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RESEARCH HIGHLIGHT

Novel molecular insights into the mechanism of GO removal by MutM

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7,8-dihydro-8-oxo-dGuanine (oxoG or GO, see Figure 1A) is one of the most abundant oxidative DNA lesions caused by exposure of DNA to reactive oxygen species. GO is highly mutagenic, frequently leading to G:C to T:A transversion, because it preferentially pairs with adenine (A) during DNA replication. To prevent genetic instability caused by GO and other mutagenic DNA base lesions, all cells express a large number of DNA glycosylases, whose functional role is to release damaged bases from DNA by cleaving the glycosidic bond [1]. There are at least two types of GO-specific DNA glycosylases, MutM and MutY in *E. coli*, and their respective functional homologs OGG1 and MYH in eukaryotic cells [1]. Deficiency in OGG1 or MYH in human cells is associated with cancers [2, 3]. Thus, persistent GO lesions are likely to have significant biological consequences, and the repair of these lesions is thought to be critical for genomic stability. Although they are all GO-specialized enzymes, MutM and OGG1 specifically remove the mutagenic GO in the GO:C base pair, and MutY and MYH specifically cleave A in the GO:A mispair. Therefore, MutM and OGG1 are truly GO-repair enzymes.

The first high-resolution co-crystal structures of MutM and OGG1 bound to GO lesions in DNA oligonucleotides revealed that both enzymes disrupt DNA helical stacking and flip/extrude the GO lesion into the enzyme active site [4, 5]. However, until recently, the molecular mechanism by which MutM and OGG1 process the GO lesion has been poorly understood. Important progress in resolving these questions has now been reported by Qi *et al.*, who recently solved and analyzed the high-resolution structures of a series of co-crystals containing catalytically inactive but recognition-competent mutants of MutM bound to GO-containing DNA substrates [6]. Their work provides molecular details and significant insight into how MutM interrogates a GO lesion, disengages the damaged base from its complementary pairing partner, and eventually extrudes the lesion into the enzyme active site for base cleavage [6]. The study also provides significant insight into the molecular basis of MutM specificity, offering an explanation for how MutM differentiates between GO- and G-containing DNA base pairs.

Extrusion of a GO-residue by MutM requires the concerted action of three MutM residues: M77, R112, and F114. In the early stages of the recognition/interrogation process, residues M77 and F114 invade the DNA helix at the 3′ end of the GO lesion, which interrupts base-stacking, kinks the DNA helix and rotates the damaged base around the glycosidic bond into a *syn* configuration. Subsequently, MutM R112 is inserted into the helical stack in the space originally occupied by GO. This movement permits R112 to physically interact with the Watson-Crick lone pair O2 on cytosine (C) opposite GO and to directly compete with the GO nucleoside for hydrogen binding to the C. As a result, R112 establishes bidentate hydrogen bonds with O2 and N3 of C, concomitantly disrupting the GO:C base pair (see Figure 1B, encounter and transition complexes). The cooperative actions of these three MutM residues extrude the GO nucleoside from the DNA helix and allow it to enter in the MutM active site (Figure 1B, extrusion complex) for the final step of the reaction, namely GO excision.

GO lesions differ from several other DNA lesions, including pyrimidine-pyrimidine dimers, alkylation damage, and base-base mismatches, in that canonical Watson-Crick DNA structure is maintained in GO-containing DNA, with no significant perturbation of local DNA conformation [7]. Because of this, the mechanism by which MutM

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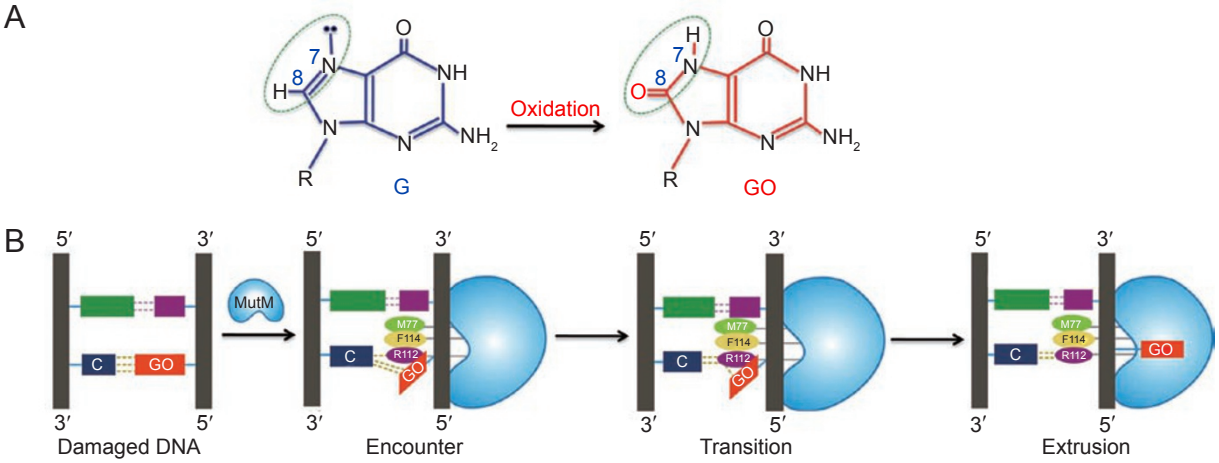

Figure 1 Mechanism of GO removal by MutM. **(A)** GO production via oxidation. The structural differences between G and GO are highlighted with a green ellipse. **(B)** Schematic illustrations of GO interrogation and extrusion by MutM.

discriminates between GO:C and G:C base pairs is not fully understood. Two striking observations made by Qi *et al.* [6] provide clues to this issue. First, striking differences in DNA backbone conformation were observed when MutM interrogated GO- and G-containing DNA substrates: G residues adopt a 2′-endo sugar pucker, while GO residues adopt a C4′-exo pucker [6]. As a result, steric clash is predicted to occur between C8 and C2′ during rotation of a G residue around the glycosidic bond, while such clash does not occur during rotation of a GO residue. This suggests that a physical barrier prevents MutM from extruding the G in a G:C base pair, thus partly explaining MutM repair specificity. Second, computational studies show that the free energy barrier at each step of the MutM reaction is lower in the MutM-GO co-complex than in the MutM-G co-complex [6]. Therefore, MutM is expected to preferentially interact with GO at every reaction step, including initial recognition/binding. A recent study by the same group provides evidence that the MutM active site can differentiate between extrahelical G and GO, so that in the event that MutM encounters an extrahelical G, the extruded base will not be cleaved [8]. Thus, MutM can also discriminate between GO and G nucleosides at the last stage of the base excision reaction. A low free energy barrier for GO processing by MutM is also consistent with the observation that MutM requires little biochemical energy during DNA translocation [9]. In this regard, MutM differs from other lesion recognition proteins such as the mismatch repair protein MutS [10] and nucleotide excision repair protein UvrABC [11].

The mechanism of several other DNA base excision repair glycosylases, including OGG1, MutY, UDG and MUD, involves nucleobase extrusion, DNA kinking and disruption of base stacking [12]. However, it is noted that unlike GO, whose extrahelical state appears to be catalyzed by M77, R112, and F114 (see above), uracil in a U:A pair flips out of the double helix in the absence of UDG [13]. Therefore, whether or not the repair mechanism of other base lesions is similar to GO removal by MutM or uracil removal by UDG remains to be determined. Nevertheless, the elegant work by Qi *et al.* [6] provides useful direction for future studies of DNA glycosylases and possibly other DNA repair enzymes.

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