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Review

Human MutY: gene structure, protein functions and interactions, and role in carcinogenesis

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Abstract. Faithful maintenance of the genome is crucial to the individual and the species. Oxidative DNA damage, such as 8-oxo-7,8-dihydroguanine (8-oxoG), poses a major threat to genomic integrity. 8-OxoG can mispair with 2′-deoxycytidine 5′-triphosphate or with 2′-deoxyadenosine triphosphate during DNA replication, forming C•8-oxoG and A•8-oxoG mispairs. Human MutY is responsible for recognition and removal of the inappropriately inserted adenine in an A•8-oxoG mispair. If unrepaired, the A•8-oxoG mispairs can result in deleterious C : G to A : T transversions. Human MutY functions in a postreplication repair pathway and is targeted to the newly synthesized daughter strand of DNA for removal of the adenine base. The human MutY protein is targeted to both the mitochondria and the nucleus and associates with the proliferating cell nuclear antigen, apurinic/apyrimidinic endonuclease 1, replication protein A and mutS homolog 6 proteins. Mutations in the human *MutY* gene and defective activity of the human MutY protein have been detected in cancer. A direct correlation between defective A•8-oxoG repair and increased levels of genomic 8-oxoG has now been established.

Key words. Human MutY; hMYH; base excision repair; colorectal cancer; DNA repair; 8-oxo-7,8-dihydroguanine; genomic instability.

Introduction

Oxidative DNA damage

Among the ~100 different types of modified bases produced, 8-oxo-7,8-dihydroguanine, or 8-oxoguanine (8-oxoG, or GO), is one of the most abundant mutagenic lesions formed from oxidative damage to cellular DNA (fig. 1). Although accurate measurement of this lesion is prone to artifact interference from sample oxidation, a value of between one and five 8-oxoG/10^6 guanine residues has been regarded as a baseline for human cells by the European Standards Committee on Oxidative DNA Damage (ESCODD) and another laboratory [1–2]. This oxidative damage can be caused by reactive oxygen species (ROS), contributing factors in the pathogenesis of several types of cancer and the etiologies of aging and other diseases [3–7]. ROS are formed as by-products during normal cellular metabolism, by genotoxic chemicals and by ionizing radiation to generate a variety of cytotoxic DNA lesions, such as abasic sites, single-strand breaks and modified bases with blocked 3′ termini [reviewed in 4]. Many of these damaged bases enhance mutagenesis because of mispairing during replication of the genome; indeed, a family of novel DNA polymerases has recently been discovered that incorporate deoxynucleotides opposite noninstructional or mutagenic bases, creating cytotoxic and mutagenic mispairs [8]. In the case of carcinogenesis, the mutations that arise from these mispairs inactivate tumor-suppressor genes and activate oncogenes. The mutagenic and carcinogenic potential of 8-oxoG in the stable *syn* conformation arises through its

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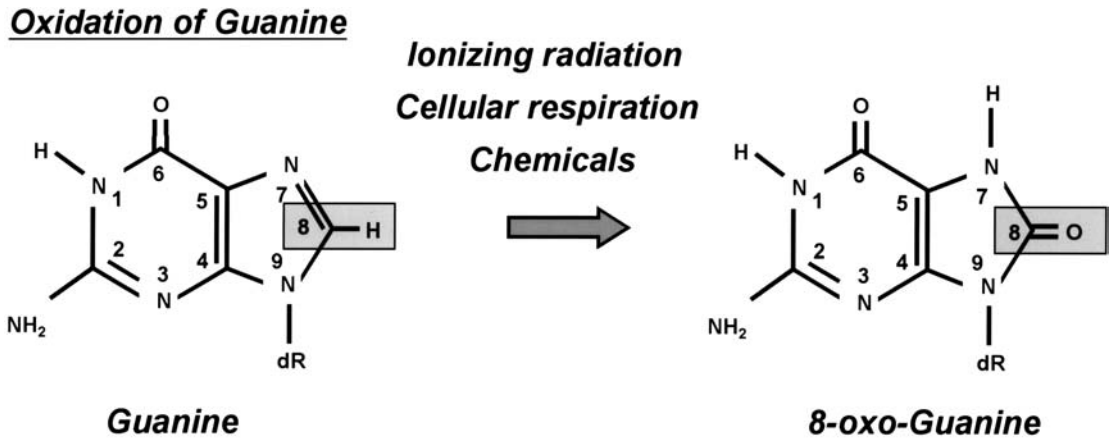

Figure 1. Structure of 8-oxoG.

readiness to bind both cytosine and adenine during replication [4]. If the C•8-oxoG intermediate is not repaired when the strands are replicated, 8-oxoG can mispair with adenine, forming an A•8-oxoG mispair that, if still unrepaired and upon further replication, may form an A:T pair in daughter cells, resulting in C:G to A:T transversions. Most significantly, if the initiating 8-oxoG lesion is not removed from the genomic DNA, it may again mispair with adenine and continue generating additional transversions.

Base excision repair and transcription-coupled repair

Base excision repair (BER) is the most important cellular process employed for removal of 8-oxoG, although both nucleotide excision repair (NER) and DNA mismatch repair (MMR) have been shown to play some role [9–11]. BER cleaves the base only, leaving the sugar-phosphate backbone intact and generating an abasic site. In *Escherichia coli* three proteins, MutY, MutM (Fpg), and MutT, the 'GO system' [12], are known to be involved in defending cellular DNA against the mutagenic effects of free 8-oxoG. In human cells the same repair system exists (fig. 2). The first level of defense is the human MutT homolog (MTH1), which has nucleoside triphosphatase activity and hydrolyzes oxidized 8-oxo-dGTP to 8-oxo-dGMP, thus functionally eliminating it from the nucleotide pool so that DNA polymerases cannot insert 8-oxoG opposite template adenine or cytosine [13] (fig. 2, step 1). Since guanylate kinase is inactive with 8-oxo-dGMP, this cannot be reutilized [14]. The second level of defense is direct repair, whereby human 8-oxoguanine DNA glycosylase protein (OGG1) removes the mutagenic 8-oxoG lesion and other oxidized purines incorporated opposite cytosines [15, 16] (fig. 2, step 2). Finally, if replication occurs, the human MutY DNA glycosylase protein removes adenine from A•8-oxoG mismatches with its adenine glycosylase activity (fig. 2, step 3) [17].

Inactivation of the *MutY* gene in *E. coli* results in a dominant base transversion mutator phenotype with 97% of transversions being C:G to A:T [18]. *MutY*-defective *E. coli* exhibit significant increases in their mutation rates (~30-fold increase in *Lac*+ reversion) (table 1), which are corrected by transformation with either *E. coli MutY* or human *MutY* cDNAs. *MutY*-defective *Schizosaccharomyces pombe MutY* also exhibits a mutator phenotype, with an ~36-fold increase in the spontaneous mutation frequency (table 1, [19]), which again was corrected by transformation with either human *MutY* or *S. pombe MutY*. The spectrum of transversions in combination with the elevated mutation rate, which can be complemented by human *MutY*, supports the notion that the *MutY* gene products protect the genome from the deleterious effects of 8-oxoG [18–20].

In quiescent G_O/G_1 cells, which do not undergo DNA replication, transcription of critical genes occurs continuously, and repair of damage in the transcribed strand must therefore be faster than in the areas of chromatin that are transcriptionally inactive. This requires additional proteins, and it has recently been shown that this transcription-coupled repair (TCR) of 8-oxoG involves BRCA1 and BRCA2 (breast cancer-associated protein), xeroderma pigmentosum group G protein (XPG) and Cockayne syndrome B protein (CSB), among others [21, 22].

Human MutY protein

The human *MutY* cDNA has been cloned and sequenced, as has that from rat and mouse [17, 23, 24], although the majority of studies to date have centered on human *MutY*. The human *MutY* gene produces up to 10 alternatively spliced transcripts [25] and encodes multiple forms of the human MutY protein, which are targeted to both the mi-

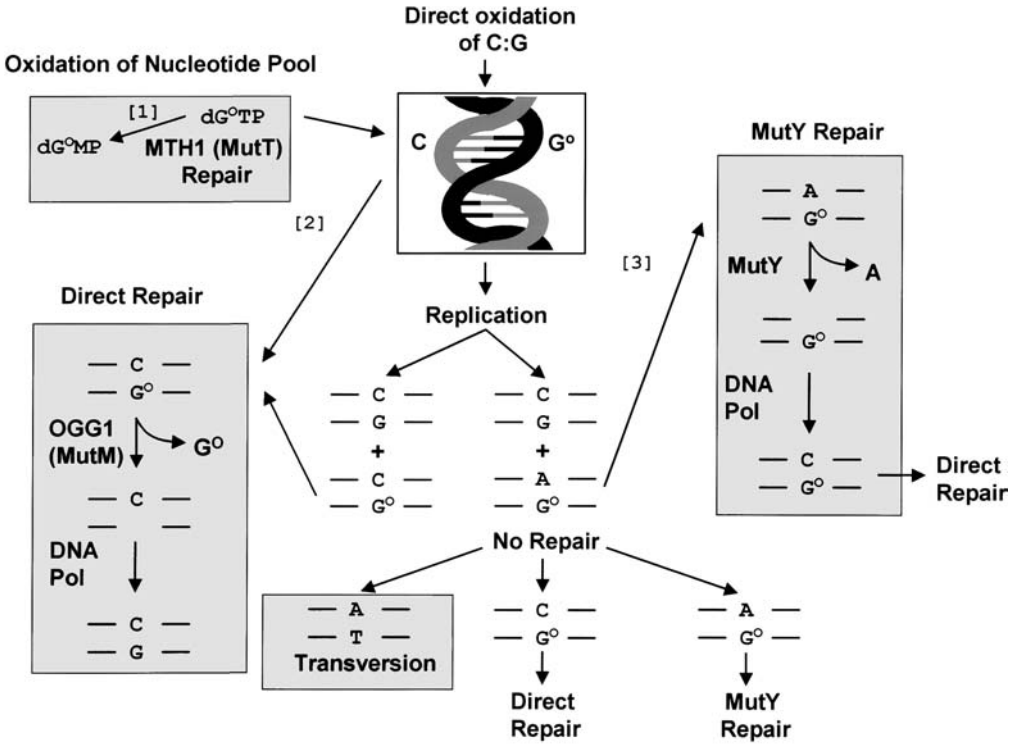

Figure 2. Base excision repair of 8-oxoG using the 'G^{O} system'. Oxidation of guanine leads to the formation of 8-oxoG in genomic DNA, converting the C : G base pair to a C•8-oxoG mispair. During replication mutagenic dG^{O}TP can also be incorporated opposite template cytosine if dGTP in the nucleotide pool becomes oxidized. MTH1 is the enzyme responsible for converting dG^{O}TP to the nonmutagenic dG^{O}MP (step 1). The C•8-oxoG mispair is directly repaired by OGG1 (step 2), but if not, 8-oxoG can mispair with adenine during replication (this does not preclude binding again with cytosine). Human MutY removes adenine from the A•8-oxoG mispair (step 3), and after subsequent processing a polymerase inserts a cytosine opposite 8-oxoG. Depending on the polymerase, an adenine can also be inserted opposite 8-oxoG. If the C•8-oxoG and A•8-oxoG mispairs are not repaired, then upon replication not only can the adenine pair with thymine, resulting in a deleterious C : G to A : T transversion, but the 8-oxoG base will not be removed from the genome. The 8-oxoG base can again mispair with adenine and cytosine, and thus the mutagenic cycle could begin again.

Table 1. Mutation rates in *MutY*-deficient bacteria and yeast complemented with eukaryotic *MutY*.

Sample	Mutant/corrected	Mutation rate	Target	References
Bacteria				
E. coli CC104 *MutY*	mutant	~20–30-fold increase	*Lac*+ revertants	[20, 26]
	mutant	~29–52-fold increase	Rif resistance	[20, 26]
with human *MutY*	corrected	~2–3-fold increase	*Lac*+ revertants	[26]
	corrected	~1-4-fold increase	Rif resistance	[26]
E. coli GBE943/DE3 *MutY*	mutant	~42-fold increase	Rif resistance	[61]
with *S.pombe MutY*	corrected	~3-fold increase	Rif resistance	[61]
Yeast				
*S. pombe MutY*Δ	mutant	~36-fold increase	FOA resistance	[94]
with *S.pombe MutY*	corrected	~1-fold increase	FOA resistance	[94]
with human *MutY*	corrected	~8-fold increase	FOA resistance	[94]
with human *MutY* (F518A/F519A)	mutant	~42-fold increase	FOA resistance	[94]

Rif, rifampicin, FOA, 5-fluoroorotic acid; *Lac, LacZ* gene.

tochondria and the nucleus [26–28]. It has been demonstrated both in vitro and in vivo that human MutY directly interacts with several proteins involved in other DNA damage-repair pathways: proliferating cell nuclear antigen (PCNA), apurinic/apyrimidinic endonuclease 1 (APE1), replication protein A (RPA) and mutS homolog 6 (MSH6) [29, 30]. Defective human MutY has recently been implicated as a possible causative agent in several types of cancer. This review encompasses and gathers recent literature to focus on the essential role of human MutY in protecting genomic integrity against the detrimental effects of oxidative DNA damage.

Structure of human *MutY* gene and human MutY protein

Human *MutY* gene and multiple isoforms of human *MutY* mRNA

Mammalian *MutY* cDNAs have been isolated from human, rat and mouse [17, 23, 24]. The human *MutY* gene contains 16 exons and is localized to the short arm of chromosome 1 between p32.1 and p34.3 [17]. Ohtsubo et al. showed that the human *MutY* gene produces three major classes of human *MutY* mRNA transcripts [25] – α, β and γ – each of which is also alternatively spliced, suggesting a total of 10 possible mature transcripts of human MutY (table 2). The existence of these various isoforms, however, remains to be confirmed in normal tissues.

The isoform human MutYα has the same 5′ sequence as the primary reference cDNA [17] and possesses four splice variants: human MutYα1, 2, 3 and 4. Human MutYα1 has a 33bp insertion (11 amino acids), while human MutYα2 has just a 3bp insertion. Human MutYα3 is the major isoform expressed in most cells and corresponds to the cDNA sequence isolated and characterized by Slupska et al. (type 1-human MutY [17]). The splice variants human MutYα1, 2 and 3 probably localize to the mitochondria, whereas human MutYα4 probably localizes to the nucleus (table 2). We recently reported that in 10 microsatellite-stable (MSS) colorectal cancer (CRC) cell lines in which the level of 8-oxoG was normal, the major primary transcript (MutYα3 isoform) was present. In addition, in one of the MSS CRC cell lines the human MutYα4 isoform was also present, whereas in another four MSS CRC cell lines, the human MutYα2 isoform was also present [31].

Human MutYβ isoform has three splice variants – human MutYβ1, 3 and 5 – which are all translated from the second AUG, 42 bases downstream from the initial AUG (table 2). All three human MutYβ variants share a common sequence at the 5′ end of their cDNAs, which is identical to that reported by Takao et al. (type 2-human MutY), with human MutYβ1 containing the same 33bp insert as human MutYα1 [26]. Human MutYγ also has three splice variants, human MutYγ2, 3 and 4. Human MutYγ3 and human MutYγ4 are similar to isoforms human MutYβ3 and human MutYα4, respectively (table 2), with human MutYγ2 and human MutYγ3 differing by the position of the third AUG. Similar to the splice variant human MutYα4, and all human MutYβ isoforms, the human MutYγ variants are also probably localized to the nucleus (table 2). However, although these transcript variants are evident, which transcript encodes which polypeptide, the relative distribution of the isoforms, immunological detection and the function of the different human MutY isoforms are still somewhat open questions.

Table 2. Human MutY Isoforms.

	Isoform	Amino acids	AUG 1, 2 or 3	Probable cellular location	Comments
Type 1	MutYα1	546 aa	1	mitochondira	33bp insert
	MutYα2	536 aa	1	mitochondira	3bp CAG insert
	MutYα3[a]	535 aa	1	mitochondira	same as Slupska et al. [17]
Type 2	MutYα4	429 aa	3	nucleus	similar to MutYγ4 isoform
	MutYβ1	532 aa	2	nucleus	same 33-bp insert as MutYα1
	MutYβ3	521 aa	2	nucleus	similar to MutYγ3 isoform
	MutYβ5	521 aa	2	nucleus	
	MutYγ2	522 aa	2	nucleus	
	MutYγ3	521 aa	2	nucleus	similar to MutYβ3 isoform
	MutYγ4	429 aa	3	nucleus	similar to MutYα4 isoform

[a] Reference Sample.
AUG 1, 2 or 3, from which translation starts; NLS, residues 505–509; MLS, residues 1–14.
Data adapted from Ohtsubo et al. [25].
cDNA not drawn to scale.

Human MutY structure

The open reading frame of full-length human *MutY* (MutYα3 isoform) (table 2) translates into a 535-amino acid (aa) protein that exhibits 78.0% identity with mouse MutY [24], 74.1% with rat MutY [23] and 41% with *E. coli* MutY proteins [17]. Both mouse MutY and rat MutY lack the first 14 amino acids that are found in type 1 human MutY (see below), such that translation starts with the second methionine (fig. 3) [17, 23–27]. This means that mouse MutY and rat MutY are both homologs of type 2-human MutY or the human MutYβ isoform [25, 26].

Although most structural studies have used *E. coli* MutY [32–36], sequence analyses of the mammalian MutY proteins reveal extensive homology and conservation with established structural domains found in *E. coli* MutY and many prokaryotic and eukaryotic base excision DNA repair proteins, the so-called helix-hairpin-helix Glu/Pro…..Asp domain (HhH-GPD) superfamily (fig. 3) [32, 37–40].

The HhH-GPD in *E. coli* MutY (aa 114–273 in human MutY) binds the phosphate backbone of the substrate and includes the highly conserved Asp138 in *E. coli* MutY (Asp222 in human MutY, aa 207 in both mouse MutY and rat MutY), which is required for nucleophilic attack of the adenine base. Human MutY, like rat MutY and mouse MutY, also contains the conserved distal MutT-like (NUDIX) domain (aa 354–486) and the iron-sulfur cluster binding domain (aa 276–292), a hallmark of the endonuclease III (endo III) family of proteins. Mammalian MutY also contains a conserved adenine recognition motif (aa 255–273 in human MutY). The crystal structure of *E. coli* MutY suggests that residues Gln182, Glu37 and Asp186 (aa Gln266, Glu120 and Glu270 in human MutY) are important for binding the adenine base. For binding the adenine partner, the major components reside in the C-terminal of *E. coli* MutY [32, 34, 36], but the N-terminal residues Gln41, Tyr82 and Arg194 are also required (aa Gln124, Tyr165 and Pro278 in human MutY). Interestingly, although the above residues and charges are generally conserved among mammalian MutY, the Arg194 position in *E. coli* MutY is instead a proline residue in human, rat and mouse (Pro278, Pro263 and Pro263, respectively). Both mouse MutY and rat MutY contain the PCNA binding motif and the putative APE1 and MSH6 binding domains (aa 509–527, 295–317 and 232–254 in human MutY) but are missing the first seven amino acids of the putative RPA binding domain (aa 8–31 in human MutY) (see below) [29, 30]. *E. coli* MutY appears to contain only a putative MSH6 binding domain, although any physical interaction between *E. coli* MutY and MutS is as yet unproven. Mouse and rat MutY both possess the nuclear localization signal (NLS) but not the mitochondrial localization signal (MLS) (see below).

Subcellular localization of human MutY

Originally, the activities of human MutY were studied in nuclear fractions [41]; however, in the mitochondria, 8-oxoG is a very abundant DNA lesion formed by exposure to ROS [42]. Since the oxidative environment of these organelles creates unfavorable conditions for DNA stability, and unlike nuclear DNA, the mitochondrial genome is not protected by histone proteins, it was proposed that the mitochondria must possess highly effective means of repairing oxidative DNA damage frequently generated in their genomes. The accumulation of oxidative DNA lesions and alterations in mitochondrial DNA (mtDNA) have been implicated in the process of aging and in several human diseases, such as carcinogenesis [3–7]. Recent studies have indicated that the mitochondria in fact contain functional base excision repair pathways responsible for the removal of oxidatively damaged DNA [43–45] and that some mammalian MutY activities were localized exclusively to the mitochondria [28]. However, the subcellular location and amount of endogenous human MutY proteins in human cells are still controversial. Elegant studies by Takao et al. showed that transfection of the full-length human *MutY* cDNA (535 aa) into COS-7 cells, HeLa cells and CHO-9 cells clearly resulted in localization of the human MutY protein to the mitochondria (type 1-human MutY) with relatively little localization to the nucleus [45]. Using immunohistochemistry and confocal microscopy, Boldogh et al. also showed that nuclear staining of human MutY was faint in HeLa cells and that most of the human MutY protein was localized to the cytoplasm [46]. However, in the same study a western blot of human MutY protein using isolated organelles suggested that more human MutY was present in the nuclear fraction than in the mitochondrial fraction. A putative N-terminal MLS (aa 1–14) and a putative C-terminal NLS (aa 505–509) have been suggested (fig. 3), and the NLS was shown to be less effective than the MLS as a protein transport signal [45]. Further studies showed that nuclear localization of the human MutY protein arose by alternative splicing, and indeed, a truncated human MutY clone missing the first 14 amino acids localized to the nucleus (type 2-human MutY)[26]. However, using similar approaches, other investigators have suggested that type 1-human MutY is in fact localized mainly to the nucleus, excluding the nucleoli [27].

It has been reported that a single protein band of human MutY protein was detected in whole cell lysates by western blot analysis [47]. However, in contrast to these results, two electrophoretically distinct human MutY isoforms have been detected in whole cell lysates of several cancer cell types by Western blot analysis [46]. The higher molecular weight band was suggested to be the type 1-human MutY protein distributed in the mitochondria, whereas the lower molecular weight band was suggested to be the type 2-human MutY protein localized to

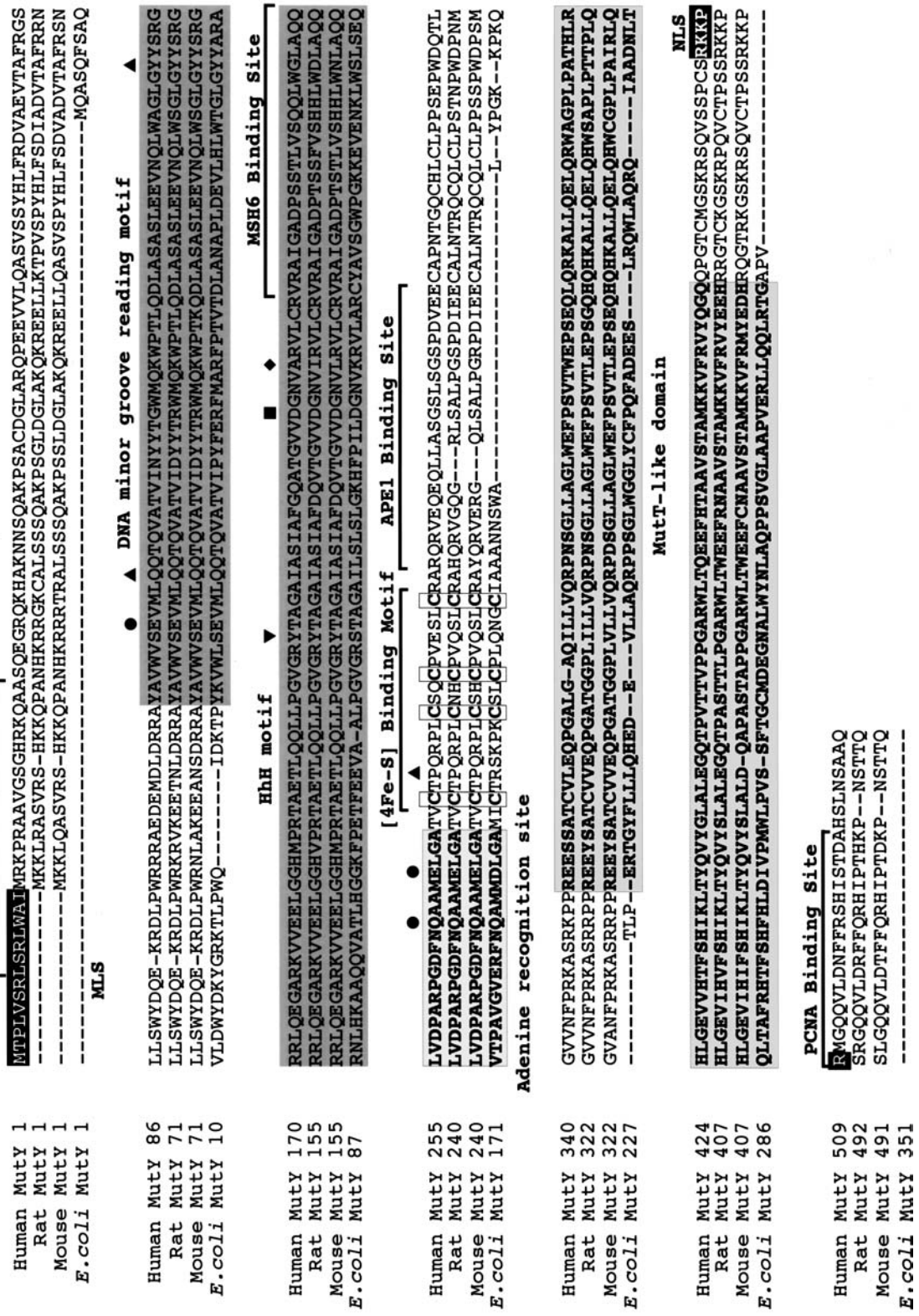

Figure 3. Alignment of the human MutY amino acid sequence (AAH03178) with rat MutY (AF478683), mouse MutY (AAG16632) and *E. coli* MutY (B38535) sequences. Numbering refers to amino acid number. The alignment to human MutY sequence shows key conserved structural domains: HhH-GPD (helix-hairpin-helix domain) (aa 114–273), an adenine recognition motif (aa 255–273), an iron-sulfur cluster binding domain (aa 276–292) and the MutT-like (NUDIX) domain (aa 354–486). The protein interaction sites for PCNA (aa 509–527), APE1 (aa 295–317), MSH6 (aa 232–254) and RPA (aa 8–31), as well as the putative mitochondrial (MLS, 1–14) and nuclear targeting signals (NLS, 505–509, black-shaded), are also shown. Shaded diamond, *E. coli* MutY lysine for Schiff-base formation; shaded triangle, aa required for binding adenine partner (usually 8-oxoG or guanine); shaded circle, aa required for binding adenine; shaded square, Asp138 in *E. coli* MutY; shaded inverted triangle, conserved serine in *E. coli* MutY. Data adapted from Lee et al. [23] and reproduced from the *Journal of Neurochemistry*, with permission.

the nucleus. In another study, one 57-kDa polypeptide was detected in the mitochondrial fraction, and two protein bands, 52 kDa and 53 kDa, were detected in the isolated nuclei of human Jurkat cells [25], although differences exist between experimental and predicted molecular masses of the isoforms. Variations among these reports may be due to differences among epitopes of human MutY, and the antibodies raised against them, as well as differences in the methods of sample preparation and in the samples analyzed.

DNA repair enzymes such as MSHα have been shown to translocate to the nucleus upon stimulation with various genotoxic chemicals [48]. It is plausible that since mitochondrial DNA has a higher rate of oxidative damage, human MutY is predominately located to the mitochondria to combat this, whereas upon cellular insult, de novo synthesis of human MutY isoforms or translocation of presynthesized human MutY to the nucleus may occur. In this respect, although human MutY has been mainly localized to the cytoplasm, at the G_1/S boundary human MutY is predominately localized to the nucleus, suggesting that human MutY expression and localization may be redistributed [46]. The subcellular localization and immunological detection of human MutY proteins, however, are contentious and remain to be definitively resolved.

Catalytic mechanism and substrate specificity of human MutY

E. coli MutY and human MutY are unique DNA glycosylases

In the bacterial system, the endoIII superfamily of DNA glycosylases are divided into two groups: monofunctional and bifunctional glycosylases. Bifunctional glycosylases, in addition to their glycosylase activity, possess strong apurinic/apyrimidinic (AP) lyase activity that cleaves the phosphodiester bond 3′ to the AP site by the reaction of β-elimination [49], whereas in monofunctional glycosylases this AP lyase activity is absent. Bifunctional DNA glycosylases, such as endoIII, can also use a highly conserved lysine (Lys120 in endoIII) to form a Schiff-base intermediate that can be covalently trapped as a protein-DNA complex in the presence of sodium borohydride [50, 51]. Monofunctional glycosylases, such as AlkA, lack this conserved lysine [39, 52].

E. coli MutY represents a unique DNA glycosylase since, although it possesses a serine residue at conserved position 120 (Ser120) [32], qualifying it as a monofunctional glycosylase, some laboratories have reported weak AP lyase activity [34, 53–55]. Furthermore, the ability to form a covalent Schiff-base intermediate with its DNA substrates has also been reported [34, 56, 57]. In contrast, human MutY does not possess either the conserved lysine or a serine-like *E. coli* MutY, but instead possesses a tyrosine residue (Tyr204), as do both mouse MutY and rat MutY (Tyr189 in both). In *E. coli* MutY, Lys142 is the base responsible for Schiff-base formation [57, 58], but at the corresponding position in human MutY (aa 226), there is an alanine (Leu211 in mouse MutY and Ile211 in rat MutY). Human MutY also appears not to possess detectable AP lyase activity [59].

Catalytic mechanism

It has been proposed that before *E. coli* MutY can catalyze cleavage of the glycosyl bond, the adenine base is first 'flipped' out of the DNA helix and sequestered into the active site [32]. Asp138 activates a water molecule by proton abstraction attacking the C1 carbon of the adenosine, releasing the mispaired adenine base. The controversial AP lyase activity seen in vitro could therefore arise from a reaction between the remaining abasic deoxyribose molecule and the nearby nucleophilic ε-amino group of activated Lys142. The attack by Lys142 could form a Schiff-base intermediate. An alternative mechanism of adenine removal has been proposed whereby Asp138 deprotonates the ε-amino group of Lys142, directly forming a nucleophile that attacks the C1 carbon and displaces the adenine base. This could also account for the observed Schiff-base intermediate [49, 58].

Substrate analysis

The rate of spontaneous mutation in *MutY*-deficient *E. coli* and *MutY*-deficient *S. pombe* is 30–40-fold higher than that observed in the wild-type organisms, with the vast majority of mutations being C:G to A:T transversions [18, 19]. The major role of *E. coli* MutY and mammalian MutY proteins is the removal of adenine from opposite mutagenic 8-oxoG so that this transversion does not occur, and to date, all isolated MutY proteins from bacteria and yeast perform this function, as well as excision of adenine mispaired with guanine [12, 60, 61]. As expected, human MutY readily cleaves adenine from the mutagenic A•8-oxoG mispair [25, 26, 59], supporting its role of protecting the genome from oxidative damage (table 3). Unexpectedly, however, there appears to be a length dependence restricting in vitro cleavage of the A•G duplex. Human MutY cleaves A•G if the duplex length is 35–45 bp but not if the length is 23 bp [59]. This length dependence does not exist for the A•8-oxoG mispair. The lack of activity of human MutY with an A•G mispair in a 23-bp duplex is unpredicted, as *S. pombe* MutY [61] and mouse MutY [59] both cleave a 20- or 23-bp duplex containing the A•G mismatch. This may reflect different affinities for this A•G mismatch among closely related species, as observed with methylpurine-DNA glycosylase from mice and humans [62]. *E. coli* MutY and to a lesser extent *S. pombe* MutY also cleave adenine from an

Table 3. Human MutY substrate specificity.

Pair/Mispair	Human MutY	*E. coli* MutY	References
A•A	N	Y[b]	[25, 59, 149]
A•C	N	Y	[12, 25, 53, 59]
A•G	Y[a]	Y	[12, 25, 53, 59]
A:T	N	N	[25, 53, 59]
C•C	N	ND	[59]
C:G	N	N	[53, 59]
C•T	N	ND	[59]
G•T	N	ND	[59]
T•T	N	ND	[59]
A•8-oxoG	Y	Y	[12, 25, 59, 150]
C•8-oxoG	N	N	[59, 150]
G•8-oxoG	Y[b]	Y	[59, 150]
T•8-oxoG	N	N	[59, 150]
2-OH-A•A	Y	N	[25, 149]
2-OH-A•C	Y	N	[25, 149]
2-OH-A•G	Y	Y	[25, 149]
2-OH-A•T	Y	N	[25, 149]

[a] Duplex length > 23 bases.
[b] Very slight cleavage.
ND, not determined; Y, yes; N, no.

A•C mismatch [61], but human MutY and Jurkat-cell extracts exhibited no glycosylase activity toward the A•C mispair [25, 59]. Surprisingly, the partially purified calf MutY has been reported to possess A•C repair activity, although this could be due to some sample contaminants [63]. These subtle differences in catalytic activity may reflect relative frequencies of mispair formation, suggesting that A•8-oxoG is the major mispair formed. Human MutY has also been shown to possess weak guanine glycosylase activity toward the G•8-oxoG mispair, although no glycosylase activity was detected toward pyrimidines mispaired with 8-oxoG [59].

In *E. coli* and mammalian cells, 2-hydroxyadenine (2-OH-A) is a highly mutagenic DNA lesion [64, 65]. Human MutY has recently been found to remove 2-OH-A from opposite all four natural bases as well as 8-oxoG [25], further illustrating the crucial role of human MutY in preserving genomic integrity.

Mitochondrial type 1-human MutY versus nuclear type 2-human MutY

Shinmura et al. cloned the two types of mitochondrial type 1-human MutY (Q324 and H324) as well as the two types of nuclear type 2-human MutY (Q310 and H310) for functional studies [66]. The rate of incision of adenine from an A•8-oxoG mispair was similar between the type 1-Q324 and 1-H324 proteins as well as between the type 2-Q310 and 2-H310 proteins, showing that the polymorphism had no major effect on glycosylase activity. Takao et al. also reported that both human MutY polymorphs (presumably type 1-Q324 and type 2-Q310) shared the same substrate specificities with 8-oxoG containing mispairs in their in vitro transcription/translation (TNT) protein expression system [26].

A native mammalian MutY has been isolated from calf-liver mitochondria, and similar to previous reports [26, 66], this MutY possessed adenine glycosylase activity toward the A•8-oxoG mispair [28], with very weak guanine glycosylase activity toward G•8-oxoG. It remains to be seen what the true specificities of nuclear and mitochondrial human MutY are, since the studies to date use unprocessed type 1-human MutY with the MLS still attached and the isolated calf mitochondrial MutY may be a degraded form of a larger calf mitochondrial MutY.

Human MutY protein interactions

Repair of nucleotides with base excision

After removal of the mispaired base by a DNA glycosylase (adenine or adenine derivatives in the case of human MutY), there are two alternative pathways for repair of the cytotoxic and mutagenic unprocessed abasic sites: a short-patch repair pathway (1 nucleotide) and a long-patch repair pathway (2–6 nucleotides) (fig. 4) [67; reviewed in 68 and 69]. It has been suggested that the type of DNA glycosylase determines the appropriate patch-size repair pathway [70, 71]; for example, repair by 3-methyladenine DNA glycosylase in HeLa cell extracts occurs via both short- and long-patch pathways, whereas repair of 8-oxoG by OGG1 and the repair of thymine or uracil opposite guanine by thymine DNA glycosylase (TDG) are mainly via the short-patch pathway [70–71].

After removal of the target base, there are two subpathways responsible for short-patch repair of the resulting AP site. The phosphodiester bond 5′ to the AP site is cleaved by APE1 if the DNA glycosylase is monofunctional and is then further processed by β-elimination of the 5′ deoxyribose phosphate residue catalyzed by polβ dRPase activity [72]. If the glycosylase is bifunctional, its AP lyase activity cleaves the phosphodiester bond 3′ to the AP site, with APE1 generating a 3′ hydroxyl group for DNA synthesis. Whether the glycosylase is monofunctional or bifunctional, the final two stages of short-patch repair involve the same enzymes. The DNA gap (1 nucleotide) is filled by polβ and then ligated by DNA ligase III and XRCC1 [69]. Since polβ binds DNA ligase I [73], and polβ and polδ can replace each other [74, 75], it has been suggested that both DNA ligase I and polδ may also function in short-patch repair. Likewise, the poly(ADP-ribose) polymerase-1 (PARP-1) may also play a role in short-patch repair, as it binds XRCC1 [76]; in fact, it has been proposed that XRCC1, DNA ligase III, pol β and PARP-1 may exist as a single multiprotein complex [77].

The long-patch repair pathway (fig. 4) repairs a 2–6 nucleotide patch and is dependent on the PCNA. In addition to a DNA glycosylase and APE1, flap endonuclease 1

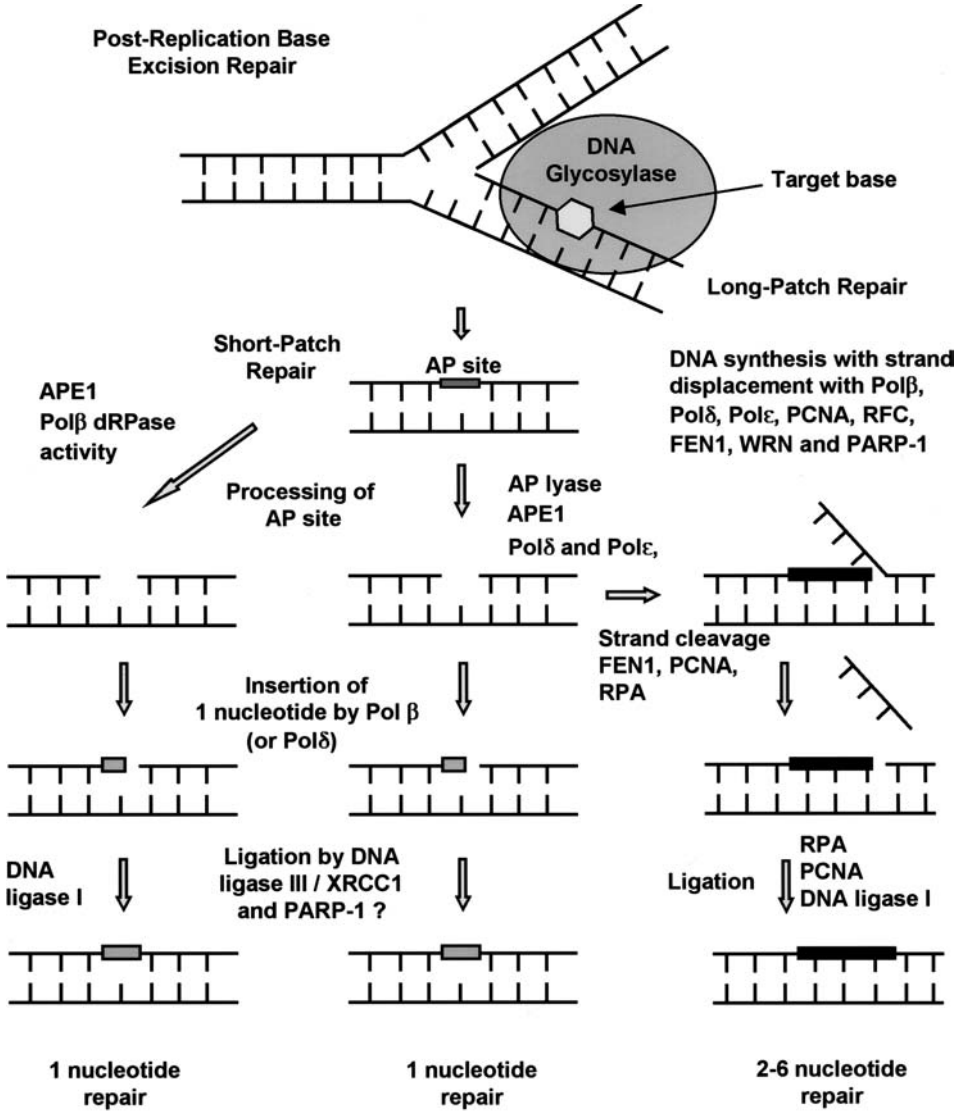

Figure 4. Removal of oxidative DNA damage with postreplication base excision repair. A glycosylase removes the target base, leaving the sugar-phosphate backbone intact. For short-patch repair of the resulting AP site, there are two sub-pathways. The phosphodiester bond 5′ to the AP site is cleaved by APE1 if the DNA glycosylase is monofunctional and is then further processed by the DNA deoxyribophosphodiesterase activity (dRPase) of polβ. If the glycosylase is bifunctional, then the AP lyase activity cleaves the phosphodiester bond 3′ to the AP site, and the resulting DNA with 3′ unsaturated aldehyde is processed by APE1, generating a 3′ hydroxyl group for DNA synthesis. Whether the glycosylase is monofunctional or bifunctional, the final two stages of short-patch repair involve the same enzymes. The DNA gap (1 nucleotide) is filled by polβ (or polδ) and then ligated by DNA ligase III (or possibly DNA ligase I) and XRCC1 in a process that may involve PARP-1. After the AP site has been processed by APE1 in long-patch repair, polβ, polδ, polε, PCNA and RFC are recruited to repair a nucleotide patch of 2–6 bases. The polymerases can be stimulated by FEN-1, WRN and PARP-1 to synthesize DNA and generate an oligonucleotide flap in the process. RPA and FEN-1 are then localized to this site, where both PCNA and RPA stimulate FEN-1 to cleave the flap structure. The newly synthesized DNA is then ligated with DNA ligase I. Data adapted with permission from Krokan et al. [69] and *FEBS Letters*.

(FEN-1), DNA polymerases β [78], δ and ε (polβ, pol δ and pol ε) and DNA ligase I are also involved [75]. Neither the DNA glycosylase or the AP endonuclease can remove a 5′ sugar phosphate and generate a 1-nucleotide gap, so to remove the 5′ sugar phosphate, the DNA polymerase first adds several nucleotides to the 3′ end of the nick and exposes the 5′ sugar phosphate as part of a single-stranded flap structure. For polδ to synthesize more than 1–2 nucleotides, FEN-1, PCNA and replication factor C (RFC) are required [79, 80]. Along with FEN-1, and more recently the Werner syndrome defective gene product (WRN), PARP-1 activates Polβ by increasing polβ strand displacement DNA synthesis [81–84]. This flap structure is recognized and excised by FEN-1 in a reaction stimulated by PCNA [85], and the newly synthesized DNA is finally ligated by DNA ligase I [75]. Recent evi-

dence from MutY protein interactions has suggested that MutY repair of A•8-oxoG involves long-patch BER.

Proliferating cell nuclear antigen

PCNA forms a homotrimer with a torus structure, allowing double-stranded DNA to pass through the inside cavity. Loading of PCNA onto DNA requires RFC, resulting in a PCNA/RFC complex known as the 'PCNA clamp' [86]. The formation of this PCNA clamp is a prerequisite for efficient DNA synthesis activity of pol δ and pol ε, but not pol α or β. As well as DNA replication [87, 88], PCNA plays an essential role in nucleotide excision repair [89, 90], base excision repair, mismatch repair, branch structure processing, cell cycle control and chromatin assembly [91–93]. In addition to a close association with DNA replication, the adenine-specific glycosylase activity of human MutY must be specifically targeted to the newly synthesized nascent DNA strand, suggesting that there may be some protein–protein interactions between human MutY and those involved in DNA replication.

In coimmunoprecipitation experiments, PCNA was initially found to associate with human MutY [29]. Boldogh et al. provided in situ colocalization evidence demonstrating that during S-phase, human MutY is specifically found at the replication foci and in close association with PCNA, suggesting that postreplicative repair of adenine, mispaired with either guanine or 8-oxoG, is associated with DNA replication [46]. Using in vitro binding studies, PCNA was shown to bind to the C-terminal region of human MutY (residues 505–527) [29]. A comparison of the human MutY amino acid sequence with that of the PCNA binding sites of other PCNA-binding proteins identified a highly conserved PCNA-binding motif, ***Q***QV***L***DN***FF,*** at residues 512–519 of human MutY (table 4, positions 1–8) [29, 91]. Generally, this motif contains a glutamine at position 1, an aliphatic residue such as leucine, isoleucine, or methionine at position 4, and a pair of aromatic residues, phenylalanine or tyrosine, at positions 7 and 8. The residues flanking the conserved motif also show a preponderance of proline residues and charged residues. Alignment of mammalian MutY protein sequences from mouse and rat with human MutY showed that putative PCNA binding sites also exist (table 4).

Binding experiments showed that *S. pombe* MutY and *S. pombe* PCNA interact in yeast [94], and although *S. pombe* MutY contains the conserved leucine (Leu441, Leu515 in human MutY) at position 4 as well as phenylalanine (Phe 444, Phe518 in human MutY) at position 7 within the PCNA binding motif, it does not contain either a glutamine at position 1 or a phenylalanine at position 8. This indicates that in yeast, the glutamine at position 1 and the phenylalanine at position 8 are dispensable for the *S. pombe* MutY and the *S. pombe* PCNA interaction. Several other PCNA-binding proteins also lack this conserved glutamine [95], although this residue has been reported as being essential for the interaction between 5′-methylcytosine DNA methyltransferase and PCNA [96]. Although position 8 of the *S. pombe* MutY PCNA binding domain is not an aromatic residue, the two phenylalanine residues at positions 7 and 8 of the PCNA-binding motif are essential for the interaction between human MutY and human PCNA as well as that of PCNA with FEN-1 and DNA ligase I [29, 97]. One factor that may en-

Table 4. Protein binding motifs in human MutY.

Interacting Protein	Protein	Organism	Position	Binding Motif
PCNA	hMutY	*H.sapiens*	509	
	rMutY[a]	*R.norvegicus*	492	
	mMutY[a]	*M.musculus*	491	
	spMutY	*S.pombe*	435	
APE1	hMutY	*H.sapiens*	295	
	rMutY[a]	*R.norvegicus*	280	
	mMutY[a]	*M.musculus*	280	
	polβ	*H.sapiens*	178	
RPA	hMutY	*H.sapiens*	8	
	rMutY[a]	*R.norvegicus*	1	
	mMutY[a]	*M.musculus*	1	
	XPA	*H.sapiens*	23	
MSH6	hMutY	*H.sapiens*	232	
	rMutY[a]	*R.norvegicus*	217	
	mMutY[a]	*M.musculus*	217	

Black boxes are conserved residues an grey boxes highlight abundant charged amino acids. [a]Putative binding site. Has not been shown experimentally. h, human; r, rat; m, mouse; sp, S.pombe; polβ, DNA polymerase β; XPA, Xeroderma pigmentosum protein A.
GenBank no. hMutY, AAH03178; rMutY, AF478683; mMutY, AAG16632; spMutY, Z69240; polβ, PO6746; XPA, P23025. Data adapted with permission from [29, 30].

hance the interactions could be the presence of clusters of charged residues flanking the conserved motif.

Experiments demonstrating that human MutY protein expression is increased in S-phase [46] and that repair of an A•8-oxoG mispair in a nonreplicating shuttle vector system is poor in mammalian cells suggest that human MutY-catalyzed repair may be replication-dependent [98]. Using an in vivo repair system, Hayashi et al. showed that DNA replication enhances the repair of the A•8-oxoG mispair and that the efficiency of replication-coupled repair was 10-fold lower in MutY-deficient murine cells than in MutY-proficient cells [99]. In MutY-deficient murine cells, expression of a mutant mouse MutY (Phe500Ala/Phe501Ala) in which the PCNA-binding motif was disrupted did not increase the repair efficiency of A•8-oxoG, suggesting that the interaction between PCNA and mouse MutY is critical for MutY-initiated replication-coupled repair of A•8-oxoG. Similar results were obtained in yeast where the human MutY mutant Phe518Ala/Phe519Ala and the *S. pombe* MutY mutant Phe444Ala, both of which cannot dock with PCNA, could not reduce the mutation frequency in an *S. pombe* MutY-deficient strain [94]. It will be interesting to see if PCNA can stimulate human MutY glycosylase activity as it does with other interacting proteins (e.g., FEN-1).

Docking of human MutY onto PCNA couples human MutY adenine excision not only to the replication machinery but also to the PCNA-dependent long-patch BER pathway. This may explain the paradox that human MutY can discriminate and thus be directed to repair misincorporated adenines on the daughter strand only. It has been suggested that PCNA may act as a molecular adaptor, coordinating and regulating the actions of DNA replication, DNA repair and cell cycle control. The mechanism by which PCNA selects the appropriate partners remains unclear.

Apurinic/apyrimidinic endonuclease 1

Human MutY catalyzes hydrolysis of the *N*-glycosylic bond of the mispaired adenine base, generating an AP site opposite the 8-oxoG base (AP•8-oxoG). Subsequent steps to repair this AP site are initiated by another BER enzyme, APE1 [100]. APE1 cleaves the sugar-phosphate backbone 5′ to the AP site, yielding a 5′-deoxyribose phosphate and 3′-hydroxyl nucleotide free ends. This abnormal abasic residue is later removed, and the gap is repaired by other components. AP sites are the most common form of DNA damage, with mammalian cells containing some 50,000–200,000 AP sites [101, 102].

Using immunoprecipitation studies, human MutY and APE1 have been shown to associate in human cell extracts, but reconstruction of a mouse MutY-APE1-DNA complex with the pure proteins has not been achieved, presumably because of the requirement of other cofactors [24, 29]. The interaction of human MutY with APE1 requires residues 295–317 containing the motif ***S/PG***XY***DV/I***, where X and Y are any amino acids (table 4, fig. 3). Residue 1 of this motif may be a serine or proline residue, and residue 6 may be a valine or isoleucine residue. Residues 2 and 5 of this 6-amino acid motif are conserved among mammalian MutY (i.e., glycine and aspartate), and 3 and 4 are any amino acids. It is also evident from the alignment that charged residues flanking this putative APE1 binding site may be important.

The interaction of human MutY with APE1 appears to be independent of DNA, whereas the interaction between pol β and APE1 requires bound DNA [103]. The mouse MutY-APE1 interaction, which is independent of APE1 activity, increases the formation of the mouse MutY-DNA complex and thus increases mouse MutY adenine glycosylase activity [24]. Interestingly, the A•G activity of *E. coli* MutY is also enhanced by *E. coli* AP endonucleases, and the interaction is localized to the C-terminus of *E. coli* MutY [104].

Although APE1 can displace both thymine-, 8-oxoguanine- and uracil-DNA glycosylases from an AP site, direct interactions of APE1 with these glycosylases have not been demonstrated [105–108]. However, the association of human MutY with the downstream enzyme APE1 is of immense biological significance. Since the AP•8-oxoG mispair created by human MutY is a substrate for OGG1, release of this cytotoxic and mutagenic mispair by human MutY would allow OGG1 to remove the 8-oxoG lesion, creating two opposing AP sites. Further processing of these opposing AP sites could result in a double strand break, thus, release prevents the formation of a double-strand break only upon physical interaction with APE1. This may explain why human MutY has high binding affinity towards A•8-oxoG and AP•8-oxoG mispairs, so that the APE1 interaction may facilitate the release of the human MutY glycosylase from the products. After the incision by APE1, pol β and FEN-1 may replace human MutY through their interaction with PCNA or APE1 to complete the repair process.

Replication protein A

RPA, also known as human ssDNA binding protein, is a trimeric protein complex involved in many cellular processes, including DNA replication, initiation and elongation, nucleotide excision repair and DNA recombination [109, 110]. Human RPA is a heterotrimer composed of a 70-kDa (RPA1), a 32-kDa (RPA2) and a 14-kDa (RPA3) subunit.

In coimmunoprecipitation experiments, RPA was initially found to associate with human MutY in HeLa cell extracts [29]. Using the 34-kDa subunit, RPA binds to the N-terminus of human MutY (residues 6–32) [29], possi-

bly by a common motif that is present in other RPA-binding proteins such as uracil DNA glycosylase (UDG2) and xeroderma pigmentosum group A protein (XPA) (table 4) [111]. Alignment of the human MutY RPA binding sequence with the N-terminal sequence of both mouse MutY and rat MutY also suggests a putative RPA binding site in these mammalian MutY, although the first 8 amino acids of the site are missing.

As is the case with PCNA, docking of human MutY onto RPA couples human MutY base excision repair of A•8-oxoG to DNA replication. In this context, it has been suggested that RPA plays an architectural role in assembly of the DNA repair complexes [111]. This may also help to explain the paradox that human MutY can be directed to repair the misincorporated adenines on the daughter strand but not on the parental strand. Alternatively, PCNA-bound human MutY may also recruit RPA so that factors essential for DNA repair and replication are localized to the site of DNA damage. This would protect the exposed single-stranded DNA region under repair and also localize RPA to stimulate FEN-1 activity in the later stages of long-patch BER [112]. It is noteworthy that UDG2 interacts with PCNA and RPA and that these proteins colocalize to the replication foci where BER is initiated to repair misincorporated uracils. The interaction between human MutY and RPA again suggests that the human MutY repair pathway requires components of the long-patch BER pathway. It will be interesting to show whether human MutY glycosylase activity is also stimulated by RPA as it is by APE1.

MutS homolog 6

The DNA mismatch repair system (MMR) enhances the fidelity of DNA replication and genetic recombination. MMR enzymes are also involved in cell cycle arrest, transcription-coupled repair and meiotic recombination [113–115]. *E. coli* MMR requires specifically the MutS, MutL and MutH proteins [115]. A homodimer of MutS recognizes base-base mismatches and short insertion-deletion loops. MutL enhances the activities of MutH, MutS and DNA helicase II, and the MutH endonuclease cleaves at the 5′ end of unmethylated GATC sequences. Eukaryotic MMR contains multiple MutS and MutL homologs; however, no MutH homolog has been identified. The MSH2/MSH6 (MutSα) heterodimer recognizes base-base mismatches and short insertion-deletion loops, whereas the MSH2/MSH3 heterodimer (MutSβ) recognizes longer insertion-deletion loops [113]. Germline mutations in human mismatch repair genes result in a mutator phenotype [114], leading to microsatellite instability and predisposition to hereditary nonpolyposis colon cancer (HNPCC) and other cancers [115–118].

Human MutY was initially found present in MSH2 and MSH6 immunoprecipitates from TK6 cell extracts. Using an in vitro binding assay with pure proteins, it was determined that human MutY directly interacts with MSH6 in MutSα and not with MSH2 [30]. The MSH6-interacting domain was localized to the region that includes residues 232–254 of human MutY and is highly conserved among mammalian MutY (table 4). Although both human MutY and MSH6 can bind to their DNA substrates, the interaction between both proteins can occur in the absence of DNA.

As with APE1, the interaction of human MutY with MutSα was shown to enhance both human MutY binding and glycosylase activity of A•8-oxoG-containing oligonucleotides. However, it was also noted that the effect of MSHα on the human MutY glycosylase activity (a 2-fold increase) was weaker than on the human MutY binding affinity toward A•8-oxoG-containing DNA substrates (an 8-fold increase). Furthermore, in the presence of a 24-fold excess of MutSα, human MutY adenine glycosylase activity was slightly inhibited. Human MutY does not have any effect on MutSα binding with A•8-oxoG-containing DNA, which correlates with previous data indicating that human MutY is the major protein to recognize A•8-oxoG-containing DNA substrate [47]. The function of this interaction is therefore to increase the human MutY glycosylase activity by enhancing DNA recognition and is thus physiologically important. The increased levels of C:G to A:T transversions in MSH6 and MMR-deficient cells may be due to an inefficient interaction with human MutY, lowering base excision repair, and may also explain the large increase in genomic 8-oxoG levels in MSH2–/– cells [10]. Any effect on MSH6 activity has yet to be reported. Since the protein expression levels of human MutY were found to be similar in four mismatch repair-deficient cell lines [30], it will be interesting to see if human MutY glycosylase activity is as well.

Proposed role of human MutY in A•8-oxoG repair

A proposed role of human MutY in replication-coupled long-patch repair of A•8-oxoG is shown in figure 5. Adenine is incorporated into the nascent DNA strand opposite 8-oxoG during DNA replication by a DNA polymerase (e.g., polδ). Human MutY recognizes the mismatch, interacts with PCNA and then binds to the mispair (fig. 5, step 1). This links 8-oxoG repair to the replication machinery. MutSα is sequestered to the site of damage via MSH6 interaction with human MutY (MutSα may also bind human MutY in the absence of PCNA, and the MutSα/human MutY complex may bind PCNA together). Polδ may dissociate from PCNA. In a reaction stimulated by MutSα, human MutY excises the adenine base from the daughter strand using its glycosylase activity (fig. 5, step 2) and remains tightly bound to the resulting AP site until APE1 binds to human MutY. Once the adenine base is released, MutSα dissociates from human

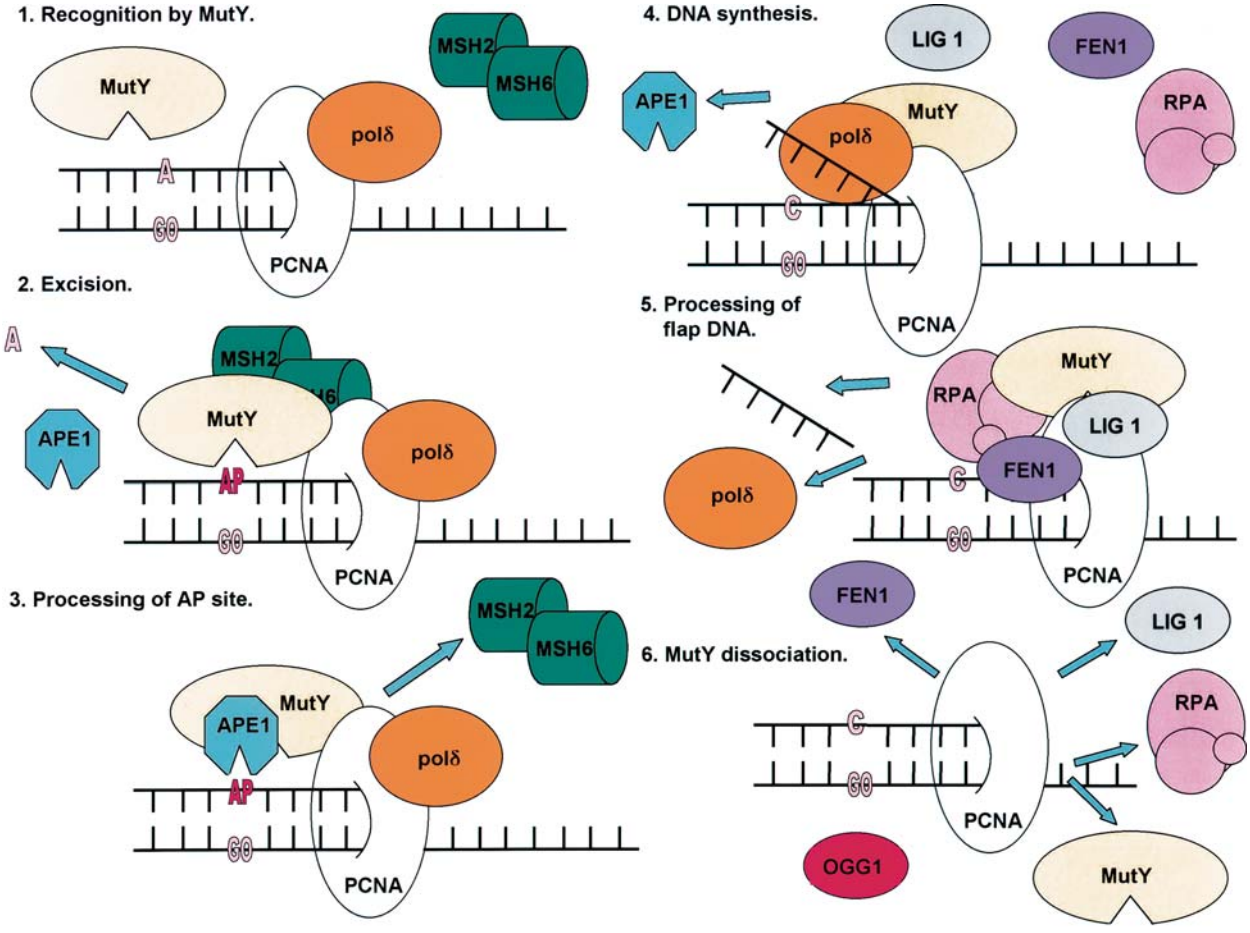

Figure 5. Proposed mechanism of human MutY replication-coupled repair of A•8-oxoG: step 1, recognition by human MutY; step 2, Excision; step 3, processing of the AP site; step 4, DNA synthesis; step 5, processing of flap structure; step 6, human MutY dissociation.

MutY. APE1 recognizes the AP site and binds human MutY, displacing human MutY so that it may access the AP site (fig. 5, step 3; APE1 may also bind in an excision complex with human MutY and MutSα in step 2 to further enhance glycosylase activity). The AP site is further processed by APE1. APE1 dissociates from human MutY, but human MutY remains bound to PCNA (fig. 5, step 4). Polδ localizes to the processed AP site for postrepair DNA synthesis at the site of damage (if the polymerase dissociates from PCNA in step 2, then either polδ or polε may be sequestered). Cytosine is incorporated opposite 8-oxoG by the polymerase. New DNA (2–6 nucleotides) is synthesized, and a flap structure is produced by strand displacement. RPA binds to human MutY, and FEN-1 binds to PCNA (fig. 5, step 5). Localization of RPA and FEN-1 to the site of DNA damage/synthesis results in RPA-stimulated FEN-1 cleavage of the flap structure. APE1 may also remain at the site of DNA damage/repair to stimulate FEN-1 via an interaction with PCNA [119]. DNA ligase I is recruited to the site when the flap structure is cleaved by FEN-1, and the nascent DNA strand is ligated. Human MutY, FEN-1 and RPA dissociate from the repair complex (fig. 5, step 6). The C•8-oxoG mispair is now a substrate for OGG1.

Defective human MutY: a role in carcinogenesis

Data documenting defective DNA repair systems in human cancers has been well established (e. g., defective nucleotide excision repair in xeroderma pigmentosum and defective MMR in HNPCC), but reports of defective base excision repair genes in human cancer have not existed until recently. In this regard, mutations in the human *MutY* gene and defective human MutY activities are just beginning to be identified in human cancers. Of potentially greater importance are the single-nucleotide polymorphisms (SNPs) observed in human *MutY*, which may increase an individual's susceptibility toward malignancy because of subtle differences in the polymorphic protein products, especially when such variations are present in key structural areas of human MutY (fig. 6). However, preliminary analysis of three of these SNPs does not support this [120].

There are several compelling pieces of data supporting a role for defective human *MutY* and human MutY in human carcinogenesis. One is from a British family described by Al-Tassan et al., who discovered an increased incidence of CRC with inherited biallelic germline *MutY* defects [120]. Because three siblings from a single family were affected by multiple colorectal adenomas and carcinoma at relatively young ages, the entire germline adenomatous polyposis coli (*APC*) cDNA was sequenced. There were no pathogenic mutations in the germline *APC* gene of these siblings; however, upon sequencing the *APC* gene in 11 tumors from this family, they discovered 18 *APC* mutations, 15 of which were C:G to A:T transversions, consistent with a defect in oxidative DNA repair. To find the cause of the increased transversions, they sequenced the entire coding regions of the *MutY, OGG1 and MTH1* genes. They identified mutations resulting in two nonconservative amino-acid variants in human MutY. The Gly382Asp mutation was also found by Shinmura et al. (discussed below) and is located in the MutT-like domain. The Tyr165Cys mutation (Tyr82 in *E. coli* MutY) resides in the HhH motif and is highly conserved in all mammalian MutY as well as in *E. coli* MutY proteins (fig. 6). Functionally, it completely abolishes adenine glycosylase activity toward the A•8-oxoG mispair. The rate of adenine removal was also decreased in the Gly382Asp mutant. Notably, the three affected siblings were all MutY compound heterozygotes with biallelic germline inactivation, whereas four unaffected siblings were wild type or possessed a single mutant allele. More recently, the same group identified seven more unrelated patients (three British Caucasian, one Pakistani and three Indian) with a large number of colorectal adenomas and carcinomas who also possessed biallelic germline mutations in human *MutY* [121]. Two patients were homozygous for the Tyr165Cys mutation, and one patient was a compound heterozygote with both Gly382Asp and Tyr165Cys mutations. Two new truncating mutations were found in four additional patients: one was homozygous for Tyr90X, and three patients were homozygous for Glu466X. In these patients with germline human *MutY* mutations, 98% of *APC* mutations were C:G to A:T transversions. No mutations were found in the coding sequences of *OGG1* or *MTH1*, so these studies are the first to demonstrate a direct link between defective human *MutY* and predisposition to colorectal cancer.

Another piece of compelling data involves 152 British patients presenting multiple colorectal adenomas (3–100) and 107 APC-mutation-negative probands with classic familial adenomatous polyposis (>100 adenomas), who were screened for germline human *MutY* mutations [122]. Of the patients with 3–100 adenomas, about 4% (6 patients) possessed biallelic germline defects in the *MutY* gene. Three patients were compound heterozygotes and 3 were presumed to be homozygotes, a total of 9 mutations. Of the mutations, Tyr165Cys and Gly382Asp accounted for 7 of the mutations, while the other 2 mutations were novel frame-shift changes: 1103delC (at codon 368) and 1419delC (at codon 473). In the *MutY* compound heterozygotes (Tyr165Cys and 1419delC, 1103delC and Gly382Asp, and Tyr165Cys and Gly382Asp), the somatic *APC* gene in each case contained G:C to T:A transversions. Since gene inactivation can arise through a wide range of mutations (e.g., almost any frameshift mutation), the fact that the Tyr165Cys and Gly382Asp mutations are repetitively isolated points to a 'founder mutation' effect despite the lack of linkage to a specific microsatellite allele at D1S2667 [122]. A similar percentage of Finnish and Danish patients with multiple adenomas were also compound heterozygotes for the mutations Tyr165Cys and Gly382Asp. Of those with more than 15 adenomas, nearly one-third possessed biallelic *MutY* mutations [122].

In the same study, 6 British patients with multiple adenomas were also found to be heterozygous for a *MutY* mutation and the wild-type allele. Two of the 6 possessed the Tyr165Cys mutation in one allele, and 2 possessed

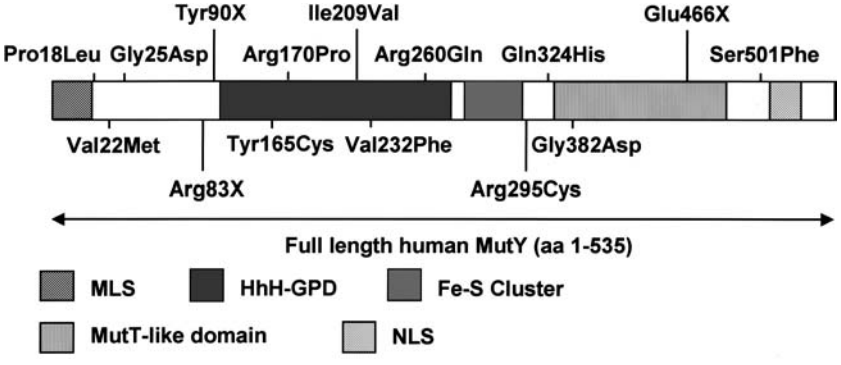

Figure 6. Some human MutY SNPs and mutations (not drawn to scale). Amino acid variants are shown that result from SNPs and human *MutY* germline mutations.

the Gly382Asp mutation. The remaining 2 possessed new mutations: Arg83X, a truncating mutation, and Arg295Cys (fig. 6), which is not a conserved residue but is present in the putative APE1 binding site [29]. Interestingly, no G:C to T:A transversions were observed in *APC* from 7 adenomas from 1 of the heterozygotes carrying the Gly382Asp mutation and a wild-type MutY allele, confirming that 1 single wild-type allele is sufficient.

Eight percent of patients who presented with classic adenomatous polyposis carried biallelic human *mutY* mutations. Tyr165Cys and Gly382Asp were again the most common mutations, but three new mutations were also found: a frameshift (252delG at codon 84), an unusual in-frame duplication (411dupATGGAT at codon 137) and a nonconservative missense change (Val232Phe). Half of these patients were heterozygous for a *mutY* mutation and the wild-type allele (Tyr165Cys in two patients, Ile209Val in one, and Gly382Asp in the other) (fig. 6).

Results from our own laboratory have identified five MSS CRC cell lines that are defective in human MutY A•8-oxoG activity and MutY protein expression [31]. Interestingly, in the same five cell lines, the genomic 8-oxoG levels are elevated more than 10-fold above control MSS CRC cell lines that possess normal levels and activities of human MutY protein. In contrast to the findings of Al-Tassan et al., these cell lines do not possess mutations in the human *MutY* gene [120], and it is likely that the reduced expression is due to promoter methylation.

Shinmura et al. identified a missense mutation, Arg170Pro (G to C transversion), in human *MutY* in the lung cancer cell line NCI-H157 [123]. Arg170 resides in the HhH-GPD motif, which is thought to be involved in mismatch specificity [32], and is highly conserved in all mammalian MutY as well as in *E. coli* MutY (fig. 3). Further analysis identified an additional eight SNPs. Although no functional data was presented, some of these SNPs are located in key structural areas in human MutY; thus, such base changes could be detrimental to the activity of human MutY. The Pro18Leu, Val22Met and Gly25Asp SNPs are present in the RPA binding site in the N-terminus of human MutY and could interfere with the localization of human MutY to the site of DNA replication. Alternatively, the localization of human MutY to the mitochondria may be impaired in a way similar to that found with the Gly12Glu OGG1 mutant in kidney cancer [124]. The Gln324His SNP has been shown to have no effect on human MutY glycosylase activity (see above). Interestingly, an OGG1 SNP (the Ser326 Cys/Cys genotype) appears to increase susceptibility to squamous-cell lung carcinoma [125], and the OGG1 Ser326 Ser/Cys polymorphism may alter the impact of some environmental factors in stomach cancer development [126]. Further studies from Yamaguchi et al. also identified a novel polymorphism creating an altered human MutYβ transcript in the lung cancer cell VMRC-LCD [127]. It was found that this transcript contained 237 bases of intron 1 due to alternative splicing caused by a G to C substitution at position IVS1+5, which resulted in the translation efficiency of polymorphic protein being 30% lower than that of the wild-type human MutY.

Conclusions

Human MutY has the important task of repairing a mutagenic DNA mispair that, if unrepaired, can generate base transversions after the next round of DNA replication. This essential role of human MutY has been masked somewhat by the promiscuity of other proteins creating backup systems for the repair of oxidative DNA damage and, in particular, 8-oxoG. However, it is now becoming evident that human MutY is engaged in a complex network of molecular interactions that extends to proteins participating in other DNA transactions, such as recombination, transcription-coupled repair, MMR and NER. Do the interactions with proteins concerned with repair of other cytotoxic DNA lesions indicate a role for human MutY outside the repair of oxidative DNA damage? Since the interaction of human MutY with MSH6 increases the adenine glycosylase activity of MutY, it is possible that this interaction is essential for maintaining genomic stability. Supporting this are the elevated levels of C:G to A:T transversions observed in MSH6-deficient cells [128]. However, remarkable results from our own laboratory have suggested that this interaction may also be important in the repair of alkylating damage and, as such, the lack of this interaction may render the cells resistant to certain alkylating mutagens [129] (A. R. Parker and J. R. Eshleman, unpublished).

Human MutY may also interact with and be a possible target of *c-myc*, a protooncogene that is rapidly induced and constitutively expressed by resting cells following mitogenic stimuli, which may contribute to the progression of a wide range of human and animal neoplasias [reviewed in 130–132]. *C-myc* binds to the PCNA binding domain of p21 to inhibit its interaction with PCNA and to activate DNA replication [133]. Since human MutY also has a PCNA binding domain, *c-myc* could possibly bind and target MutY. Deregulated *c-myc* expression could therefore inhibit MutY binding to PCNA and thus inhibit repair of 8-oxoG, allowing unscheduled DNA replication to proceed. It is noteworthy that induction of *c-myc* expression causes an induction of ROS [134]. Any attempt to further define the central role of human MutY in protecting the genome will probably require identifying more MutY protein partners and almost certainly require a comprehensive two-hybrid screen against a cDNA library. Thus, the emerging complexity of the interactions of human MutY could be a reflection of its fundamental role in mutation avoidance.

An intriguing question is what are the physiological roles of the proposed MutY isoforms? There is very little data documenting MutY expression/isoforms from normal tissue, and so tumor cells are commonly used. Of the MutY isoforms, only MutYα1–3 are predicted to localize to the mitochondria. The other seven are predicted to localize to the nucleus, which is unexpected because the nuclear MutY isoforms do not appear to possess an RPA binding site, previously suggested to aid localization of MutY to the site of DNA damage/replication. Furthermore, results from our own laboratory have suggested that the isoforms predominantly present in four MSS CRC cell lines are MutYα2 and α3, suggesting that no major nuclear MutY isoform is present in these cell lines [31]. This may be a significant observation, and the type of isoforms present may represent a predisposition to cancer, but it is essential to first determine what isoforms are present in normal human tissue to serve as a baseline. Given the crucial role of human MutY in protecting genomic stability, understanding and determining the precise physiological functions of human MutY may also have clinical implications. MutY-defective human and yeast cells exhibit increased sensitivity toward ROS-forming agents such as hydrogen peroxide [19, 129], and since radiotherapy is a major type of treatment for human cancers, the data suggest that cells with defective MutY could be utilized clinically as a radiosensitizer. Diagnosis of defective *MutY* in human cancer is not clinically routine, but since defective *MutY* and MutY are emerging as possible candidates for colorectal carcinogenesis [31, 120–122], this observed sensitivity to ROS-forming agents may have immense potential therapeutic implications for CRC. Inhibition of base excision repair has been previously used as a target for sensitizing colonic tumor cells to antitumor methylating drugs [135, 136], as well as for radiosensitizing hamster ovary cells [137]. An alternative approach may be to make the tumors *MutY*-defective using molecular approaches such as antisense RNA [138].

Furthermore, organelle targeting of the human *MutY* cDNA may be a viable strategy for either protecting normal cells during cancer therapy or sensitizing tumor cells to treatment. A variety of diseases arise through specific mutations in genomic DNA [3–7], which may result from exposure to ROS. Controlled localization of *MutY* cDNA to either the mitochondria or the nucleus may help to protect the cell from the mutagenic effects of 8-oxoG and other DNA damage, as has been demonstrated with localization of *OGG1* and *FPG* cDNA [139–141]. Alternatively, the increase in MutY could also be employed to increase the rate of production of AP sites that are cytotoxic to the cell and can, therefore, enhance cell death [100].

The interest in human *MutY* with regard to carcinogenesis and the etiologies of other diseases is only just beginning. The recent discovery of a novel family of error-prone DNA polymerases [8] such as polκ, which preferentially inserts adenine opposite 8-oxoG, has increased the interest in base excision repair of DNA, since these polymerases are now found overexpressed or underexpressed in cancers, which can lead to increased rates of transversions. Increased oxidative DNA modifications have been found in aging animals, Parkinson's disease, Cockayne syndrome and Alzheimer's disease, to name but a few [3–7]. The 8-oxoG lesion has also been found elevated in several different cancer types (e.g., hepatocellular, lung and colorectal carcinoma [142–144]) and has been implicated in breast and gastric carcinogenesis [145, 146]. It is noteworthy that C:G to A:T transversions are the most frequent somatic mutations in the tumor suppressor gene *p53* in human lung and liver cancers [147] and are abundant in the *Ras* oncogenes in non-small-cell lung cancer [148].

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