity on A/C or A/hoU. The increased G:C to A:T transitions in *mutY* mutants were previously interpreted as a failure of A/C mismatch repair (101,102). Because the MutY glycosylase activity on A/hoU (29) is similar to its weak activity on A/C mismatches (59,102) and hoU is an oxidized form of cytosine (103), the increased G:C to A:T transitions in *mutY* mutants may be attributed to its deficiency in A/hoU repair. When hoU/G mismatches are not repaired by Endo VIII, adenines are frequently incorporated opposite hoU bases during DNA replication (104). An A/hoU mismatch can subsequently cause a G:C to A:T transition during a second round of DNA replication. The glycosylase activity of MutY on A/hoU may be involved in increasing fidelity removing replication by the adenine misincorporated opposite hoU if a guanine is incorporated opposite the template hoU during MutY repair synthesis.

Cells with a single mutation in the *mutY* and *mutM* genes are moderate mutators, with approximately 10and 40-fold higher mutation frequencies than wild-type cells, respectively (101,102,105). However, the mutation rates of *mutY* and *mutM* double mutants are 3-orders of magnitude higher than the wild-type cells (55). The *nei* mutants exhibit no mutator phenotype and *nei mutY* double mutants have the same mutation frequencies as single *mutY* mutants (13). However, *nei mutY mutM* triple mutants have significant higher mutation frequencies than *mutY mutM* double mutants (13). The *mutT* mutants have about 1000fold higher mutation frequencies of A:T to C:G mutations (106). However, *mutTmutY* double mutants have 2-fold lower mutation rates of A:T to C:G mutations than single *mutT* strains (107).

4.4. MutY interacting proteins 4.4.1. MutY and AP endonucleases

AP sites generated by DNA glycosylases are potentially mutagenic due to lack of base coding information and blockage of DNA synthesis (108,109). To prevent this toxic effect, MutY, like other DNA glycosylases, binds tightly to its AP site products (80,84,85) until other components are recruited to carry out the next repair step. It has been suggested that the base excision repair pathway may involve highly coordinated processes that are governed by protein-protein and protein-DNA interactions (9,110,111). The affinity of MutY to AP/GO is particularly strong and is mediated by its Cterminal domain (80,84,87). The AP endonucleases, Endo IV and Exo III, have been shown to enhance the rate of product release of MutY with an A/G substrate, however, neither AP endonucleases enhance MutY turnover with an A/GO substrate (30).

4.4.2. Interaction of MutY with MutM and Endo VIII

There are interplays between the pathways involved in repair of oxidized purines and of oxidized pyrimidines. We and others have shown that MutY can inhibit MutM glycosylase activity on GO/AP, GO/C, GO/T, and GO/G mismatches (8,80). The extent of these inhibition effects is correlated to MutY binding affinity to these substrates. The biological significance for this modulation of MutM activity by MutY has been discussed in section 4.1.

MutY may have some role in modulating Endo VIII on repair of oxidized pyrimidines. Endo VIII has been shown to serve as a backup pathway to repair 8-oxoG in the absence of MutM and MutY because *nei mutY mutM* triple

mutants have significant higher mutation frequencies than *mutY* and *mutM* double mutants (13). However, its major role is to repair oxidized pyrimidines (13,112). The repair of hoU and hydantoins (the oxidation product of GO) by Endo VIII is inhibited by MutY (29,113). However, Hazra et al. (8) have shown that Endo VIII GO glycosylase activity on GO/G, GO/A and GO/C substrates is not affected by MutY and that Endo VIII does not affect MutY activity on A/GO and G/GO. This is surprising based on the tight binding of MutY to A/GO and AP/GO (47,80,84,87). We showed that Endo VIII can promote beta/delta-elimination on AP/G and weakly promote beta/delta-elimination on AP/GO, when AP/G and AP/GO are products of MutY reaction (29). In addition, we showed that Endo VIII can promote MutY dissociation from AP/G, but not from AP/GO (29). So far, it remains unclear how MutY dissociates from AP/GO in vivo.

5. EUKARYOTIC MYH

The MutY homologs from fission yeast *S. pombe* (15,114), mouse (34,115), calf thymus (116), and human (16,117) have been characterized. SpMYH consists of 461 residues that shows 28% and 31% identity with *E. coli* MutY and hMYH, respectively (114). Due to the high homology between SpMYH and hMYH, *S. pombe* has been used as a model organism to study MYH pathway. The mouse MYH has provided a good alternative to hMYH because mMYH has a better expression level than hMYH (16,18) and they share 86% similarity (34). Based on the physical interaction with PCNA and RPA (31,33,118) and *in vitro* repair assays (119,120), it is suggested that hMYH is involved in a long patch base excision repair pathway.

5.1. Different forms of mammalian MYH

A cDNA of the human mutY gene (hMYH) with an open reading frame encoding a 535 residue (59 kDa protein) was first reported by Slupska et al. (121). Ohtsubo et al. (17) then isolated ten different forms of cDNA for hMYH, which are classified into three types (alpha, beta, and gamma). Type alpha3 hMYH corresponds to the cDNA sequence identified by Slupska et al. (121). Mammalian MYH is significantly larger than the bacterial protein (17,34,121,122). The extended N-terminal domain is involved in mitochondrial targeting of MYH and interaction with replication protein A (RPA) while the C-terminal domain contains the nuclear localization sequence and PCNA interacting motif (17,19,20,33,123). Interestingly, the first 62 residues of type alpha1-alpha3 hMYH (17) are not present in the S. pombe MYH sequence (114). Moreover, both the rat and mouse transcripts cloned to date (34,122,124) lack sequences encoding for the first 14 N-terminal amino acids, which are present in human type alpha1-alpha3 MYH transcripts (17,121). In the human sequence, these 14 amino acids are a part of a putative mitochondria-targeting signal (19,123) as well as the proposed RPA binding motif (33). In this respect, the rodent cDNAs, are similar to type beta human transcripts, which start with the second methionine in hMYH alpha1-alpha3 (17).

Takao *et al.* have shown that there are two types of hMYH proteins: a mitochondrial form (Type 1, residues

1–535) and a nuclear form (Type 2, residues 15–535) (19). However, Tsai-Wu *et al.* showed that Type 1 is localized to the nucleus (20). Ohtsubo *et al.* (17) showed that human cells have at least three isoforms (52-, 53-, 57-kDa) of hMYH and the 57 kDa Type 1 form is specifically detected in the mitochondrial fraction. These controversial results remain to be resolved. In addition, there are evidence that hMYH may be phosphorylated *in vivo* (125). Defective phosphorylation has been implicated in MYH dysfunction in colorectal cancer cell lines (125). *In vitro*, MYH glycosylase activity is modified by phosphorylation (16). Potential acetylation and phosphorylation sites in mammalian MYH sequences are clustered in both termini.

5.2. Substrate specificity of MYH 5.2.1. *S. pombe* MYH

Similar to EcMutY protein, purified recombinant SpMYH expressed in *E. coli* has adenine DNA glycosylase activity on A/G- and A/GO-containing DNA (31,114,126). However, both enzymes have different salt requirements and slightly different substrate specificities. SpMYH has greater glycosylase activity on 2-aminopurine/G and A/2aminopurine but weaker activity on A/C than EcMutY (31,114). Partially purified SpMYH also removes G from G/GO at a lower efficiency than with A/GO and A/G (15). EcMutY and SpMYH also have different substrate binding affinity (114). Although SpMYH has great affinity to A/GOcontaining DNA as MutY, the binding affinity to A/Gcontaining DNA is substantially lower for SpMYH than MutY.

5.2.2. Murine MYH

As mentioned above, both the rat and mouse transcripts (34,122,124) lack sequences encoding the first 14 N-terminal amino acids, which are present in human type alpha(1–3) MYH transcripts (17,121). Yang et al. (34) have expressed a truncated mouse (m) MYH lacking the first 28 N-terminal amino acids in E. coli. Similar to EcMutY, mMYH excises A from A/GO and A/G substrates and binds tightly to its product containing an AP/GO (34). A further detailed analysis of the same truncated mMYH by Pope and David (115) indicates that the intrinsic rates of adenine removal from both A/GO and A/G substrates by mMYH are approximately 10-fold slower than those of EcMutY. However, similar to EcMutY, the rate of adenine removal is approximately nine-fold faster with an A/GOthan an A/G-containing substrate. In contrast to EcMutY, mMYH removes 2-hydroxyadenine mispaired with GO or G in duplex DNA efficiently. Furthermore, compared to MutY, mMYH is less sensitive to the structure of the base mispaired with GO or G.

To investigate the function(s) of the N-terminus of rat (r) MYH function, Ma *et al.* (127) assayed several Nterminal truncated forms. Deletion of 75 amino acids, which perturbs the catalytic core that is conserved with EcMutY, abolished excision activity. In contrast, rMYH with deletions of either 25 or 50 amino acids retain glycosylase activity. Rat MYH (delete50) has lower K_m and weaker binding with A/GO mismatches and favors the formation of excision products with 3'-OH termini than the intact protein. It should be noted that their studies are carried out in nuclear extracts containing the tested proteins (127) whose properties may differ from those of bacterially overexpressed (34,115).

5.2.3. Calf MYH

A 65-kDa protein as well as a functional, but apparently degraded, form of 36 kDa is partially purified from calf thymus nuclear extracts (116). Calf MYH specifically removes mispaired adenines from A/G, A/GO, and A/C mismatches. The glycosylase activity on A/C mismatches was 30-fold lower and the activity on A/GO mismatches was two-fold lower than that on A/G mismatches in the steady state assays. A specific binding complex is formed between calf MYH and A/G and A/GO substrates. Binding is sevenfold greater on A/GO substrates than on A/G substrates.

A 35-40 kDa MYH protein, which is smaller than that of the nuclear MYH, has been purified from calf liver mitochondria (mt). The purified mtMYH cross-reacts with antibodies against both MutY and human MutY homolog (hMYH) (128). The purified mtMYH exhibited DNA glycosylase activity by removing the adenine mispaired with GO, G, or C and weakly removing guanine mispaired with GO. The protein binds to A/GO, G/GO and T/GO, weakly with C/GO, but not with A/G and A/C mismatches.

5.2.4. Human MYH

The first eukaryotic MYH activity on A/G mismatches is detected in HeLa nuclear extracts (21). After the cloning of cDNA of hMYH (121), the hMYH protein has been expressed in E. coli (16,18,20), in an in vitro transcription-translation system (19), and in baculovirus expression system (129). Because of the low expression level and low solubility (16-18), hMYH cannot be purified to high homogeneity thus far. The recombinant hMYH expressed in E. coli and in an in vitro transcriptiontranslation system has adenine DNA glycosylase activity on A/8-oxoG-containing DNA substrates, but very weak activity on A/G-containing DNA substrates (16,18-20). However, the hMYH protein expressed in a baculovirus system has efficient binding and adenine DNA glycosylase activities on both A/G- and A/8-oxoG-containing DNA substrates at low salt (1-50 mM) concentrations (129). In contrast, at 150 mM salt concentration, hMYH glycosylase and DNA binding on A/G mismatches are weaker than on A/GO mismatches (129). Human MYH also can remove 2hydroxyadenines paired with A, G, and GO (17).

There is indication that hMYH may be modified *in vivo*. The substrate specificities of native and recombinant hMYH have been compared (16). Human cell extracts possess the adenine DNA glycosylase activity of hMYH and can form protein–DNA complexes with both A/G and A/8-oxoG mismatches. However, recombinant hMYH expressed in bacteria has much weaker glycosylase and substrate-binding activities towards A/G mismatches than native hMYH. Phosphorylation of native hMYH may explain, in part, this difference of the substrate specificities because dephosphorylation of native hMYH reduces the glycosylase activity on A/G more extensively than on A/8oxoG mismatches (16). Defective phosphorylation has been implicated in hMYH dysfunction in colorectal cell lines (125).



Figure 4. Mutations of the *hMYH* gene identified in MAP cases. The two most common *hMYH* mutations (Y165C and G382D) which account for near 80% of MAP in Caucasians are in larger font. Four of these variants of hMYH (Y165C, G382D, R227W and V232F, shown in red) have been characterized biochemically. Functional domains of hMYH are indicated. The conserved Asp222 required for hMYH catalytic activity is shown by a red line inside the MYH coding box. The binding sites of hRPA (residues 6-32, in orange box), hMSH6 (residues 232-254, in yellow box), hAPE1 (residues 295-318, in green box), and hPCNA (residues 505-527, in blue box) of hMYH are also shown (32,33). This figure is modified from Sampson *et al.* (170) with permission from Biochemistry Society.

5.3. Interactions between MYH and other repair pathways

The MYH activity can be modulated by other proteins. Physical interactions between MYH and APE1 have been demonstrated (33) and APE1 can enhance the glycosylase activity of MYH (34). The hAPE1 binding site is mapped to amino acid residues 295-318 of hMYH (33) (Figure 4). Interestingly, the enhancement of MYH catalytic activity by APE1 is independent of its endonuclease activity (34). In addition, hMYH and hAPE1 are present in the same protein–DNA complex formed by the HeLa extracts and A/GO-containing DNA (16).

We have shown that MYH is directly associated with hMutS-alpha (MSH2/MSH6) via hMSH6 (32). The hMutS-alpha binding site is mapped to amino acid residues 232-254 of hMYH (Figure 4), a region conserved in the MutY family. The glycosylase and DNA binding activities of MYH with an A/8- oxoG mismatch can be stimulated by MutS-alpha (32). These results suggest that proteinprotein interactions may be a means by which hMYH repair and mismatch repair cooperate in reducing replicative errors caused by oxidized bases.

5.4. Interaction between MYH and DNA replication

We have shown that MYH is directly associated with proliferating cell nuclear antigen (PCNA) and replication protein A (RPA) (31,33). The hAPE1 binding site is mapped to amino acid residues 295-318 of hMYH (33) (33) (Figure 4). Interestingly, both the rat and mouse transcripts

cloned to date (34,122,124) lack sequences encoding the first 14 N-terminal amino acids of hMYH and the S. pombe MYH (114) lacks the first 62 N-terminal amino acids of hMYH. It will be interesting to see whether mouse, rat, and S. pombe MYH interact with RPA. The hPCNA binding activity is located at the C-terminus of hMYH containing residues 505-527 (33) (Figure 4). The conserved PCNA binding motif QXXLXXFF is found in human and mouse MutY homologs (34,121). Parker et al. (33) have shown that hMYH is directly associated with hPCNA and Boldogh et al. (118) have shown that hMYH colocalizes with hPCNA to the replication foci. Although S. pombe MYH (114) does not contain a perfect PCNA binding motif, SpMYH interacts with SpPCNA (31). Moreover, SpMYH interacts with hPCNA and SpPCNA interacts with hMYH (31). Thus, glutamine at position 1 and phenylalanine at position 8 are dispensable for SpMYH and SpPCNA interaction.

In order to prevent mutations, MYH adenine glycosylase activity must be directed to the newly synthesized strand, but not the template strand, during DNA synthesis. It has been suggested that hMYH repair is coupled to DNA replication through docking with hPCNA and hRPA (31,33,118). In such a model, MYH can remove adenines on the daughter strands mismatched with guanines or 8-oxoG (Figure 1, reaction 3) as a result of DNA replication errors, but cannot excise the adenines on the template strands (Figure 1, reaction 5). Boldogh *et al.* (118) have demonstrated that hMYH in the nucleus co-



Figure 5. DNA glycosylases may act as adaptors for checkpoint sensors. ATM/ATR, Rad17-RFC, and the 9-1-1 complexes may serves as sensors for DNA damage response to arrest cell cycle, enhance DNA repair, and induce apoptosis. After stress, ATM or ATR is activated and can transduce the DNA damage signal by phosphorylating Chk1, BRCA1, p53, and other proteins in a 9-1-1 complex and Rad17 dependent manner. Recent results support a model that DNA glycosylases recognize the lesions and recruit Rad9/Rad1/Hus1 and Rad17/RFC to the site of damage. The base excision repair activity is then further enhanced by Rad9/Rad1/Hus1 (indicated by an open arrow).

cocalizes with BrdU at replication foci and with proliferating cell nuclear antigen (PCNA). The levels of hMYH in the nucleus increase 3- to 4- fold in S phase compared to early G1. We have provided direct evidence that the interaction between SpMYH and SpPCNA of S. pombe is important for SpMYH biological function in mutation avoidance (31). A mutant form of SpMYH, which has normal glycosylase activity but cannot interact with SpPCNA, is partially defective in vivo. While the SpMYH-defective cells expressing hMYH have partially reduced mutation frequency, the F518AF519A mutant hMYH containing mutations in its PCNA binding motif could not reduce the mutation frequency of SpMYHdefective cells. Hayashi et al. (119) have shown that DNA replication enhances the MYH-dependent repair of A/GO mismatches in vivo. They also showed that interaction between PCNA and MYH is critical for MYH-initiated A/GO repair. In contrast, the interaction between mMYH and mPCNA is found to be unimportant for mutation avoidance in mouse ES cells (130).

Parlanti *et al.* (120) have shown that human cell extracts perform BER on both DNA strands of an A/GO mismatch. First, A/GO mispairs are converted to C/GO that may be mediated by the MYH BER pathway. The resulting C/GO is then corrected to C/G that is consistent with a hOGG1-mediated BER. Repair synthesis on either strand is completely inhibited by aphidicolin suggesting that the two BER events are likely to be mediated by POLdelta/epsilon. Although POLdelta prefers to incorporate dCTP (14% misincorporation of dATP opposite GO on the template), the extension past A/GO mispair predominates (131). However, binding of hMYH to DNA polymerases beta and delta is not detected (33).

5.5. Interactions between MYH and cell cycle checkpoint proteins

DNA repair is coordinated with cell-cycle progression and DNA-damage checkpoints (6,132). Cell cycle checkpoints are surveillance mechanisms that monitor the cell's state and preserve genome integrity (38,133-135). The loss of proper response to DNA damage controlled by these checkpoints can lead to genomic instability, and has been implicated in carcinogenesis. The signal transduction pathways triggered by DNA damage involve many components, including sensors, transducers, and effectors (Figure 5). Human ATM (ataxia telangiectasia mutated) and ATR (ATM- and Rad3-related protein) are phosphoinositol phosphate 3 (PI-3) kinaserelated kinases, and are central components of the DNA damage response (38). After stress, ATM or ATR is activated and can transduce the DNA damage signal by phosphorylating Chk1 and other proteins in a Rad9/Rad1/Hus1 (9-1-1 complex) and Rad17 (a component of clamp loader) dependent manner. Rad17 protein is homologous to the largest subunit of replication factor C (RFC), and forms the alterative clamp loader with RFC2-5. Rad9, Rad1, and Hus1 form a heterotrimeric complex (the 9-1-1 complex) that exhibits structural similarity with the homotrimeric clamp, PCNA (36,37,136). The 9-1-1 complex is loaded onto DNA by Rad17-RFC (137-139).

ATM/ATR, the 9-1-1 complex, and Rad17 are proposed to act at an early step to sense DNA damage (140) (Figure 5). The recruitment mechanism of these checkpoint sensor proteins to DNA lesions is poorly understood. There are two models to address how these sensors are recruited to the damaged sites. In the first model, these checkpoint proteins may detect a common intermediate, such as single-stranded DNA coated by replication protein A (RPA), which is processed by various DNA repair pathways (135). RPA has been shown to directly interact with the 9-1-1 complex (141). In the second model, these checkpoint proteins may require a series of "adaptors" to recognize DNA damage (Figure 5). Such adaptor proteins may be DNA damage recognition proteins involved in base excision repair, mismatch repair, nucleotide excision repair, and double-strand break repair (142 - 145).

Recent findings showed a link between the 9-1-1 complex and the BER pathway. We have shown that the fission yeast *S. pombe* 9-1-1 complex is associated with SpMYH, and that the DNA damage-induced

phosphorylation of SpHus1 is dependent on SpMYH expression (35). Our unpublished results showed that hMYH is also associated with the checkpoint proteins, Rad9, Rad1, and Hus1. Recently, the 9-1-1 complex has been shown to interact with and stimulate other components of base excision repair, including polymerase beta (146), flap endonuclease 1 (FEN1) (147,148), RPA (141), and DNA ligase 1 (LIG1) (149) (Figure 3). These findings indicate a new role for the 9-1-1 complex. At the lesion sites, the complex not only serves as a damage sensor to activate checkpoint control, but it is also a component of BER pathway and may act as a platform for the different factors involved in BER (150) (Figure 3). The results support a model that checkpoint proteins require a series of "adaptors" to recognize DNA damage. In this model, a DNA glycosylase first recognizes specific DNA lesions, and then recruits Rad9/Rad1/Hus1 to initiate the signal response pathways that control cell cycle arrest, apoptosis, and direct activation of DNA repair (Figure 5).

5.6. MYH knockout yeast and mouse

The first demonstration that eukaryotic MYH plays an important role in cellular protection against oxidative DNA damage was performed in S. pombe cells (126). The SpMYH knockout strain displays a 36-fold higher mutation frequency than the wild type strain. The strong mutator phenotype of a SpMYH mutant stain suggests that SpMYH, like E. coli MutY, is critical for oxidative DNA repair and other DNA repair pathways cannot substitute for its functions. Because S. pombe does not contain any MutM or OGG1 homolog (Table 1), a single *SpMYH* mutant may behave like a *mutYmutM* double mutant of E. coli whose mutation rate is 3-orders of magnitude higher than the wild-type cells (55). Disruption of SpMYH also causes increased sensitivity to H₂O₂, but not to UV-irradiation. Expression of SpMYH in the knockout cells restores the adenine glycosylase activity, reduces the mutation frequency, and elevates the resistance to H_2O_2 . The SpMYH mutant strain expressing catalytic inactive SpMYH(D172N) is still a mutator. Moreover, when SpMYH(D172N) mutant is expressed in the wild type cells, the mutation frequency is even higher than that of the parental strains. Thus, a mutant SpMYH that retains substrate-binding activity but is defective in glycosylase activity exhibits a dominant negative effect.

In contrast to S. pombe, knockouts of Myh or Ogg1 gene in mice have unexpectedly mild consequences (151-153). This has been explained by redundant repair pathways to cope with oxidative stress in the mouse cell. It has been reported that *Myh* knockout mice develop higher frequency of intestine tumor after 18 months old (152). MYH-null mouse embryonic stem (ES) cells have a spontaneous mutation rate in the Hprt locus 2-fold higher than the wild type cells (130). The expression of wild type mMYH restores the increased spontaneous mutation rates of the MYH-null ES cells to the wild type level (130). The combined deficiency in *Mvh* and *Ogg1* predisposes mice to tumors, predominantly lung and ovarian tumors, and lymphomas (153). It is interesting that the types of tumors developed in the double knockout mice is different from that found in human MAP with colon cancer. Subsequent analyses identified G:C to T:A mutations in 75% of the lung tumors at an activating hot spot, codon 12, of the Kras oncogene. Moreover, malignant lung tumors were increased with combined heterozygosity of *Msh2* (153). Thus, oxidative DNA damage appears to play a causal role in carcinogenesis.

5.7. MYH deficiency and colorectal cancer

Failure to repair oxidation damage is predicted to lead to genome instability and degenerative conditions including aging and cancer (4). Among many DNA glycosylases, deficiency of MYH and NEIL1 are associated with human disease. Mutations in the *hNEIL1* gene may be involved in gastric cancer (154). Biallelic germ-line mutations in the human MYH gene are associated with recessive inheritance of multiple colorectal adenomas and carcinoma (22-26). This new genetic predisposition to cancer is referred to as MYH-associated polyposis (MAP). MAP is similar to but slightly differs from familial adenomatous polyposis coli (FAP) in its mode of transmission, later age of onset, a less florid form of polyposis, and fewer extra colonic manifestations. MYH mutations can cause G:C to T:A mutations of the adenomatous polyposis coli (APC), K-ras, and other genes that control cellular proliferation in the colon (22,27,28). The mutations in APC gene found in MAP occur at hot spots containing GAA sequences (22). Screening of hMYH mutation in FAP-like patients without inherited APC mutation has shown that biallelic mutations in the *hMYH* gene account for approximately 25% of such cases (24-26,155-158). More than 20 mutations in the hMYH gene have been identified in MAP patients to date (Figure 4). Human MYH may have functions other than mutation avoidance. A patient with rheumatic disease contains autoantibodies to hMYH (159). The levels of GO-repair enzymes including MYH increase after ischemiareperfusion of spinal cord (159,160).

The two most common hMYH mutations, which account for approximately 80% of MAP in Caucasians, are Y165C and G382D. The biochemical effects of Y165C and G382D mutations have been defined (161-164). In vivo, expression of hMYH(Y165C) mutant is unable to complement E. coli mutY mutation (162). The Y165C of hMYH (164) and the equivalent Y150C mMYH (163) expressed in E. coli, as well as the equivalent Y82C of E. coli MutY (161) are defective in DNA glycosylase activity on A/GO mismatches. The Y150C mMYH has a large decrease in the rate of adenine removal from both A/GO and A/G mismatches (163). The mMYH(Y150C) mutant exhibits a significantly reduced binding affinity to DNA. In addition, while hAPE1 stimulates the glycosylase activities of wild-type mMYH with an A/GO substrate, the glycosylase activity of mMYH(Y150C) is inhibited by hAPE1 (163). The importance of Y165 of hMYH in catalysis can be explained by the X-ray structure of BsMutY bound to an A/GO mismatch-containing duplex (62). The corresponding Y88 of BsMutY is intercalated on the 5'-side of the GO, and the OH group of Y88 hydrogen bonds with S308, which in turn hydrogen bonds with the N7H of the GO. The intercalation of this tyrosine into the duplex has been implied to interrogate undamaged DNA and search for lesions (165).

The G382 of hMYH is highly conserved among members in MutY family, thus suggesting its importance role in the activity of MutY and its homologues. The BsMY-DNA structure reveals that the corresponding G260 initiates a turn that makes contact to the phosphate backbone 5' to the GO (62), and therefore a mutation at this position likely affects the GO recognition role of the C-terminal domain (47,80,84,87). *In vivo*, expression of hMYH(G382D) mutant is unable to fully complement *E. coli mutY* mutation (162) and expression of mMYH(G365D) protein cannot suppress the elevated spontaneous mutation rate of the MYH-null ES mouse cells (130). Thus, the germ-line mutation G382D in *hMYH* gene is likely to be responsible for the MAP phenotype.

However, there are inconsistent results about the biochemical activities of hMYH(G382D) protein on A/GO mismatches. While hMYH(G382D) expressed in E. coli has been shown to be inactive towards A/GO mismatches (164), the equivalent EcMutY(G253D) is only partially inactive on A/GO mismatches (161,162) and mMYH(G365D) shows a decreased catalytic rate with an A/G but not on A/GO mismatches (163,166,167). Recently, Ushijima et al. (167) showed that mMYH(G365D) has substantially reduced glycosylase activity on 2-OH-A opposite of guanine. Their results imply that this reduced repair capacity of the mutant hMYH(G382D) is the cause of MAP. The mMYH(G365D) variant has a significantly reduced binding affinity to DNA (163,166). Tominaga et al. (166) showed that mutant mMYH(G365D) cannot prevent mAPEX1 from incising the generated AP site paired with GO and cannot prevent OGG1 from excising 8-oxoG opposite the generated AP site. However, Pope et al. (163) showed that APE1 can stimulate product formation by mMYH(G365D) with an A/GO substrate. The conflict between these results has yet to be solved. Nevertheless, these results suggest that the product binding affinity of MYH is to avoid inappropriate and potentially mutagenic activities by enzymes such as hOGG1 (166).

Two additional variants of hMYH (R227W and V232F) associated with MAP have recently been characterized (117). The hMYH(R227W) severely reduces the ability to bind an A/8-oxoG mispair and to catalyze adenine excision from A/8-oxoG mismatches. In addition, the R227W mutant also displays an impaired function to complement the phenotype of E. coli mutY. hMYH(V232F) is also deficient in DNA binding and glycosylase activities, although the biochemical differences between the mutant and wild type enzymes are less pronounced than for hMYH(R227W). The hMYH(V232F) mutant enzyme may be able to effectively recognize mispairs and catalyze some reactions, but it exhibits a lower affinity to DNA substrates than the wild type protein (as observed for the mouse G382D homolog) (163,166). Although mutations (R227W and V232F) lie adjacent to the hMSH6 binding domain (Figure 4), both mutant proteins retain the ability to interact with hMSH6.

6. CONCLUSIONS AND PERSPECTIVES

Since the discovery of the linkage of germ-line mutations in the hMYH gene with MAP in 2002, studies on

MutY and MYH repair have been greatly stimulated. Oxidative DNA damage plays a causal role in mutation load and carcinogenesis and is removed by several repair pathways that are usually redundant. It is believed that BER involves highly coordinated processes that are governed by protein-protein and protein-DNA interactions, and that these processes may involve a hand-off mechanism of the cytotoxic intermediates. The important questions to address are how the cell completes the repair pathway without releasing toxic intermediates, such as AP sites or strand breaks, and how it coordinates different DNA repair pathways for different lesions in relation to DNA replication and the cell cycle. So far, how MutY and MYH dissociate from AP/GO is not clear. Unidentified factors may be involved to facilitate this process.

DNA glycosylases must interrogate millions of base pairs of undamaged DNA in order to locate the lesion. The nature of this search process remains poorly understood. Recently, the structure of MutM with normal DNA provides some clues to this search (165). It has been proposed that MutY may use a dimer form to scan the DNA and locate the mismatch (95). A unique property of MutY and MYH is that they excise a normal A from A/GO, A/G, A/C and A/5-hydroxyuracil as well as excise G from G/GO. In contrast to EcMutY, hMYH can remove 2hydroxyadenines paired with A, G, and GO. The biological significance for MutY/MYH to bind T/GO, G/GO, and C/GO requires further investigation. There may be some catalytic and binding substrates that remain to be identified.

S. pombe and mouse have provided good models to study MYH repair through knockout cells and animals. However, the tumor type developed in the Myh and Ogg1 double knockout mice is different from that found in human MAP with colon cancer. There are also conflicting results regarding the biological significance of the interaction of MYH and PCNA in S. pombe and mouse systems. The interaction is shown to be important in S. *pombe* (31), but is found insignificant in mouse cells (130). The roles of RPA, MutS homologs, and perhaps other replication proteins in directing MYH repair to the newly synthesized strand are worth investigating in both systems. Although POLdelta/epsilon have been implicated to carry out the repair synthesis in the MYH BER pathway, no physical interaction between hMYH and DNA polymerases beta and delta is detected. The involvement of other transletion DNA polymerases cannot be ruled out.

MYH has been shown to physically interact with many proteins involved in BER, MMR, replication, and checkpoint control. The *in vitro* and *in vivo* activities of MYH can be modulated by APE1, PCNA, and MSH2/MSH6. Some of these interacting proteins may be involved to facilitate the turnover of MYH from AP/GO products. A defect in these interactions may contribute to the cause of MAP. Unfortunately, biochemical analyses of mutant MYH proteins associated with MAP are limited and none has been identified to defect in such protein-protein interactions. It is also possible that damage recognition involves a large complex, such as the BRCA1-associated genome surveillance complex (BASC), which contains DNA repair, checkpoint, and replication proteins, including BRCA1, MSH2, MSH6, MLH1, ATM, RAD50, and RFC (168). DNA damage response may be coordinated with ongoing DNA repair.

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8. REFERENCES

1. Friedberg,E.C., Walker,G.C., Siede,W., Wood,R.D., Schultz,R.A. and Ellenberger,T. (2005) *DNA Repair and Mutagenesis*. ASM Press, Washington, D. C.

2. Krokan H. E., H. Nilsen, F. Skorpen, M. Otterlei & G. Slupphaug: Base excision repair of DNA in mammalian cells. *FEBS Lett* 476, 73-77 (2000)

3. Lindahl T. & R. D. Wood: Quality control by DNA repair. *Science* 286, 1897-1905 (1999)

4. Loeb L. A. & F. C. Christians: Multiple mutations in human cancers. *Mutat Res* 350, 279-286 (1996)

5. Friedberg E. C.: DNA damage and repair. *Nature* 421, 436-440 (2003)

6. Sancar A., L. A. Lindsey-Boltz, K. Unsal-Kacmaz & S. Linn: Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* 73, 39-85 (2004)

7. Bellacosa A.: Functional interactions and signaling properties of mammalian DNA mismatch repair proteins. *Cell Death Differ* 8, 1076-1092 (2001)

8. Hazra T. K., J. W. Hill, T. Izumi & S. Mitra: Multiple DNA glycosylases for repair of 8-oxoguanine and their potential *in vivo* functions. *Prog Nucleic Acid Res Mol Biol* 68, 193-205 (2001)

9. Lu A.-L., X. Li, Y. Gu, P. M. Wright & D.-Y. Chang: Repair of oxidative DNA damage. *Cell Biochem Biophy* 35, 141-170 (2001)

10. Michaels M. L. & J. H. Miller: The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxo-guanine). *J Bacteriol* 174, 6321-6325 (1992)

11. Tchou J. & A. P. Grollman: Repair of DNA containing the oxidatively-damaged base 8-hydroxyguanine. *Mutat Res* 299, 277-287 (1993)

12. Modrich P. & R. S. Lahue: Mismatch repair in replication fidelity, genetic recombination and cancer biology. *Annu Rev Biochem* 65, 101-133 (1996)

13. Blaisdell J. O., Z. Hatahet & S. S. Wallace: A novel role for *Escherichia coli* endonuclease VIII in prevention of spontaneous G-->T transversions. *J Bacteriol* 181, 6396-6402 (1999)

14. Cheadle J. P. & J. R. Sampson: Exposing the MYH about base excision repair and human inherited disease. *Hum Mol Genet* 12 Spec No 2, R159-R165 (2003)

15. Doi T., S. Yonekura, K. Tano, S. Yasuhira, S. Yonei & Q. M. Zhang: The Shizosaccharomyces pombe homolog (SpMYH) of the Escherichia coli MutY is required for removal of guanine from 8-oxoguanine/guanine mispairs to

prevent G:C to C:G transversions. J Radiat Res (Tokyo) 46, 205-214 (2005)

16. Gu Y. & A.-L. Lu: Differential DNA recognition and glycosylase activity of the native human MutY homolog (hMYH) and recombinant hMYH expressed in bacteria. *Nucleic Acids Res* 29, 2666-2674 (2001)

17. Ohtsubo T., K. Nishioka, Y. Imaiso, S. Iwai, H. Shimokawa, H. Oda, T. Fujiwara & Y. Nakabeppu: Identification of human MutY homolog (hMYH) as a repair enzyme for 2- hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. *Nucleic Acids Res* 28, 1355-1364 (2000)

18. Slupska M. M., W. M. Luther, J. H. Chiang, H. Yang & J. H. Miller: Functional expression of hMYH, a human homolog of the *Escherichia coli* MutY protein. *J Bacteriol* 181, 6210-6213 (1999)

19. Takao M., Q. M. Zhang, S. Yonei & A. Yasui: Differential subcellular localization of human MutY homolog (hMYH) and the functional activity of adenine:8-oxoguanine DNA glycosylase. *Nucleic Acids Res* 27, 3638-3644 (1999)

20. Tsai-Wu J.-J., H.-T. Su, Y.-L. Wu, S.-M. Hsu & C. H. H. Wu: Nuclear localization of the human MutY homologue hMYH. *J Cell Biochem* 77, 666-677 (2000)

21. Yeh Y.-C., D.-Y. Chang, J. Masin & A.-L. Lu: Two nicking enzymes systems specific for mismatch-containing DNA in nuclear extracts from human cells. *J Biol Chem* 266, 6480-6484 (1991)

22. Al Tassan N., N. H. Chmiel, J. Maynard, N. Fleming, A. L. Livingston, G. T. Williams, A. K. Hodges, D. R. Davies, S. S. David, J. R. Sampson *et al.*: Inherited variants of MYH associated with somatic G:C to T:A mutations in colorectal tumors. *Nat Genet* 30, 227-232 (2002)

23. Halford S. E., A. J. Rowan, L. Lipton, O. M. Sieber, K. Pack, H. J. Thomas, S. V. Hodgson, W. F. Bodmer & I. P. Tomlinson: Germline mutations but not somatic changes at the MYH locus contribute to the pathogenesis of unselected colorectal cancers. *Am J Pathol* 162, 1545-1548 (2003)

24. Jones S., P. Emmerson, J. Maynard, J. M. Best, S. Jordan, G. T. Williams, J. R. Sampson & J. P. Cheadle: Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C-->T:A mutations. *Hum Mol Genet* 11, 2961-2967 (2002)

25. Sieber O. M., L. Lipton, M. Crabtree, K. Heinimann, P. Fidalgo, R. K. Phillips, M. L. Bisgaard, T. F. Orntoft, L. A. Aaltonen, S. V. Hodgson *et al.*: Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N Engl J Med* 348, 791-799 (2003)

26. Sampson J. R., S. Dolwani, S. Jones, D. Eccles, A. Ellis, D. G. Evans, I. Frayling, S. Jordan, E. R. Maher, T. Mak *et al.*: Autosomal recessive colorectal adenomatous polyposis due to inherited mutations of MYH. *Lancet* 362, 39-41 (2003)

27. Jones S., S. Lambert, G. T. Williams, J. M. Best, J. R. Sampson & J. P. Cheadle: Increased frequency of the k-ras G12C mutation in MYH polyposis colorectal adenomas. *Br J Cancer* 90, 1591-1593 (2004)

28. Lipton L., S. E. Halford, V. Johnson, M. R. Novelli, A. Jones, C. Cummings, E. Barclay, O. Sieber, A. Sadat, M. L. Bisgaard *et al.*: Carcinogenesis in MYH-associated polyposis follows a distinct genetic pathway. *Cancer Res* 63, 7595-7599 (2003)

29. Lu A. L., C. Y. Lee, L. Li & X. Li: Physical and functional interactions between Escherichia coli MutY and endonuclease VIII. *Biochem J* 393, 381-387 (2006)

30. Pope M. A., S. L. Porello & S. S. David: *Escherichia coli* apurinic-apyrimidinic endonucleases enhance the turnover of the adenine glycosylase MutY with G:A Substrates. *J Biol Chem* 277, 22605-22615 (2002)

31. Chang D. Y. & A.-L. Lu: Functional interaction of MutY homolog (MYH) with proliferating cell nuclear antigen (PCNA) in fission yeast, *Schizosaccharomyces pombe. J Biol Chem* 277, 11853-11858 (2002)

32. Gu Y., A. Parker, T. M. Wilson, H. Bai, D. Y. Chang & A. L. Lu: Human MutY homolog (hMYH), a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins hMSH2/hMSH6. *J Biol Chem* 277, 11135-11142 (2002)

33. Parker A., Y. Gu, W. Mahoney, S.-H. Lee, K. K. Singh & A.-L. Lu: Human homolog of the MutY protein (hMYH) physically interacts with protein involved in long-patch DNA base excision repair. *J Biol Chem* 276, 5547-5555 (2001)

34. Yang H., W. M. Clendenin, D. Wong, B. Demple, M. M. Slupska, J. H. Chiang & J. H. Miller: Enhanced activity of adenine-DNA glycosylase (Myh) by apurinic/apyrimidinic endonuclease (Ape1) in mammalian base excision repair of an A/GO mismatch. *Nucleic Acids Res* 29, 743-752 (2001)

35. Chang D. Y. & A. L. Lu: Interaction of Checkpoint Proteins Hus1/Rad1/Rad9 with DNA Base Excision Repair Enzyme MutY Homolog in Fission Yeast, *Schizosaccharomyces pombe. J Biol Chem* 280, 408-417 (2005)

36. Shiomi Y., A. Shinozaki, D. Nakada, K. Sugimoto, J. Usukura, C. Obuse & T. Tsurimoto: Clamp and clamp loader structures of the human checkpoint protein complexes, Rad9-1-1 and Rad17-RFC. *Genes to Cells* 7, 861-868 (2002)

37. Venclovas C. & M. P. Thelen: Structure-based predictions of Rad1, Rad9, Hus1 and Rad17 participation in sliding clamp and clamp-loading complexes. *Nucleic Acids Res* 28, 2481-2493 (2000)

38. Zhou B. B. & S. J. Elledge: The DNA damage response: putting checkpoints in perspective. *Nature* 408, 433-439 (2000)

39. Mol C. D., S. S. Parikh, C. D. Putnam, T. P. Lo & J. A. Tainer: DNA repair mechanisms for the recognition and removal of damaged DNA bases. *Annu REv Biophys Biomol Struct* 28, 101-128 (1999)

40. McCullough A. K., M. L. Dodson & R. S. Lloyd: Initiation of base excision repair: glycosylase mechanisms and structures. *Annu Rev Biochem* 68, 255-285 (1999)

41. Sun B., K. A. Latham, M. L. Dodson & R. S. Lloyd: Studies on the catalytic mechanism of five DNA glycosylases: probing for enzyme-DNA imino intermediates. *J Biol Chem* 270, 19501-19508 (1995)

42. Labahn J., A. Scharer, A. Long, K. Ezaz-Nikpay, G. L. Verdine & T. E. Ellenberger: Structural basis for the excision repair of alkylation-damaged DNA. *Cell* 86, 321-329 (1996)

43. Yamagata Y., M. Kato, K. Odawara, Y. Tokuno, Y. Nakashima, N. Matsushima, K. Yasumura, K. Tomita, K. Ihara, Y. Fujii *et al.*: Three-dimensional structure of a DNA

repair enzyme, 3-methyladenine DNA glycosylase II, from *Escherichia coli. Cell* 86, 311-319 (1996)

44. Thayer M. M., H. Ahern, D. Xing, R. P. Cunningham & J. A. Tainer: Novel DNA binding motifs in the DNA repair enzyme endonuclease III crystal structure. *EMBO J* 14, 4108-4120 (1995)

45. Boiteux S. & J. P. Radicella: Base excision repair of 8hydroxyguanine protects DNA from endogenous oxidative stress. *Biochimie* 81, 59-67 (1999)

46. O'Connor T. R. & J. Laval: Physical association of the 2,6-diamino-4-hydroxy-5N- formamidopyrimidine-DNA glycosylase of *Escherichia coli* and an activity nicking DNA at apurinic/apyrimidinic sites. *Proc Natl Acad Sci U S A* 86, 5222-5226 (1989)

47. Gogos A., J. Cillo, N. D. Clarke & A.-L. Lu: Specific recognition of A/G and A/8-oxoG mismatches by *Escherichia coli* MutY:removal of the C-terminal domain preferentially affects A/8-oxoG recognition. *Biochemistry* 35, 16665-16671 (1996)

48. Lu A.-L., D. S. Yuen & J. Cillo: Catalytic mechanism and DNA substrate recognition of *Escherichia coli* MutY protein. *J Biol Chem* 271, 24138-24143 (1996)

49. Manuel R. C. & R. S. Lloyd: Cloning, overexpression, and biochemical characterization of the catalytic domain of MutY. *Biochemistry* 36, 11140-11152 (1997)

50. Williams S. D. & S. S. David: Evidence that MutY is a monofunctional glycosylase capable of forming a covalent Schiff base intermediate with substrate DNA. *Nucleic Acids Res* 26, 5123-5133 (1998)

51. Wright P. M., J. Yu, J. Cillo & A.-L. Lu: The active site of the *Escherichia coli* MutY DNA adenine glycosylase. *J Biol Chem* 274, 29011-29018 (1999)

52. Zharkov D. O. & A. P. Grollman: MutY DNA glycosylase: base release and intermediate complex formation. *Biochemistry* 37, 12384-12394 (1998)

53. Au K. G., S. Clark, J. H. Miller & P. Modrich: *Escherichia coli mutY* gene encodes an adenine glycosylase active on G/A mispairs. *Proc Natl Acad Sci U S A* 86, 8877-8881 (1989)

54. Bulychev N. V., C. V. Varaprasad, G. Dorman, J. H. Miller, M. Eisenberg & A. P. Grollman: Substrate specificity of *Escherichia coli* MutY protein. *Biochemistry* 35, 13147-13156 (1996)

55. Michaels M. L., C. Cruz, A. P. Grollman & J. H. Miller: Evidence that MutM and MutY combine to prevent mutations by an oxidatively damaged form of guanine in DNA. *Proc Natl Acad Sci U S A* 89, 7022-7025 (1992)

56. Michaels M. L., J. Tchou, A. P. Grollman & J. H. Miller: A repair system for 8-oxo-7,8-dihydrodeoxyguanine (8-hydroxyguanine). *Biochemistry* 31, 10964-10968 (1992) 57. Lu A.-L., J.-J. Tsai-Wu & J. Cillo: DNA determinants and substrate specificities of *Escherichia coli* MutY. *J Biol Chem* 270, 23582-23588 (1995)

58. Manuel R. C., E. W. Czerwinski & R. S. Lloyd: Identification of the structural and functional domains of MutY, an *Escherichia coli* DNA mismatch repair enzyme. *J Biol Chem* 271, 16218-16226 (1996)

59. Tsai-Wu J.-J., H.-F. Liu & A.-L. Lu: *Escherichia coli* MutY protein has both N-glycosylase and apurinic/apyrimidinic endonuclease activities on AC and AG mispairs. *Proc Natl Acad Sci U S A* 89, 8779-8783 (1992) 60. Williams S. D. & S. S. David: Formation of a Schiff base intermediate is not required for the adenine glycosylase activity of *Escherichia coli* MutY. *Biochemistry* 38, 15417-15424 (1999)

61. Zharkov D. O., R. Gilboa, I. Yagil, J. H. Kycia, S. E. Gerchman, G. Shoham & A. P. Grollman: Role for lysine 142 in the excision of adenine from A:G mispairs by MutY DNA glycosylase of *Escherichia coli*. *Biochemistry* 39, 14768-14778 (2000)

62. Fromme J. C., A. Banerjee, S. J. Huang & G. L. Verdine: Structural basis for removal of adenine mispaired with 8-oxoguanine by MutY adenine DNA glycosylase. *Nature* 427, 652-656 (2004)

63. Guan Y., R. C. Manuel, A. S. Arvai, S. S. Parikh, C. D. Mol, J. H. Miller, S. Lloyd & J. A. Tainer: MutY catalytic core, mutant and bound adenine structures define specificity for DNA repair enzyme superfamily. *Nat Struct Biol* 5, 1058-1064 (1998)

64. Dianov G. & T. Lindahl: Reconstitution of the DNA base excision-repair pathway. *Curr Biol* 4, 1069-1076 (1994)

65. Radicella J. P., E. A. Clark, S. Chen & M. S. Fox: Patch length of localized repair event: role of DNA polymerase I in *mutY*-dependent mismatch repair. *J Bacteriol* 175, 7732-7736 (1993)

66. Tsai-Wu J.-J. & A.-L. Lu: *Escherichia coli mutY*dependent mismatch repair involves DNA polymerase I and a short repair tract. *Mol Gen Genet* 244, 444-450 (1994)

67. Frosina G., E. Cappelli, P. Fortini & E. Dogliotti: *In vitro* base excision repair assay using mammalian cell extracts. *Methods Mol Biol* 113, 301-315 (1999)

68. Krokan H. E., R. Standal & G. Slupphaug: DNA glycosylases in the base excision repair of DNA. *Biochem J* 325, 1-16 (1997)

69. Matsumoto Y., K. Kim, J. Hurwitz, R. Gary, D. S. Levine, A. E. Tomkinson & M. S. Park: Reconstitution of proliferating cell nuclear antigen-dependent repair of apurinic/apyrimidinic sites with purified human proteins. *J Biol Chem* 274, 33703-33708 (1999)

70. Srivastava D. K., B. J. Berg, R. Prasad, J. T. Molina, W. A. Beard, A. E. Tomkinson & S. H. Wilson: Mammalian abasic site base excision repair. Identification of the reaction sequence and rate-determining steps. *J Biol Chem* 273, 21203-21209 (1998)

71. Pascucci B., M. Strucki, Z. O. Jonsson, E. Dogliotti & U. Hubscher: Long patch base excision repair with purified human proteins: DNA ligase I as patch size mediator for DNA polymerases d and e. *J Biol Chem* 274, 33696-33702 (1999)

72. DeMott M. S., S. Zigman & R. A. Bambara: Replication protein A stimulates long patch DNA base excision repair. *J Biol Chem* 273, 27492-27498 (1998)

73. Dianov G. L., B. R. Jensen, M. K. Kenny & V. A. Bohr: Replication protein A stimulates proliferating cell nuclear antigen- dependent repair of abasic sites in DNA by human cell extracts. *Biochemistry* 38, 11021-11025 (1999) 74. Dianov G. L., R. Prasad, S. H. Wilson & V. A. Bohr: Role of DNA polymerase beta in the excision step of long patch mammalian base excision repair. *J Biol Chem* 274, 13741-13743 (1999)

75. Fortini P., E. Parlanti, O. M. Sidorkina, J. Laval & E. Dogliotti: The type of DNA glycosylase determines the base excision repair pathway in mammalian cells. *J Biol Chem* 274, 15230-15236 (1999)

76. Lu A. L., M. J. Cuipa, M. S. Ip & W. G. Shanabruch: Specific A/G-to-C.G mismatch repair in *Salmonella typhimurium* LT2 requires the *mutB* gene product. *J Bacteriol* 172, 1232-1240 (1990)

77. Li X. & A. L. Lu: Molecular Cloning and Functional Analysis of the MutY Homolog of *Deinococcus radiodurans. J Bacteriol* 183, 6151-6158 (2001)

78. Au K. G., M. Cabrera, J. H. Miller & P. Modrich: *Escherichia coli mutY* gene product is required for specific A/G to C:G mismatch correction. *Proc Natl Acad Sci U S A* 85, 9163-9166 (1988)

79. Lu A.-L. & D.-Y. Chang: A novel nucleotide excision repair for the conversion of an A/G mismatch to C/G base pair in *E. coli. Cell* 54, 805-812 (1988)

80. Li X., P. M. Wright & A.-L. Lu: The C-terminal domain of MutY glycosylase determines the 7,8-dihydro-8-oxo-guanine specificity and is crucial for mutation avoidance. *J Biol Chem* 275, 8448-8455 (2000)

81. Zhang Q. M., N. Ishikawa, T. Nakahara & S. Yonei: *Esherichia coli* MutY protein has a guanine-DNA glycosylase that acts on 7,8-dihydro-8-oxoguanine:guanine mispair to prevent spontaneous G:C to C:G transversions. *Nucleic Acids Res* 26, 4669-4675 (1998)

82. Tchou J., V. Bodepudi, S. Shibutani, I. Antoshechkin, J. H. Miller, A. P. Grollman & F. Johnson: Substrate specificity of Fpg protein: recognition and cleavage of oxidative damaged DNA. *J Biol Chem* 269, 15318-15324 (1994)

83. Bridges B. A., M. Sekiguchi & T. Tajiri: Effect of *mutY* and *mutM/fpg-1* mutations on starvation-associated mutation in *Escherichia coli*: implications for the role of 7,8-dihydro-8-oxoguanine. *Mol Gen Genet* 251, 352-357 (1996)

84. Chmiel N. H., M. P. Golinelli, A. W. Francis & S. S. David: Efficient recognition of substrates and substrate analogs by the adenine glycosylase MutY requires the C-terminal domain. *Nucleic Acids Res* 29, 553-564 (2001)

85. Porello S. L., A. E. Leyes & S. S. David: Singleturnover and pre-steady-state kinetics of the reaction of the adenine glycosylase MutY with mismatch-containing DNA substrates. *Biochemistry* 37, 14756-14764 (1998)

86. Michaels M. L., L. Pham, Y. Nghiem, C. Cruz & J. H. Miller: MutY, an adenine glycosylase active on G-A mispairs, has homology to endonuclease III. *Nucleic Acids Res* 18, 3841-3845 (1990)

87. Noll D. M., A. Gogos, J. A. Granek & N. D. Clarke: The C-terminal domain of the adenine-DNA glycosylase MutY confers specificity for 8-oxoguanine.adenine mispairs and may have evolved from MutT, an 8-oxodGTPase. *Biochemistry* 38, 6374-6379 (1999)

88. Tsai-Wu J.-J., J. P. Radicella & A.-L. Lu: Nucleotide sequence of the *Escherichia coli micA* gene required for A/G-specific mismatch repair: Identity of MicA and MutY. *J Bacteriol* 173, 1902-1910 (1991)

89. Nash H. M., S. D. Bruner, O. D. Scharer, T. Kawate, T. A. Addona, E. Spooner, W. S. Lane & G. L. Verdine: Cloning of a yeast 8-oxoguanine DNA glycosylase reveals

the existence of a base-excision DNA-repair protein superfamily. *Curr Biol* 6, 968-980 (1996)

90. Bruner S. D., D. P. Norman & G. L. Verdine: Structural basis for recognition and repair of the endogenous mutagen 8- oxoguanine in DNA. *Nature* 403, 859-866 (2000)

91. Chepanoske C. L., M. P. Golinelli, S. D. Williams & S. S. David: Positively charged residues within the iron-sulfur cluster loop of *E. coli* MutY participate in damage recognition and removal. *Arch Biochem Biophys* 380, 11-19 (2000)

92. Golinelli M.-P., N. H. Chmiel & S. S. David: Sitedirected mutagenesis of the cysteine ligands to the [4Fe-4S] of *Escherichia coli* MutY. *Biochemistry* 38, 6997-7007 (1999)

93. Lu A. L. & P. M. Wright: Characterization of an *Escherichia coli* mutant MutY with a cysteine to alanine mutation at the iron-sulfur cluster domain. *Biochemistry* 42, 3742-3750 (2003)

94. Messick T. É., N. H. Chmiel, M. P. Golinelli, M. R. Langer, L. Joshua-Tor & S. S. David: Noncysteinyl coordination to the [4Fe-4S]²⁺ cluster of the DNA repair adenine glycosylase MutY introduced via site-directed mutagenesis. Structural characterization of an unusual histidinyl-coordinated cluster. *Biochemistry* 41, 3931-3942 (2002)

95. Lee C. Y., H. Bai, R. Houle, G. M. Wilson & A. L. Lu: An *Escherichia coli* MutY mutant without the six-helix barrel domain is a dimer in solution and assembles cooperatively into multisubunit complexes with DNA. *J Biol Chem* 279, 52653-52663 (2004)

96. Li L. & A. L. Lu: The C-terminal domain of *Escherichia coli* MutY is involved in DNA binding and glycosylase activities. *Nucleic Acids Res* 31, 3038-3049 (2003)

97. Volk D. E., P. G. House, V. Thiviyanathan, B. A. Luxon, S. Zhang, R. S. Lloyd & D. G. Gorenstein: Structural similarities between MutT and the C-Terminal Domain of MutY. *Biochemistry* 39, 7331-7336 (2000)

98. Volk D. E., V. Thiviyanathan, P. G. House, R. S. Lloyd & D. G. Gorenstein: ¹H, ¹³C and ¹⁵ N resonance assignments of the C-terminal domain of MutY: an adenine glycosylase active on G:A mismatches. *J Biomol NMR* 14, 385-386 (1999)

99. Abeygunawardana C., D. J. Weber, A. G. Gittis, D. N. Frick, J. Lin, A. F. Miller, M. J. Bessman & A. S. Mildvan: Solution structure of the MutT enzyme, a nucleoside triphosphate pyrophosphohydrolase. *Biochemistry* 34, 14997-15005 (1995)

100. Lin J., C. Abeygunawardana, D. N. Frick, M. J. Bessman & A. S. Mildvan: Solution structure of the quaternary MutT-M2+-AMPCPP-M2+ complex and mechanism of its pyrophosphohydrolase action. *Biochemistry* 36, 1199-1211 (1997)

101. Nghiem Y., M. Cabrera, C. G. Cupples & J. H. Miller: The *mutY* gene: a mutator locus in *Escherichia coli* that generates G:C to T:A transversions. *Proc Natl Acad Sci U S A* 85, 2709-2713 (1988)

102. Radicella J. P., E. A. Clark & M. S. Fox: Some mismatch repair activities in *Escherichia coli*. *Proc Natl Acad Sci U S A* 85, 9674-9678 (1988)

103. Dizdaroglu M.: Characterization of free radicalinduced damage to DNA by the combined use of enzymatic hydrolysis and gas chromatography-mass spectrometry. J Chromatogr 367, 357-366 (1986)

104. Purmal A. A., Y. W. Kow & S. S. Wallace: Major oxidative products of cytosine, 5-hydroxycytosine and 5-hydroxyuracil, exhibit sequence context-dependent mispairing *in vitro*. *Nucleic Acids Res* 22, 72-78 (1994)

105. Michaels M. L., L. Pham, C. Cruz & J. H. Miller: MutM, a protein that prevents G^C to T^A transversions, is formamidopyrimidine-DNA glycosylase. *Nucleic Acids Res* 19, 3629-3632 (1991)

106. Akiyama M., H. Maki, M. Sekiguchi & T. Horiuchi: A specific role of MutT protein: To prevent dG dA mispairing in DNA replication. *Proc Natl Acad Sci U S A* 86, 3949-3952 (1989)

107. Vidmar J. J. & C. G. Cupples: MutY repair is mutagenic in *mutT* strains of *Escherichia coli*. *Canad J Microbiol* 39, 892-894 (1993)

108. Barzilay G. & I. D. Hickson: Structure and function of apurinic/apyrimidinic endonucleases. *BioEssays* 17, 713-719 (1995)

109. Randall S. K., R. Eritja, B. E. Kaplan, J. Petruska & M. F. Goodman: Nucleotide insertion kinetics opposite abasic lesions in DNA. *J Biol Chem* 262, 6864-6870 (1987) 110. Mol C. D., T. Izumi, S. Mitra & J. A. Tainer: DNA-bound structures and mutants reveal abasic DNA binding by APE1 and DNA repair coordination. *Nature* 403, 451-456 (2000)

111. Wilson S. H. & T. A. Kunkel: Passing the baton in base excision repair [news]. *Nat Struct Biol* 7, 176-178 (2000)

112. Jiang D., Z. Hatahet, R. J. Melamede, Y. W. Kow & S. S. Wallace: Characterization of Escherichia coli endonuclease VIII. *J Biol Chem* 272, 32230-32239 (1997)

113. Hazra T. K., J. G. Muller, R. C. Manuel, C. J. Burrows, R. S. Lloyd & S. Mitra: Repair of hydantoins, one electron oxidation product of 8-oxoguanine, by DNA glycosylases of *Escherichia coli*. *Nucleic Acids Res* 29, 1967-1974 (2001)

114. Lu A.-L. & W. P. Fawcett: Characterization of the recombinant MutY homolog, an adenine DNA glycosylase, from *Schizosacchromyces pombe. J Biol Chem* 273, 25098-25105 (1998)

115. Pope M. A. & S. S. David: DNA damage recognition and repair by the murine MutY homologue. *DNA Repair* (*Amst*) 4, 91-102 (2005)

116. McGoldrick J. P., Y.-C. Yeh, M. Solomon, J. M. Essigmann & A.-L. Lu: Characterization of a mammalian homolog of the *Escherichia coli* MutY mismatch repair protein. *Mol Cell Biol* 15, 989-996 (1995)

117. Bai H., S. Jones, X. Guan, T. M. Wilson, J. R. Sampson, J. P. Cheadle & A. L. Lu: Functional characterization of two human MutY homolog (hMYH) missense mutations (R227W and V232F) that lie within the putative hMSH6 binding domain and are associated with hMYH polyposis. *Nucleic Acids Res* 33, 597-604 (2005)

118. Boldogh I., D. Milligan, M. S. Lee, H. Bassett, R. S. Lloyd & A. K. McCullough: hMYH cell cycle-dependent expression, subcellular localization and association with replication foci: evidence suggesting replication- coupled repair of adenine:8-oxoguanine mispairs. *Nucleic Acids Res* 29, 2802-2809 (2001)

119. Hayashi H., Y. Tominaga, S. Hirano, A. E. McKenna, Y. Nakabeppu & Y. Matsumoto: Replication-associated repair of adenine:8-oxoguanine mispairs by MYH. *Curr Biol* 12, 335-339 (2002)

120. Parlanti E., P. Fortini, P. MacPherson, J. Laval & E. Dogliotti: Base excision repair of adenine/8-oxoguanine mispairs by an aphidicolin-sensitive DNA polymerase in human cell extracts. *Oncogene* 21, 5204-5212 (2002)

121. Slupska M. M., C. Baikalov, W. M. Luther, J.-H. Chiang, Y.-F. Wei & J. H. Miller: Cloning and sequencing a human homolog (*hMYH*) of the *Escherichia coli mutY* gene whose function is required for the repair of oxidative DNA damage. *J Bacteriol* 178, 3885-3892 (1996)

122. Lee H. M., C. Wang, Z. Hu, G. H. Greeley, W. Makalowski, H. L. Hellmich & E. W. Englander: Hypoxia induces mitochondrial DNA damage and stimulates expression of a DNA repair enzyme, the Escherichia coli MutY DNA glycosylase homolog (MYH), *in vivo*, in the rat brain. *J Neurochem* 80, 928-937 (2002)

123. Takao M., H. Aburatani, K. Kobayashi & A. Yasui: Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage. *Nucleic Acids Res* 26, 2917-2922 (1998)

124. Ichinoe A., M. Behmanesh, Y. Tominaga, Y. Ushijima, S. Hirano, Y. Sakai, D. Tsuchimoto, K. Sakumi, N. Wake & Y. Nakabeppu: Identification and characterization of two forms of mouse MUTYH proteins encoded by alternatively spliced transcripts. *Nucleic Acids Res* 32, 477-487 (2004)

125. Parker A. R., R. N. O'Meally, F. Sahin, G. H. Su, F. K. Racke, W. G. Nelson, T. L. DeWeese & J. R. Eshleman: Defective human MutY phosphorylation exists in colorectal cancer cell lines with wild-type MutY alleles. *J Biol Chem* 278, 47937-47945 (2003)

126. Chang D.-Y., Y. Gu & A.-L. Lu: Fission yeast (*Schizosaccharomyces pombe*) cells defective in the MutY-homologous glycosylase activity have a mutator phenotype and are sensitive to hydrogen peroxide. *Mol Genet Genomics* 266, 336-342 (2001)

127. Ma H., H. M. Lee & E. W. Englander: N-terminus of the rat adenine glycosylase MYH affects excision rates and processing of MYH-generated abasic sites. *Nucleic Acids Res* 32, 4332-4339 (2004)

128. Parker A., Y. Gu & A. L. Lu: Purification and characterization of a mammalian homolog of *Escherichia coli* MutY mismatch repair protein from calf liver mitochondria. *Nucleic Acids Res* 28, 3206-3215 (2000)

129. Shinmura K., S. Yamaguchi, T. Saitoh, M. Takeuchi-Sasaki, S. R. Kim, T. Nohmi & J. Yokota: Adenine excisional repair function of MYH protein on the adenine:8- hydroxyguanine base pair in double-stranded DNA. *Nucleic Acids Res* 28, 4912-4918 (2000)

130. Hirano S., Y. Tominaga, A. Ichinoe, Y. Ushijima, D. Tsuchimoto, Y. Honda-Ohnishi, T. Ohtsubo, K. Sakumi & Y. Nakabeppu: Mutator phenotype of MUTYH-null mouse embryonic stem cells. *J Biol Chem* 278, 38121-38124 (2003)

131. Einolf H. J. & F. P. Guengerich: Fidelity of Nucleotide Insertion at 8-Oxo-7,8-dihydroguanine by Mammalian DNA Polymerase delta. STEADY-STATE AND PRE-STEADY-STATE KINETIC ANALYSIS. *J Biol Chem* 276, 3764-3771 (2001)

132. Bartek J., C. Lukas & J. Lukas: Checking on DNA damage in S phase. *Nat Rev Mol Cell Biol* 5, 792-804 (2004)

133. Canman C. E.: Replication checkpoint: Preventing mitotic catastrophe. *Curr Biol* 11, R121-R124 (2001)

134. Longhese M. P., M. Foiani, M. Muzi-Falconi, G. Lucchini & P. Plevani: DNA damage checkpoint in budding yeast. *EMBO J* 17, 5525-5528 (1998)

135. Zou L. & S. J. Elledge: Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 300, 1542-1548 (2003)

136. Burtelow M. A., P. M. Roos-Mattjus, M. Rauen, J. R. Babendure & L. M. Karnitz: Reconstitution and molecular analysis of the hRad9-hHus1-hRad1 (9-1-1) DNA damage responsive checkpoint complex. *J Biol Chem* 276, 25903-25909 (2001)

137. Bermudez V. P., L. A. Lindsey-Boltz, A. J. Cesare, Y. Maniwa, J. D. Griffith, J. Hurwitz & A. Sancar: Loading of the human 9-1-1 checkpoint complex onto DNA by the checkpoint clamp loader hRad17-replication factor C complex *in vitro*. *Proc Natl Acad Sci U S A* 100, 1633-1638 (2003)

138. Ellison V. & B. Stillman: Biochemical characterization of DNA damage checkpoint complexes: clamp loader and clamp complexes with specificity for 5' recessed DNA. *PLoS Biol* 1, E33 (2003)

139. Majka J. & P. M. Burgers: Yeast Rad17/Mec3/Ddc1: a sliding clamp for the DNA damage checkpoint. *Proc Natl Acad Sci U S A* 100, 2249-2254 (2003)

140. Melo J. A., J. Cohen & D. P. Toczyski: Two checkpoint complexes are indenpendently recuited to sites of DNA damage *in vivo. Genes Dev* 15, 2809-2821 (2001)

141. Wu X., S. M. Shell & Y. Zou: Interaction and colocalization of Rad9/Rad1/Hus1 checkpoint complex with replication protein A in human cells. *Oncogene* 24, 4728-4735 (2005)

142. Brown K. D., A. Rathi, R. Kamath, D. I. Beardsley, Q. Zhan, J. L. Mannino & R. Baskaran: The mismatch repair system is required for S-phase checkpoint activation. *Nat Genet* 33, 80-84 (2003)

143. Giannattasio M., F. Lazzaro, M. P. Longhese, P. Plevani & M. Muzi-Falconi: Physical and functional interactions between nucleotide excision repair and DNA damage checkpoint. *EMBO J* 23, 429-438 (2004)

144. Lavin M. F.: The Mre11 complex and ATM: a twoway functional interaction in recognising and signaling DNA double strand breaks. *DNA Repair (Amst)* 3, 1515-1520 (2004)

145. Wang Y. & J. Qin: MSH2 and ATR form a signaling module and regulate two branches of the damage response to DNA methylation. *Proc Natl Acad Sci U S A* 100, 15387-15392 (2003)

146. Toueille M., N. El Andaloussi, I. Frouin, R. Freire, D. Funk, I. Shevelev, E. Friedrich-Heineken, G. Villani, M. O. Hottiger & U. Hubscher: The human Rad9/Rad1/Hus1 damage sensor clamp interacts with DNA polymerase beta and increases its DNA substrate utilisation efficiency: implications for DNA repair. *Nucleic Acids Res* 32, 3316-3324 (2004)

147. Friedrich-Heineken E., M. Toueille, B. Tannler, C. Burki, E. Ferrari, M. O. Hottiger & U. Hubscher: The two DNA clamps Rad9/Rad1/Hus1 complex and proliferating

cell nuclear antigen differentially regulate flap endonuclease 1 activity. *J Mol Biol* 353, 980-989 (2005)

148. Wang W., P. Brandt, M. L. Rossi, L. Lindsey-Boltz, V. Podust, E. Fanning, A. Sancar & R. A. Bambara: The human Rad9-Rad1-Hus1 checkpoint complex stimulates flap endonuclease 1. *Proc Natl Acad Sci U S A* 101, 16762-16767 (2004)

149. Smirnova E., M. Toueille, E. Markkanen & U. Hubscher: The human checkpoint sensor and alternative DNA clamp Rad9-Rad1-Hus1 modulates the activity of DNA ligase I, a component of the long-patch base excision repair machinery. *Biochem J* 389, 13-17 (2005)

150. Helt C. E., W. Wang, P. C. Keng & R. A. Bambara: Evidence that DNA damage detection machinery participates in DNA repair. *Cell Cycle* 4, 529-532 (2005)

151. Klungland A., I. Rosewell, S. Hollenbach, E. Larsen, G. Daly, B. Epe, E. Seeberg, T. Lindahl & D. E. Barnes: Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc Natl Acad Sci U S A* 96, 13300-13305 (1999)

152. Tsuzuki T., K. Yamauchi, T. Isoda, A. Egashira, S. Kura & Y. Nakatsu: Spontaneous tumorigenesis and mutagenesis in DNA repair-deficient mice-Significance of error-avoiding mechanism for oxidative DNA damge in mutagenesis. *Environ Mutagen Res* 27, 101-110 (2005)

153. Xie Y., H. Yang, C. Cunanan, K. Okamoto, D. Shibata, J. Pan, D. E. Barnes, T. Lindahl, M. McIlhatton, R. Fishel *et al.*: Deficiencies in mouse Myh and Ogg1 result in tumor predisposition and G to T mutations in codon 12 of the K-ras oncogene in lung tumors. *Cancer Res* 64, 3096-3102 (2004)

154. Shinmura K., H. Tao, M. Goto, H. Igarashi, T. Taniguchi, M. Maekawa, T. Takezaki & H. Sugimura: Inactivating mutations of the human base excision repair gene NEIL1 in gastric cancer. *Carcinogenesis* 25, 2311-2317 (2004)

155. Eliason K., B. C. Hendrickson, T. Judkins, M. Norton, B. Leclair, E. Lyon, B. Ward, W. Noll & T. Scholl: The potential for increased clinical sensitivity in genetic testing for polyposis colorectal cancer through the analysis of MYH mutations in North American patients. *J Med Genet* 42, 95-96 (2005)

156. Isidro G., F. Laranjeira, A. Pires, J. Leite, F. Regateiro, Castro e Sousa, J. Soares, C. Castro, J. Giria, M. J. Brito *et al.*: Germline MUTYH (MYH) mutations in Portuguese individuals with multiple colorectal adenomas. *Hum Mutat* 24, 353-354 (2004)

157. Tao H., K. Shinmura, T. Hanaoka, S. Natsukawa, K. Shaura, Y. Koizumi, Y. Kasuga, T. Ozawa, T. Tsujinaka, Z. Li *et al.*: A novel splice-site variant of the base excision repair gene MYH is associated with production of an aberrant mRNA transcript encoding a truncated MYH protein not localized in the nucleus. *Carcinogenesis* 25, 1859-1866 (2004)

158. Venesio T., S. Molatore, F. Cattaneo, A. Arrigoni, M. Risio & G. N. Ranzani: High frequency of MYH gene mutations in a subset of patients with familial adenomatous polyposis. *Gastroenterology* 126, 1681-1685 (2004)

159. Lai F. P., Y. Tsukada, H. Ichikawa, K. Dunster, J. W. Sentry & B. H. Toh: Autoantibody to DNA excision repair enzyme hMYH in a patient with rheumatic disease. *Clin Immunol* 99, 291-297 (2001)

160. Lin R., G. Roseborough, Y. Dong, G. M. Williams & C. Wei: DNA damage and repair system in spinal cord ischemia. *J Vasc Surg* 37, 847-858 (2003)

161. Al Tassan N., T. Eisen, J. Maynard, H. Bridle, B. Shah, C. Fleischmann, J. R. Sampson, J. P. Cheadle & R. S. Houlston: Inherited variants in MYH are unlikely to contribute to the risk of lung carcinoma. *Hum Genet* 114, 207-210 (2004)

162. Chmiel N. H., A. L. Livingston & S. S. David: Insight into the functional consequences of inherited variants of the hMYH adenine glycosylase associated with colorectal cancer: complementation assays with hMYH variants and pre-steady-state kinetics of the corresponding mutated E.coli enzymes. *J Mol Biol* 327, 431-443 (2003)

163. Pope M. A., N. H. Chmiel & S. S. David: Insight into the functional consequences of hMYH variants associated with colorectal cancer: distinct differences in the adenine glycosylase activity and the response to AP endonucleases of Y150C and G365D murine MYH. *DNA Repair (Amst)* 4, 315-325 (2005)

164. Wooden S. H., H. M. Bassett, T. G. Wood & A. K. McCullough: Identification of critical residues required for the mutation avoidance function of human MutY (hMYH) and implications in colorectal cancer. *Cancer Lett* 205, 89-95 (2004)

165. Banerjee A., W. L. Santos & G. L. Verdine: Structure of a DNA glycosylase searching for lesions. *Science* 311, 1153-1157 (2006)

166. Tominaga Y., Y. Ushijima, D. Tsuchimoto, M. Mishima, M. Shirakawa, S. Hirano, K. Sakumi & Y. Nakabeppu: MUTYH prevents OGG1 or APEX1 from inappropriately processing its substrate or reaction product with its C-terminal domain. *Nucleic Acids Res* 32, 3198-3211 (2004)

167. Ushijima Y., Y. Tominaga, T. Miura, D. Tsuchimoto, K. Sakumi & Y. Nakabeppu: A functional analysis of the DNA glycosylase activity of mouse MUTYH protein excising 2-hydroxyadenine opposite guanine in DNA. *Nucleic Acids Res* 33, 672-682 (2005)

168. Wang Y., D. Cortez, P. Yazdi, N. Neff, S. J. Elledge & J. Qin: BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev* 14, 927-939 (2000)

169. Lu,A.-L: Isolation and analyses of MutY and MutY homologs (MYH). In DNA repair (J. L. Campbell and P. Modrich, eds.), *Methods in Enzymology* 408, in press, (2006)

170. Sampson J. R., S. Jones, S. Dolwani & J. P. Cheadle: MutYH (MYH) and colorectal cancer. *Biochem Soc Trans* 33, 679-683 (2005)

Abbreviations: The 9-1-1 complex, Rad9, Rad1, and Hus1 heterotrimer complex; AP, apurimic/apyrimidinic; APC, adenomatous polyposis coli; APE1, AP endonucleaseATM, ataxia telangiectasia mutated protein; ATR, ATM- and Rad3-related protein; BASC, BRCA1-associated genome surveillance complex; BER, DNA base excision repair, Bs, Bacillus stearothermophilus; dG^oTP, 8-oxo-dGTP; dG°MP. 8-oxo-dGMP; dRPase, DNA deoxyribophosphodiesterase; Ec, Escherichia coli; Endo IV, E. coli endonuclease IV; Endo VIII (Nei protein), E. coli endonuclease VIII; ES, mouse embryonic stem cells;

Exo III, *E. coli* exodonuclease III; FCL, iron-sulfur cluster loop; FEN1, flap endonuclease 1; GO or 8-oxoG, 7,8dihydro-8-oxo-guanine; h, human; HhH, helix-hairpinhelix; hoU, 5-hydroxyuracil; IR, ionizing radiation; K_d , apparent dissociation constant; LIG1, DNA ligase 1; m, mouse; MMR, DNA mismatch repair; MSH, MutS homolog; hMutS-alpha, MSH2/MSH6 complex; hMTH1, MutT homolog; MYH or MUTYH, MutY homolog; NEIL1, NEI-like protein 1; OGG1, 8-oxoG glycosylase; PCNA, proliferating cell nuclear antigen; PI-3, phosphoinositol phosphate 3; POLbeta, DNA polymerase beta; POLdelta, DNA polymerase delta; POLepsilon, DNA polymerase epsilon; r, rat; RFC, replication factor C; RPA, replication protein A; ROS, reactive oxygen species; Sc, *Saccaromyces cerevisiae*; Sp, *Schizosaccharomyces pombe*.

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