Involvement of the nucleotide excision repair proteins in the removal of oxidative DNA base damage in mammalian cells*

Minireview

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Oxidative DNA base damage produced primarily by reactive oxygen species is assumed to be the most important endogenous damage. Lack of its repair may contribute to mutagenesis, carcinogenesis and aging. It is supposed that most oxidative DNA base damage is removed by the base excision repair pathway; although it was shown recently that other DNA repair pathways could be involved. This review is focused on the role of nucleotide excision repair (NER) and transcription-coupled repair (TCR) in the removal of oxidative DNA base damage in mammalian cells.

Key words: Reactive oxygen species, oxidative DNA base damage, base excision repair, nucleotide excision repair, transcription-coupled repair.

Reactive oxygen species (ROS) that are formed in cells as products of the normal aerobic metabolism, or because of many different environmental influences, damage DNA as well as other biomolecules, such as proteins and lipids. ROS causing oxidative damage may be divided into two categories: free oxygen radicals and non-radical ROS. ROS radicals include hydroxyl radical ($OH^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), superoxide radical ($O_2^{\cdot -}$). Non-radical ROS consist of singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$) and hydrogen peroxide (H_2O_2) (reviewed in [12]). Various endogenous sources of ROS have been identified in living organisms, i.e. the mitochondrial respiratory chain, the primary immune system, degrading fatty acids and other molecules by peroxisomes (reviewed in [2]). Organisms are also exposed to ROS from external sources which include visible light, ultraviolet, X- and γ-radiation and ozone [56]. Many compounds of pro-oxidant nature, such as quinines, capable of redox cycling are delivered to the organism through the diet. Cigarette smoke contains an array of free radicals, i.e. ROS, nitric oxide, which contribute to the increased risk of lung cancer among smokers [54]. ROS-induced cellular changes have been also implicated in a multitude of diseases, including cardiovascular dysfunction, arthritis, and cancer, as well as in the aging process [6, 13, 28, 62].

Many defense mechanisms within the organism have evolved to limit the levels of reactive oxidants and the damage they inflict. The antioxidant defense system in most cells consists of two components, the antioxidant enzymes (i.e. superoxide dismutase, catalase, glutathione peroxidase), and the low molecular weight antioxidants component (i.e. vitamins A and E, ascorbate, glutathione, thioredoxin). Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defense mechanisms, causing damage to biomolecules [2].

Cells also possess multiple of repair pathways, which remove and repair the wide range of DNA damage. If the damage is unrepaired, it can lead to harmful biological consequences in organisms, including cell death, mutations and malignant transformation [72]. Detailed knowledge of repair of oxidative DNA damage might lead to drug developments and clinical applications.

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Oxidative DNA damage

Oxidative DNA damage may be formed through a direct insult on nucleotides in the helix or through incorporation of damaged nucleoside triphosphates during replication [1]. Estimates of background level of oxidative base damage in human cells vary enormously, i.e. from 300 down to 0.4 molecules of 8-oxoguanine per 10^6 guanines [16].

The reaction of ROS with DNA results in many kinds of oxidative DNA modifications, such as oxidized base, abasic site (AP), bulky adducts, DNA-protein crosslinks, strand breaks and variety of sugar lesions. Nearly 100 different base and sugar modifications have been identified [18]. The bulky adducts comprise several modifications that disturb the conformation of the helix. Whereas the oxidative base damage and strand brakes are usually formed by a direct attack of ROS on DNA, the bulky adducts probably result from secondary products produced in reactions with lipids and proteins [47].

Oxidative DNA base damage. The most common oxidized pyrimidine base is 5,6-dihydro-5,6-dihydroxythymine (thymine glycol, Tg). Tg causes significant distortion of the duplex DNA molecule. It is a strong block to DNA replication when presented in double-stranded DNA and therefore is lethal. In a single-stranded DNA template, Tg is poorly mutagenic and results predominantly in T→C mutations. Most oxidation products of cytosine are analogous to those detected for thymine. The cytosine glycol is highly unstable and readily deaminates or dehydrates (reviewed in [71]).

Purines can undergo oxidation of C8 ring atom forming 7,8-dihydro-8-oxoguanine (8-oxoG) and imidazole-ring fragmented lesions, i.e. formamido-pyrimidines (FaPy). 8-oxoG is the most studied and characterized oxidative base damage. This lesion has strong miscoding properties and both bacterial and eukaryotic DNA polymerases insert A opposite 8-oxoG with high frequencies. Consequently, the presence of 8-oxoG residues in the template during DNA replication induces G:C→T:A transversions. In contrast, FaPy residues represent blocks to DNA replication and have mostly cytotoxic effects (reviewed in [18]). Other abundant oxidatively modified purines and pyrimidines include 8-oxoadenine, 2-hydroxyadenine, FapyAdenine, 5-hydroxylmethyluracil, 5-hydroxycytosine (reviewed in [71]). In addition, a large number of other modifications of bases and sugar have been identified (reviewed in [18]).

Base excision repair

The BER pathway is thought to be the principal pathway to repair oxidized DNA bases. The first step of BER consists of recognition and removal of the altered base accomplished by DNA glycosylases. DNA glycosylases are generally small, monomeric proteins that hydrolyze the N-C1' glycosylic bond between the deoxyribose sugar moiety and the target abnormal DNA base, thus releasing a free base and leaving an AP site in DNA that is cytotoxic and mutagenic and must be further processed [5].

The next step following hydrolysis is the excision of resulting AP site by Ape1/Ref-1 via Mg^{2+}-stimulated mechanism (reviewed in [22]). Ape1/Ref-1 nicks the phosphodiester backbone 5' to the AP site leaving a 3'-hydroxyl group and a 5'-deoxyribose phosphate (dRP) group flanking the nucleotide gap [20, 46].

BER can proceed via two alternative sub-pathways, short-patch or long-patch ones (reviewed in [50]). In mammalian cells, the majority of oxidized bases are processed through a single-nucleotide patch mechanism that is DNA polymerase β-dependent pathway. DNA polymerase β (*pol*β) has an intrinsic dRPase activity as well as the DNA polymerase activity [53]. It performs excision of the 5'-dRP moiety remaining after Ape1/Ref-1 incision and inserts one nucleotide [43, 52, 61]. This is followed by sealing of the remaining nick presumably by the DNA ligase III in complex with the scaffold protein XRCC1 (X-ray repair cross complementing protein 1) [63].

The second sub-pathway has been discovered in eukaryotes and is a proposed minor pathway. Unlike short-patch repair, resynthesis of the corresponding nucleotides could be realized by DNA polymerases β, δ or ε, proliferating cell nuclear antigen (PCNA), replication factor C (RF-C), and possibly other undetermined factors [26, 33]. Following nucleotide addition, FEN 1 acts to remove the dRP containing displaced strand in patches approximately between 2-10 nucleotides. Complete repair occurs when DNA ligase I or possibly DNA ligase III/XRCC1 restores the phosphodiester backbone.

Mammalian DNA glycosylases. Most of the DNA glycosylases are highly specific and can excise various types of modified bases. Currently, they can be classified as monofunctional or bifunctional, according to whether they possess an AP lyase activity. Monofunctional glycosylases remove the modified base leaving a natural AP site. In the case of bifunctional ones, AP site generated by the glycosylase activity is further incised at the 3' side via β-elimination, which produces an obstructive 3'-end (reviewed in [36]).

The DNA glycosylases specific for oxidatively damaged bases have been well studied in the model organism *Escherichia coli* and most of these glycosylases are bifunctional.

Oxidized pyrimidines in mammalian cells, in particular thymine glycol and cytosine glycol, are removed from DNA by glycosylase NTH1 (homologue of *E.coli* EndoIII encoded by the *nth* gene) [4]. The human as well as the mouse genes coding for the DNA glycosylase NTH1 have been

cloned [4]. The hNTH1 can remove five different oxidized pyrimidines: 5-hydroxycytosine, thymine glycol, 5-hydroxy-6-hydrothymine, 5,6-dihydroxycytosine, and 5-hydroxyuracil [19]. The NER protein, XPG, enhances the binding activity of hNTH1 to DNA containing Tg. This is one of *in vitro* evidences that some of the nucleotide excision proteins have a role in BER pathway [7].

8-oxoguanine, an abundant and arguably the most critical mutagenic lesion, is repaired primarily by 8-oxoG-DNA glycosylase (OGG1) in mammalian cells (homologue of *E. coli* MutM/Fpg). OGG1 is essential for the repair of 8-oxoG in the non-transcribed strand of a gene. In contrast, the removal of 8-oxoG in the transcribed strand is OGG1-independent *in vivo* [39]. The incision of 8-oxoG and the AP-lyase activity are most efficient when the base is located opposite a cytosine [8]. The human MYH protein, on the other hand, prefers 8-oxoG paired with G and A [59].

Several human DNA glycosylases that belong to the formamidopyrimidine-DNA glycosylase (Fpg)/Nei family have been described recently [29]. One of them, hNEIL1, efficiently excises FaPy, oxidised pyrimidines and, weakly, 8-oxoG. Another enzyme, hNEIL2, appears to have a rather limited substrate range, preferring cytosine-derived lesions only [30].

Knockout mice. In view of the mutagenic and toxic consequences of oxidative damage to bases in DNA, it is expected that deficiency in their repair should have significant pathophysiological effects. Several mice lacking BER enzymes have been generated using mouse knockout technology (reviewed in [24]). However, knockout mice deficient in above mentioned DNA glycosylases show no overt phenotypic abnormalities, have no predisposition to cancer, in spite of accumulation of mutagenic and toxic base lesions in their genomes [34, 45, 67]. This could be an evidence of the existence of a backup repair systems for lacking DNA glycosylases. In contrast to the glycosylases, mice defective in genes involved in BER downstream of DNA glycosylases (APE1, Polβ, DNA ligase I and XRCC1) all show embryonic lethality (reviewed in [25]), probably because of an accumulation of repair intermediates, in particular cytotoxic AP sites and dRP moieties.

Transcription-coupled repair and NER

TCR is an evolutionary conserved process not only in eukaryotes, but also in prokaryotes. It was originally demonstrated for UV-induced lesions [44]. TCR was first thought to be a specific sub-pathway of the NER pathway for repair of UV-induced DNA damage. However, over the past few years, it has become clear that oxidative DNA lesions removed by BER pathway are also repaired through TCR [40]. Thus, TCR could be redefined as a discrete pathway for initiating rapid removal of transcription-blocking lesions by either NER or BER.

A signal for rapid recruitment of the repair machinery is blockage of RNA polymerase II elongation by a lesion in the DNA template. Thus, only those lesions that block RNA polymerase will be subject to TCR. Subsequent to transcription-coupled lesion recognition, various repair pathways, like NER and BER, are assembled to verify and remove the lesion. Within NER, TCR functions in addition to global genome repair (GGR) pathway. GGR is responsible for removal of DNA lesions all over the genome. GGR-dependent lesion recognition is initiated by the XPC-hHR23B heterodimer [64] and/or DDB protein [70]. The next step for both TCR and GGR is unwinding of the DNA helix around the lesion by the transcription-repair factor TFIIH [57, 68] via its two DNA helicases, XPB (3'→5') and XPD (5'→3'). The XPA and RPA proteins properly position the structure-specific XPG and ERCC1/XPF endonucleases that incise 3' and 5' of the damage, respectively [48, 60]. Finally, DNA fragment containing damage is released and the gap is filled by a newly synthesized DNA strand and ligated [3].

The fundamental importance of TCR is evident from the finding that people, who are compromised in their ability to perform the reaction due to mutations in TCR genes, are afflicted with a severe hereditary disorder called Cockayne's syndrome (CS) [9]. CS is a rare autosomal recessive disease that shows diverse clinical symptoms including photosensitivity, severe mental retardation, developmental defects, growth failure, but no predisposition to UV-induced skin cancer (reviewed in [14]). CS is classified into two genetic complementation groups (CSA and CSB) [49].

The CSA protein is the WD-repeat protein [31]. The CSB protein has ATPase and DNA binding activities [58]. It can have role in chromatin remodeling or maintenance, activation or repression of transcription [15] . CSA and CSB proteins interact with each other *in vitro* and have further interactions with NER proteins. CSA interacts with the TFIIH p44 subunit and CSB interacts with XPA and XPG proteins [31, 58, 32].

In very rare cases, complementation analyses have assigned some CS patients to Xeroderma pigmentosum (XP) groups B, D, or G. Some of these XP/CS patients also present an XP phenotype. The diagnostic features of XP consist of dry scaly skin, abnormal pigmentation, photosensitivity and marked predisposition to skin cancer (reviewed in [14]).

The XPB protein interacts with the XPD and XPG proteins and is required for the 5' cleavage during NER [23]. *XPB* gene is essential for cell viability [51]. Contrarily to the XPB helicase, the helicase activity of XPD is indispensable for NER but is dispensable for transcription, although the presence of the protein plays an essential role to promoter escape [11].

XPG is another multifunctional protein that is, like TFIIH, required for NER. It also plays a role in transcription-coupled repair of base damage such as thymine glycols [17]. This is its second important function in addition to its role in NER [38]. Patients from the XP-G complementation group clinically exhibit heterogeneous symptoms, from mild to very severe. About half of the described XP-G patients exhibit also CS symptoms.

Transcription-coupled repair of oxidative DNA damage

One of the first indications that TCR might also direct BER in addition to NER was the finding of more rapid repair of lesions produced by ionizing radiation in the transcribed strand compared to the non-transcribed strand of the active *DHFR* gene in normal human cells [38].

In view of the ubiquitous occurrence of oxidative damage (e.g. thymine glycol and 8-oxoG), the biological impact of these lesions may be significant. Oxidative damage is shown to be removed by TCR in cells from normal individuals and from XP-A, XP-F, and XP-G patients who have NER defects but not from XP-G patients who have severe CS. Interestingly, cell lines from XP/CS patients with mutations in *XPB*, *XPD* and *XPG* genes show a transcription-coupled repair defect of oxidative lesions [17].

The TCR defect in XP-D/CS and XP-B/CS cells includes inability to preferentially remove Tg from transcribed strand as shown by studies of H_2O_2-treated cells using an antibody specific for Tg, whereas XP-D cells were normal in this regard. It is concluded that TCR of Tg but not its global genome repair requires the TFIIH subunits XPB and XPD [40]. Evidence that XPG is required for TCR of Tg as well as being essential for its global removal has been provided [17]. This pathway is independent of the XPG incision function in NER, but it probably requires protein-protein interactions between XPG and TFIIH and perhaps other proteins.

The 8-oxoG is also repaired by TCR. It was demonstrated by using an SV40-based shuttle vector containing a single 8-oxoG in the 3'-untranslated region of the large T antigen gene. Transcription of the sequence containing the lesion was determined by the presence or absence of the SV40 early promoter in the vector, which was transfected into various human cell strains and then recovered after incubation for analysis of persistence of the lesion by replication in repair-defective bacteria. CSB, XP-G/CS, XP-B/CS and XP-D/CS cells were blocked for repair of 8-oxoG and there were no detectable removal even after 3 days. However, the removal of this lesion from nontranscribed strand was identical with removal in normal cells [40].

The data about the existence of TCR of oxidative damage in rodent cells are controversial. Thorslund et al [69] found no preferential repair of 8-oxoG in transcribed genes compared to non-transcribed regions and did not detect any strand-bias in the repair of the endogenous housekeeping gene, dihydrofolate reductase (*DHFR*). Moreover, while in human cells unrepaired 8-oxoG blocks transcription by RNA polymerase II [40], hamster cells are capable of transcribing DNA containing 8-oxoG introduced by a treatment with photosensitizer plus light [69]. However, when the removal of 8-oxoG from plasmids that contains a single 8-oxoG.C base pair in a sequence that can be transcribed or non-transcribed in the Chinese hamster ovary cells was analyzed [10], the results showed that 8-oxoG located in transcribed sequence is removed faster than in a non-transcribed one, indicating TCR of 8-oxoG in rodent cells. The results also showed that hamster cells efficiently repair DNA molecules that contain an Ogg1-incised AP site, which is the first intermediate in the course of BER of 8-oxoG.

To assess the role of the hOGG1 DNA glycosylase in TCR of 8-oxoguanine, the removal of this lesion in wild type and ogg1-/- null mouse embryo fibroblast cell lines has been investigated. 8-oxoG was not removed from the nontranscribed strand in the homozygous ogg1-/- null mouse cell line whereas there was still efficient 8-oxoG repair in the transcribed strand. The expression of the mouse OGG1 protein in the homozygous ogg1-/- cell lines restored the ability to remove 8-oxoG in the nontranscribed strand. This might indicate the existence of an OGG1-independent pathway for TCR of 8-oxoG *in vivo* [39].

The involvement of other proteins in TCR of oxidative damage, in addition to CSB, XPB, XPD and XPG, was also shown. Their role in this process has not been clearly understood. It was found that mouse embryonic stem cells deficient in BRCA1 were defective in the ability to carry out TCR of oxidative DNA damage, and were hypersensitive to ionizing radiation and hydrogen peroxide [41]. These results suggest that BRCA1 participates, directly or indirectly, in TCR of oxidative DNA damage. BRCA1 is not required for global removal of oxidized base.

Overlapping functions for oxidative base damage repair pathways

There is growing amount of data that organisms have developed overlapping DNA repair pathways to protect DNA from unavoidable damage. For example, in *E. coli* UvrABC-mediated nucleotide excision repair also plays a role in the repair of oxidized DNA bases. Both *in vitro* and *in vivo*, the UvrABC complex recognizes and excises Tg residues from DNA [35, 42]. In *S. cerevisiae* single BER or single NER mutants demonstrate wild-type sensitivity to oxidizing agents. However, double (or multiple) BER and NER mutants are more sensitive comparing with respective single mutants. These data suggest that BER and NER have

overlapping specificities and act synergistically to repair oxidative damage in yeast [21, 27, 66].

In contrast to bacterial and yeast cells there is no report of mammalian cells being deficient in more than one repair pathway. Hence, it is still unknown whether other repair processes will compensate for a lost or saturated pathway in mammalian cells. Global genome repair of oxidative lesions in human cells is normal in all NER mutant cell lines tested so far (i.e. *XPA, XPB/CS, XPD, XPD/CS, XPG*) including CSA and CSB cell lines defective in TCR (reviewed in [37]). However, in hamster cells global genome repair of 8-oxoG requires a functional *CSB* gene product [65]. Only limited data about the DNA repair of oxidative lesions in NER deficient hamster cell lines are available. Using the cell free extract (CFE) from wild-type and NER deficient hamster cell lines (*ERCC1, ERCC2/XPD, ERCC3/XPB, ERCC4/XPF, ERCC5/XPG*) Reardon et al [55] found that CFE from wild-type cells excised both Tg and 8-oxoG lesions while CFEs from mutant cell line were as defective in removing 8-oxoG and Tg as they are in removing thymine dimers.

Conclusion

Majority of oxidative DNA damage in mammalian cells whether caused by endogenous or exogenous agents is either cytotoxic or mutagenic. The biological importance of its removal is underline by the existence of several DNA repair processes. Base excision repair is the major repair pathway of oxidative DNA damage. In addition, nucleotide excision repair and transcription-coupled repair are important back-up systems. Their substrate specificities and repair functions are overlapping. Moreover, several repair enzymes are involved in more then one repair pathways. The mammalian cells (and organisms) thus possess the complex system of genome maintenance to produce healthy offspring.

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