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NUCLEOTIDE EXCISION REPAIR FROM DNA DAMAGE PROCESSING TO HUMAN DISEASE

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ABSTRACT

A network of DNA damage surveillance systems warrants genomic stability under conditions where cells and organisms are continuously exposed to DNA damaging agents. This network includes DNA repair pathways, but also signaling pathways that activate cell cycle checkpoints, apoptosis, transcription, and chromatin remodeling. Among the various repair pathways, nucleotide excision repair (NER) is a highly versatile and evolutionary conserved pathway with an intriguing wide substrate specificity; this pathway removes structurally unrelated bulky DNA lesions from the genome such as sunlight induced photolesions, bulky adducts formed by polycyclic aromatic hydrocarbons, cisplatin intrastrand crosslinks and alkylation products. The common features of these lesions are the variable degree of DNA helix distortion inflicted and their potency to block replication and transcription. The importance of functional NER for human health is highlighted by the existence of rare autosomal recessive human disorders such as xeroderma pigmentosum (XP). Affected individuals, characterized by a defect in NER, suffer from hypersensitivity to sunlight and display strongly enhanced cancer susceptibility in sunlight exposed parts of the skin. Mammalian NER involves multiple proteins (in excess of 30) and carries out the repair reaction in a highly orchestrated fashion.

In this book chapter we discuss the current knowledge of the molecular mechanisms underlying NER, its relation with human disease and the translation of knowledge to clinical use.

INTRODUCTION

Through evolution a network of DNA damage surveillance systems has evolved to warrant genomic stability under conditions where cells and organisms are continuously exposed to genotoxic agents present within the environment or exerted by endogenous processes. This network not only includes DNA repair pathways, but also signaling pathways that activate cell cycle checkpoints, apoptosis, transcription, and chromatin remodelling. The mechanisms by which eukaryotic cells sense DNA damage and activate signaling pathways are still poorly understood. One of the challenges is to understand how cells are capable to sense, recognize and repair low levels of different DNA lesions in their genomes at various stages of the cell cycle and in different chromatin environments.

A limited set of DNA repair pathways is capable to repair the large variety of structurally different DNA lesions that are formed in the genome. Nucleotide excision repair (NER) is a highly versatile and evolutionary conserved repair pathway that removes structurally unrelated bulky DNA lesions from the genome such as bulky adducts formed by polycyclic aromatic hydrocarbons, cisplatin intrastrand crosslinks and alkylation products. The common features of these lesions are the variable degree of DNA helix distortion inflicted and their potency to block replication and transcription elongation. In fact, NER is the only repair pathway in humans to remove the toxic and mutagenic photodimers from sunlight exposed parts of the skin. Mammalian NER involves multiple proteins (in excess of 30) and carries out the repair reaction in a highly orchestrated fashion involving a number of defined steps: (I) lesion recognition, (II) DNA unwinding and lesion demarcation, (III) dual incision and release of the incised fragment and (IV) gap filling by repair synthesis and ligation.

Two mechanistically distinct NER subpathways have been identified: Global genome NER (GG-NER) is capable of repairing DNA lesions in chromatin of different compaction levels and different functional states throughout the cell cycle. A subpathway of NER designated transcription-coupled repair (TC-NER) enables efficient repair of RNA polymerase II (RNAPII) blocking DNA lesions and allows quick resumption of transcription. The existence of three rare autosomal recessive human disorders i.e. xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD) all associated with sensitivity to sunlight and NER deficiency, highlights the importance of functional NER for human health. Cells from XP patients are sensitive to UV (ultraviolet)-light and chemicals inducing bulky DNA lesions, and complementation studies revealed eight genes involved in the disease (XPA–XPG and XP Variant; see also chapter by A Lehmann). Complementation studies have revealed two CS complementation groups, CS-A and CS-B. In addition to XP and CS patients, a third group of UV sensitive and cancer prone patients has been identified that encompasses individuals exhibiting both XP and CS symptoms.

Although NER removes a variety of structurally unrelated lesions from the genome, we will concentrate on NER in UV-irradiated mammalian cells since UV-induced pyrimidine-pyrimidone (6-4) photoproducts (6-4 PP) and cyclobutane pyrimidine dimers (CPD) (figure 1) are the lesions most intensively studied and as such paradigmatic for NER.

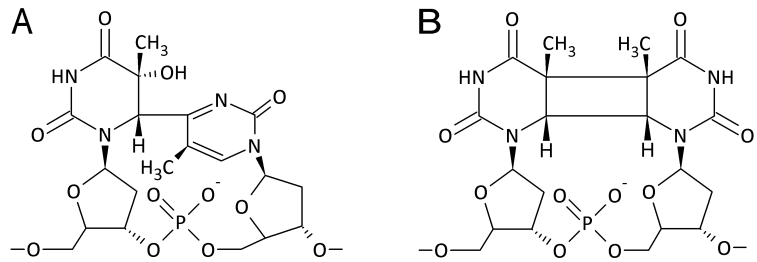

Figure 1: Structure of UV induced DNA photolesions. (A) pyrimidine-pyrimidone (6-4) photoproduct (6-4PP), (B) cis-syn cyclobutane thymine (pyrimidine) dimmer (CPD)

2.1 GLOBAL GENOME REPAIR (GG-NER)

Cells of all XP patients (except XP variant) were found to be defective in global genome repair of UV-induced photolesions. The identification of the different XP complementation groups led to the isolation of the XP genes and encoded proteins and allowed reconstitution of the process *in vitro* using purified proteins and naked DNA harboring a specific lesion. The first steps in NER, i.e. DNA lesion recognition and dual incision, require all XP factors (XPC, XPA, XPG, ERCC1-XPF) together with other factors (RPA, TFIIH). In addition, the *in vitro* reaction requires RF-C, PCNA, DNA polymerase ε and DNA ligase I for repair synthesis (Aboussekhra et al., 1995). This scheme represents the proteins to perform NER *in vitro*; additional factors are required for in vivo NER on chromatinized DNA templates.

2.1.1 DNA lesion recognition in GG-NER

Although XPA was originally proposed to be the principal damage recognition protein, it is now well established that GG-NER is initiated by the XPC protein which forms a trimeric complex with hHR23B (the human homologue of Rad23) and CEN2 (Masutani et al., 1994;Volker et al., 2001;Sugasawa et al., 1998;Araki et al., 2001). *In vivo* the recruitment of NER proteins to UV damage is abolished in XPC deficient cells, indicating that assembly of the NER complex is strictly XPC dependent. Mobility studies on GFP-tagged XPC suggest that the majority of XPC-hHR23B molecules (>90%) transiently interact non-specifically with genomic DNA (Hoogstraten et al., 2003;Politi et al., 2005;Hoogstraten et al., 2003). In fact, the general affinity of the complex for DNA and its specific affinity for photolesions such as CPD and 6-4PP are relatively low *in vivo* (Moser et al., 2005). The capacity of XPC-hHR23B to recognize a broad spectrum of structurally unrelated lesions might be understood from the observation that XPC binds to the accessible non-damaged DNA strand opposite to a DNA injury (Sugasawa and Hanaoka, 2007;Min and Pavletich, 2007).

Although the XPC-hHR23B complex acts as the principle initiator of NER, its action is preceded by the heterodimeric UV-DDB complex consisting of the p48 and p127 proteins, products of the DDB2 and DDB1 genes respectively (Keeney et al., 1993). In fact, repair of CPDs requires functional UV-DDB (Tang et al., 2000) and, in addition, UV-DDB significantly stimulates the repair of 6-4 PPs particularly at low UV doses (Hwang et al., 1999;Moser

et al., 2005). Microinjection of purified UV-DDB was originally found to restore the repair defect of XP-E cells as measured by unscheduled DNA synthesis. Subsequently it was found that the XP group E phenotype is caused by mutations in the DDB2 gene, encoding the p48 protein (XPE). The general affinity of UV-DDB for DNA is much higher (100–1000-fold) than that of XPC-hHR23B, while the specific affinity for 6-4PP is comparable (Batty et al., 2000). DDB2 is part of a functional CUL4A-based E3 ubiquitin ligase through its interaction with DDB1 (Groisman et al., 2003) and also binds to UV lesions as an active E3 ubiquitin ligase independent of XPC. UV irradiation activates the E3 ubiquitin ligase activity of the DDB2 complex by the binding of the ubiquitin-like protein Nedd 8 to the Cullin 4A. Several substrates for ubiquitylation were identified, including DDB2 itself, XPC and histones H2A, H3 and H4 (Kapetanaki et al., 2006;Sugasawa et al., 2005;Wang et al., 2006). The current view is that ubiquitylation (at least partly) facilitates NER: ubiquitylation of XPC enhances its affinity for DNA (both damaged and non-damaged DNA) whereas ubiquitylation of histones facilitates the access of repair proteins to DNA damage in chromatin by weakening the histone-DNA association. Interestingly and less well understood, ubiquitylated DDB2 is quickly targeted for degradation after UV (Sugasawa et al., 2005;Rapic-Otrin et al., 2002) even in the presence of large numbers of unrepaired photolesions.

As a general mechanism it was proposed (Moser *et al.*, 2005) that UV-DDB forms a stable complex when bound to DNA damage such as UV-induced 6-4PP, allowing subsequent repair proteins, starting with XPC-hHR23B, to accumulate and to verify the lesion, ultimately resulting in efficient repair. The fraction of 6-4PP that can be bound by UV-DDB is limited due to the low cellular quantity and fast UV dependent degradation of DDB2. In cells lacking UV-DDB a slow XPC-hHR23B dependent pathway is capable of repairing 6-4PP whereas repair of CPD is virtually absent.

2.1.2 Assembly of the preincision complex

Upon the recognition of lesions by a concerted action of UV-DDB and XPC-hHR23B, the latter recruits the multiprotein transcription factor TFIIH via direct protein-protein interactions (figure 2). The TFIIH complex is composed of a seven-subunit core containing two XP factors (XPB, XPD, TTD, p34, p44, p52, p62) and a three-subunit kinase complex (Cdk7, cyclin H and MAT1) termed the CAK unit. The complex exhibits dual functions i.e. it plays a role in RNAPI and RNAPII driven transcription as well as in NER. The XPB subunit of TFIIH is an ATP-dependent helicase that mediates unwinding of promoter DNA in a 3'-5' orientation during transcription initiation, whereas the XPD subunit of TFIIH is a 5'-3'ATP dependent helicase. Interestingly, the unwinding step of the damaged DNA during NER requires only the XPD helicase activity, whereas the ATPase activity of XPB is dispensable for NER (Coin et al., 2007). A two-step mechanism underlies the opening of the damaged DNA to allow assembly of the NER preincision complex: TFIIH mediates the initial opening after which RPA, XPA and XPG bind to obtain full opening of approximately 30 nucleotides around the lesion (Evans et al., 1997). XPA stimulates the ATPase activity of TFIIH whereas RPA and XPG stabilize the repair intermediate and contribute to full opening around the lesion. Since TFIIH functions in both transcription initiation and NER, it has been proposed that the recruitment of TFIIH to UV damage abolishes transcription initiation in UV-

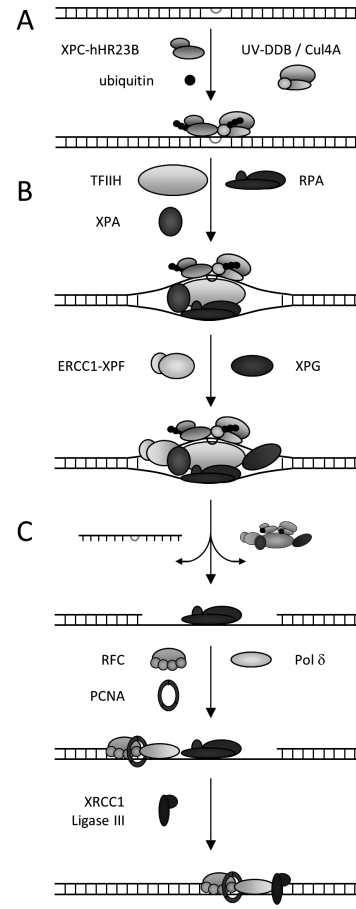

Figure 2: Model for global genome NER. (A) DNA damage recognition by UV-DDB and XPC-hHR23B. Ubiquitylation of damaged bound XPC and DDB2 is mediated via DDB1/Cul4A. (B) Assembly of the preincision complex. TFIIH is recruited to the lesion by the XPC-hHR23B complex, opening up the DNA though its helicase activity. The association of RPA further stimulates the unwinding. XPA binding contributes to damage verification and recruits the ERCC1-XPF complex. The ERCC1-XPF and XPG endonucleases incise the damaged DNA strand both 5' and 3' of the lesion. In addition its incision activity XPG also serves to stabilize the open DNA bubble structure. (C) Gap filling and ligation. Following dual incision the single stranded DNA patch is filled by the concerted action of RFC, PCNA and DNA polymerase δ, whereas XRCC1/Ligase III performs the final DNA ligation step. Note that in cycling cells DNA polymerase ε and DNA Ligase I also contribute to repair.

irradiated cells by a trans mechanism (Mone et al., 2001;Mullenders, 1998). However, inhibition of transcription by UV irradiation only occurs by direct interference of damage with the transcription machinery (Mone *et al.*, 2001) and does not otherwise affect the engagement of TFIIH in transcription (Hoogstraten et al., 2002).

Replication protein A (RPA) consists of three subunits and is an abundant single-stranded DNA-binding protein that binds optimally to approximately 30 nucleotides (de Laat et al., 1998). During the formation of the preincision complex RPA associates with the undamaged DNA strand partially unwound by TFIIH (~8-10 nucleotides) and subsequently extends it association to a 30-nucleotide region. This action leads to a separation of the DNA strands around the lesion. Importantly, RPA has been shown to interact with several core NER proteins including XPA, XPG and ERCC1-XPF. In living cells RPA can assemble into the pre-incision complex (consisting of XPC, TFIIH and XPG) in the absence of XPA. This complex, however, is insufficient to stimulate the 3' incision by XPG and incapable to recruit the 5' XRCC1-XPF endonuclease; the latter event requires recruitment of XPA in order to assemble the complete pre-incision complex (Rademakers et al., 2003). XPA is essential

for GG-NER and it is likely that RPA is required to recruit the XPA protein although direct *in vivo* evidence is lacking. The multiple protein interactions of XPA i.e. the association with RPA, and XRCC1-XPF, were demonstrated by the finding that the N-terminus of XPA binds to RPA and ERCC1, whereas the C-terminus interacts with TFIIH (Park et al., 1995) consistent with a central role for XPA in the formation of the NER pre-incision complex. Most notably, XPA deficient cells completely lack incision activity, indicating that XPA plays an important role in the coordination of dual incision. The observation that XPA binds preferentially to bent or kinked DNA duplexes (Camenisch et al., 2006;Camenisch et al., 2007) sheds light on its function in NER and links initial damage recognition by UV-DDB and XPC-hR23B to XPA recruitment. Both XPC and DDB2 introduce kinks in the DNA upon binding and hence might stimulate binding of XPA (Janicijevic et al., 2003). The binding of XPA most likely contributes to DNA damage verification in the pre-incision complex: the interaction between RPA bound to the undamaged strand and XPA with the kinked DNA duplex, provide the molecular tools that allow identification of the DNA lesion in the pre-incision complex.

RPA plays a key role at the interface of the pre-and post incision step of NER as the protein precipitates in chromatin immunoprecipitation (ChIP) reactions with antibodies raised against pre-incision and post-incision proteins (Moser et al., 2007). Analysis of the assembly and disassembly of repair proteins on immobilized damaged-DNA templates *in vitro* revealed that RPA remains bound after dual incision and initiates the assembly of DNA synthesis factors such as PCNA (Riedl et al., 2003).

2.1.3 Dual incision step

The two structure specific endonucleases XPG and ERCC1-XPF are involved in dual incision 3' and 5' of the lesion respectively. In the presence of both proteins, both 5' and 3' uncoupled incisions have been observed indicating that both incisions are made simultaneously (Moggs et al., 1996). The XPG protein specifically incises DNA at the side of the junction between single-stranded DNA and double stranded DNA (O'Donovan et al., 1994) approximately 2-8 nucleotides from the 3' side of the lesion. The protein interacts with RPA and TFIIH and the recruitment of XPG to the preincision complex was shown to depend on functional TFIIH. However, the presence of XPG in the pre-incision complex was shown to be required for stabilizing the open DNA bubble structure containing the DNA lesion, allowing binding and 5' incision by XRCC1-XPF (Wakasugi et al., 1997). Hence, XPG also has a structural role in NER and this goes along with the recent finding that XPG may act as a major stabilizing factor by associating with TFIIH (Ito et al., 2007), although dynamic measurements support separate moieties rather than a joined complex *in vivo* (Zotter et al., 2006). In cells of XPG patients with a combined XP and CS phenotype, XPG fails to associate with TFIIH and as a consequence the CAK subunit dissociates from core TFIIH. Deletion mutant analysis of XPG revealed that the so-called spacer region within the protein (which is not required for endonuclease activity) contributes to the substrate specificity of XPG and is required for the interaction with TFIIH and for NER activity *in vitro* and *in vivo* (Dunand-Sauthier et al., 2005).

The 5' junction between single-stranded and double-stranded DNA is cleaved by the heterodimeric endonuclease ERCC1-XPF approximately 15-24 nucleotides away from the 5' side of the lesion (Matsunaga et al., 1995). The two proteins cannot be isolated as separate entities indicating that complex formation underlies the stability of the dimeric endonuclease (Sijbers et al., 1996). Both *in vivo* and *in vitro* it has been shown that the interaction of ERCC1-XPF with XPA is essential for NER and that XPA recruits ERCC1-XPF to the pre-incision complex (Volker *et al.*, 2001). The incision activity of ERCC1-XPF is stimulated by direct interactions with RPA in model substrates (de Laat et al., 1998). Mutations in XPF are associated with mild XP; however, a unique XP-F patient with a severe phenotype was recently described displaying signs of accelerated aging (Niedernhofer et al., 2006). Moreover, mutations in ERCC1 have so far only been reported for one patient with severe clinical features but only a mild repair defect at the cellular level (Jaspers et al., 2007). These latter findings suggest additional functions for ERCC1 and XPF. Indeed, ERCC1-XPF is involved in several other processes such as homologous recombination, repair of interstrand cross-links and telomere maintenance.

2.1.4 The post-incision step in NER

Dual incision and removal of the lesion containing single stranded DNA fragment is followed by gap filling and ligation, generally termed repair synthesis. The transition between dual incision and repair synthesis needs to be coordinated to omit activation of the DNA damage signaling and to prevent recombination, the formation of deletions etc. Conceivably the incision reactions might not occur simultaneously and might be initiated by ERCC1-XPF to start DNA synthesis before XPG cutting takes place (Gillet and Scharer, 2006). An alternative mechanism to prevent undesired processing is that one key factor is partner in the pre-and post-incision stages of NER and remains bound to the DNA. The two stages of NER can be separated *in vitro* (Riedl *et al.*, 2003) and pre-and post- incision complexes have been isolated from living cells (Moser *et al.*, 2007). These analyses revealed RPA as common factor in the reaction and showed that RPA remains associated with the DNA upon dual incision. In addition to RPA, repair synthesis requires RF-C, PCNA, DNA polymerases ε and δ as well as Ligase I *in vitro* (Aboussekhra et al., 1995;Shivji et al., 1992;Shivji et al., 1995); the recruitment of the post-incision factors is entirely depending on dual incision. PCNA is a homotrimeric sliding clamp that encircles the DNA and acts as a template to allocate DNA polymerases ε and δ to the DNA (Maga and Hubscher, 2003). Loading of PCNA on the DNA requires the clamp loader RF-C and ATP. Recent *in vivo* experiments showed that predominantly DNA polymerase δ is recruited to repair patches upon UV irradiation in replicating and quiescent cells and that the role of DNA polymerase ε is restricted to S-phase cells (Moser *et al.*, 2007). Moreover, the surprising finding was recently made that under certain conditions (G_0 cells, DNA synthesis inhibitors) the translesion synthesis DNA polymerase κ may also play a role in repair synthesis during NER (Ogi and Lehmann, 2006), emphasizing the need to confirm the roles of these late factors in NER *in vivo*.

The NER reaction is completed by ligation of the 5' end of the newly synthesized DNA to the original sequence. Although Ligase I is sufficient for sealing nicks during *in vitro*

repair synthesis, XRCC1-LigIIIα appears to be indispensable for ligation of NER-induced breaks. Two distinct complexes were identified that differentially carry out gap filling in NER (Moser *et al.*, 2007). XRCC1-LigIIIα and DNA polymerase δ co-localize and interact with NER components in a UV- and incision-dependent manner throughout the cell cycle. In contrast, DNA Ligase I and DNA polymerase ε are recruited to UV-damage sites only in proliferating cells. These findings indicate that cells have differential requirements for ligases and polymerases in repair synthesis depending on the cell cycle.

Finally, the progression of NER seems to be controlled and requires the completion of the post-incision step in NER. Inhibition of DNA polymerases δ and ε in non-dividing normal human cells by the DNA polymerase inhibitors HU/AraC leads to accumulation of DNA strand breaks, DNA damage signaling (H2AX signaling) but also to strong retardation of repair of UV induced photolesions such as 6-4PP (Moser *et al.*, 2007). Obviously, efficient gap filling by DNA synthesis and ligation of the repair patch is required to drive NER to completeness and implicates either the existence of efficient cellular control mechanisms or factors that limit the number of (pre-) incision events.

2.1.5 Damage signaling in GG-NER

It has been long acknowledged that exposure of cells to UV light not only activates NER, but also modulates other DNA damage responses impacting cell cycle progression and apoptosis. The exact mechanisms underlying the decision in cell fate have long remained obscure. However, recent works have begun to uncover the molecular mechanisms determining cell fate following UV exposure and demonstrate links between NER and other pathways in the DNA damage response. One of the most prominent players in the UV-induced DNA damage response would be the ataxia telangiectasia mutated and Rad3-related (ATR) protein. ATR is a member of the phosphoinositol-3-kinase like kinase family that also includes the ataxia telangiectasia mutated (ATM) protein. It has now become clear that both proteins act as one of the earliest components in the damage response, their main function being the phosphorylation various proteins to effectively propagate the damage signaling. While ATM and ATR share many substrates for phosphorylation, the activating structures for these kinases themselves differ. ATM is activated by double stranded DNA breaks (Savitsky et al., 1995), whereas it is RPA bound to single stranded DNA what activates the ATR kinase at stalled replication forks (Zou and Elledge, 2003). Although it was initially believed that the capacity to activate ATR was restricted to cells in S phase, it was later demonstrated that H2AX is phosphorylated in an ATR dependent manner following UV exposure of non-replicating cells (O'Driscoll et al., 2003). The origin of this signaling lies in the formation of single stranded DNA patches following the excision of the damage containing oligo by GG-NER (Marini et al., 2006;Marti et al., 2006;Matsumoto et al., 2007;O'Driscoll et al., 2003). Such RPA containing ssDNA patches would resemble the structures formed after replication fork stalling and hence allow ATR signaling via a common mechanism. This allows not only the phosphorylation of the many substrates of this kinase, but also serves as a prerequisite for ubiquitylation of histone H2A (Bergink et al., 2006).

Normally the activation of ATR is associated with cell cycle checkpoint arrest and NER dependent activation of ATR indeed is able to induce cell cycle arrest outside the S phase (Stiff et al., 2008). Whether other processes are affected by this signaling is currently unknown. However, one possibility would be that ATR activation could regulate the levels of checkpoint protein p53. As a transcriptional regulator p53 mediates the expression of genes involved in DNA repair, cell cycle arrest and apoptosis. The fact that both DDB2 and XPC expression are regulated in a p53 dependent manner allows for the possibility that ATR activation could enhance the NER capacity following damage induction. Although the existence of such a regulatory mechanism remains to be demonstrated, support comes from the observation that cells lacking functional p53 have a deficiency in GG-NER (Ford and Hanawalt, 1995). Recent experiments indicate that ATR is indeed important for efficient repair of photolesions as ATR deficient cells are profoundly defective in GG-NER but, surprisingly, only during S-phase (Auclair et al., 2008). The mechanism underlying this cell cycle specific regulation of repair remains to be clarified. Although GG-NER mediated signaling is now well established, the first demonstration that UV exposure induced a checkpoint response (Yamaizumi and Sugano, 1994) was in TC-NER deficient cells. Here checkpoint activation does not depend on processing of UV lesions by NER, but rather it is the absence of repair that results in enhanced checkpoint activation. DNA lesions that block RNA polymerase II, such as UV lesions, cause a dramatic increase in the levels of both normal and phosphorylated p53 when cells are deficient in TC-NER. Despite the fact that p53 induction has long been associated with stalled transcription, the molecular mechanisms that underlie it remain enigmatic. The identification of ATR as the kinase that, in conjunction with RPA, phosphorylates p53 has begun to shed some light on this matter (Derheimer et al., 2007). However, this discovery itself raises a question about the mechanism of ATR activation at stalled RNA polymerases. The archetypical activating structure for ATR is believed to be a single stranded DNA gap and activation of the kinase depends on additional proteins (e.g. TopBP1, Rad9) that are independently recruited to such substrates (Delacroix et al., 2007;Kumagai et al., 2006). It is questionable whether a stalled RNA polymerase confers a structure that resembles gapped DNA, and as such it would be of interest to investigate the participation of other factors normally associated with ATR signaling in the context of RNA polymerase II mediated signaling.

2.1.6 Chromatin structure and NER

In general, the condensed structure of chromatin poses problems to DNA metabolizing processes; notably, NER in a chromatin context is severely inhibited compared to naked DNA (Hara et al., 2000). To overcome this barrier, different mechanisms have evolved to remodel chromatin enhancing the accessibility of damaged DNA for repair proteins. In addition, following removal of the lesion and completing of the post incision stage of NER, cells need to restore the original chromatin structure to maintain the epigenetic information (Green and Almouzni, 2002). Finally, there is clear evidence that repair efficiencies differ greatly in various chromatin environments but the underlying mechanism is not well understood (Mullenders et al., 1991). Two major mechanisms may alter chromatin structure: posttranslational modification of histone tails and ATP dependent chromatin remodelling.

As mentioned in section 1.1, the role of UV-DDB in NER has revealed unexpected complexities as this complex associates with proteins that are involved in chromatin remodeling (acetylation) and ubiquitylation (Groisman et al., 2003;Datta et al., 2001); the latter activity is related to the participation of DDB1 and DDB2 in a large complex making up a ubiquitin ligase together with Cul4A and Roc1. The ligase activity of this complex is regulated by the COP9 signalosome (CSN). The ubiquitin ligase activity is stimulated by UV (at least with respect to GG-NER) leading to poly-ubiquitylation and subsequent degradation of DDB2 itself; importantly, ubiquitylation of XPC does not serve as a signal for degradation, but merely stimulates the activity of XPC–HR23B by an unknown mechanism. It is conceivably that the ubiquitylation of histones and DDB2 may lead to increased accessibility of the site of damage by removal and/or loosening of DNA–histone contacts and the displacement of UV-DDB from the lesion.

One of the important changes after UV irradiation is the appearance of hyperacetylated histones, most notably shown by the inhibition of histone deacetylases (HDAC) that trigger genome-wide histone hyperacetylation at both histone H3 and H4 upon UV irradiation. Recently, histone acetyl transferases (HAT) such as the HAT p300 (Fousteri et al., 2006) and Gcn5 (Yu et al., 2005) have been suggested to play a role in increasing the accessibility of chromatin to NER proteins. A role for p300 in NER is suggested by interactions of p300 with the repair factors UV-DDB (Rapic-Otrin *et al.*, 2002) and PCNA (Hasan et al., 2001), stimulation of repair by p300 *in vitro* (Frit et al., 2002) and enhancement of NER by the histone deacetylase inhibitor sodium butyrate (Ramanathan and Smerdon, 1989). Genetic approaches (Smerdon et al., 1982;Mullenders et al., 1986) revealed that histone acetylation is not only important for GG-NER but also for TC-NER, although transcriptionally active genes themselves are enriched for acetylated histones.

In vitro NER assays using chromatin substrates with defined lesions, generally reveal that repair is slow in the nucleosomal DNA with no movement or disruption of nucleosomes (Gaillard et al., 2003). Repair measurement of a defined DNA lesion (i.e. 6-4PP) located in a dinucleosome chromatin template demonstrated that ATP-dependent remodeling might enhance the pre-and postincision steps of NER as dual incision is facilitated by ACF, an ATP-dependent chromatin remodeling factor (Ura et al., 2001). The ACF protein moves nucleosomes rather than displacing them. Also incubation with the nucleosome remodeling complex SWI/SNF and ATP altered the conformation of nucleosomal DNA and promoted more homogeneous repair by nucleosome sliding, thereby increasing accessibility to DNA (Gaillard *et al.*, 2003;Hara and Sancar, 2003). *In vivo* data in yeast suggest that SWI/SNF has a significant role in modulating the accessibility of UV induced photolesions for the NER repair machinery thereby enhancing repair (Yu *et al.*, 2005). Interestingly, the SWI/SNF complex and the abovementioned Gcn5 histone acetyl transferase facilitate chromatin modifications independent of functional NER, indicating that chromatin remodeling precedes NER. In spite of this, and unexpectedly, the homologues of XPC-HR23B in yeast (Rad4-Rad23) directly interact with the SWI/SNF remodeling complex via two subunits and this interaction was shown to be enhanced following UV irradiation (Gong et al., 2006). Taken together the limited data available to date, suggest important roles for histone modifying enzymes such as HATs and ATP-dependent chromatin remodelers,

yet mechanistic understanding of their impact on NER awaits further experimentation particularly to clarify their roles in mammalian NER.

Several studies have provided evidence for the involvement of the acidic HMG proteins that destabilize higher order chromatin structures, in the response to bulky DNA lesions. High mobility group protein B1 (HMGB1) binds to and bends damaged DNA and recent evidence demonstrates that mouse cells lacking HMGB1 are hypersensitive to the toxic effects of UVC radiation and may display reduced NER (Lange et al., 2008). HMGN1 was demonstrated to be recruited to TCR complexes (Fousteri *et al.*, 2006) and is exclusively involved in TC-NER as HMGN1-deficient mouse cells showed decreased rates of CPDs removal in actively transcribed genes (Birger et al., 2003). HMGN1 proteins directly compete for DNA binding sites with histone H1, elevate the level of histone H3 acetylation (Lim et al., 2005) and modulate the level of histone H3 phosphorylation (Lim et al., 2004). It is feasible that the loss of H1 in concert with histone modifications might enhance the DNA damage response following UV irradiation, but surprisingly this only affects TC-NER.

The current models of NER propose that chromatin structure is transiently disrupted during the various stages of repair to facilitate access of the repair machinery to DNA lesions and to carry out the subsequent steps. As a final step it is then necessary to restore the preexisting chromatin structure. A central question is whether chromatin restoration involves recycling of parental histones or new histone incorporation. The chromatin assembly factor (CAF-1), a key factor involved in histone deposition, plays a role in the restoration of chromatin following gap filling and ligation. In living cells this protein is recruited to sites of UV-induced DNA damage in a NER-dependent manner (Green and Almouzni, 2002); a process that is possibly mediated by PCNA (Gerard et al., 2006). The role of CAF-1 as chromatin assembly factor was further highlighted by the observation that histone H3.1 was assembled *de novo* at repair sites, reflecting a chromatin restoration step following NER (Polo et al., 2006). Hence, chromatin restoration after DNA damage is more than recycling of histones and may represent an imprint for newly repaired chromatin.

Taken together, it appears that repair proteins, DNA and histone binding proteins and chromatin modifiers play a key role in modulating the accessibility of nucleosomal DNA to the repair machinery as well as in the restoration of the chromatin state following repair. However, it is also evident that we are only beginning to understand the modifications that are required to allow NER in different chromatin environments.

2.2 TRANSCRIPTION COUPLED REPAIR

As pointed out, stalled transcription elongation by a DNA lesion is counteracted by the activation of a specialized NER subpathway named transcription coupled repair (TC-NER). A hallmark of TC-NER is the accelerated repair of DNA lesions (most notably demonstrated for UV-induced CPD) in the transcribed strand of active genes and the inability of TC-NER deficient cells to resume DNA damage-inhibited DNA and RNA synthesis (van Oosterwijk et al., 1996;Mayne and Lehmann, 1982;van Oosterwijk et al., 1996). Obviously, the elongating RNA polymerase II (RNAPIIo) when stalled at a lesion efficiently triggers the recruitment of TC-NER specific factors and NER proteins. Once the lesion has been recognized, all

subsequent steps leading to assembly of a functional NER complex require the same NER core factors as described for GG-NER (figure 3). TC-NER is a strongly conserved repair pathway identified in a variety of organisms including bacteria, yeast and mammals. In UV-irradiated E. coli cells, a 130 kDa protein encoded by the mfd gene (termed TRCF: transcription-repair coupling factor) was found to be essential for TC-NER (Selby et al., 1991). This protein releases the RNA polymerase and transcript from the DNA in an ATP dependent manner and also facilitates repair of DNA damage by attracting NER factors, in particular UvrA. Also in mammalian cells specific factors for TC-NER have been identified. Measurements of UV–photolesions in transcriptionally active genes of cells derived from various UV sensitive patients identified impaired TC-NER in cells from individuals suffering from Cockayne syndrome (CS). CS is a rare disorder that is associated with a wide variety of clinical symptoms including dwarfism, mental retardation, cataract and eye abnormalities as well as photosensitivity, but no enhanced susceptibility to cancer. As a consequence, these patients die at an early age and CS has been classified as a premature aging syndrome. Complementation studies have identified two CS complementation groups, CS-A and CS-B. A third group encompasses patients with mutations in XPB, XPD or XPG genes exhibiting both XP and CS symptoms. The CSB gene encodes a 168 kDa protein that contains helicase domains (strong homology to similar domains in SNF2-like proteins) and that displays DNA-dependent ATPase and DNA binding activity, but no helicase activity. Also, the bacterial and yeast counterparts of CSB, i.e. Mfd and Rad26 respectively, are DNA dependent ATPases. In addition, CSB has nucleosome remodelling activity and binds to core histone proteins *in vitro* (Citterio et al., 2000) and transcriptome analysis of CS-B cells revealed deregulation of gene expression similar to that caused by agents that disrupt chromatin structure (Newman et al., 2006).

The CSA protein contains WD-40 repeats (a motif involved in protein-protein interactions) and is part of an E3-ubiquitin ligase (E3-ub ligase) complex consisting of DDB1, Cullin 4A and ROC1/Rbx1 proteins (Groisman *et al.*, 2003). In response to UV the COP9 signalosome (CSN) was found to associate with the CSA complex resulting in the inactivation of the ubiquitin ligase activity of the CSA complex in TC-NER.

XAB2 is an XPA binding protein and an essential factor in TCR, but so far mutations in XAB2 have not been associated with UV sensitive patients. The protein is involved in pre-mRNA splicing and transcription, interacts with chromatin bound stalled RNAPIIo complex in a UV- and CS-dependent manner and might function as a scaffold for protein complex formation in TC-NER (Kuraoka et al., 2008). Finally, deficiency in HMGN1 (a nucleosome binding protein) leads to UV-B sensitivity in HMGN1 knock out mice and impairs TC-NER in UV-C irradiated mouse embryonic fibroblasts. Interestingly, HMGN1 interacts with UV-stalled RNAPIIo and this interaction depends on CS proteins (Fousteri *et al.*, 2006).

2.2.1 Molecular models for TC-NER

The additional involvement of RNAPII in TC-NER replaces the requirement for XPC-HR23B and UV-DDB to identify DNA lesions, as is the case in GG-NER. Instead the system utilises a factor that is capable to couple blockage of transcription by DNA damage to

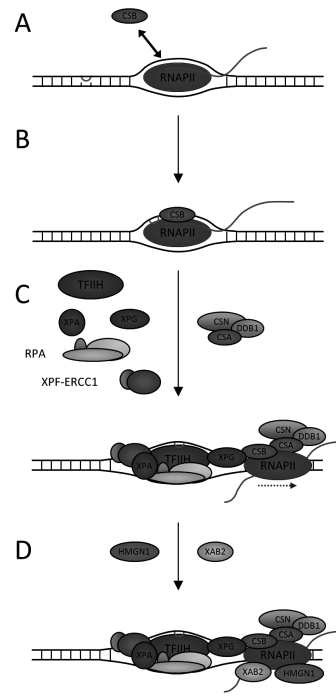

Figure 3: Model for transcription coupled NER. (A) During transcription CSB dynamically interacts with elongating RNAPII. (B) Stalling of RNAPII on DNA lesions stabilizes the interaction of CSB with the polymerase. (C) The stalled RNAPII/CSB complex allows for the recruitment of the core NER factors as well as the CSA/DDB1/CSN ubiquitin ligase complex. Conformational changes to the stalled RNAPII complex imposed by TFIIH and XPG would allow access to the damaged DNA strand. (D) The association of CSA with the stalled polymerase enables the recruitment of additional repair factors like HMGN1 and XAB2.

efficient DNA damage recognition and repair. The transcription-repair coupling factor in mammalian cells appears to be CSB: a DNA dependent ATPase that interacts with RNAPII even in undamaged cells (van Gool et al., 1997). Mu and Sancar (Mu and Sancar, 1997) showed that purified human excision repair factors and a DNA substrate analogous to a transcription bubble terminating at a CPD, are capable to excise the lesion independent of XPC. Hence the transcription bubble may substitute for XPC function, which in GG-NER causes the two damaged base pairs to flip out of the double helix.

Persistent blockage of transcription activates a stress response leading to stabilization of p53 and specific modifications of p53 at Ser 15 providing a strong signal for apoptosis in cultured cells and in the epidermis of mice (Ljungman et al., 1999;van Oosten et al., 2000). To relieve the strong apoptotic signal the cell has to remove the transcription blockage; however, the stalled RNAPII is likely to shield the DNA lesion and prevents access to the NER machinery. Two scenarios exist to cope with this problem. One potential mechanism would be that the RNAPII is displaced from the DNA or removed by ubiquitylation and subsequent degradation by the proteosome thereby making the lesion available for the NER machinery. This mechanism has been described for bacterial TC-NER as Mfd releases RNAP and recruits repair proteins (Selby and Sancar, 1994). In mammals such a scenario would require the recruitment of NER proteins by the action of CS proteins.

Another possible mechanism is that TC-NER occurs without displacement/removal of RNAPIIo but requires conformational changes of RNAPII to allow access to the DNA lesion and resume transcription. Particularly the XPG endonuclease in concert with the

basal transcription factor TFIIH have been implicated in an ATP dependent remodelling of the arrested RNAPII, allowing incision 3' of the lesion without the need for CSB (Sarker et al., 2005). Although *in vitro* experiments indicate a prominent role of XPG in the early stages of TC-NER, recruitment of XPG to stalled RNAPII in intact cells requires functional CSB (Fousteri *et al.*, 2006). Upon binding to stalled RNAPIIo, CSB functions as a coupling factor that mediates the recruitment of subsequent NER repair factors TFIIH, XPG, RPA and ERCC1-XPF. Indeed, live cell imaging revealed that GFP-tagged CSB interacts with the transcription machinery in the presence of DNA damage. Recruitment of CSA is CSB dependent and required for binding of both HMGN1 and XAB2 but is dispensable for the recruitment of pre-incision NER proteins. The emerging picture of TC-NER is rather complex and not well understood at the molecular level. Most strikingly, repair of transcription blocking lesions in mammalian cells occurs without displacement of the stalled RNAPIIo and requires at least two essential assembly factors with differential modes of action: CSB as a repair–transcription coupling factor to attract the core NER pre-incision factors and CSA to recruit chromatin remodelers. However, the precise role of CSB ATP-ase activity and the CSA associated the E3-ubiquitin ligase complex in TC-NER are not known.

2.3 NER DEFICIENCIES AND CANCER

As pointed out, inherited defects in the NER pathway are manifested in at least three different diseases: XP, CS and the photosensitive form of trichothiodystrophy (TTD). Of these, only patients with XP are prone to sunlight-induced skin cancer, although patients with CS and the photosensitive form of trichothiodystrophy (TTD) are clearly UV-sensitive. For most cancers the causative agent is unknown but skin cancer is a notorious exception. In fact, XP is a paradigm for a causal link between defective DNA repair and exposure to an exogenous (environmental) component i.e. sunlight, as XP patients have a >1000-fold increased risk to develop skin tumors primarily at sun-exposed sites of their body. Mutation analysis of TTD revealed a complicated genotype as patients have been identified with mutations in the XPB, XPD and TTDA genes, all components of the TFIIH complex. Since TFIIH functions both in DNA repair and transcription it is assumed that photosensitive TTD patients have a defect in both processes; these patients are characterized by sulphur-deficient brittle hair and nails, ichthyosis, neurological/developmental abnormalities and short life span. Finally, patients exist that belong to the XP-B, XP-D or XP-G complementation group that display severe features of CS (early death and neurological/developmental abnormalities) and XP (skin lesions and skin cancer).

As mentioned above, the most overt phenotype of XP patients is their enhanced susceptibility to develop skin cancer including basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) but also melanomas. Increased cancer susceptibility is not only seen at the sun-exposed parts of the body but is also evidenced by a low incidence of internal tumors. Since epidemiological data on the relationship between skin cancers and ambient solar UV radiation are very restricted, animal models i.e. (transgenic) mice, have been used to study the process of UV carcinogenesis in depth and to gain quantitative data on tumor development and dose, time and wavelength of the UV radiation and genetic

make-up. Transgenic hairless mice (to facilitate UVB irradiation and the identification of tumors) mimicking the human XP phenotype have been extremely useful in studying the role of (exogenously-induced) DNA damage in mutagenesis, carcinogenesis and aging. The protective role of GG-NER and TC-NER against the acute (i.e. erythema, apoptosis, cell cycle arrest) and long term (i.e. skin cancer, aging) effects of genotoxic (UV-B light, bulky chemicals) exposure has been dissected in mouse models with defined mutations in NER genes, i.e. XPE (DDB2), XPA (defective in GG-NER and TC-NER), XPC (defective in GG-NER) or CSB (defective in TC-NER) deficient mice. DDB2$^{-/-}$ mice are deficient in GG-NER of CPD, but otherwise TC-NER proficient. XPA$^{-/-}$ and CSB$^{-/-}$ mice appeared to be 10-fold more sensitive to the acute toxic effects of UV-B light (erythema/edema of the skin) and to the polycyclic aromatic hydrocarbon DMBA (lethality) compared to normal, XPC$^{-/-}$ or DDB2$^{-/-}$ mice (Wijnhoven et al., 2001;Berg et al., 1998). The difference in UV-B sensitivity relates to enhanced apoptosis and severe cell cycle arrest of epidermal keratinocytes in XPA$^{-/-}$ and CSB$^{-/-}$ mice (van Oosten *et al.*, 2000;Stout et al., 2005). These results highlight TC-NER as a profound survival pathway and identify TC-NER as the principal defense mechanism towards the deleterious effects of transcription blocking DNA lesions by counteracting apoptosis and cell cycle arrest. However, this increased survival occurs at expense of increased mutagenesis manifested by the fast appearance of epidermal patches expressing mutant p53 in UV-B irradiated XPC$^{-/-}$ mice (Rebel et al., 2005) but also by increased spontaneous mutagenesis in lymphocytes (Wijnhoven *et al.*, 2001). Mutation spectrum analysis showed that almost all UV-B light induced mutations in rodent and human were at dipyrimidine positions with C→T transition mutations being the most prominent. The latter is caused by three factors. Firstly, DNA polymerase η preferentially incorporates adenine residues opposite to non-instructional lesions. Secondly, 5-methylcytosines within CPD lesions display accelerated deamination rates, resulting in base changes to uracil. Moreover, CPDs are formed preferentially at dipyrimidines containing 5-methylcytosine when cells are irradiated with UV-B or sunlight. Finally, CC→TT double transitions are caused in vivo exclusively by UV-induced pre-mutagenic lesions in XPA and XPC deficient mice and human XPC patients (Spatz et al., 2001). Also in tumors isolated from UV-B irradiated mice (with high frequencies of p53 mutations) defective GG-NER and TC-NER resulted in increased mutations in p53 through UV-targeted dipyrimidine sites but strikingly, only XPA$^{-/-}$ and CSB$^{-/-}$ mice developed benign papillomas before squamous cell carcinomas (SCC). These papillomas carried mutations in the 12th Hras codon with a dipyrimidine site in the transcribed strand; such mutations were not observed in the UV-induced SCCs. Evidently, proficient TC-NER prevents Hras mutagenesis and therefore prevents the development of papillomas.

Taken together, GG-NER and TC-NER protect against UV-B induced skin cancer in mice. Although the mouse cancer data reveals remarkable similarities with skin cancer susceptibility in human, striking differences exist as well. Most notably, the XPA$^{-/-}$ mice, in contrast to XP patients, do not develop melanoma whereas CSB$^{-/-}$ mice, but not CSB patients, are skin cancer prone. The latter is related to the poorly expressed GG-NER system in rodents. Unlike human cells, rodent epidermal cells express DDB2 at a low level. Mice ectopically expressing DDB2 display delayed onset of squamous cell carcinoma following

chronic UV-B light exposure and at the cellular level enhanced repair of UV-photolesions (Alekseev et al., 2005;Pines et al., 2008) whereas DDB2$^{-/-}$ mice were hypersensitive to UV-induced skin carcinogenesis. Ectopic expression of DDB2 in CSB$^{-/-}$ mice counteracts the cancer proness of UVB exposed CSB$^{-/-}$ mice indicating that GG-NER serves as a back-up system for TC-NER deficiency (Pines et al., in preparation).

2.4 PERSPECTIVES

Molecular, cellular and animal studies over the last three decades have greatly improved our understanding of the interplay between cellular processes (DNA damage, NER and transcription) and human disease. However, much is to be learned about the exact functions of key players in NER and the mechanisms by which eukaryotic cells sense DNA damage in their genome and which signals activate and regulate NER. The mammalian genome is protected against genotoxic insults by a network of DNA damage response (DDR) mechanisms initiated by sensing of DNA damage or damage-induced chromatin alterations through specific sensors. The next stage in the process is to transmit the signal to transducers that are able to pass the signal to effectors that control various protective pathways i.e. different DNA repair pathways, cell cycle checkpoints, apoptosis, transcription and chromatin remodeling. Hence, full understanding of mammalian NER not only requires insights into the mechanisms of NER but also the DNA damage signaling cascade.

Faithful DNA damage processing in various chromatin environments requires process control at each individual step including regulation of the expression of NER factors, regulation of NER protein activity by post-translational modifications, remodeling of chromatin at sites of DNA damage, monitoring progress and completeness of repair and checking integrity of chromatin after damage removal. Presently, little is known about these regulatory processes and how NER is connected with DNA damage signaling pathways i.e. specific sensors of DNA damage (such as UV-induced photolesions) and transducers able to pass the signal to downstream effectors i.e. transcription, chromatin remodeling and protein modification. Which are the factors that control initiation, progression and completion of the NER process? Which factors enable GG-NER activity in different chromatin environments such as heterochromatic and euchromatic regions? The ultimate goal is to use this information to further improve the mathematical modeling of NER. The current model based on in vivo kinetic data (Politi *et al.*, 2005) unveils that a sequential assembly mechanism appears remarkably advantageous in terms of repair efficiency and suggests that random assembly and preassembly are kinetically unfavorable.

Multiple gene products are implicated in TC-NER but we lack knowledge of the signals that regulate TC-NER and we do not know the precise function of key components in TC-NER. Most notably, it is not clear why chromatin remodeling would be required for TC-NER in addition to the structural changes that are needed to allow transcription of actively transcribed chromatin-embedded DNA substrates. Currently it is not well understood which factors or processes are required to resume transcription although it is clear that besides TC-NER other mechanisms play a role (Rockx et al., 2000). Of particular interest to resolve is the fate of stalled RNAPII when TC-NER fails to operate and to find

out which types of oxidative DNA damage (induced by metabolic processes) can inhibit transcription *in vivo*. A stalled RNAPII transcription machinery senses DNA damage and leads to a strong signal for apoptosis. Moreover, there is increasing evidence that during S-phase collisions of replication forks with transcription complexes stalled at DNA lesions, are a very mutagenic event (Hendriks et al., 2008). Hence, it is of pivotal importance to dissect the contributions of impaired TC-NER and transcription defects in the aetiology of the progeroid, neurodevelopmental disorder of CS.

Knowledge of the NER pathway and repair proteins has lead to the identification of inherited polymorphisms of NER genes (SNPs). These SNPs may contribute to variations in DNA repair capacity and genetic susceptibility to cancer. Numerous published data provide emerging evidence that polymorphisms in NER genes may contribute to the genetic susceptibility to cancers in man. However, many of the studies are of limited value because of the limited size of the study populations. It is obvious that large and well-designed population-based studies are warranted to identify NER genes as biomarkers to screen high-risk populations for early detection of cancer. Knowledge of the NER pathway and repair proteins can also be applied as basis for enzyme therapy to counteract sunlight induced skin cancer. The bacterial DNA repair enzyme T4 endonuclease V packaged in an engineered delivery vehicle was shown to be capable of reversing the defective repair in xeroderma pigmentosum cells (Yarosh, 2002). Moreover, expression of the CPD-photolyase in mouse epidermis is an effective tool to combat UV-B induced non-melanoma skin cancer (Jans et al., 2005). These findings directly proof that enhancement of repair activity can be used as a therapeutic tool to protect against UVB induced skin cancer although NER proteins have not been applied so far.

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