Free Radical Biology and Medicine 107 (2017) 202–215

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Repair of 8-oxoG:A mismatches by the MUTYH glycosylase: Mechanism, metals and medicine☆

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ARTICLE INFO

Keywords:
Base excision repair
Glycosylase
8-oxoguanine
MutY
MUTYH
MUTYH-associated polyposis
Fe-S clusters

ABSTRACT

Reactive oxygen and nitrogen species (RONS) may infringe on the passing of pristine genetic information by inducing DNA inter- and intra-strand crosslinks, protein-DNA crosslinks, and chemical alterations to the sugar or base moieties of DNA. 8-Oxo-7,8-dihydroguanine (8-oxoG) is one of the most prevalent DNA lesions formed by RONS and is repaired through the base excision repair (BER) pathway involving the DNA repair glycosylases OGG1 and MUTYH in eukaryotes. MUTYH removes adenine (A) from 8-oxoG:A mispairs, thus mitigating the potential of G:C to T:A transversion mutations from occurring in the genome. The paramount role of MUTYH in guarding the genome is well established in the etiology of a colorectal cancer predisposition syndrome involving variants of MUTYH, referred to as MUTYH-associated polyposis (MAP). In this review, we highlight recent advances in understanding how MUTYH structure and related function participate in the manifestation of human disease such as MAP. Here we focus on the importance of MUTYH's metal cofactor sites, including a recently discovered "Zinc linchpin" motif, as well as updates to the catalytic mechanism. Finally, we touch on the insight gleaned from studies with MAP-associated MUTYH variants and recent advances in understanding the multifaceted roles of MUTYH in the cell, both in the prevention of mutagenesis and tumorigenesis.

1. Introduction: MutY's Nobel Heritage

In the late 1980s, the laboratory of 2015 Chemistry Nobel Laureate Paul Modrich discovered an activity in *E. coli* that was able to restore G:A mismatches to G:C base-pairs [1]. This activity was independent of methylation status of the template strand, and therefore could not be attributed to mismatch repair (MMR). Around the same time, Miller and co-workers identified a mutator locus in *E. coli* (*Ec*) that generates G:C to T:A transversion mutations, which they termed as the *mutY* gene [2]. Together Miller and Modrich determined that the *mutY* gene product was an adenine glycosylase [3], similar to the base excision repair (BER) Uracil-DNA glycosylase (UDG) [4], discovered by 2015 Chemistry Nobel Laureate Tomas Lindahl [5]. Using a combination of biochemistry and genetics, Miller, Michaels and co-workers demonstrated that MutY plays an important role in the prevention of mutations caused by the oxidatively damaged guanine lesion, 8-oxo-7,8-dihydroguanine (8-oxoG) by removing adenine (A) from 8-oxoG:A base pairs that form due to the miscoding properties of 8-oxoG [2,6–10]. The knowledge of these fundamental features of MutY were crucial in the discovery of a colorectal cancer (CRC) predisposition syndrome involving variants of the human MutY homolog, MUTYH, in a disease referred to as MUTYH-associated polyposis (MAP) [11,12].

The importance of MUTYH in MAP, and increased interest in targeting DNA repair enzymes as new therapeutic strategies, have upped the ante in understanding the inherent chemistry of MutY/MUTYH and other BER glycosylases [13]. In this review, we will

Abbreviations: G, guanine; C, cytosine; A, adenine; T, thymine; MMR, mismatch repair; BER, base excision repair; UDG, uracil DNA glycosylase; 8-oxoG, 8-oxo-7,8-dihydroguanine; CRC, colorectal cancer; MUTYH, MutY homolog; MAP, MUTYH-associated polyposis; RONS, reactive oxygen and nitrogen species; AP, apurinic-apyrimidinic or abasic; APE1, apurinic/apyrimidinic endonuclease; Fpg, formamidopyrimidine DNA glycosylase; OGG1, 8-oxoguanine glycosylase 1; NUDT1, nudix hydroylase; IDC, interdomain connector; *Gs*, *Geobacillus Stearothermophilus*; *Ec*, *Escherichia coli*; LRC, lesion recognition complex; WT, wild type; FA, *arabino* 2′-fluoro-2′-deoxyadenosine; FLRC, fluorine lesion recognition complex; HhH, helix-hairpin-helix; FCL, iron-sulfur cluster loop; TSAC, transition state analog complex; 1N, azaribose transition state analog; TS, transition state; KIE, kinetic isotope effect; SSB, single-strand break; FAP, familial adenomatous polyposis; APC, adenomatous polyposis coli; LS, Lynch syndrome; MSH2, MutS homolog 2; MEF, mouse embryonic fibroblast; GFP, green fluorescent protein; PCNA, proliferating cell nuclear antigen; RPA, replication protein A; 9-1-1, Rad9-Hus1-Rad1; SDM, site directed mutagenesis; CT, charge transport; hMSH6, human MutS homolog 6; PARP, poly (ADP-ribose) polymerase; AIF, apoptosis inducing factor; KO, knock out

☆ This article is one of a series of papers on the subject of oxidative DNA damage & repair that have been published as a special issue of Free Radical Biology & Medicine to commemorate the Nobel Prize won by Prof. Tomas Lindahl. A detailed introduction and synopsis of all the articles in the special issue can be found in the following paper by Cadet & Davies [193].

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http://dx.doi.org/10.1016/j.freeradbiomed.2017.01.008
Received 17 November 2016; Received in revised form 1 January 2017; Accepted 4 January 2017
Available online 10 January 2017

highlight recent insights into the structural and functional properties of MutY, MUTYH and MAP variants. For example, recent investigations of MAP variants in the David laboratory resulted in the discovery of a second metal binding site in mammalian homologs of MUTYH [14]. Furthermore, despite MutY's discovery almost 30 years ago, new features of MutY base excision have been revealed prompting revisions to the accepted mechanism for MutY, implicating that similar revisions may be appropriate for related glycosylases [15]. We also look beyond MAP and call attention to MUTYH's roles in other diseases, including intriguing links between MUTYH and neurological disorders [16–19]. These various aspects of MUTYH collectively accentuate how the structure and function of MutY and MUTYH elegantly tie to their activities in the cell and the ever-unraveling roles they play in protecting life from DNA damage. Of note, in this review, we highlight primarily new studies and therefore direct the reader to previous reviews on MutY, MUTYH and MAP for additional details [12,20–27].

2. Oxidatively damaged DNA and base excision repair

2.1. Oxidatively damaged DNA and 8-oxoG

Replicating cells continue life by passing on genetic information in the form of DNA. If the information within DNA is compromised by DNA base modifications resulting from reactions with chemical species, such as reactive oxygen and nitrogen species (RONS), malignant cellular phenotypes may result, ultimately leading to diseases such as cancer [28–31]. RONS arise from cellular exposure to xenobiotic toxins, as well as ionizing radiation and ultraviolet light. However, RONS are more frequently formed as a consequence of cellular respiration, inflammatory responses, and by-products of cellular signaling and lipid peroxidation [reviewed by 32].

DNA damage resulting from RONS manifests in the form of DNA inter- and intra-strand crosslinks, protein-DNA crosslinks, and chemical alterations to the sugar or base moieties [12,33,34]. The most well studied oxidative base modification to DNA is 8-oxoG, which occurs within the human genome approximately once per million guanine residues [12,35,36]. The prevalence of 8-oxoG in genomic DNA is due to the low redox potential of guanine, making it highly susceptible to oxidation [37–39]. The correlation between oxidative stress and high levels of 8-oxoG has resulted in the use of 8-oxoG as a cellular biomarker [40–42].

The formation of 8-oxoG via oxidation results in the addition of an oxo group at the C8 position and a hydrogen atom at the N7 position of guanine (Fig. 1). When 8-oxoG is oriented in the normal *anti* conformation within DNA, the 8-oxo group sterically clashes and unfavorably interacts with the O4′ position of the DNA sugar. Because of this, 8-oxoG preferentially adopts the *syn* conformation during DNA replication offering a Hoogsteen face that resembles that of the Watson-Crick face of thymine [43,44]. Because of this dual-coding ability, replicative polymerases often misincorporate A opposite 8-oxoG [12,45]. If this 8-oxoG:A mispair persists within DNA without being repaired, a G:C to T:A transversion mutation permanently incorporates into the genome. For example, G:C to T:A mutations have been shown to be the predominant type of somatic mutation in protein kinase genes in lung, breast and colorectal cancers [46].

2.2. The GO repair pathway

Tomas Lindahl's insight that the high level of spontaneous cytosine deamination to uracil must be repaired in order to preserve life resulted in the seminal discovery of UDG and the BER pathway [5,47–49]. The BER pathway mitigates the mutagenic and toxic properties of a wide array of DNA nucleobase modifications [12,50]. The key players of this pathway are a cadre of DNA glycosylases that scour the genome and initiate the repair of specific types of base modifications in DNA by hydrolyzing the N-glycosidic bond between the damaged base and the 2′-deoxyribose sugar, affording apurinic/apyrimidinic (AP) sites within DNA [4]. Mono-functional glycosylases pass the AP site to a 5′ AP endonuclease (such as APE1 in humans) that hydrolyzes the phosphodiester DNA backbone at the AP site. Further trimming of the remaining sugar fragments allows for gap-filling by a DNA polymerase to replace the excised nucleotide. Lastly, DNA ligase provides the finishing touches of repair by sealing the phosphodiester backbone [51]. The mammalian BER pathway is more complex than its bacterial counterpart and has two distinct sub pathways: short-patch and long-patch BER, which differ in the number of nucleotides incorporated during the polymerase step and the various enzymes involved [52–55].

Miller and Michaels coined the term “GO” (for “Guanine Oxidation”) repair pathway to describe the combined efforts of MutM (Fpg), MutY and MutT in mitigating the mutagenic properties of 8-oxoG in bacteria (Fig. 2) [7,8,10,12,56]. Later work established a similar GO repair pathway in human cells featuring the functional equivalents

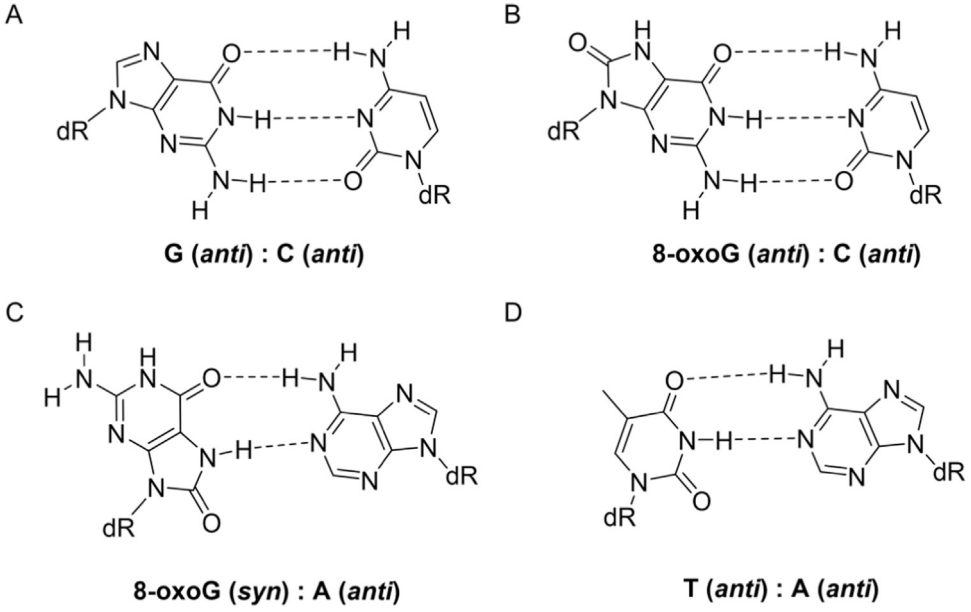

Fig. 1. The DNA contexts of 8-oxoG. When a normal G:C base pair (A) is damaged to form an 8-oxoG:C base pair (B), 8-oxoG may adopt a *syn* conformation about its glycosidic bond to form a stable 8-oxoG:A base pair after a round of replication (C). Following a subsequent round of replication, where A is in the template DNA strand, a T:A base pair results (D).