For MutY, It's All about the OG

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MutY and its human ortholog, MUTYH, repair a specific form of DNA damage: adenine mis-paired with the oxidatively modified form of deoxyguanosine, 8-oxo-7,8-dihydro-2′-deoxyguanosine. In a recent issue of *Chemistry & Biology*, Brinkmeyer et al. utilized mutant forms of MutY to reveal the critical residues in MutY that are required for this selectivity and specificity.

Reactive oxygen species (ROS) contribute both positively and negatively to human health. In healthy cells, ROS are critical intracellular signaling molecules, such as during the innate immune response to bacterial infection (West et al., 2011). However, disregulation of ROS metabolism leads to the accumulation of DNA-damaging agents such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, which derive from endogenous sources via cellular metabolism (e.g., oxidative phosphorylation, microsomes, and NADPH oxidases) and exogenous sources such as asbestos, cigarette smoke, UV light, ionizing radiation, pesticides, and related neurotoxins such as paraquat. Depending on the extent and type of ROS exposure, all macromolecules in the cell are subject to ROS-mediated damage, including proteins, lipids, RNA, and DNA. The DNA-damaging effects of ROS have a clear impact on cellular function, genome stability, and human health, thus leading to cancer, cardiovascular and pulmonary diseases, triplet-repeat expansion, organ dysfunction, and neurodegenerative diseases (Hoeijmakers, 2009). As such, it is therefore not surprising that all cells have developed intricate and redundant DNA repair pathways to prevent the deleterious effects that can arise from oxidatively damaged DNA (Svilar et al., 2011).

The oxidatively modified form of deoxyguanosine in DNA is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG or OG) (Figure 1A) and was first identified more than 25 years ago (Kasai and Nishimura, 1984). Although now known to be one of many oxidatively modified forms of deoxyguanosine (Svilar et al., 2011), OG is the most extensively studied oxidative lesion of guanine and is repaired by the DNA glycosylase and base excision repair (BER) enzyme OGG1 in humans (Figure 1B) (Almeida and Sobol, 2007). The OG lesion does not cause a significant block to DNA replication in mammalian cells and, in general, the OG DNA lesion is minimally cytotoxic. Failure to repair the OG lesion by OGG1 is not necessarily associated with disease or the onset of cancer. Although there have been some reports suggesting an increase in oxidative stress-induced cancers associated with defects in OGG1, the presence of the OG lesion is highly mutagenic and the predominant phenotype is due to the mutations that are caused by replication past the OG lesion (Figure 1B). The increase in mutations, mostly G to T transversion mutations, arises from efficient DNA synthesis past the lesion and insertion of dAMP opposite the OG DNA lesion followed by a second round of replication (David et al., 2007). The nuclear and mitochondrial replicative and repair DNA polymerases can insert both dAMP and dCMP opposite OG, including DNA polymerase δ, κ, β, λ, and γ (Svilar et al., 2011). To avoid the deleterious G to T transversion mutations, the ***OG-A*** mis-pair can be corrected by another round of BER, this time initiated by MUTYH (Figure 1B). Removal of adenine in the ***OG-A*** mis-pair is followed by repair via the BER pathway that involves DNA synthesis and preferential insertion of the correct C base mediated by DNA polymerase β (Polß), as suggested recently in a structural analysis of a Polß ternary complex with OG/dCTP (Krahn et al., 2003). Therefore, BER-mediated repair initiated by MUTYH preferentially leads to the ***OG-C*** base pair that would then be available for repair initiated by OGG1, preventing the accumulation of G to T transversion mutations (Figure 1B). This combined action of OGG1 to remove OG in the ***OG-C*** mis-pair or MUTYH to remove the A in the ***OG-A*** mis-pair followed by a second round of OGG1-mediated repair, is commonly referred to as the GO pathway (Michaels and Miller, 1992), where the cell has developed an iterative process to prevent mutations following formation of OG in DNA. Clinically, it appears that MutY (MUTYH) is the most significant of the repair proteins in the GO pathway. Mutations or defects in MUTYH are associated with an increase in colorectal cancer, now referred to as MUTYH-associated polyposis (MAP) (Chow et al., 2004), and as expected, tumors that derive from defects in MUTYH have an increase in G to T mutations.

MUTYH activity is essential to prevent oxidative damage-induced mutations and the onset of MAP, and therefore, a detailed understanding of the molecular mechanism of MutY (MUTYH) and the amino acid residues involved in catalysis and lesion recognition will help define the human mutations that may predispose to disease (MAP). Thus, David et al. (2007) have made significant contributions toward understanding the detailed molecular mechanism of MutY- and MUTYH-mediated lesion removal. In the recent issue of *Chemistry & Biology*, Brinkmeyer et al. (2012) utilize (in vitro and in vivo) mutant forms of MutY, mutated in the glycosylase active site or the OG binding domain, to reveal the critical residues in MutY that are required for the selectivity and specificity of the enzyme to remove the "A" residue in an

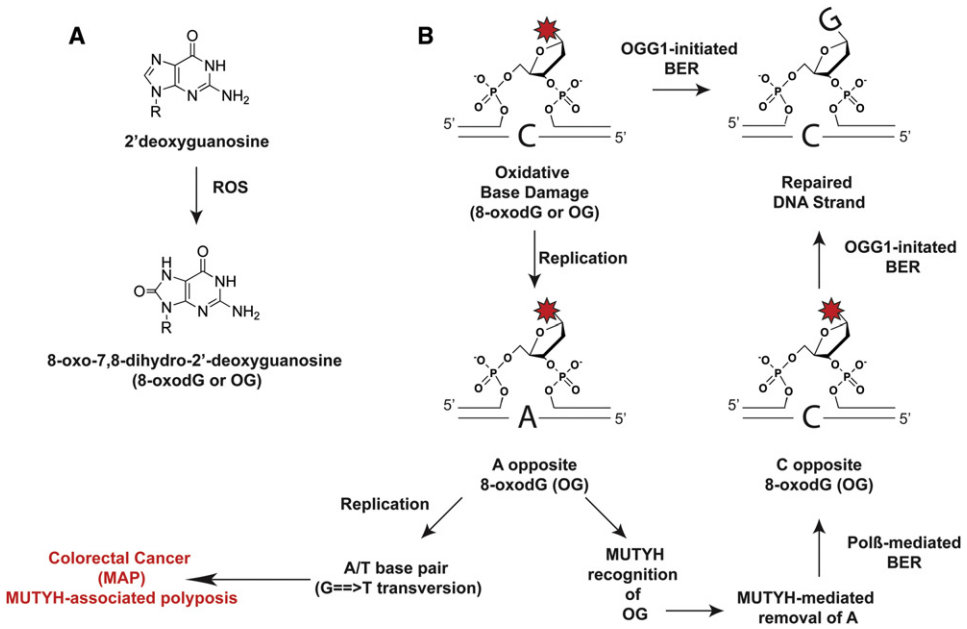

Figure 1. The Pivotal Role of MutY or MUTYH in the GO Pathway to Prevent Genomic or Mitochondrial DNA Mutations Mediated by Oxidatively Damaged Deoxyguanosine

(A) The chemical structure of 2′deoxyguanosine and the oxidatively-modified DNA lesion 8-oxo-7,8-dihydro-2′deoxyguanosine (OG).

(B) Depiction of the GO pathway following formation of OG in DNA. The OG lesion can be directly repaired by BER, removing OG and replacing the lesion with the normal G base. However, if the OG lesion is not repaired and cells undergo replication, the resulting ***OG-A*** mis-pair is also a substrate for BER, initiated by MutY (MUTYH in humans). As suggested by Brinkmeyer et al. (2012), repair of the A opposite the OG lesion in vivo requires initial recognition of the OG lesion by MutY, facilitated by the C-terminal OG recognition domain (residues 226–350), and the subsequent base removal via the glycosylase activity (requiring residues Asp138 and Glu37). Preferential insertion of C opposite the OG lesion by polymerase β (Polß) yields the ***OG-C*** mis-pair, a substrate for OGG1-mediated BER. The availability of two separate BER processing steps avoids the accumulation of G to T transversion mutations following replication of the ***OG-A*** mis-pair, preventing the onset of colorectal cancer.

OG-A mis-pair and prevent oxidative damage-induced mutations. This study focuses on two critical active site residues in MutY from *Escherichia coli*: Asp138 and Glu37. Previous structural analyses suggested that Asp138 and Glu37 were key catalytic active site amino acid residues and corresponded to two of the most common MUTYH variants associated with MAP onset. Kinetic analysis with purified enzymes confirmed the critical role of amino acid residue Asp138 in the reaction mechanism and demonstrated that Asp138 must be deprotonated to stabilize the oxacarbenium ion transition state intermediate. These studies also confirmed the significance of Glu37 in the enzymatic reaction because the activity of the Glu37Asp mutant was reduced 200-fold, as compared to the wild-type (WT) enzyme. To confirm that these two active site residues are essential in vivo, Brinkmeyer et al. (2012) developed a cellular ***OG-A*** repair assay in which an ***OG-A*** containing reporter plasmid was transfected into *E. coli* strains expressing the WT MutY or mutant MutY proteins with alterations in either Asp138 or Gly37 or a deletion of the C-terminal OG binding domain (residues 226–350). Analysis of the recovered reporter plasmid provided a quantitative readout of MutY-mediated repair. Similar to the in vitro kinetic analyses, the in vivo ***OG-A*** repair assay further supports the critical role of Asp138 and Glu37 in the enzymatic activity of MutY. However, it was also noted that the Glu37Asp MutY mutant, with 200-fold diminished enzymatic activity in vitro, exhibited close to half the activity of the WT enzyme in vivo, suggesting that even severely compromised glycosylase activity could mediate repair of the ***OG-A*** mis-pair within the cellular environment. Conversely, the MutYΔ226–350 mutants could not facilitate any measurable repair in vivo, confirming the need for OG recognition in MutY-mediated repair of the ***OG-A*** mis-pair. A similar observation was also observed in a rifampicin complementation assay. Overall, these authors suggest that mutations in the OG binding/recognition domain of MutY, and by inference, the human counterpart MUTYH, may predispose to disease (MAP) to a greater extent than those mutants within the enzyme active site.

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