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Removal of oxidatively generated DNA damage by overlapping repair pathways

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ABSTRACT

It is generally believed that the mammalian nucleotide excision repair pathway removes DNA helix-distorting bulky DNA lesions, while small non-bulky lesions are repaired by base excision repair (BER). However, recent work demonstrates that the oxidativly generated guanine oxidation products, spiroimininodihydantoin (Sp), 5-guanidinohydantoin (Gh), and certain intrastrand cross-linked lesions, are good substrates of NER and BER pathways that compete with one another in human cell extracts. The oxidation of guanine by peroxynitrite is known to generate 5-guanidino-4-nitroimidazole (NIm) which is structurally similar to Gh, except that the 4-nitro group in NIm is replaced by a keto group in Gh. However, unlike Gh, NIm is an excellent substrate of BER, but not of NER. These and other related results are reviewed and discussed in this article.

1. Introduction – oxidatively generated DNA damage is genotoxic

Environmental factors (infectious agents, asbestos, tobacco, UV light) are known to contribute to the development of chronic inflammation in human tissues [1,2]. Reactive oxygen and nitrogen species (ROS and RNS, respectively) are overproduced at sites of chronic inflammation and induce persistent DNA damage that, if not properly repaired, can ultimately lead to the initiation and promotion of cell proliferation and cancer [3]. Epidemiological and clinical studies suggest that about 25% of all cancer cases worldwide are linked to chronic inflammation. Patients suffering from chronic inflammation are at a much higher risk of developing cancers [4]. A chronic imbalance between DNA damage and repair increases the risk of genomic instability, and it is therefore important to understand the mechanisms of the repair pathways that remove oxidatively generated DNA lesions from the genome. In this contribution, we consider some recent results that indicate that some of these DNA lesions can be excised by different overlapping repair pathways such as base excision, nucleotide incision, and nucleotide excision repair.

2. Mechanisms of DNA repair

2.1. Base excision repair (BER)

The repair of oxidatively generated lesions is critical for maintaining genomic stability during oxidative stress [5]. Existing paradigms suggest that base excision repair (BER) mechanisms are responsible for the removal of non-bulky oxidatively generated DNA lesions [6]. The mechanisms of BER are highly conserved from bacteria to humans [7,8] and involve the distinct enzymatic reactions depicted in Fig. 1A.

BER proteins recognize damaged nucleobase by first binding to the damaged site and then cleaving the N-glycosyl bond to release the damaged base, thus forming an abasic site as shown in Fig. 1A [9]. In the case of monofunctional glycosylases, the abasic site is cleaved by an apurinic (AP) human endonuclease (APE1) to form fragments with 3′-OH and 5′-deoxyribose phosphate (5′-dRP) ends [10]. Polymerase β (Pol β) subsequently adds a nucleotide to the 3′-OH end using the base in the complementary strand as the template. The 5′-dRP is concurrently removed by the AP lyase activity associated with Pol β (Fig. 1A), and finally the nick is sealed by DNA ligase III/XRCC1 [11]. On the other hand, bifunctional glycosylases in addition possess AP lyase activity, which cleaves the abasic site in DNA resulting in the formation of a single-strand break containing either a phosphate (P) group (β, δ-

Abbreviations: BER, base excision repair; NER, nucleotide excision repair; NIR, nucleotide incision repair; ROS, reactive oxygen species; RNS, reactive nitrogen species; GG, global genomic; TC, transcription coupled; cdG, 5′,8-cyclo-2′-deoxyguanosine; cdA, 5′,8-cyclo-2′-deoxyadenosine; NIm, 5-guanidino-4-nitroimidazole; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-oxoG, 8-oxo-7,8-dihydroguanine; G*-T*, intrastrand guanine(C8)-thymine(N3) cross-link; Sp, spiroiminodihydantoin; Gh, 5-guanidinohydantoin; Ia, iminoallantoin

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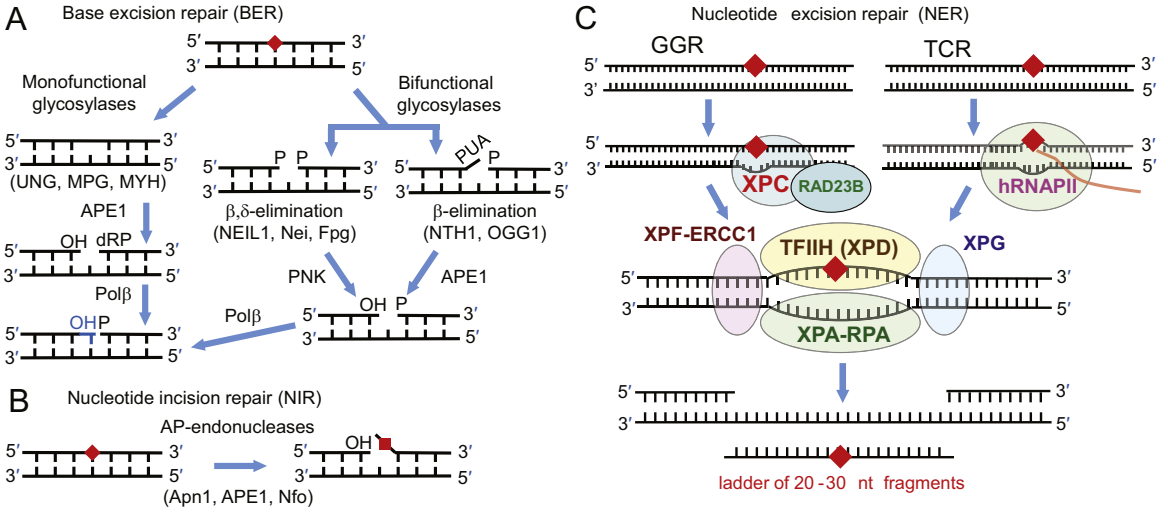

Fig. 1. Excision of damaged nucleobases by (A) BER, (B) NIR (the lesion is not excised in this pathway), and (C) NER mechanisms.

elimination), or an α,β-unsaturated aldehyde PUA, (β-elimination) at the 3′-end [12,13]. The 3′-PUA and 3′-phosphate groups are further removed by the diesterase activity of APE1 and the phosphatase activity of polynucleotide kinase (PNK) to form the same gapped product as in the case of the monofunctional glycosylase mechanism [14,15]. This single nucleotide gap is filled by Pol β (Fig. 1A), and the nick is sealed by DNA ligase III/XRCC1 as in the case of the monofunctional glycosylases [11].

2.2. Nucleotide incision repair (NIR)

An alternative nucleotide incision repair pathway involving the AP endonucleases of *E. coli* Nfo, yeast Apn1 and human APE1, nick DNA on the 5-side of the damaged base, thus generating fragments with 3′-OH ends, as well as a damaged nucleotide at the 5′-end [16,17] (Fig. 1B). It has been suggested that the NIR pathway can serve as a backup system for BER if the appropriate glycosylase is missing or inefficient. Once the AP endonuclease has produced the initial incision, the full regeneration and repair of the incised strand can occur as long as the other, subsequent repair factors are also available [18].

2.3. Nucleotide excision repair (NER)

The mammalian global genomic nucleotide excision repair system (GG-NER) recognizes the distortions in the DNA double helix caused by the DNA lesions, rather than the lesions themselves [19] and the full repair of the DNA damage requires the sequential action of more than 30 proteins [20,21]. In mammalian GG-NER, the recognition of DNA damage is achieved by the protein heterodimer XPC-RAD23B (Fig. 1C). The resulting XPC-RAD23B – damaged DNA complex recruits the ten-protein factor TFIIH, XPA, XPF and XPG that cooperate to excise the characteristic ~24 – 30 nucleotide (nt) dual incision products that contain the lesion and are the hallmarks of successful NER [22,23]. By contrast, transcription coupled nucleotide excision repair (TC-NER) is initiated when human RNA polymerase II (hRNAPII) is stalled by DNA lesions. The stalled polymerase serves as the signal for recruiting the NER factor (TFIIH, etc.) and other NER factors that lead to the NER double incisions and, ultimately, the filling of the ~24–30 nt gap created by the dual incision and the removal of the damage-bearing 24–30 nt fragments. However, TC-NER removes DNA lesions only from the transcribed strand of active genes [24], and thus repairs a smaller, but critical fraction of cellular DNA damage.

3. Guanine is the major target of reactive oxygen and nitrogen species

The primary target of oxidatively generated damage in DNA is guanine [25], the most easily oxidizable nucleic acid base in DNA [26]. The oxidation of guanine is typically initiated either by one-electron abstraction or by the addition of hydroxyl radicals (Fig. 2) [27].

The radical intermediates formed are highly reactive and rapidly transform to stable end-products. The most abundant and best known oxidatively generated guanine lesion is 8-oxoG that is ubiquitous in cellular DNA [25,28]. Other oxidation products include the diastereomeric 5,8′-cyclo-2′-deoxypurine lesions [29–31]. In gamma irradiated and aerated aqueous nucleoside solutions these lesions are formed at ~40 times lower concentrations than 8-oxoG; however, the levels of cdG and cdA were found to increase by factor of 10–20 as the oxygen concentration was diminished [32]. Alternatively radical intermediates can transform to 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) [25,27,33]. Formation of FapyG has been detected upon exposure of DNA in aerated aqueous solutions to either hydroxyl radicals or one-electron oxidants [34,35]. Furthermore, FapyG is produced in two-fold higher yield than 8-oxoG in gamma irradiated cellular DNA [36]. Radicals derived from the one-electron oxidation of guanine remain strong electrophiles and readily add to neighboring thymines to form guanine(C8)-thymine(N3) crosslinks (G*-T*) in air-equilibrated solutions (Fig. 2) [37]. Other forms of cross-linked DNA lesions include G[C8-5m]T and G [8–5] C lesions [29,30,38–42]. Guanine radicals readily combine with oxyl radicals to form stable end-products [43], while combination with nitrogen dioxide radicals leads to the formation of 5-guanidino-4-nitroimidazole (NIm) [44,45]. NIm was found among a number of other products of guanine oxidation by peroxynitrite [46,47] and serves as a marker of inflammation-related oxidation mechanisms [48].

It is well established that 8-oxoG is more easily oxidized than the parent guanine base [49]. Its further oxidation by diverse oxyl radicals ($CO_3^{\bullet-}$, $^{\bullet}NO_2$, $SO_4^{\bullet-}$, $RO^{\bullet}$) [45,50–53] and peroxynitrite [47], can lead to the formation of the diastereomeric spiroiminodihyadantoin (Sp) and 5-guanidinohydantoin (Gh) lesions [54–61]. The Sp and Gh lesions have been detected in mice with infection-induced colitis at concentration levels of about one percent, relative to the more abundant 8-oxoG levels [62]. Due to the presence of chiral carbon atoms, the Sp and Gh nucleobases exist as *R* and *S* diastereomers. Oligonucleotides containing single, site-specifically inserted *S*-Sp and *R*-Sp lesions can be isolated by anion-exchange HPLC [63]. In aqueous solutions, the Gh diastereomers are easily interconvertible, and can isomerize to iminoallantoin (Ia) (Fig. 2) [55]. In DNA, the isomeriza-