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Oxidative DNA Damage and Nucleotide Excision Repair

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Abstract

Significance: Oxidative DNA damage is repaired by multiple, overlapping DNA repair pathways. Accumulating evidence supports the hypothesis that nucleotide excision repair (NER), besides base excision repair (BER), is also involved in neutralizing oxidative DNA damage. **Recent Advances:** NER includes two distinct sub-pathways: transcription-coupled NER (TC-NER) and global genome repair (GG-NER). The CSA and CSB proteins initiate the onset of TC-NER. Recent findings show that not only CSB, but also CSA is involved in the repair of oxidative DNA lesions, in the nucleus as well as in mitochondria. The XPG protein is also of importance for the removal of oxidative DNA lesions, as it may enhance the initial step of BER. Substantial evidence exists that support a role for XPC in NER and BER. XPC deficiency not only results in decreased repair of oxidative lesions, but has also been linked to disturbed redox homeostasis. **Critical Issues:** The role of NER proteins in the regulation of the cellular response to oxidative (mitochondrial and nuclear) DNA damage may be the underlying mechanism of the pathology of accelerated aging in Cockayne syndrome patients, a driving force for internal cancer development in XP-A and XP-C patients, and a contributor to the mixed exhibited phenotypes of XP-G patients. **Future Directions:** Accumulating evidence indicates that DNA repair factors can be involved in multiple DNA repair pathways. However, the distinct detailed mechanism and consequences of these additional functions remain to be elucidated and can possibly shine a light on clinically related issues. *Antioxid. Redox Signal.* 18, 2409–2419.

Introduction

Oxidative stress originates from an imbalance between the generation of reactive oxygen species (ROS) and their scavenging by multiple cellular antioxidant defenses. Excessive amounts of ROS are potentially deleterious to cells because of interactions with cellular macromolecules, such as nucleic acids, proteins, and lipids (14, 64). These cellular components are protected against oxidation by evolutionary conserved defense mechanisms. Nevertheless, the damage to molecules such as DNA resulting from oxidative stress is extensive and is recognized as a causal feature in many diseases and in the aging process (7, 70).

The most important ROS are superoxide anion ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) (74). A well-established source of endogenous ROS is normal aerobic metabolism. Oxidative stress can also occur due to the generation of oxygen-derived free radicals from exposure to environmental stressors, including ionizing and nonionizing

radiation, and certain chemicals (14). Oxidative damage to DNA has been estimated as 10^4 hits per cell per day in humans (29). ROS can induce a number of covalent modifications to DNA, which encompass single-nucleobase lesions, strand breaks, inter- and intrastrand cross-links, along with protein-DNA cross-links. The most prevalent damage to purines is 7,8-dihydro-8-oxoguanine, more commonly named 8-oxoguanine or 8-oxoG; while the most common damage to pyrimidines is the formation of thymine glycol (74). These are nonhelix distorting (nonbulky) lesions. An example of bulky modified bases are the 8,5'-cyclopurine-2'-deoxynucleosides.

Primary defense systems to manage the prevention of ROS include detoxification enzymes such as glutathione peroxidase, catalase, and superoxide dismutase; radical scavenging by antioxidants; and reduction of radicals by redoxins. Another important defense mechanism that is used for limiting mutagenesis, cytostasis, and cytotoxicity due to oxidative stress is DNA repair. Multiple, overlapping, DNA repair pathways are responsible for removal of the damage. Base excision repair

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FIG. 1. Nucleotide excision repair. Schematic overview of the nucleotide excision repair (NER) pathway. Damaged DNA is recognized by either initial factors of transcription-coupled repair (CSA and CSB) or global genome repair (a.o. XPC), which constitute the two different repair pathways in NER. After DNA damage recognition, the repair route progresses along the same way. After helix unwinding and verification of the damage, incisions are made to remove the faulty stretch of DNA. Finally, DNA synthesis and subsequent ligation reproduce the correct DNA sequence. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

(BER) is considered the primary mechanism involved in oxidative DNA damage repair. Growing evidence, however, indicates that the nucleotide excision repair (NER) pathway is also involved in neutralizing oxidative DNA damage (7, 74). The possible functions of several NER proteins in oxidative DNA damage repair are discussed in detail in this review.

Nucleotide Excision Repair

The NER pathway consists of more than 30 proteins, which in concert can deal with a broad spectrum of (mostly) structurally unrelated bulky DNA lesions, which can arise from either endogenous or exogenous agents. Bulky lesions can originate on exposure to several damaging agents. For instance, UV radiation (sunshine) is a DNA-damaging agent that mainly produces helix-distorting cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6,4)-pyrimidone products (6-4PP). Exposure to various chemicals or alkylating agents can also result in bulky DNA adducts formation; for example, polycyclic aromatic hydrocarbons (present in cigarette smoke or charcoaled meat). More recently, however, NER is also believed to be involved in eliminating oxidative DNA damage.

NER includes two sub-pathways, which mechanistically initiate in a divergent manner, but after damage recognition, both pathways proceed along the same molecular route (see Fig. 1). The sub-pathways are designated Transcription-Coupled NER (TC-NER) and Global Genome NER (GG-NER). TC-NER is responsible for eliminating lesions in the transcribed strand of active genes. This repair process takes care of lesions blocking the transcription machinery. GG-NER recognizes and removes lesions throughout the entire genome, and is considered a relatively slow and somewhat more inefficient process, as it scans the whole genome for DNA damage.

Both pathways can generally be divided into 4 phases: (i) DNA damage recognition, (ii) recruitment of the preincision complex and DNA unwinding, (iii) creating dual incisions flanking the DNA lesion site and subsequent excision of the damaged fragment, and (iv) DNA repair synthesis and ligation (Fig. 1). In the next sections, we will touch on these different phases.

DNA damage recognition

TC-NER and GG-NER differ in the initial damage recognition step (Fig. 1). The initial structural components that are recognized after DNA damage are helical distortions and alterations.

In TC-NER, a stalled RNA polymerase II (RNA polII) during transcription events sets the NER machinery in motion to remove and repair the blocking lesion. Both the Cockayne syndrome complementation group A (CSA) and B (CSB) proteins not only play a crucial role in the onset of transcription-coupled repair, but are also implicated in RNA polII transcription functions. The CSB protein, a SWI/SNF ATPase, interacts with RNA polII (86); while CSA does not (85). In TC-NER, CSB is thought to be responsible for displacement of the stalled RNA polymerase. The function of CSA remains to be fully elucidated, but among others, it seems to be implicated in TC-NER during elongation of the transcription process (34, 44). A bifunctional damage detection and ubiquitin ligase complex (CSA-DDB1-CUL4-RBX1 E3 ligase, also known as CRL4^{CSA}) is recruited to the lesion, where it promotes DNA repair and the consequential transcription restart (25). However, the complex also serves in GG-NER (CRL4^{DDB2}), but the substrate receptor in this case is DDB2 instead of CSA (25). Very recently, an additional TC-NER factor, UV-sensitive

syndrome protein A (UVSSA), has been discovered and characterized (56, 68, 95). UVSSA interacts with elongating RNA polymerase II and is implicated in stabilizing CSB by delivering the deubiquitinating enzyme USP7 to TC-NER complexes (68). The recruitment of the consecutive components of the NER machinery (also known as the preincision complex) to the damaged site (5, 94) occurs in a CSB-dependent manner (27, 28). CSA is dispensable for attraction of NER proteins; however, in cooperation with CSB, it has been suggested to be required to recruit the chromatin remodeling factors XAB2, HMG1, and TFIIS (27, 28).

In GG-NER, the XPC/hHR23B complex (including centrin2), along with the UV-Damaged DNA Binding (UV-DDB) protein (assembled by the DDB1 (p127) and DDB2/XPE (p48) subunits), is involved in lesion recognition (22). The XPC/hHR23B complex is also essential for recruiting the preincision complex, as is the case for CSB in TC-NER. The UV-DDB complex is a part of the DDB2-DDB1-CUL4-RBX1 E3 ligase complex (CRL4^{DDB2}), which acts in the repair of UV-induced DNA lesions in chromatin (69).

Various helix-distorting base lesions that do not share a common chemical structure are recognized by XPC. Biochemical studies have revealed that XPC recognizes a specific secondary DNA structure rather than the lesions themselves (55, 80, 82). Min and Pavletich (55) suggested that XPC binds opposite to the lesion site and flips the damaged bases out of the helix structure, providing an explanation for the broad specificity of GG-NER damage recognition. XPC itself has affinity for DNA and is able to initiate GG-NER *in vitro*, but its functionality is enhanced when hHR23b and centrin2 are added (4, 57). XPC (together with DDB1 and DDB2) appears to scan the DNA for distortions by migrating over the DNA, repeatedly binding and dissociating from the double helix (41). The binding affinity of XPC to the DNA seems to correlate with the extent of helical distortion; for this reason, specificity for bulkier 6-4PP adducts is higher than for CPDs (81). More recent studies have indicated that the UV-DDB protein complex facilitates the recognition of lesions (such as CPDs) that are less well recognized by the XPC-hHR23B complex (26).

The XPC protein contains several binding domains: for DNA binding, binding to hHR23B, Centrin2, 8-oxoguanine glycosylase (OGG1), or p62/SQSTM1 and transcription factor II H (TFIIH) (12, 79). TFIIH is a multifunctional transcription initiation factor but is also a core NER component (Fig. 1).

DNA helix unwinding

TC-NER and GG-NER converge into the same pathway after DNA damage recognition and subsequent recruitment of the TFIIH complex. The complex is essential for the continuation of the NER pathway and is responsible for unwinding the DNA helix after damage recognition by XPC/hHR23B and/or stalled RNA polymerase. The TFIIH complex is built up by 10 proteins: XPB, XPD, p62, p52, p44, p34, p8, and the CDK-activating kinase (CAK) complex: MAT1, CDK7, and Cyclin H. TFIIH forms an open bubble structure in the DNA helix (31, 32). The partial unwinding of the DNA duplex is promoted by the DNA helicases XPB and XPD, facilitating the preincision complex to enter the lesion site (60) (Fig. 1). The XPA, RPA, and XPG proteins are additional factors of the preincision complex and on recruitment, all of them are assembled around the damaged site (96) (Fig. 1).

XPA is believed to be responsible for lesion verification. It also acts as an organizational factor, along with the single-strand DNA binding complex RPA, stimulating accurate positioning of the repair proteins around the lesion. Both XPA and RPA are believed to protect the undamaged strand for incorrect incision (17, 38) and see to the complete opening of the damaged DNA. It has been suggested that the latter step is essential for the initiation of incision/excision of the damaged DNA stretch (2, 13). RPA interacts with the endonucleases XPG and the ERCC1-XPF dimer, which are required for the dual incision of the damaged strand (Fig. 1). Correct positioning of the endonucleases is facilitated by RPA (51, 62).

Incision, DNA repair synthesis, and ligation

DNA incisions are made by the endonucleases ERCC1-XPF and XPG (Fig. 1). A general consensus of the mechanism is that the concerted actions of XPG and ERCC1-XPF result in the removal of the damaged site by the excision of a 24–32 nucleotide single-strand fragment (39). The presence of XPG might be necessary for ERCC1-XPF activity. It is generally believed that ERCC1-XPF is responsible for carrying out the initial incision at the 5' end (91) and is later followed by the 3' incision by XPG, even though XPG is thought to be recruited first by the TFIIH complex (96). The damaged oligomer is released, and the gap is filled by DNA polymerases (Pol δ , Pol ϵ , and Pol κ) (Fig. 1). The process is facilitated by proliferating cell nuclear antigen, RPA, and replication factor C. Finally, the 3' nick is closed by DNA ligase. From studies using human cell-free extracts, an alternative "cut-patch-cut-patch" mechanism has also been proposed for the dual incision and resynthesis process (77).

NER factor functionality in prevention or repair of oxidative DNA damage

We will discuss the two sub-pathways of NER, TC-NER, and GG-NER, in relation to the repair of oxidative DNA damage. Both *in vivo* and *in vitro* evidence as well as human and mammalian data are provided in this review if available.

Transcription-coupled repair: CSA, CSB, and XPG

The CSA and CSB proteins play a crucial role in the onset of TC-NER. Most purposeful studies conducted on the role of cockayne syndrome (CS) proteins in relation to oxidative stress have been performed with cells or mice deficient in CSB. Murine cells defective in CSB appeared hypersensitive to ionizing radiation and other oxidants (19). CSB deficiency also causes increased sensitivity to oxidative DNA damage in mice (18). In addition, the *Csb*-deficient mouse retina was found to be hypersensitive to ionizing radiation (33). Fibroblasts derived from CS-B patients are more sensitive to oxidants and are impaired in the repair of oxidatively induced DNA lesions (63, 90).

The role of CSA in oxidative DNA damage response is less unambiguous. *Csa*^{-/-} mouse embryonic fibroblasts and keratinocytes as well as *Csa*^{-/-} mice failed to show an increased sensitivity to oxidative stressors (18). These findings might suggest that CSA is not involved in an oxidative DNA damage response. However, more recent studies using human cells indicate not only cells deficient in CSB, but also cells deficient in CSA are more sensitive to treatment with H₂O₂ (76). In addition, primary fibroblasts and keratinocytes from

FIG. 2. Base excision repair (BER). Schematic overview of the BER pathway. Shown is a general model of the short-patch (left) and long-patch (right) BER pathways. Short-patch repair replaces the lesion with a single nucleotide; long-patch repair replaces the lesion with approximately 2 to 10 nucleotides. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

CS-A patients were shown to be hypersensitive to potassium bromate, a specific inducer of oxidative damage (16). Recently, inactivation of CSA and CSB in human cells was demonstrated to result in an altered redox status, causing the accumulation of DNA damage. In these CS cells, both oxidative and energy cell metabolism were affected (63). Taken together, these findings indicate that the function of CSA may differ between species. In contrast to mice, CSA appears to be involved in an oxidative DNA damage response in humans.

At the molecular level, the CSB protein has been shown to physically interact with poly-ADP ribose-polymerase 1 (PARP1), a nuclear enzyme that binds with high affinity to and is activated by DNA single-strand breaks. Oxidative stress induces post-translational modifications of CSB by PARP1 (88). More importantly, the CS proteins are also recruited to the mitochondrion on oxidative stress. Endogenous ROS are largely formed during oxidative phosphorylation in the mitochondria. One of the most common oxidative DNA lesions is 8-oxoG. Mitochondria efficiently repair oxidative DNA damage such as 8-oxoG, primarily by the BER pathway (Fig. 2) (3, 20, 84). NER provides backup to BER when glycosylases are defective in the nucleus, but NER systems are absent from mammalian mitochondria. In mammalian cells, specific DNA glycosylases carry out the first step of BER: recognition and removal of the damaged base. The glycosylase responsible for the repair of 8-oxoG is the OGG1. Release of the damaged base results in the formation of an apurinic/apyrimidinic (AP) site. These are then cleaved by AP endonucleases (APE1, 2 in Fig. 2). The resulting single-strand break can then be processed by either short-patch (where a single nucleotide is replaced) or long-patch BER (where 2–10 new nucleotides are synthesized) (Fig. 2).

In humans, CSB has a regulatory effect on OGG1 expression for maintaining OGG1 enzyme levels and function. Deficiency

in CSB leads to deficient transcription of the OGG1 gene and, thus, to deficient repair of 8-oxoguanine in DNA (21, 42). CSB has been suggested to play a role in the repair of 8-oxoG in mitochondrial DNA (mtDNA) in human and mouse cells (78). Protein levels of CSA and CSB in mitochondria of human cells are increased on oxidative stress. CSA was also found to be recruited to the nuclear matrix in a CSB-dependent manner on H_2O_2 treatment *in vitro* using human cells, which suggested that CSA is also relevant to transcription-coupled repair of oxidative DNA damage (44). Results of Kamenisch and colleagues indicated that the proteins directly interact with mtDNA and OGG1 (43), where they stabilize repair complexes at the mitochondrial membrane (1). In addition, mtDNA damage has been shown to accumulate in CSB-defective cells (61).

CS is characterized by developmental defects, neurodegeneration, and accelerated aging (Fig. 3; Table 1) (37). The involvement of CSA and CSB in the repair of oxidative DNA lesions may, in part, explain the symptoms that are typical for patients with Cockayne syndrome, but the symptoms have also been linked to deregulated mitochondrial activity (43) or transcriptional response (10, 24). The pathology of accelerated aging may be due to the accumulation of DNA damage in mitochondria. Several lines of evidence support this theory, but the exact relationship is not yet fully clear. Various mouse models have been generated that harbor increased levels of mtDNA mutations; some models display multiple symptoms of accelerated aging (47). Taken together, a large body of evidence supports a significant role for the CS proteins in mitochondrial BER regulation. However, further research is needed to elucidate the specific roles of CSA and CSB herein.

A possible role for XPG has also been suggested in transcription-coupled repair of oxidative lesions (48). This endonuclease is responsible for the 3' incision of the DNA lesion. Little is known about this putative role of XPG in oxidative

FIG. 3. Picture of a patient with Cockayne syndrome (CS) at the age of 10 years, with his 11-year-old sister. Note the typical CS appearance with deep set eyes, prominent ears, and cachexia. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

DNA damage response. Recent studies with *Xpg*-deficient mouse dermal fibroblasts fail to show sensitivity to high doses of oxidative damage-inducing agents such as H_2O_2 (Hoeijmakers, personal communication). In contrast, *in vivo* these *Xpg*-deficient mice express higher levels of antioxidant protein NQO1 in liver, indicating that accumulation of (other) endogenous oxidative DNA damage occurs in the absence of functional XPG protein. The interaction of XPG with the core TC-NER factor CSB and possibly also TFIIH might be the underlying mechanism. Other support for the involvement of XPG in oxidative DNA damage repair comes from studies with fibroblasts derived from an XP-G patient, which appeared to be deficient in the repair of oxidative DNA damage (92). The XPG protein has been demonstrated to stimulate the initial step of BER in the repair of thymine glycol *in vitro*, although BER activity was reconstituted here by recombinant proteins. XPG herein promoted binding of hNth1, the human counterpart of *E. coli* endonuclease III, to damaged DNA (48). This indicates that, similar to other repair factors such as the CS proteins, XPG might have functions outside of NER.

TABLE 1. CLINICAL FEATURES OF COCKAYNE SYNDROME, XERODERMA PIGMENTOSUM, AND XERODERMA PIGMENTOSUM WITH NEUROLOGICAL DISEASE

Feature	CS	XP	XP+
Skin			
Skin sun sensitivity	Yes	Yes	Severe
Increased skin pigmentation	No	Yes	Yes
Sunlight-induced skin cancer	No	Yes	Yes
Eyes			
Photophobia	Yes	Yes	Yes
Conjunctival growths	No	Yes	Yes
Cancer (anterior eye/lids)	No	Yes	Yes
Congenital cataracts	Yes	No	No
Pigmentary retinal degeneration	Yes	No	No
Somatic			
Short stature	Yes	No	No/Yes
Immature sexual development	Yes	No	No
Nervous system			
Sensorineural deafness	Yes	No	Yes
Developmental delay	Yes	No	Yes
Progressive neurological degeneration	Yes	No	Yes
Dysmyelination	Yes	No	Yes
Cerebral atrophy	Yes	No	No
Cerebellar atrophy	Yes	No	Yes
Calcification (basal ganglia)	Yes	No	No
Disease mechanism			
Nucleotide excision repair defect	Yes	Yes	Yes
Base excision repair affected	Poorly	XPC?	XPG?
Reaction to exo- or endogenous damaging agents	Yes	Yes-severe	Yes-severe
Developmental defect	Yes-severe	No	Yes
Accelerated aging	Yes	No	Yes
Cancer proneness	No	Yes	Yes

Adapted/modified from Kraemer *et al.* (50).

CS, Cockayne syndrome; XP, Xeroderma pigmentosum; XP+, Xeroderma pigmentosum with neurological disease.

In humans, the effect of impaired XPG functioning remains to be fully elucidated, as variants of the XPG protein may lead to divergent complex phenotypes in humans. Some patients develop Xeroderma pigmentosum (XP), a rare autosomal inherited and a pure cancer prone disease. Others develop CS symptoms or a combined XP/CS phenotype. Patients with a (partial) XPG defect are rare: Only 14 have a XP phenotype, 3 have a CS-like disorder, and 7 developed XP in combination with severe CS (23, 87). In humans, XPG genes containing missense mutations, resulting in a stable XPG protein, appear to predominantly give rise to the XP phenotype; while XP/CS is the result of a truncated and/or production of an unstable XPG protein (23, 58, 87). In mice, variants having mutations in conserved N-terminal regions are devoid of any NER activity and, in general, show an XP phenotype (89). On the other hand, domains of the C-terminal part of XPG could be attributed to functions in TC-NER. As such, mice missing the last exon 15 of the *Xpg* gene have been shown to develop a CS phenotype (73).

Global genome repair: XPC and XPA

In GG-NER, XPC is involved in the recognition of a variety of bulky DNA-distorting lesions. The *Xpc*-deficient mouse model

supplied valuable information that pointed toward the possible involvement of the XPC protein in the removal of oxidative DNA damage. Hollander and colleagues demonstrated that *Xpc*-deficient mice in a mixed genetic background (75% C57BL/6J, 25% 129) exhibited a very large increase in lung tumor incidence compared with wild-type controls. A potential reason for this phenotype might be continuous exposure to oxidative stressors and subsequent oxidative DNA damage in the lung tissue (40). Both at the time of death and at the intercurrent age of 16–17 months 100% of the *Xpc*^{-/-} mice harbored lung tumors. In a C57BL/6J congenic background, we also observed an increase in lung tumor incidence in *Xpc*-deficient mice. In contrast, *Xpa*-deficient mice spontaneously had a significantly increased tumor incidence in the liver, but not in the lung (54). Moreover, an elevated level of mutations was found in lung tissue in *Xpc*^{-/-} mice during aging, which correlated to the increase in lung tumors in these mice, and is most likely caused by unrepaired oxidative DNA damage. No such increase in mutational load was found in wild-type and *Xpa*-deficient mice. Since *Xpa*-deficient mice seemed unaffected in lung tumor incidence and mutational load, these results pointed toward an additional function of XPC in mice in response to oxidative DNA damage.

In vitro experiments using mouse embryonic fibroblasts revealed that cells derived from *Xpc*-deficient mice were more sensitive, in terms of survival and mutation accumulation, to oxygen exposure than to cells derived from *Xpa*-deficient or wild-type mice (54). We showed that 9 month exposure to the pro-oxidant diethylhexyl phthalate through the diet resulted in elevated levels of mutations in *Xpc*-deficient liver tissue, which were not visible in wild-type and *Xpa*-deficient liver tissue or on 3 month exposure in all three genotypes (unpublished results). In addition, short-term exposure (1 and 2 weeks) to equine estrogen, inducing 8-oxodG adducts, did not demonstrate enhanced sensitivity to 8-oxodG DNA adduct accumulation in repair-deficient *Xpc* mice (59).

Studies using human primary keratinocytes and fibroblasts from XP-C patients showed that XPC protects human skin cells from the killing effects of oxidants such as potassium bromate or those induced by X-rays (15). Furthermore, the same study provided evidence that XPC is involved in the repair of 8,5'-cyclopurine 2'-deoxynucleosides and major oxidized DNA bases such as 8-oxoG (15). Human XP-C fibroblasts have also been demonstrated to be impaired in repair of oxidative DNA damage induced by methylene blue plus visible light (45). Furthermore, silencing of XPC in a human glioma cell line increased arsenic-induced cell death. Arsenic exerts its cytotoxicity via the generation of ROS and inhibition of DNA repair. XPC silencing did not interfere with repair of arsenic-induced DNA damage, but caused increased arsenic susceptibility by disturbing redox homeostasis, as was suggested by Liu *et al.* (52). In addition, in normal human keratinocytes, the down-regulation of XPC resulted in increased intracellular ROS levels, genomic and mtDNA oxidation, and altered metabolism (65). It could, therefore, also be possible that, besides decreased repair of oxidative lesions, the hypersensitivity to oxidative stress in cells defective in XPC can at least partly be due to altered oxidative metabolism, resulting in an increased production of ROS.

The repair mechanisms involved are most likely not only NER, but also BER. It has been postulated that the XPC-hHR23B complex acts as a co-factor in the short-patch BER pathway by stimulating OGG1 activity (15). Analysis of the

biochemical properties behind mutations in the *XPC* gene found in XP patients demonstrated a direct interaction between the N-terminal part that encompasses the P334 surrounding region of XPC and OGG1. The XPC/P334H mutation weakens the interaction with OGG1, resulting in a decreased capacity to regulate the glycosylase activity (6). In addition, the hHR23B factor was found to interact with BER protein 3-methyladenine DNA glycosylase (49). XPC was also proposed to interact with thymine DNA glycosylase (TDG), supporting the hypothesis that XPC may also be involved in long-patch BER (Fig. 2) (72). XPC appears nonessential for BER, but might contribute to its effectiveness. D'Errico and colleagues have proposed a mechanism in which XPC-hHR23B might bend DNA at sites of damage and, thus, facilitate loading and turnover of DNA glycosylases by direct protein-protein interaction or by competition with the DNA substrate (15). The possibly more supportive role of XPC in the removal or prevention of oxidative DNA damage proposed by several earlier groups is accentuated by the phenotype of XP-C patients. XP-C patients who are diagnosed early and are well protected from sunlight mostly show no evidence for any significant pathology that might indicate abnormal responses to oxidative damage, although primary internal tumors have been identified in two young XP-C patients (30). In addition, one early diagnosed XP-C patient (XP1M1), harboring the (P334H) mutation, has been diagnosed with neuropathology. As mentioned earlier, evidence was provided that this P334H substitution can prevent stimulation of BER factor OGG1 (6). Another XP-C patient (XP21BE) with neurological abnormalities has been identified, but neuropathology might be caused by another genetic defect other than impaired XPC functioning (46). Furthermore, potential allelic loss of XPC was observed in many of the human lung tumors investigated by Hollander *et al.* (40). The late onset of adverse health effects due to lung tumors in *Xpc* mice suggests that lung or other internal tumors driven by oxidative DNA damage would be apparent only in older XP-C patients.

Based on the findings from studies with *Xpa*-deficient mice, XPA seems not to play an important role in oxidative DNA damage repair other than the repair of oxidative bulky lesions. Extensive reviews by Brooks (9, 10) bundled the evidence that the oxidatively induced, bulky 8,5'-cyclopurine-2'-deoxynucleosides can act as a substrate for NER. These lesions can, in part, explain the neurodegeneration found in several XP patients, among whom are XP-A patients. More recently, cells from XP-A patients exhibited defective repair of 8,5'-(S)-cyclo-2'-deoxyadenosine, a free radical-induced endogenous DNA lesion (36). More recent studies furthermore indicate that intra-strand crosslink lesions such as G[8-5]C, G[8-5m]mC, and G[8-5m]T can be caused by oxidative DNA damage and are a substrate for NER (35, 93). The latter study also demonstrated that XPA-deficient human brain tissue and liver tissue of *Xpa* mice contained higher levels of G[8-5 m]T lesions than the corresponding normal controls (93). In addition, an increased susceptibility to oxidative stress-induced genotoxicity was detected in primary fibroblasts from an XP-A patient after treatment with sodium arsenite and H₂O₂ (53). A recent report shows that fibroblasts derived from XP-C and XP-A patients are deficient in the repair of both bulky photoproducts and oxidative DNA damage. Both types of DNA damage induced more mutations in cells defective in XPA than in control cells (92).

It is possible that oxidative DNA damage is a contributing or possibly even a driving factor to cancer development in XP

FIG. 4. Northern African xeroderma pigmentosum (XP)-C patient at the age of 23 years with numerous hyperpigmented macules on his face. Nodular basal cell cancer is present on his left nasal root. Pigmented basal cell cancer is present on his left cheek. His eyes show cornea scarring from unprotected sun exposure. Adapted from Bradford *et al.* (8). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

patients. XP is characterized by skin disease with accompanying cancer predisposition and neurodegeneration caused by deficiencies in one of the XP proteins involved in NER. XP in humans is accompanied by a severe and early onset of skin cancer; the mean latency time of cutaneous neoplasms is 8 years (Fig. 4, table 1). This may overshadow potential tumors in other tissues such as the liver and lung, which were found in NER-deficient mice (54). The risk of developing skin cancer is more

than a 1,000-fold increased in XP patients (49). In Western Europe and the United States, the incidence frequency is approximately 1:250,000; rates are higher in Japan (1:40,000). Internal tissues are not exposed to UV, and exposure to chemicals that induce bulky adducts is probably low. Lungs are known to be exposed to higher levels of oxidative stress. In addition, increased oxidative stress levels have been implied in colorectal, bladder, and lung carcinogenesis (11, 66, 67, 71, 75, 83). However, these and other cell types can also come into contact with harmful (environmental) agents or their reactive metabolites that can cause DNA damage. Internal tumors of XP-C patients have been analyzed, and results indicate that mutations are most likely caused by unrepaired oxidative DNA lesions (30).

Innovation

In the last decade, accumulating evidence has been published that implicates multiple nucleotide excision repair proteins in the regulation of oxidative DNA damage repair. Either by regulation of base excision repair factors, involvement in redox homeostasis, or the actual repair of bulky oxidative DNA lesions. The additional functionality of these factors can, in part, explain the pathology exhibited by xeroderma pigmentosum (XP), cockayne syndrome (CS), or XP/CS patients.

Conclusions

In this review, we present an overview of the (possible) functions of NER proteins in the repair of oxidative DNA damage. Repair of DNA lesions induced by oxidative stress requires the activity and interaction of different DNA repair pathways, including BER and NER. A large body of evidence suggests that several proteins primarily involved in NER also play a role in BER. Depending on the type and structure of the lesion present, NER proteins will involve either the NER or the BER pathway (Fig. 5).

Transcription-coupled repair of (oxidative) DNA lesions requires functional CSA and CSB. Deficiency in either of these proteins will result in a defective TC-NER (Fig. 5, transcription-coupled NER). Consequently, bulky lesions in actively transcribed genes in the nucleus will be left unrepaired. Besides this

FIG. 5. Schematic overview of possible functions of NER proteins in the major excision repair pathways NER and BER in removal of oxidative DNA damage.

function as NER component, the CS proteins also seem to have a regulatory effect on BER, and, thus, also on the removal of nonbulky oxidative lesions. This is of particular importance for mitochondria, as NER is notably absent in these organelles. Loss of functional CSA or CSB may cause accumulation of oxidative damage in mtDNA, which could contribute to the accelerated aging phenotype (including neuropathology) that CS patients display (Fig. 5, transcription-coupled BER).

The endonuclease XPG has also been implicated in transcription-coupled repair of oxidative lesions. The underlying mechanism could be the interaction with CSB and TFIIH to ensure the recruitment of XPG. For lesions repaired through NER, the interaction with TFIIH is necessary to localize XPG to sites of repair. In BER, XPG potentially functions as a co-factor by promoting the binding of DNA glycosylase Nth (and perhaps other glycosylases) to damaged DNA (Fig. 5, transcription-coupled BER). The recruitment of XPG in BER could also be facilitated by an interaction with CSB via domains in the C-terminal part of XPG. Reduced or lack of affinity for CSB may explain the CS phenotype of XP-G patients with truncations at the C-terminus of the protein.

In global genome repair, XPC plays a key role as a damage recognition protein. Helix-distorting, oxidative lesions are recognized by the XPC-hHR23B complex and repaired via NER. Defects in XPC will leave lesions in the nontranscribed strand unrepaired, which could be the main cause for the greatly increased skin cancer susceptibility of XP patients (Fig. 5, global genome NER). By interacting with various DNA glycosylases, XPC appears to be also involved in short-patch and long-patch BER. The latter mechanism may be of importance for the repair of endogenous oxidative DNA lesions in nontranscribed DNA. Failure to repair these particular lesions may give rise to the few reported internal tumors found in XP-C patients (Fig. 5, global genome BER).

Since XPA is a core factor of NER, XP patients who belong to complementation group A are devoid of both TC-NER and GG-NER activity. To date, no data that indicate a link between XPA and BER have been reported. This suggests that the BER pathway is still fully functional in XP-A patients. However, this will not prevent the accumulation of mutations and thereby the development of tumors in XP patients (Fig. 5, NER). Next, the neuropathology as reported in XP-A patients possibly arises from a defect in TC-NER. Interestingly, however, in XP-A patients, this is not accompanied by other severe accelerated aging phenotypes, such as in the XP-B and XP-D complementation groups (50). Future studies should be engaged in defining in greater detail the exact role of the various NER proteins in the NER and BER pathways in response to oxidative DNA damage. Elucidating the complex mechanisms that underlie the NER-related phenotypes, such as cancer, accelerated aging, and neuropathology, is still unresolved, creating an interesting challenge for future research.

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Abbreviations Used

6-4PP	= pyrimidine-(6,4)-pyrimidone products
8-oxoG	= 7,8-dihydro-8-oxoguanine
BER	= base excision repair
CPD	= cyclobutane pyrimidine dimers
CS	= Cockayne syndrome
DDB	= DNA damage-binding
GG-NER	= global genome NER
H ₂ O ₂	= hydrogen peroxide
hNth	= human equivalent of <i>E. coli</i> endonuclease III
mtDNA	= mitochondrial DNA
NER	= nucleotide excision repair
OGG1	= 8-oxoguanine glycosylase
PARP1	= poly-ADP ribose-polymerase 1
PCNA	= proliferating cell nuclear antigen
RNA polII	= RNA polymerase II
ROS	= reactive oxygen species
TC-NER	= transcription-coupled NER
TDG	= thymine DNA glycosylase
TFIIH	= transcription factor II H
UVSSA	= UV-sensitive syndrome protein A
XP	= xeroderma pigmentosum