Photochemical & Photobiological Sciences

PERSPECTIVE



**Cite this:** *Photochem. Photobiol. Sci.*, 2018, **17**, 1842

# Solar UV damage to cellular DNA: from mechanisms to biological effects

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Received 30th April 2018,
Accepted 23rd July 2018

DOI: 10.1039/c8pp00182k

rsc.li/pps

Solar ultraviolet (UV) radiation generates bulky photodimers at di-pyrimidine sites that pose stress to cells and organisms by hindering DNA replication and transcription. In addition, solar UV also induces various types of oxidative DNA lesions and single strand DNA breaks. Relieving toxicity and maintenance of genomic integrity are of clinical importance in relation to erythema/edema and diseases such as cancer, neurodegeneration and premature ageing, respectively. Following solar UV radiation, a network of DNA damage response mechanisms triggers a signal transduction cascade to regulate various genome-protection pathways including DNA damage repair, cell cycle control, apoptosis, transcription and chromatin remodeling. The effects of UVC and UVB radiation on cellular DNA are predominantly accounted for by the formation of photodimers at di-pyrimidine sites. These photodimers are mutagenic: UVC, UVB and also UVA radiation induce a broadly similar pattern of transition mutations at di-pyrimidine sites. The mutagenic potency of solar UV is counteracted by efficient repair of photodimers involving global genome nucleotide excision repair (GG-NER) and transcription-coupled nucleotide excision repair (TC-NER); the latter is a specialized repair pathway to remove transcription-blocking photodimers and restore UV-inhibited transcription. On the molecular level these processes are facilitated and regulated by various post-translational modifications of NER factors and the chromatin substrate. Inherited defects in NER are manifested in different diseases including xeroderma pigmentosum (XP), Cockayne syndrome (CS), UV sensitive syndrome (UVsS) and the photosensitive form of trichothiodystrophy (TTD). XP patients are prone to sunlight-induced skin cancer. UVB irradiated XP and CS knockout mouse models unveiled that only TC-NER counteracts erythema/edema, whereas both GG-NER and TC-NER protect against UVB-induced cancer. Additionally, UVA radiation induces mutations characterized by oxidation-linked signature at non-di-pyrimidine sites. The biological relevance of oxidation damage is demonstrated by the cancer susceptibility of UVB-irradiated mice deficient in repair of oxidation damage, *i.e.*, 8-oxoguanine.

## Introduction

Cells and organisms are continuously exposed to genotoxic agents either present within the environment or exerted by endogenous processes. Maintenance of genomic integrity under conditions of genotoxic stress is a prerequisite for proper cell function and of clinical importance in relation to cancer, neurodegeneration and premature ageing. The genome of all organisms is protected against genotoxic insults by a network of DNA damage response (DDR) mechanisms triggered by DNA lesions and specific sensors. Ultimately, the initiation of signal transduction cascades regulates various genome-protection pathways including DNA damage repair, cell cycle control, apoptosis, transcription and chromatin remodelling. We still poorly understand the mechanisms of DNA damage sensing and activation of signalling pathways, particularly, how cells sense, recognize and repair low levels of DNA lesions in their genomes at various stages of the cell cycle and in different chromatin environments.

Sunlight is a very strong genotoxic stressor and solar UV radiation is acknowledged as the primary cause of photocarcinogenesis and therefore contributes to the development of skin cancer *i.e.*, squamous cell carcinoma, basal cell carcinoma and melanoma. UVB (280–315 nm) radiation causes covalent linkages to form between adjacent pyrimidine bases (photodimers), creating primarily cyclobutane pyrimidine dimers (CPDs) and pyrimidine-6,4-pyrimidone photoproducts (6,4PPs). Nucleotide excision repair (NER) is an evolutionary conserved repair pathway that repairs DNA lesions with variable degree of distortion of the DNA helix and with the potency to block replication and transcription. Notably, NER is the only repair pathway in man to remove the toxic and mutagenic photodimers in a highly orchestrated fashion involving multiple proteins and defined steps.[1] Two mechanistically dis-

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tinct NER subpathways have been identified: global genome NER (GG-NER) is capable of repairing photodimers in chromatin of different compaction levels and different functional states throughout the cell cycle. A subpathway of NER, termed transcription-coupled repair (TC-NER), enables quick resumption of UV-inhibited transcription by efficient repair of the photodimers that arrest elongating RNA Polymerase II (RNAPIIo).[2]

The relevance of NER for human health is manifested by the severe clinical consequences associated with inherited NER defects including skin toxicity, developmental and neurological abnormalities, premature ageing and extreme cancer susceptibility. The NER defects lead to the rare autosomal inherited diseases, xeroderma pigmentosum (XP), Cockayne syndrome (CS), triochothiodystrophy (TTD) and Cerebro-oculo-facio-skeletal (COFS) syndrome, but also to the much milder disorder UV sensitive syndrome (UVsS).[3] Most of the core NER machinery has been described and recent studies concentrate on molecular mechanisms that either facilitate NER in the context of chromatin or fine-tune the process by promoting post-translational modifications of NER factors and chromatin substrate.

Although this review primarily focuses on UVC/UVB induced photolesions, their repair (NER) and biological consequences, it is important to emphasise the role of UVA (315–400 nm) radiation. UVA accounts for the large proportion of the solar UV radiation and most likely contributes to human skin cancer risk. In contrast to UVC (<280 nm) radiation, UVA damages DNA indirectly, largely through cellular chromophores that act as photosensitisers to generate reactive oxygen species (ROS)[4]. Oxidative stress by UVA (and photosensitisers) generates damage to biomolecules, notably DNA nucleobases and proteins including among others NER proteins.[5] Oxidation of the latter leads to impairment of the NER reaction and consequently contributes to biological effects. Finally, although much less efficient than UVA radiation, also UVB can generate oxidized purine and pyrimidine bases together with DNA single-strand breaks to DNA in isolated cells and skin.[6]

In this review paper I will summarize (i) UV-induced DNA damage signalling, (ii) the repair of photodimers by NER and (iii) the impact of NER deficiencies on the cellular responses of organisms to sunlight/UVB induced toxicity, mutations and cancer. These topics will be complemented with description of effects of UVA radiation.

## Cellular signalling to photodimers

An important response to UV radiation is the upregulation of the checkpoint protein p53, not only in cycling but also in non-cycling cells.[7,8] Studies using cells with genetic defects in various NER genes have led to better understanding of UV-mediated checkpoint signalling. Impairment of TC-NER in nondividing cells results in high p53 expression following treatment with UVC,[7] indicating that (persistent) arrest of elongating RNAPIIo initiates a signaling response.[9,10] Also processing of UV-photodimers by GG-NER triggers a checkpoint signalling.[7,11,12] ATR together with ATM are key kinases that orchestrate DNA damage signalling.[13] RPA containing single stranded DNA gaps formed during NER are substrates for the ATR/ATRIP complex, thereby initiating UV damage signalling manifested by H2AX phosphorylation (γH2AX) and ubiquitylation of histone H2A.[12,14] Moreover it was shown that a single 5′ incision at a site undergoing NER can provoke ATR dependent signalling and γH2AX formation even when repair synthesis is inhibited.[15] Yet, there is evidence that in the absence of both GG-NER and TC-NER, DNA damage checkpoints are activated in nondividing cells. Recent experiments[7] showed that nondividing cells defective in NER, displayed persistent ATR-dependent signalling after UVC irradiation, resulting in activation of the G1 checkpoint. This response prevents the transition of UV-irradiated cells from G1 to S phase upon stimulation of quiescent cells to divide.

High-dose UVB exposure of repair-proficient human cells (*i.e.*, under conditions that saturate NER) leads to retarded or incomplete repair of UV photolesions and also activates a signaling response. Human skin epidermis from healthy individuals includes nondividing basal cells that contain high levels of unrepaired damage.[16] Differences in the expression of repair genes might account for the accumulation of DNA damage in these epidermal cells. Also, non-cycling mammalian cells including melanocytes and keratinocytes, can be stimulated to divide by external stressors such as UVB radiation[17] and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)[18] in the presence of DNA damage. Therefore, this newly identified pathway might also be relevant for the general population by preventing quiescent cells with high levels of photodimers to enter the cell cycle.

The mechanism underlying the UV-induced signaling response in NER deficient cells depends on an alternative mechanism that involves the endonuclease APE1.[4] When 6,4PPs are not efficiently removed, these lesions are targeted by APE1 generating single strand breaks that activate the ATR pathway. ATR has been implicated in the UVB-induced signaling following transcription blockage:[19] UVC-exposed cells that lack TC-NER, such as CSB and XPA cells,[20] displayed a rapid, very pronounced and persistent accumulation of the phosphorylated checkpoint protein p53. Hence, stalled RNA polymerase complexes might adopt a chromatin configuration facilitating RPA mediated activation of the ATR kinase.

## Global genome repair (GG-NER) of photodimers

Cells of all XP patients (except XP variant) are defective in global genome repair of solar UV-induced photolesions. The identification of the XP genes and encoded proteins led to detailed understanding of the various steps in NER. The first step (pre-incision step) includes the DNA lesion recognition, the opening of the damaged DNA to allow assembly of the NER pre-incision complex and dual incision complex for-

mation, together requiring all XP factors (XPE, XPC, XPA, XPG, ERCC1/XPF) and other factors (RPA, TFIIH containing XPB and XPD).

The second step (post-incision step) of the reaction requires RF-C, PCNA, DNA polymerase ε and δ and DNA ligase 1/3 for repair synthesis and ligation to fill in the gap after dual incision.[21,22] Recognition of damage is one of the rate-limiting steps in NER;[23] in GG-NER this step requires the interplay between two DNA damage sensor complexes *i.e.*, UV-DDB and XPC-HR23B-Centrin2. *In vivo*, the recruitment of NER proteins to photodimers is abolished in XPC-deficient cells, indicating that assembly of the NER complex is strictly XPC-dependent. UV-DDB consists of the heterodimer DDB1-DDB2 and is linked to XP: mutations in DDB2 give rise to xeroderma pigmentosum (XPE). Moreover, UV-DDB is part of the CUL4A-DDB1-RBX1 ubiquitin ligase complex (CRLDDB2) that facilitates XPC binding to UV-damaged chromatin;[24,25] particularly it is needed for repair of the major photodimer, CPD. DDB2 regulates the retention time of both UV damage-recognition complexes UV-DDB and XPC-HR23B[26] at the site of the photodimer. The underlying mechanism is that CRLDDB2 promotes the ubiquitination of XPC and itself upon UVB irradiation, thereby increasing stability of XPC at photodimers.[27] The residence time of DDB2 at photodimers is regulated by competing post-translational modifications, *i.e.*, poly-ADP ribosylation (PARylation) and ubiquitination of DDB2. These modifications occur at the same part of the protein with the former inhibiting the latter, therefore increasing the half-life of DDB2.[26] Upon UVC irradiation, XPC appears to be tightly regulated by multiple post-translational modifications, *i.e.*, SUMOylation and ubiquitinylation.[28] These modifications behave cooperatively resulting in the dissociation of HR23B and stabilization of XPC at the photodimer.

Upon recognition of the photodimer, the NER repair complex is built-up by XPC, which recruits the multiprotein transcription factor, TFIIH, *via* direct protein–protein interactions. TFIIH mediates the initial opening of the DNA after which RPA, XPA and XPG bind to obtain full opening of ~30 nucleotides around the lesion. The TFIIH complex is composed of a seven-subunit core containing two XP factors (XPB, XPD) and TTD, p34, p44, p52, p62 and a three-subunit kinase complex Cdk7, cyclin H and MAT1. The XPB subunit of TFIIH is an ATP-dependent helicase that mediates unwinding of DNA in a 3′-5′ orientation during transcription initiation, whereas the XPD subunit of TFIIH is a 5′-3′ATP-dependent helicase. However, unwinding of the damaged DNA during NER is mediated by virtue of the XPD helicase activity only, whereas the ATPase activity of XPB is indispensable for NER. Nevertheless it is obvious that the repair reaction is driven by the ATPase activity of XPB, in combination with the helicase activity of XPD. A two-step mechanism underlies the opening of the damaged DNA to allow assembly of the NER pre-incision complex: TFIIH mediates the initial opening after which RPA, XPA and XPG bind to obtain full opening of ~30 nucleotides around the lesion.[29] During the pre-incision complex formation, RPA associates with the undamaged DNA strand partially unwound by TFIIH, leading to a separation of the DNA strands around the lesion. Thereby RPA interacts with several core NER proteins including XPA, XPG and ERCC1-XPF to assemble the pre-incision complex.[30] The two structure specific endonucleases XPG and ERCC1-XPF are involved in dual incision 3′ and 5′ of the lesion, respectively. The post-incision step in NER is completed by repair synthesis and ligation. In addition to RPA, this step requires RF-C, PCNA, DNA polymerases ε and δ as well as Ligases I and 3.[21,22]

Replication protein A (RPA) plays a key role at the interface of the pre-and post-incision steps of NER and the coordination of these steps. Tight regulation of new incision events is essential to prevent generation of DNA strand-breaks that could lead to illegitimate recombinogenic events.[31] RPA appears to regulate the incision events: the protein is recruited prior to incision and remains associated at the site of repair after recruitment of post-incision factors and is the sole NER protein involved in pre- and post-incision steps[31,32] (Fig. 1). New incision events at photodimers are only initiated after finishing ongoing repair, thereby making RPA available. Interestingly, RPA is clearly a limiting factor in the NER reaction as excessive sequestration of the protein at stalled replication forks leads to defective NER due to scarceness of RPA available to NER.[32] Likewise, ROS might diminish NER capacity by causing damage to RPA. ROS generated by UVA radiation in combination with exogenous photosensitizers causes extensive protein oxidation including oxidation of NER repair factors. Notably, cellular RPA is surprisingly vulnerable to oxidation and its oxidized forms are associated with impaired NER and photosensitivity.[5]

Like other DNA metabolising processes, NER is to a large extent regulated by chromatin status. Highly compacted heterochromatin poses a challenge for efficient NER, indicated by slow repair of UV photodimers in this chromatin. Chromatin remodeling promotes transient chromatin decompaction and might facilitate access of the repair machinery to sites of damage. Although UV damage *per se* results in histone eviction,[33] local chromatin decompaction is achieved by post-translational modifications of core histones. The first factor to recognize UV photolesions, UV-DDB, facilitates local chromatin unfolding by chromatin decompaction and/or histone eviction.[34] DDB2-induced chromatin PARylation facilitates the recruitment of the chromatin remodeler ALC1,[26] which can further restructure UV-damage-containing nucleosomes.

## Transcription coupled repair (TC-NER) of photodimers

Arrest of transcription elongation by RNAPIIo blocking DNA lesions, such as UV photodimers, is counteracted by the activation of the specialized NER subpathway transcription coupled repair (TC-NER). A hallmark of TC-NER is the fast repair of DNA lesions in the transcribed strand of active genes and the ability of TC-NER-proficient cells to resume DNA damage-inhibited DNA and RNA synthesis.[35] Impaired

 

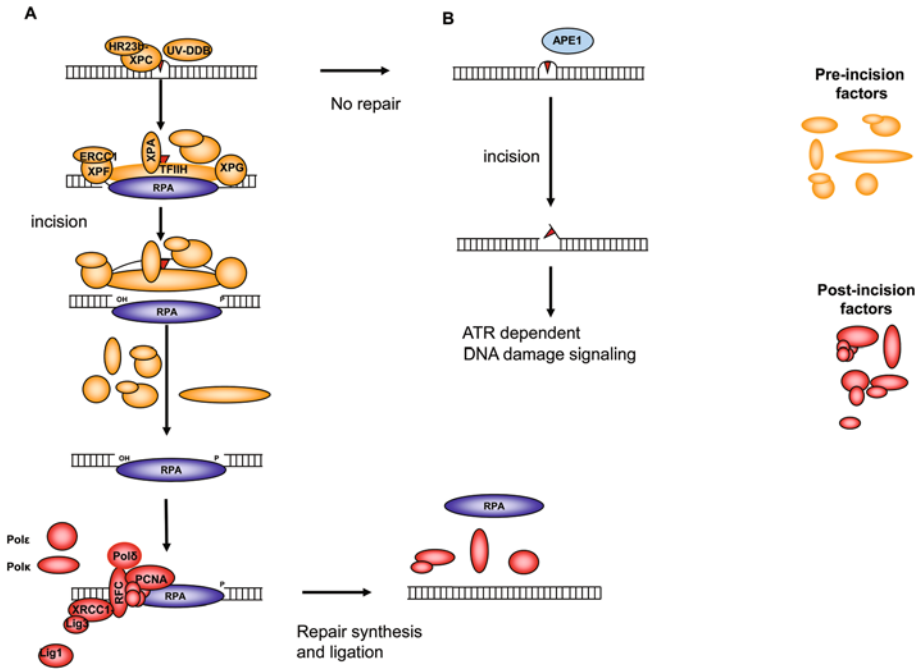


**Fig. 1** GG-NER mediated and aberrant incisions. (A) Following recognition of photodimers by UV-DDB and XPC-HR23B, local opening of DNA by TFIIH is required to recruit XPA, RPA, XPG, and XPF/ERCC1. The undamaged single-stranded DNA is covered by RPA and dual incision is followed by the release of core NER factors. The post-incision step of NER is performed by RFC stable loading PCNA onto the incised DNA, the recruitment of various DNA polymerases and DNA ligases to fill in and ligate the gap, respectively. (B) In GG-NER repair-deficient cells, photodimers (primarily 6,4PPs) are incised by APE1 slowly creating a gap without further repair, leading to persistent ATR-dependent DNA damage signaling. Figure adapted from ref. 28.

TC-NER was identified in cells from patients suffering from Cockayne syndrome (CS), a rare disorder associated with a wide variety of clinical symptoms including dwarfism, intellectual disability, eye abnormalities as well as photosensitivity, but no enhanced susceptibility to cancer.[36] Two CS complementation groups, CSA and CSB, have been identified and a third group encompasses patients with mutations in XPB, XPD or XPG genes exhibiting both XP and CS symptoms. Defects in TC-NER are also associated with UV sensitive syndrome (UVsS); this syndrome shares skin photosensitivity with CS, but lacks the severe growth and developmental defects. The causative gene for UVsS is UVSSA,[37–39] a new player in TC-NER.

The CSB gene encodes a 168 kDa protein that displays DNA-dependent ATPase, DNA binding and nucleosome remodeling activities.[40] The CSA protein contains WD-40 repeats and is part of an E3-ubiquitin ligase (CRLCSA) complex consisting of DDB1, Cullin 4A and ROC1/Rbx1 proteins.[41] Recently a stable interaction between CSA and the TRiC chaperonin was found, ensuring stability of CSA and the DDB1-dependent assembly into the CRLCSA complex. Loss of TRiC leads to cellular mislocalization and depletion of CSA, as well as impaired transcription recovery following UV damage.[42] The function of UVSSA has been partly clarified by the observed interaction with the deubiquitinating enzyme ubiquitin-specific protease 7 (USP7). UVSSA and USP7 appeared to regulate the proteasome-dependent degradation of CSB. The function of the UVSSA-USP7 complex in CSB stabilization would be a UVSSA-mediated positioning of USP7 in close proximity to CSB in the TC-NER complex, preventing the proteasomal degradation of CSB by exerting USP7's deubiquitinating activity on CSB.

The involvement of elongating RNA polymerase II (RNAPIIo) in TC-NER replaces the requirement for XPC-HR23B and UV-DDB to identify UV photodimers. Instead the system utilizes CSB by virtue of its interaction with RNAPIIo even in undamaged cells.[43] Current models describe the recruitment of CSB upon stalling of RNAPIIo to the site of damage, their transient interaction being stabilized in an ATP-dependent manner.[44–46] This step is followed by the CSB-dependent recruitment of CSA/CRLCSA and further formation of the TC-NER complex. One of the presumed functions of CRLCSA is the removal of RNAPIIo by its ubiquitination and ultimate degradation under conditions of slow or no repair of RNAPIIo blocking lesions.[46]

As indicated, persistent blockage of RNAPIIo by photodimers 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 UV irradiated mice.[20,47] Removal of the transcription blockage is required to diminish the apoptotic signal, but this process might be problematic as a stalled RNAPIIo likely poses hindrance and limited access of the DNA lesion to NER proteins. A mechanism to solve this problem is the displacement of RNAPIIo from the DNA or its degradation. A different mechanism is that TC-NER occurs by conformational changes

of RNAPIIo to expose the DNA lesion to the repair machinery and resume transcription. *In vitro* studies revealed a key role of the XPG endonuclease and TFIIH in remodeling of the arrested RNAPIIo to allow 3′ cutting of the lesion, even without the need of CSB.[48] However, recruitment of XPG to stalled RNAPIIo in intact cells requires functional CSB, and live cell imaging revealed that GFP-tagged CSB interacts with the transcription machinery in the presence of DNA damage. CSB is a member of the SWI2/SNF2 family of DNA-dependent ATPases and is capable of remodeling nucleosomes and hence may induce local changes in chromatin allowing conformational changes of the stalled RNAPIIo.[49] Interestingly, a recent study shows that Rad26 (the yeast homolog of CSB) binds to the DNA upstream of RNAPIIo, promoting the forward movement of RNAPIIo (backtracking) and making the lesion accessible to repair proteins.[50]

Assessment of the kinetics of DNA repair and RNA synthesis recovery in human cells following UV-irradiation by nascent RNA Bru-seq and quantitative long PCR put light on the relationship between TC-NER and transcription recovery.[51] The principal finding was that transcription elongation was inhibited by UV radiation and that recovery of RNA synthesis occurred as a wave in the 5′-3′ direction with slow recovery at the 3′ end of long genes. The (initial) lack of recovery of transcription in the 3′ end of large genes would be consistent with a model suggesting that RNAPIIo blocked at lesions cannot resume RNA synthesis even after removal of the blocking photodimers. Such a conclusion would favor a model in which removal of the transcription blockage is primarily important to diminish the apoptotic signal that leads to cell death. It also points to the importance of transcription initiation to allow transcription recovery after UV irradiation and the identification of the factors involved.[49]

## Solar UV radiation and TC-NER: a survival pathway with antimutagenic properties

TC-NER functions as a defense mechanism for cells and organisms to solar UV radiation by fast removal of transcription-blocking photodimers to escape from lethal effects of inhibited transcription. The blockage of RNAPIIo activates a robust stress response and apoptosis.[20,47] In TC-NER-proficient cells and mice, the pathway protects against UV-induced apoptosis in a p53-dependent manner, however the apoptotic response in TC-NER-deficient cells and mouse epidermis did not strongly rely on p53.

The protective role of TC-NER against genotoxic exposure has been most convincingly demonstrated in UVB-irradiated hairless mice with defined mutations in NER genes, *i.e.*, XPE, XPA, XPC or CSB-deficient mice. *XPA*−/− mice are deficient in both GG-NER and TC-NER, whereas *XPC*−/− and *CSB*−/− mice are defective in GG-NER and TC-NER, respectively. *XPE*−/− mice are only deficient in GG-NER of CPD and are TC-NER-proficient. When hairless mice were exposed to UVB radiation and the minimal erythema dose (MED) was estimated, *XPA*−/− and *CSB*−/− mice appeared to be 10-fold more sensitive to induction of erythema/edema than WT, *XPC*−/− or *XPE*−/− mice[52,53] (Fig. 2). Similar observations were made in XP patients exposed to sunlight.[55] These findings suggest that blockage of transcription by photodimers is a key event in the development of UV erythema and edema. The difference in sensitivity between the various genotypes coincided with a pronounced difference in apoptosis and cell cycle progression: the *XPA*−/− and *CSB*−/− mice appeared to display UVB-induced apoptosis at much lower dose than the *XPC*−/−, *XPE*−/− and WT mice.[52,53] Similar observations were made when mice were exposed to the polycyclic aromatic hydrocarbon DMBA (a potent rodent mutagen and carcinogen) that induces DNA lesions targeted by NER[56] (Fig. 2).

The fast repair of CPD from the transcribed strand of expressed genes compared to the non-transcribed strand impacts the frequency and nature of mutations induced by photodimers. Mutational analysis showed that almost all mutations induced by UVC radiation in rodent cells were found at di-pyrimidine positions in the non-transcribed strand.[57] In contrast, most mutations in CSB-deficient cells were found at di-pyrimidine sites in the transcribed strand, illustrating the protective role of TC-NER against UV-induced mutations.[58] Also tumors isolated from UVB-irradiated TC-NER-deficient mice (*CSB*−/− and *XPA*−/− mice) revealed increased mutations in p53 through UV-targeted di-pyrimidine sites in the transcribed DNA strand. Insight into the mechanism underlying the strong bias of mutations particularly in NER-deficient cells has been a real challenge. Recent studies[59] revealed that gene transcription itself increases UV-induced mutagenesis (transcription associated mutagenesis, TAM) in mouse embryonic stem cells in a strand-specific manner (Fig. 3). Importantly, this process occurred *via* selected deamination of cytosines to uracil in photodimers in the transcribed strand. This is most likely due to enhanced single strandedness of photodimers at arrested transcription complexes.[59] These data also revealed a key role of TC-NER in preventing the mutagenic consequences of TAM. Additionally, transcription of UVB-irradiated mouse ES cells induced another class of TAM mutations, *i.e.*, intragenic deletions. In S-phase, the cell replication forks may encounter arrested RNA polymerase complexes; such an encounter might lead to collapsed forks provoking transcription-associated recombination and resulting in DNA strand break formation and subsequently deletions following error prone DNA break repair.

## Photodimers, NER and cancer

As mentioned above, inherited defects in NER are manifested in different diseases: XP, CS, UVsS and the photosensitive form of trichothiodystrophy (TTD). Only patients with XP are prone to sunlight-induced skin cancer (>1000-fold increased risk), although CS, UVsS and TTD patients are clearly UV-sen-

 

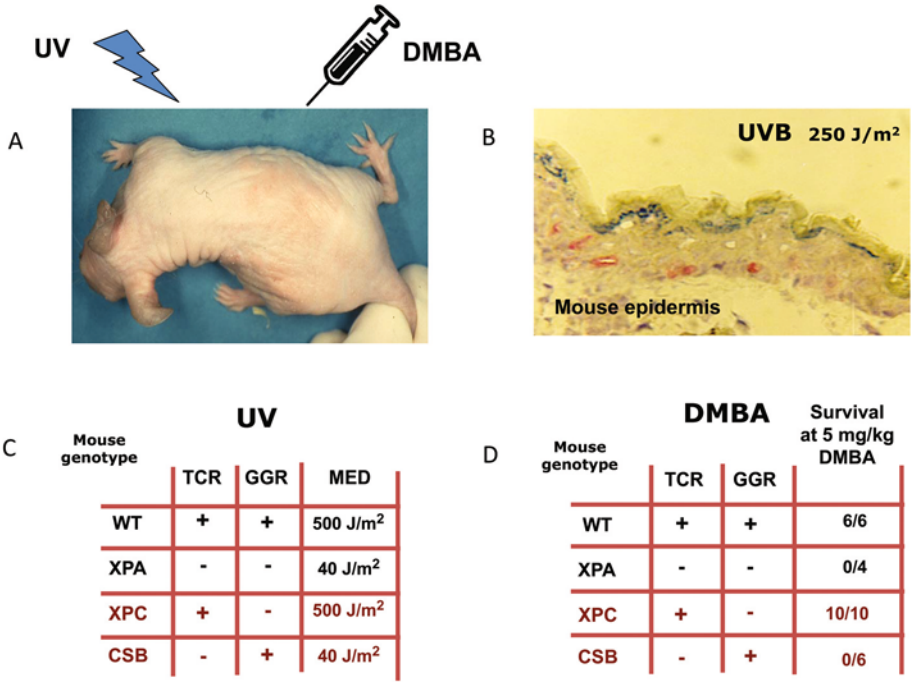


**Fig. 2** TC-NER protects against acute toxicity of UVB radiation and DMBA. (A) Exposure of hairless mice to UVB radiation or DMBA. (B) Induction of apoptotic cells in the mouse epidermis after UVB (adapted from ref. 44). (C) and (D) Induction of erythema (C) and lethality (D) in various mouse genotypes.

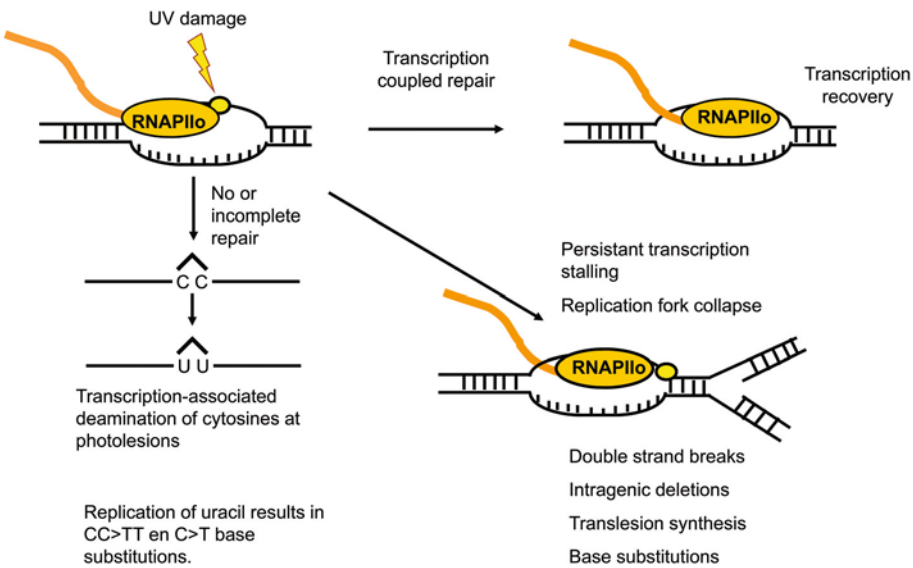


**Fig. 3** Mutagenic consequences of transcription arrest by photodimers. Photodimers are very effective in blocking elongating RNAPIIo. Unrepaired or slowly repaired photodimers in transcription bubbles undergo rapid deamination at cytosine due to single strandedness, leading to transitions and a strand bias in mutagenesis. This situation is characteristic for GG-NER deficient XPA or TC-NER deficient CSB cells (see ref. 59). Moreover, encounter of replication forks might result in induction of DNA strand breaks and intragenic deletions.

sitive. Genetic analysis of TTD uncovered patients with mutations in the XPB, XPD or TTDA genes, all components of the TFIIH complex. At the extreme, some patients belonging to the XP-B, XP-D or XP-G complementation group display severe features of both CS (early death and neurological/developmental abnormalities) and XP (skin lesions and skin cancer).

The most overt phenotype of XP patients is their enhanced susceptibility to develop skin cancer, including basal cell carcinomas (BCCs), squamous cell carcinomas (SCCs) and melano-

mas. Epidemiological data on the relationship between skin cancer and solar UV exposure are limited, and hence animal models including transgenic mice, have been used to study UV-induced skin cancer. The focus of these studies is to gain quantitative data on development and genetic make-up of tumors, dose, time, wavelength of the UV radiation and on the impact of genotype. The protective role of GG-NER and TC-NER against the acute toxic (erythema, apoptosis, cell cycle arrest) and long term (skin cancer, ageing) effects of UVB radiation has been dissected in XP models, *i.e.*, XPE−/−, XPA−/−, XPC−/− and CSB−/− deficient mice.[50,51,54] As described, the protective role of TC-NER to toxic effects is manifested by a 10-fold greater sensitivity of XPA−/− and CSB−/− mice to UVB radiation-induced erythema compared to WT and XPC−/− mice. This highlights TC-NER as a key survival pathway to protect against apoptosis and cell cycle arrest.

However, the increased survival in the absence of functional GG-NER occurred at the expense of mutations, illustrated by epidermal patches expressing mutant P53 in UVB-irradiated XPC−/− mice.[60] UVB radiation-induced mutations in rodent and human cultured cells or tumors were primarily C → T transitions targeted to di-pyrimidine positions. Three factors contribute to the UVB spectrum: the DNA polymerase eta preferentially incorporates A opposite to non-instructional lesions;[61] (methyl) cytosines within photodimers undergo accelerated deamination[59] and UVB or sunlight-induced CPDs are formed preferentially at dipyrimidines containing 5-methylcytosine.[62] Finally, CC → TT double transitions are caused *in vivo* by UV-induced photodimer lesions in XPA, XPC and XP-V-deficient mice and human XPA, XPC and XP-V cells/patients.[63,64] UVB-induced tumors isolated from GG-NER and/or TC-NER deficient mice revealed 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.

Meta-analysis of mutation data including endogenous genes in UV-irradiated mammalian cells or transgenes in mouse skin revealed that mutations induced by UVC, UVB or UVA radiation are broadly similar with a high percentage of transition mutations at di-pyrimidine sites.[65] However, it is also obvious that a percentage of UVA-induced mutations are characterized by transversions at non-dipyrimidine sites resembling mutations induced by oxidative agents like hydrogen peroxide. Indeed, data obtained three decades ago showed that the relative wavelength dependence for cell killing and mutagenesis did not correlate with photodimer formation: cell killing and mutagenesis exceeded photodimer frequency in the UVA range indicating a role of oxidative damage.[66] It is now obvious that UVB and UVA-induced oxidative DNA damage, *i.e.*, 8-oxoguanine, can contribute to skin cancer. Ogg1 knockout mice that are lacking the functional repair enzyme, 8-oxoguanine-DNA glycosylase, accumulate 8-oxoguanine in epidermal cells of the skin after chronic UVB irradiation. Compared to wildtype mice, Ogg1 knockout mice are susceptible to tumor formation, indicating that oxidative DNA damage induced by UVB (and sunlight) contributes to skin carcinogenesis.[67]

Although the mouse cancer data reveal remarkable similarities with skin cancer susceptibility in humans, striking differences exist as well. In general, suberythemogenic doses of UVB do not induce melanoma in wildtype mice but intermittent UV overexposure does induce melanoma.[68] The XPA−/− mice that do not tolerate overexposure, do not develop melanoma in contrast to XP patients, whereas CSB−/− mice but not CSB patients are prone to skin cancer. The skin cancer susceptibility of CSB−/− mice appeared to be related to the poorly expressed GG-NER system in rodents. Unlike human cells, rodent epidermal cells express DDB2 (a subunit of UV-DDB) at a low level. Mice ectopically expressing DDB2 displayed delayed onset of SSC following chronic UVB radiation and at the cellular level showed improved repair of UV-photolesions particularly CPD;[52,69] in contrast, DDB2−/− mice were hypersensitive to UV-induced skin carcinogenesis. Expression of DDB2 in CSB−/− mice appeared to generate a ‘ humanized’ mouse model by counteracting the strand specificity of p53 mutations (rodent signature) and cancer proneness of UVB exposed CSB−/− mice. This leads to the important conclusion that GG-NER serves as a back-up system for TC-NER deficiency in man.[69]

## Summary

The mammalian genome is protected against genotoxic insults (photodimers, oxidatively induced base damage and single strand breaks) induced by solar UV by a network of DNA damage response (DDR) mechanisms that counteract toxicity and safeguard genome integrity. In man, nucleotide excision repair (NER) is the sole pathway for high fidelity repair of photodimers. Most of the core NER proteins have been identified and linked to human disease; research is now shifting to fine-tuning of the process. Particularly, post-translational modifications of NER proteins and chromatin substrate appear to be important to optimize NER in the context of restrictive chromatin. It is expected that novel factors that regulate post-translational modifications, will emerge in the response to solar UV.

Even in the absence of functional NER, UVC radiation of nondividing human cells induces incisions at sites of photodimers by the action of the endonuclease Ape1. This leads to the formation of single-strand DNA breaks, checkpoint activation and a pronounced cell cycle arrest. This pathway will prevent non-dividing basal cells in human skin epidermis from healthy individuals known to accumulate high levels of photodimers, to enter the S-phase upon growth stimulation.

In contrast to other XP patients, XPC patients proficient in TC-NER but deficient in GG-NER, do not express severe

 

erythema/edema after solar UV. These patients are therefore diagnosed later, and are less likely to adhere to solar UV protection and more likely to have skin cancer diagnosed at an earlier age. This situation also holds for XPE and XP-V patients.

Besides photodimers, UVB and UVA induce oxidative DNA damage including 8-oxoguanine, that can contribute to skin cancer as shown by the (modest) cancer susceptibility of UVB irradiated Ogg1 knockout mice incapable of repairing 8-oxoguanine. In addition, UVA might impair NER by oxidatively damaging key NER proteins and in combination with photosensitizers that might lead to photosensitivity and increased skin cancer risk.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This work was supported from grants by Leiden University, Leiden University Medical Center LUMC, Netherlands Organisation for Scientific Research NWO, Dutch Cancer Society KWF, EU and l'Oreal to LM. I thank the postdoctoral and PhD students and staff members who contributed the various studies described in this paper. Particularly I would like to thank Frank de Gruijl (LUMC, the Netherlands) for the longstanding and productive collaboration and introducing me to Jan van der Leun.

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