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Review

Recognition of DNA Damage in Mammals

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DNA damage by UV and environmental agents are the major cause of genomic instability that needs to be repaired, otherwise it give rise to cancer. Accordingly, mammalian cells operate several DNA repair pathways that are not only responsible for identifying various types of DNA damage but also involved in removing DNA damage. In mammals, nucleotide excision repair (NER) machinery is responsible for most, if not all, of the bulky adducts caused by UV and chemical agents. Although most of the proteins involved in NER pathway have been identified, only recently have we begun to gain some insight into the mechanism by which proteins recognize damaged DNA. Binding of *Xeroderma pigmentosum* group C protein (XPC)-hHR23B complex to damaged DNA is the initial damage recognition step in NER, which leads to the recruitment of XPA and RPA to form a damage recognition complex. Formation of damage recognition complex not only stabilizes low affinity binding of XPA to the damaged DNA, but also induces structural distortion, both of which are likely necessary for the recruitment of TFIIH and two structure-specific endonucleases for dual incision.

Keywords: DNA damage, Nucleotide excision repair, Replication protein A, XPA, and XPC-hHR23B

DNA damage recognition in nucleotide excision repair

Nucleotide excision repair (NER) action involves interaction of multiple proteins locating the damaged DNA sites, removal of short oligonucleotides containing DNA adducts, and synthesizing a replacement patch. In mammals, NER requires over 20 polypeptides including damage recognition/structure distortion factors [*Xeroderma pigmentosum* group A protein (XPA), XPC-hHR23B, replication protein A (RPA, also known as human single-stranded DNA binding protein, hSSB), and XPE], TFIIH containing two DNA helicases (XPB and XPD) that separate the strands to create an open preincision complex, two structure-specific endonucleases, ERCC1-XPF and XPG, and the enzymes necessary for filling-in the gap [DNA polymerase δ/ε, proliferating cell nuclear antigen (PCNA), replication factor C (RF-C), and RPA] (see Fig. 1).

An ongoing challenge is to understand how DNA damage is recognized and distinguished from non-damaged sites during NER. Recognition of DNA damage is the initial, but complicated event for NER. It involves multiple proteins: XPA, RPA, XPC-hHR23B, XPE and TFIIH, all of which can independently bind to damaged DNA and may have a role in damage recognition (Sancar, 1996; Dawn and Wood, 2000). Although some characteristics of the damage recognition proteins are known, the molecular mechanism of how these proteins function at the damaged DNA site is not clear. A recent *in vitro* study suggested that XPC-hHR23B is an initiator of global genomic repair, but not of transcription-coupled repair (Sugasawa *et al.*, 1998; Sugasawa *et al.*, 2001). This is based on the findings that preincubation of UV-damaged plasmid DNA with XPC was preferentially repaired in an *in vitro* kinetic experiment and also that XPC has considerable preference for binding to UV-damaged DNA in the presence of non-damaged competitor DNA (Sugasawa *et al.*, 1998). It should be noted however that the binding of XPC-hHR23B to UV-damaged DNA in the absence of non-damaged DNA showed only moderate preference for damaged DNA (Guzder *et al.*, 1998; Jansen *et al.*, 1998; Wakasugi and Sancar, 1999).

There is current discussion as to whether XPA or XPC functions first in the recognition of damage or helix distortion. Both RPA and XPA preferentially bind to cisplatin- or UV-damaged DNA (Jones and Wood, 1993; He *et al.*, 1995; Lee *et al.*, 1995; Li *et al.*, 1995; Burns *et al.*, 1996; Patrick and Turchi, 1998) and form a stable complex on damaged DNA (Wang *et al.*, 2000). Interaction between XPA and RPA is not only crucial for damage-recognition but also for recruiting other repair factors such as XPG, ERCC1-XPF, and TFIIH to

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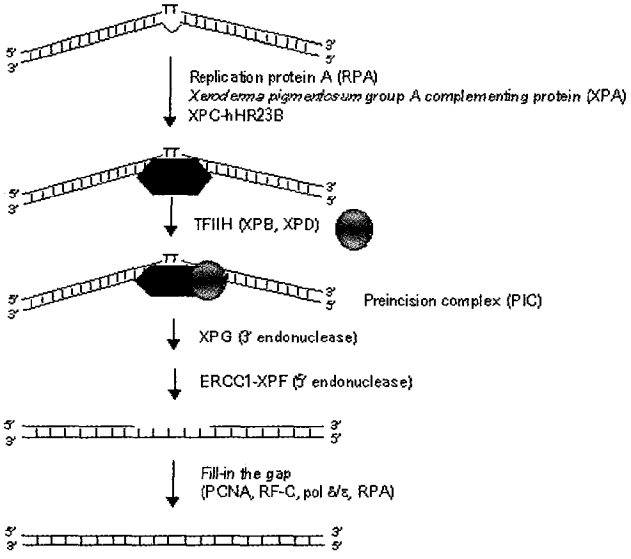

Fig. 1. A model for the nucleotide excision repair of UV-damaged DNA.

the damaged site (He *et al.*, 1995; Park *et al.*, 1995; Nocentini *et al.*, 1997; Bessho *et al.*, 1997; de Laat *et al.*, 1998). The XPA-DNA interaction is relatively weak and characterized by rapid dissociation, whereas RPA formed a 100-fold more stable complex with UV-damaged DNA (Wang *et al.*, 2000). A mutant RPA lacking XPA interaction domain leads to destabilization of XPA-damaged DNA complex, implicating a role for RPA in the early stage of DNA repair.

XPE (also known as a UV damage-specific DNA binding protein (UV-DDB) is another UV-damage recognition protein found in human and monkey cells (Payne and Chu, 1994; Nichols *et al.*, 2000). XPE binds to various damaged DNA induced by UV, cisplatin, depurination, and nitrogen mustard (Payne and Chu, 1994). Recent *in vivo* and *in vitro* studies showed that UV-DDB stimulates the excision of cyclobutant pyrimidine dimer, but not (6-4) photoproducts (Wakasuki *et al.*, 2001). However, XP-E mutant cells show the mildest NER defect among the XP groups and an *in vitro* NER reaction can be reconstituted without XPE (Aboussekhra *et al.*, 1995; Kazantsev *et al.*, 1998; Nichols *et al.*, 2000), suggesting that XPE may not be an essential factor for NER. In addition to the repair factors described above, high mobility group (HMG) domain protein and human Ku autoantigen (Ku70-Ku80 complex, a regulatory subunit of DNA-dependent protein kinase) bind to cisplatin-DNA adducts (Hughes *et al.*, 1992; Turchi and Henkels, 1996), although they are not essential for NER activity. The binding of HMG protein to cisplatin-damaged DNA inhibited an *in vitro* excision repair activity, suggesting that HMG domain protein may influence DNA repair *in vivo* (Huang *et al.*, 1994).

DNA structure requirements

NER machinery is responsible for recognizing and removing most of the chemically modified bulky adducts, whereas non-bulky base adducts such as those caused by hydrolysis, oxygen free radicals, and simple alkylating agents are recognized and repaired by the base excision repair (BER). In NER, presence of non-complimentary bases alone does not cause oligonucleotides excision. The loss of hydrogen bonding between two helical strands is an essential signal for the recruitment of NER enzymes. Oligonucleotides excision also requires chemical modification of DNA. For example, cisplatin covalently binds to the N7 positions of guanine and adenine bases and mainly forms 1, 2-intrastrand d(GpG) or d(ApG) adducts. The 1,3-intrastrand d(GpTpG) cisplatin is more structurally distorted than 1,2-intrastrand adducts and is therefore recognized and repaired more efficiently (Bellon *et al.*, 1991; Patrick and Turchi, 2001). Inclusion of non-complementary residue opposite the 1, 2-intrastrand d(GpG) adducts also increases the structural distortion of DNA and also increases repair efficiency significantly (Moggs *et al.*, 1997; Mu *et al.*, 1997). These findings suggest that local disruption of Watson-Crick base pairing as well as chemical modification of bases are indispensable components of the molecular signal that attracts human NER enzymes to the damaged DNA sites (Buschta-Hedayat *et al.*, 1999).

The cisplatin-DNA adduct containing 1, 2-intrastrand d(GpG) cross-link not only generates structural distortion of DNA, but also induces DNA unwinding and DNA bending (>40°) toward the major groove (Rice *et al.*, 1988). All these changes in DNA structure are likely important for damage recognition in mammals since XPA binds most efficiently to rigidly bent duplexes but not to single-stranded DNA (Missura *et al.*, 2001), while RPA preferentially recognizes single-stranded DNA site rather than bent DNA. The association of XPA with RPA may generate dual sensorship that detects both backbone and base pair distortion of DNA. Since the affinity of XPA for bent duplexes is not compatible with its function in recognition of nucleotide lesions, XPA in collaboration with RPA may be involved in monitoring the integrity of the Watson-Crick base-pairing to verify the damage-specific localization of repair complexes (Buschta-Hedayat *et al.*, 1999; Missura *et al.*, 2001).

Damage recognition proteins

Requirement of multiple damage recognition factors in NER indicates that XPC-hHR23B, XPA, RPA, and TFIIH may all have unique role in damage recognition and the early stage of repair. It is still in debate however how these damage recognition proteins function at the damaged DNA site. XPA is a multifunctional zinc-finger protein involved in the damage recognition step of NER (Robins *et al.*, 1991; Guzder *et al.*, 1993; Jones *et al.*, 1993). Its zinc-finger motif is essential for its DNA binding activity as well as its function in NER (Matsuda *et al.*, 1995). XPA preferentially binds to (6-4) photoproduct of UV-irradiated DNA, although it exhibits a low affinity binding to UV-damaged DNA (Wang *et al.*,

Table 1. Mammalian proteins involved in early stage of nucleotide excision repair

Repair factor	(yeast homolog)	Size (kDa)	Role in repair
XPA	(RAD14)	31-kDa	Damage recognition, Interaction with RPA
RPA	(RFA1, 2, 3)	70, 34,11-kDa	Damage recognition, DNA binding Interaction with XPA
XPC-hHR23B	(RAD4)	125-kDa (XPC)	Damage recognition, DNA binding
	(RAD23)	58-kDa (HR23B)	Stabilzation of XPC, control of DNA repair?
TFIIH	(RAD25)	89-kDa (XPB)	DNA helicase
	(RAD3)	80-kDa (XPD)	DNA helicase
	(TFB-1)	62-kDa	Core TFIIH subunit
	(SSL1)	44-kDa	Core TFIIH subunit
	(KIN28)	41-kDa (Cdk7)	cyclin-dependent protein kinas
	(CCL1)	38-kDa	cyclin H
		34-kDa (CAK)	Cdk activating kinase
XPF	(RAD1)	112-kDa (XPF)	5'-incision
	(RAD10)	33-kDa (ERCC1)	5'-incision
XPG	(RAD2)	135-kDa (ERCC5)	3'-incision

2000). XPA's binding to damaged DNA is stabilized through interaction with RPA on damaged DNA (Stigger *et al.*, 1998; Wang *et al.*, 2000). XPA also plays a role in recruiting TFIIH to the damaged site for subsequent incision/excision step (Park *et al.*, 1995; Nocentini *et al.*, 1997).

RPA is a heterotrimeric protein complex consisting of 70-, 34-, and 11-kDa subunits involved in DNA replication, repair, and recombination (Wold, 1997). RPA is a 4-cysteine type zinc-finger protein and a mutation at any zinc-finger cysteine abolishes its function in repair (Stigger *et al.*, 1998; Dong *et al.*, 1999), suggesting a unique role for the zinc-finger domain in early stage of repair. In contrast to XPA, RPA's zinc-finger domain is not essential for DNA binding activity, but is involved in regulation of RPA's DNA binding activity through redox change [Lin *et al.*, 1998; Park *et al.*, 1999). RPA preferentially binds to UV-damaged DNA and its interaction with UV-irradiated DNA was not affected by prior enzymatic photoreactivation of DNA, suggesting a preferred binding of RPA to the (6-4) photoproduct (Burns *et al.*, 1996). It is interesting to note that RPA, unlike XPA, may bind to duplex cisplatin-damaged DNA via generating single-stranded DNA at the lesion (Patrick *et al.*, 1999). The findings that RPA stimulates the XPA-DNA interaction through interaction with XPA on damaged DNA and also interacts with two endonucleases, XPG and ERCC1-XPF, suggest that the XPA-RPA complex, once formed on damaged DNA, recruits together XPG, ERCC1-XPF, and TFIIH to the damaged site for subsequent incision/excision step (He *et al.*, 1995; Matsunaga *et al.*, 1996). RPA may also be involved in a later stage of NER, gap-filling, that requires PCNA, RF-C, and DNA polymerase δ (or ε) (Aboussekhra *et al.*, 1995).

XPC-HR23B is a human homolog of yeast Rad4 and Rad23 proteins, respectively, and has been shown to exhibit strong affinity for damaged DNA (Sugasawa *et al.*, 1998; Batty *et al.*, 2000), as does the yeast counterpart, Rad4-Rad23 complex (Jansen *et al.*, 1998). The 106-kDa XPC protein forms a stable complex with 43-kDa HR23B (Masutani *et al.*, 1994; Shivji *et al.*, 1994). In yeast, Rad23 without Rad4 does not show any DNA binding under conditions where Rad4-Rad23 complex does (Guzder *et al.*, 1998), suggesting that Rad4 may be responsible for recognition of UV-damaged DNA. Rad23 (HR23B) is essential for XPC function in NER and may be necessary for the solubility of Rad4 (Guzder *et al.*, 1998). Recent studies also indicate that HR23B may be involved in regulation of repair activity. For example, Rad23 contains ubiquitin-associated (UBA) domain that may play a role in controlling NER through proteosome-mediated ubiquitin-dependent degradation of repair factors (Gilletts *et al.*, 2001; Chen *et al.*, 2001). HR23B also interacts with base excision repair factor, MPG, suggesting that HR23B may have a unique role in DNA repair accommodating various repair pathways (Miao *et al.*, 2000).

Evidence suggests that XPC-HR23B is involved in the recruitment of TFIIH to the damaged site through physical interaction with TFIIH (Yokoi *et al.*, 2000). XPC-HR23B may collaborate with XPA in recruiting TFIIH since XPA also interacts with TFIIH on damaged DNA (Park *et al.*, 1995; Nocentini *et al.*, 1997). TFIIH, once loaded on the damaged DNA, may play a role in distinguishing the damaged strand from the non-damaged one since the translocation along a DNA strand by TFIIH DNA helicases is hampered by encountering a lesion (Naegeli *et al.*, 1992; Naegeli *et al.*, 1993). Local unwinding of the damaged DNA region by TFIIH likely generates junction between single-stranded DNA and duplex DNA that is recognized by two structure-specific endonucleases, XPG and ERCC1-XPF, for dual incision on the damaged strand.

Interaction of damage recognition proteins with DNA lesions

Both RPA and XPA preferentially bind to DNA lesions induced by cisplatin or UV-irradiation (Jones *et al.*, 1993; Guzder *et al.*, 1993; Asahina *et al.*, 1994; He *et al.*, 1995; Burns *et al.*, 1996; Wagasuki *et al.*, 1999; Wang *et al.*, 2000). RPA binds to (6-4) photoproduct-containing DNA with nearly 2-fold higher affinity relative to *cis-syn* cyclobutane dimer and nearly 4-fold greater than non-damaged DNA (Wang *et al.*, 2000). Although XPA showed preferential binding to (6-4) photoproduct-containing DNA, its interaction with damaged DNA was significantly weaker ($K_D = 2.13 \times 10^{-8}$ M) than RPA (2.02×10^{-10} M). Real-time analysis showed that XPA quickly dissociated from damaged DNA, while RPA remained associated with damaged DNA (Wang *et al.*, 2000; Wang *et al.*, 2001). Low affinity binding of XPA to damaged DNA is stabilized by RPA. A mutant RPA lacking XPA interaction domain failed to stabilize XPA-damaged DNA interaction, indicating that a role for RPA in NER may be to stabilize the interaction between XPA and damaged DNA through protein-protein interaction. Interaction of XPA with RPA on damaged DNA is not only crucial for damage-recognition and but may also play a role in subsequent steps in recruiting other DNA repair proteins such as XPG, ERCC1-XPF, and TFIIH to the damaged site (He *et al.*, 1995; Park *et al.*, 1995; Lee *et al.*, 1995; Li *et al.*, 1995; Saijo *et al.*, 1996; Stigger *et al.*, 1998).

The fact that both XPA and RPA showed only moderate preference to damaged DNA over non-damaged DNA raises a question as to whether XPA and/or RPA are responsible for recognition of damaged DNA *in vivo*. XPC-hHR23B showed a remarkable preference to UV-damaged circular DNA particularly in the presence of non-damaged competitor DNA (Sugasawa *et al.*, 1998; Sugasawa *et al.*, 2001). Preferential binding of XPC-hHR23B to damaged circular DNA was likely due to its higher binding affinity to UV-damaged DNA than to a non-damaged counterpart, but is also possible that high affinity binding may be due to structural alteration caused by UV-damage on circular DNA, but not on duplex DNA fragment. It is interesting that XPC-hHR23B, like RPA, physically interacts with XPA and stabilizes the XPA-damaged DNA interaction (Wang *et al.*, 2001). In fact, XPC-hHR23B and RPA exhibited remarkably similar biochemical properties: their binding to damaged DNA, physical interaction with XPA, and stabilization of XPA on damaged DNA. Nonetheless, preincubation of damaged DNA with XPC-hHR23B but not with RPA significantly enhanced XPA-damaged DNA interaction, suggesting that damage recognition complex may be formed in an ordered process, such that XPC-hHR23B interacts with damaged DNA before addition of XPA and RPA to form a stable damage recognition complex (Sugasawa *et al.*, 2001). A recent immunohistochemistry study also strongly supports a role for XPC as a global initiator in repair (Volker *et al.*, 2001) while suggesting a role for XPA and RPA as repair mediator proteins. Biochemical basis for the interaction of XPC and damaged DNA needs to be addressed to clarify the discrepancy.

It is highly speculated that three damage recognition proteins (XPA, RPA, and XPC-hHR23B) form a complex at the damaged DNA site, although there is no direct evidence on that. A potential implication of forming damage recognition complex on damaged DNA is to position the preincision machinery for dual incision reaction at damaged DNA strand. In DNA replication, origin binding protein such as SV40 T-antigen form a multiprotein complex with RPA and DNA polymerase (pol) α-primase complex for the proper positioning of pol α-primase to initiate DNA synthesis at the specific site (Hurwitz *et al.*, 1990; Bullock *et al.*, 1991). Likewise, the formation of multiprotein complex at damaged site may be necessary for proper positioning of two endonucleases XPG and ERCC1-XPF for accurate incision at the 3' and 5' sites of the damaged strand (de Laat *et al.*, 1998).

Protein-induced structural distortion of damaged DNA

In DNA replication, binding of origin recognition factor(s) to the replication origin leads to a significant structural distortion at AT-rich region, which is necessary for loading of DNA helicase(s) (Borowiec *et al.*, 1990 and the references therein). During NER, distortion of damaged DNA may also be essential for loading of two DNA helicases (XPB and XPD of the TFIIH complex) to the damaged site. Structural distortion at damaged DNA is not only crucial for the formation of preincision complex (PIC), but also necessary for dual incisions. Since the formation of PIC at damaged site requires RPA, XPA, XPC-hHR23B, and TFIIH in the expense of ATP hydrolysis (Evans *et al.*, 1997a; Evans *et al.*, 1997b; Mu *et al.*, 1997a), it is conceivable that multiple repair factors contribute to a structural distortion of damaged DNA. RPA is a leading candidate for structural distortion of damaged DNA. RPA's zinc-finger motif is essential for its function in NER (Stigger *et al.*, 1998). The failure of a zinc-finger mutant in supporting NER was not due to its role in stabilization of XPA-damaged DNA complex (Park *et al.*, 1999; Wang *et al.*, 2000), suggesting that RPA may have a unique role in early stage of repair. Tryptic digestion pattern and far-UV CD spectra analysis indicated that RPA undergoes a significant conformational change upon binding to DNA in its zinc-finger motif-dependent manner (Wang *et al.*, 2001). RPA's zinc-finger motif may mediate the transition of RPA-XPA interaction into a stable RPA-XPA-damaged DNA complex through its conformational change (Park *et al.*, 1999; Wang *et al.*, 2001). Alternatively, both RPA and XPA, two zinc-finger proteins, undergo conformational changes upon binding to the damaged DNA (Wang *et al.*, 2001) to further distort the damaged DNA. It is also interesting to note that requirement for XPC-hHR23B in dual incisions can be bypassed by the use of a bubble-like DNA substrate containing a thymidine

dimer (Mu *et al.*, 1997b), suggesting that XPC-hHR23B may also play a role in inducing a structural distortion and/or local opening of the damaged DNA site.

Concluding Remarks

The NER machinery is primarily responsible for the repair of various intrastrand cross-linked DNA (Sancar, 1996; Wood, 1996), although some NER factors such as ERCC1-XPF may also participate in the repair of interstrand cross-linked damage (Kuraoka *et al.*, 2000; Mu *et al.*, 2000). Although the details of damage recognition process is still not clear, all three damage recognition factors, XPA, RPA, and XPC-hHR23B, play an essential role in damage recognition and recruiting other repair factors to the damaged site for dual incision. Protein-induced structural distortion at the damaged DNA is likely necessary for the loading of TFIIH complex (XPB and XPD helicases) to initiate unwinding of both 3'- and 5'-sides of DNA damage. Therefore, a new challenge in early stage of repair is to unravel the role of damage recognition factors in further helix destabilization at damaged DNA site.

Another future challenge is to understand how damage recognition factors effectively recognize DNA damage on chromatin as compared to that on naked DNA as the chromosomal DNA *in vivo* is assembled into a compact nucleosome structure. We do not know the answer, however, chromatin accessibility factors such as GADD45 may play a role in mediating the recognition of damaged DNA on chromatin (Carrier *et al.*, 1999). Also, various DNA damages induce chromatin modifications such as phosphorylation of histone H2AX and acetylation of histone H3 (Paull *et al.*, 2000; Brand *et al.*, 2001). These modifications would likely facilitate the access of damage recognition proteins and/or DNA repair machinery to lesions.

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