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Formation of UV-induced DNA damage contributing to skin cancer development

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Abstract. UV-induced DNA damage plays a key role in the initiation phase of skin cancer. When left unrepaired or when damaged cells are not eliminated by apoptosis, DNA lesions express their mutagenic properties, leading to the activation of proto-oncogene or the inactivation of tumor suppression genes. The chemical nature and the amount of DNA damage strongly depend on the wavelength of the incident photons. The most energetic part of the solar spectrum at the Earth's surface (UVB, 280-320 nm) leads to the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (64PPs). Less energetic but 20-time more intense UVA (320-400 nm) also induces the formation of CPDs together with a wide variety of oxidatively generated lesions such as single strand breaks and oxidized bases. Among those, 8-oxo-7,8-dihydroguanine (8-oxoGua) is the most frequent since it can be produced by several mechanisms. Data available on the respective yield of DNA photoproducts in cells and skin show that exposure to sunlight mostly induces pyrimidine dimers, which explains the mutational signature found in skin tumors, with lower amounts of 8-oxoGua and strand breaks. The present review aims at describing the basic photochemistry of DNA and discussing the quantitative formation of the different UV-induced DNA lesions reported in the literature. Additional information on mutagenesis, repair and photoprotection are briefly provided.

Introduction

Overexposure to sunlight, and in particular to the ultraviolet (UV) portion of its spectrum, is unambiguously linked to the onset of skin cancer^{1,2} as well as photoaging and ocular pathologies. Solar UV has two main components classified as "Class I carcinogens" by IARC³: UVB (290-320 nm) and UVA (320-400 nm) emitted in an about 1:20 ratio that varies according to latitude and season. A causative relationship, at least in terms of mutation induction, has been established between UVB and the induction of non-melanoma skin cancers (NMSC)⁴⁻⁶ that consist of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Evidence has also been provided for

a major role of sunlight in malignant melanoma (MM)⁷ but the respective contribution of UVA and UVB remains less understood. Depletion of stratospheric ozone that is accompanied by an enhanced contribution of UVB radiation and concomitantly a higher incidence of both NMSC and MM, has been identified as another critical environmental factor⁸. Solar UV is not the only concern for skin cancer since artificial tanning equipments represent another risk. These devices emit intense UV radiations richer than sunlight in UVA and evidence is growing for their involvement in MM and to a lesser extent SCC⁹⁻¹¹.

UV radiation is a complete carcinogen. Its initiating properties arise from its ability to damage DNA and induce mutations in proto-oncogenes and tumor suppressor genes such as p53^{12,13}. The main damaging

process involves direct absorption of UVB and to a lesser extent of UVA photons that trigger dimerization of pyrimidine bases¹⁴⁻¹⁶. UVA radiation also damages DNA in an oxygen-dependent mode that involves photosensitization^{15, 17}. The resulting reactive oxygen and nitrogen species lead to base lesions such as 8-oxo-7,8-dihydroguanine (8-oxoGua) and single strand breaks (SSBs)^{15, 18-20}. Efficient repair of DNA damage before replication is a requisite to prevent carcinogenesis to occur. The importance of repair of DNA photoproducts is illustrated in the photosensitive xeroderma pigmentosum patients who in most cases suffer from a deficiency in the nucleotide excision repair (NER) mediated removal of overwhelming bipyrimidine photoproducts and exhibit an increase by up to ten thousand-fold of the risk of skin cancer on UV exposed zones together with a significantly reduced lifetime expectancy²¹⁻²³. The present survey is aimed at critically reviewing the direct and sensitized photoreactions of cellular DNA induced by UVB, UVA and solar radiations. In addition information is also provided on several more biological end-points including DNA repair and photodamage prevention.

Pyrimidine dimers

Dimeric photoproducts involving adjacent pyrimidine bases are the most frequent UV-induced lesions in cellular DNA¹⁸. Cyclobutane thymine dimer has been first identified almost 60 years ago^{24, 25}. Since then, very large amounts of data have been gathered on the formation of other bipyrimidine photoproducts and their chemical properties^{26, 27}. Their relevance to skin cancer has been clearly established by the observation of an overwhelming majority of mutations at bipyrimidine sites in specific genes of skin tumors, in particular in non-melanoma skin cancers^{28, 29}. Next generation sequencing confirmed the accumulation of UV-specific mutations to a high rate in melanoma³⁰. The biological significance of pyrimidine dimers in UV mutagenesis is further shown by the coincidence between hotspots for their formation and occurrence of mutations³¹. While oxidatively generated DNA lesions are produced by photosensitized processes, pyrimidine dimers arise mostly from direct absorption of UV photons. The maximal absorption of DNA is at 260 nm, namely in the UVC range. However, absorption of DNA remains significant in the UVB range with values of 20 and 3 % compared to 260 for 290 and 300 nm, respectively. UVA photons are much more weakly absorbed but may also induce the formation of cyclobutane pyrimidine dimers (CPDs), either by direct excitation or via photosensitization.

DNA photochemistry

In the last two decades, the spectacular advances in time-resolved spectroscopy and the development of novel theoretical approaches have drastically modified our perception of electronic excitation processes in DNA photochemistry. In particular, the role of stacking and interaction between bases as well as the dynamics of DNA were shown to play key roles. This is in particular revealed by the comparison between the fate of excited states of bases either in DNA or as monomers in aqueous solution. In the latter case, the $\pi\pi^*$ excited states produced after absorption of UV photons deactivate on a ps time scale back to ground state³². A similar rapid process also takes place in DNA but together with other pathways of longer time scale^{33, 34}, some of them leading to photochemical reactions. The vast majority of the resulting DNA damage involves dimerization of adjacent pyrimidine bases. A role for C5-methylation of cytosine has also to be considered. Formation of minor DNA lesions such as adenine dimer^{35, 36} or adenine-thymine dimer³⁷⁻³⁹ has been reported as minor photoreactions but will not be discussed here.

Cyclobutane pyrimidine dimers

One consequence of the formation of excited states is the formation of DNA photoproducts. Immediately after UV absorption by DNA, the excitation energy can be delocalized over a few bases into Frenkel excitons^{40, 41} as the result of stacking between adjacent bases. When the stacking is so strong that orbitals overlap, excited states acquire a charge transfer state character (CT states)⁴². Both species can lead to DNA damage. The Frenkel excitons were proposed on the basis of theoretical calculations to be involved in the formation of CPDs. These dimeric photoproducts arise from a [2+2] cycloaddition that takes place through an energy barrier-less process⁴³ on the ps time scale⁴⁴. In contrast to what was long believed, triplet excited states seem to play a limited role in the formation of bipyrimidine photoproducts. An upper estimation of 10 % has been proposed. This is based on the observation that the quantum yield of CPDs is constant over a wide range of UVC and UVB wavelengths while the yield triplet state decreases⁴³. The limited role of triplet states in the formation of CPDs has also been shown at the dinucleoside monophosphate level where they account only for approximately 3% of the CPDs⁴⁵. Another interesting result of recent spectroscopic studies is the ability of UVA photons to directly induce excited states in DNA. Absorption of UVA photons by DNA bases does not take place in monomers but become significant in double stranded systems⁴⁶. Spectroscopic studies have shown that the produced excited states are mostly CT states⁴⁷. The possibility of formation of CPDs from such

states is not documented. However, a combined spectroscopic and theoretical study has shown that vibrational motion can convert them into Frenkel excitons⁴⁸. The observation that dimer formation is favored in double- compared to single-stranded DNA⁴⁷ could be explained by this property. It should though be stressed that the UVA photochemistry of DNA is quantitatively less significant than that of UVC and UVB since the molecular absorption coefficient of DNA in the UVA range is 1000 time lower than at 260 nm.

CPDs are formed at each of the four bipyrimidine doublets. The structure and the main properties of CPDs have been extensively studied in monomeric systems, dinucleoside monophosphates and isolated DNA. CPDs can be formed as several diastereoisomers which were isolated and characterized upon photolysis of isolated bases and nucleosides^{16,49,50}. However, the geometrical constraints encountered in DNA restrict the formation of possible isomers (Fig. 1). First, only *syn* isomers, with the two pyrimidine rings in a parallel orientation can be produced. In double stranded B-DNA, the stacking of the bases also forces the bases to be located on the same side of the cyclobutane ring, in a *cis* isomery^{51, 52}. In single-stranded DNA or destabilized double helix, *trans* isomers with the bases on opposite sides of the cyclobutane ring can be also produced^{51, 52}. It was also proposed that interstrand *anti* isomers, with the bases in antiparallel orientation, could be produced in quadruplexes⁵³. In B-form double-stranded mammalian DNA, TT; TC, CT and CC *cis,syn* CPDs are produced approximately in 100:50:25:10 ratios following exposure to UVC and UVB⁵¹. These ratios are slightly affected by the irradiation conditions such as the temperature or the ionic strength^{52, 54, 55}. However, the parameter that exhibits the most drastic effect on the ratio between CPDs is the GC base pair content⁵⁶. In bacterial DNA with high percentage of GC, TT CPD is a minor photoproduct. It can however be considered as the major UV-induced photoproducts upon UVB and UVC irradiation of isolated mammalian DNA^{49, 51}. This trend is even stronger in UVA-irradiated isolated DNA where no 64PP is detected by highly specific chromatographic assays while TT CPD represents 90% of the CPDs⁴⁶.

CPDs exhibit several specific characteristics. In particular, although the modified pyrimidine bases no longer absorb UVB, CPDs keep a residual absorption in the UVC range with a maximum at 230 nm. At these wavelengths, absorption leads to the reversion of the CPD with a high efficiency^{57, 58}. As a consequence, prolonged irradiation with UVC, typically the 254 nm light used in many experimental works, leads at high

dose to an equilibrium between formation and reversion of CPDs, especially for C-containing CPDs⁵¹.

Pyrimidine (6-4) photoproducts and their Dewar valence isomers

Pyrimidine (6-4) pyrimidone photoproducts (64PPs) are not directly produced from the excited states. Time-resolved studies have shown that their spectroscopic signature appears after 4 ms⁵⁹, namely 1000 time more slowly than CPDs⁴⁴. This is explained by the formation of a cyclic reaction intermediate exhibiting an oxetane structure when the 3'-end pyrimidine is a thymine (Fig. 2). When the 3'-end base is a cytosine, the intermediate is an azetidone. Unambiguous evidence for the formation of a cyclic intermediate has been provided by the isolation and the characterization of a thietane as the precursor of the 64PP between thymine and 4-thiothymine⁶⁰. In all cases, the cyclic intermediates decompose into the final photoproducts that bear a pyrimidone ring on the 3'-end and in which the C4 substituent of the 3'-end base is shifted to the 5 position of the 5'-end base. Involvement of a Paternò-Büchi reaction, namely a biradical process, has been proposed for the formation of the cyclic intermediate. More recently, involvement of a charge transfer excited states was proposed through either the triplet⁶¹ or the singlet channel⁴³. Interestingly, the latter theoretical work predicted a high energy barrier that could explain why the quantum yield of 64PPs decreases over the UVC/UVB range and why 64PPs are produced in very low amount or not at all in the UVA range. In UVC- and UVB-irradiated double-stranded DNA, 64PPs are less frequent than CPDs^{51, 62}. They are produced in a 1:3 to 1:5 ratio. However, this value depends on the bipyrimidine sites. At TT, 64PP is ten times less frequent than CPDs⁵¹. The ratio is even lower at CT where 64PP is hardly detectable. CC 64PP is also much less frequent than the corresponding CPD and its formation has been reported mostly in isolated DNA. In contrast, TC 64PP is the most frequent 64PP^{51, 63}, produced in a yield almost as large as that of TC CPD, making it the third most frequent photoproduct following UVB and UVC irradiation.

The most striking features of 64PPs are associated with the presence of the pyrimidone ring. First, 64PPs exhibit an absorption maximum in the UVB range (325 nm for TpT and dCpT 64PPs, and 315 nm for TpdC and dCpdC 64PPs). When excited at these wavelengths, 64PPs are fluorescent. For derivatives of dinucleoside monophosphates, which mimic some of the geometrical constraints encountered in DNA, the emission is maximal at 390-400 nm and the quantum yield is in the range of 1 to 3 %⁶⁴. Irradiation of 64PPs at 320 nm triggers another process: the

photoisomerization into a Dewar valence isomer first characterized in TpT⁶⁵ (Fig. 3). The latter compound arises from an intramolecular 4π electrocyclisation and takes place within 130 ps⁶⁶. Similar photoreactions were also reported for C- and 5mC-containing 64PPs⁶⁷⁻⁶⁹. The published quantum yields of photoisomerization range between 2⁵⁸ and 8%⁶⁶ in model systems. This value is lower in DNA because of the presence of adjacent bases, and in particular G⁷⁰. Dewars were detected at the four bipyrimidine sites in isolated DNA exposed to UVB radiation⁵¹. Large doses of UVB are necessary to reach a high ratio between the yield of Dewars and that of 64PPs. In contrast, combination of UVB and UVA exposure leads to a much larger photoisomerization yield. This can be explained by the absorption properties of DNA and 64PPs⁷¹. The presence of a pyrimidone ring is also the basis of a recently proposed Trojan horse process. Pyrimidones have been shown to exhibit a high energy and well populated triplet state. When added to a solution of plasmid DNA, both 5-methylpyrimidone⁷² and an analog of TT 64PP⁷³ were found to favor the formation of additional CPDs upon exposure to a lamp emitting within the 300-400 nm range. Theoretical works show that pyrimidone could be a sensitizer when incorporated within DNA⁷⁴. It remains to be shown experimentally whether the photosensitizing properties of the pyrimidone moieties of 64PPs present within double-stranded DNA can be expressed before conversion into Dewars.

Photoproducts of 5-methylcytosine

5-Methylcytosine (5mC) is an important epigenetic factor mostly present in CpG islands in genomic DNA. These frequently methylated sites are UV mutational hotspots and have been shown, mostly by ligation-mediated polymerase chain reaction (LMPCR), to be also hot spots for the formation of CPDs⁷⁵⁻⁷⁷. Dimeric photoproducts involving 5mC and either T or C have been isolated in dinucleoside monophosphates^{67, 78}. More recently, the effect of the methylation site of cytosine was investigated in a trinucleotide bearing a TX (with X=C, 5mC or N4-methylC, a bacterial minor base) sequence flanked with a 3'-G⁷⁹. Compared to that of TCG, photolysis of T5mCG leads to an increased formation of CPDs, which is more significant with UVB than UVC. Conversely, the yields of 64PPs and Dewars decrease as a consequence of methylation. Opposite trends were observed for TN4mCG⁷⁹. The results obtained with T5mCG were explained by conformational changes rather by absorption properties of 5mC as often proposed⁸⁰.

Dimers by UVA and photosensitization

Other processes exist that lead in particular to the formation of CPDs. They involve absorption of the UV energy by other molecules with subsequent transfer to DNA that thus indirectly reaches an excited state. The most common of this photosensitization pathway is known as triplet-triplet energy transfer (TTET)⁸¹. TTET takes place with compounds absorbing UVA and exhibiting a large yield of intersystem crossing. In addition, the energy level of the first triplet excited state has to be higher than that of DNA components. Under these conditions, the triplet excited state of bases, and in particular thymine⁸², can be populated, opening the way to occurrence of photoreactions. As a matter of fact, it is well known that TTET, both in small model systems (bases, nucleoside, and dinucleoside monophosphates) and DNA, leads to the formation of CPDs but not of 64PPs and Dewars. TT CPD is the major photoproduct, representing at least 90% of the lesions, much more than following UVB and UVC irradiation where its proportion among CPDs is approximately 50% (Fig. 4). Two main classes of molecules have been studied for their potency at mediating TTET. The first one are the aromatic ketones such as acetophenone or benzophenone⁸³. This chemical structure is often encountered in some non-steroidal anti-inflammatory drugs. The second and more recently investigated class of TTET photosensitizers are the fluoroquinolones⁸⁴. These compounds are drugs used as anti-bacterial agents that have been shown to induce a strong photosensitivity in treated patients.

While the role of triplet state thymine is unquestionable in model systems^{45, 85}, recent findings suggest that excitation of a single isolated thymine base may not be the only mechanism involved in the photosensitized formation of CPDs. First, photosensitization of DNA with different proportion of TT, TC and CT sites led to a constant over-reactivity of TT⁸⁶. A random excitation of thymine bases should have resulted in a low yield of TT CPD in T-poor DNA, which is not observed. In addition, CC CPDs were also detected, although in low yield, which is not compatible with the energy level of the triplet state of this base. It is thus likely that, like for the direct excitation of DNA, collective effects are involved in the photosensitized formation of CPDs. A possibility is the occurrence of the Dexter energy transfer that would lead to a charge transfer state and explain the similarity with the formation of CPDs by UVA irradiation. This hypothesis remains yet to be investigated. Another work involving a series of benzophenone derivatives tethered to two thymines allowed proposing a role for delocalized triplet states in the TTET mechanism⁸⁷.

An alternative process of CPD formation has been recently described⁸⁸. It also involves energy transfer but, unlike classical photosensitization, it occurs after rather than during UV exposure. UVA-irradiated melanocytes continue to accumulate CPDs after termination of irradiation as the result of a chemiexcitation process. CPD levels are maximal around two hours after UVA exposure and are thereafter decreased by DNA repair. This was observed for TT, TC and CT CPDs. Interestingly, this process did not take place in keratinocytes, suggesting a role played by melanin. Accordingly, treatment of isolated DNA with irradiated precursors of melanin led to the formation of CPDs. Altogether, the proposed mechanism involves the formation of oxidation products of melanin and its precursors, likely by peroxynitrite, which may migrate to the nucleus. There, they decompose into small molecules generated in an excited state, for example activated carbonyls, that transfer their energy to DNA and induce the formation of CPDs through chemiexcitation⁸⁸. More chemical studies are necessary to understand this novel pathway that raises new issues in terms of prevention and photoprotection.

Biological properties of pyrimidine dimers

The observation that most mutations in skin tumors are located at bipyrimidine sites points to strong mutagenic properties of pyrimidine dimers. A wide series of biochemical studies have been devoted to the unravelling of the underlying properties. In the case of CPDs, a major reaction to consider is deamination. This hydrolytic process converts cytosine and its derivatives into uracil. The reaction rate is very low for unmodified cytosine but is drastically increased when the C5-C6 bond is saturated like in CPDs. Recent DFT calculations have suggested that 2 water molecules are necessary to achieve the deamination process. A first mechanism, which can be extended to CT and TC CPDs, was established for 5,6-dihydrocytosine⁸⁹ (Fig. 5) while another was proposed for the deamination of CC CPD⁹⁰. CPDs deaminate with half-reaction times of a few hours in the case of the *cis,syn* diastereoisomers of the dinucleoside monophosphates TpdC and dCpT⁹¹⁻⁹⁴. In DNA, the deamination rate is slightly lower⁹⁵⁻⁹⁷ but plays a major role in the mutagenic properties of cytosine-containing CPDs. The well-established scheme for mutagenesis is the initial formation of TC, CT and CC CPDs that deaminate into TU, UT and UU CPDs. During replication, U residues in CPDs code like T and lead to the incorporation of A⁹⁸⁻¹⁰¹ which in turns codes for T in the next replication. The resulting transition mutations are the hallmarks of UV mutagenesis, namely T→C at TC sites and the tandem mutation CC→TT.

Mutations at CT sites are much less frequent, maybe as the result of the more efficient repair of this CPD¹⁰². *In vitro* experiments have also shown that TT CPD is more a blocking than a mutagenic photoproduct¹⁰³, mostly because the two thymine rings involved in the dimer keep their coding properties. Deamination of C-containing CPDs was shown to depend on the sequence, with an increased rate at TCG triplets¹⁰⁴. Interestingly, this property explained the sequence specificity of the mutations found in UVC-irradiated mouse skin¹⁰⁵. In cells, deamination of CPDs is also increased during transcription¹⁰⁶. Deamination of 5mC-containing photoproducts is slower than that of related unmethylated cytosine lesions. However, deamination is increased by a factor 25 when flanked by G, as in CpG islands^{104, 107}. This trend is increased by the acceleration of deamination in nucleosome for both Tm5C and TC CPDs¹⁰⁸.

Like CPDs, 64PPs may undergo deamination. However, this reaction is not possible on the 3'-end of the photoproduct since the base is no longer a pyrimidine and the C4-amino group has migrated to the C5 position of the 5'-end base. Deamination of 5'-end cytosine thus remains possible in CC and CT 64PPs. Interestingly, it was found that the deamination rate of the CT 64PP was much lower than that of the corresponding *cis,syn* CPD⁹¹. The most frequent 64PP, TC 64PP, does not deaminate. In spite of this limited involvement of deamination in the fate of 64PPs, the latter photoproducts can be highly mutagenic. This is mostly explained by their major impact on the DNA structure that strongly modifies the network of hydrogen bonding during replication. In particular, bonding with G to the 3'-end moiety and A to 5'-end base of 64PPs is favored. As a result, TT 64PP is highly mutagenic and leads to T→C transitions¹⁰⁹, while TC 64PP is much less. In contrast, more frequent incorporation of A opposite the pyrimidone moiety takes place in Dewars. Consequently, TT Dewar is much less mutagenic¹¹⁰ than its TC analog which induces C→T transitions¹¹¹.

Knowledge of the mutagenic properties of the different classes of photoproducts is not enough to establish their respective contributions to solar mutagenesis. Repair has to be considered since it determines the lifetime of DNA damage in the genome and therefore the possibility to express their mutagenic potential. All investigations point to a much more efficient repair through the global nucleotide excision repair (NER) pathway of 64PPs and Dewars than CPDs¹¹²⁻¹¹⁴. This is explained by the larger impact of the former photoproducts on the DNA structure, making them more easily detectable by the repair machinery,

in particular XPC. The contribution of 64PPs to UV mutagenesis is thus expected to be lower than that of CPDs. Accordingly, expression of photolyase enzymes specific for either class of pyrimidine dimer in mammalian cells showed that removal of CPDs prevent mutagenesis while that of 64PPs has no impact ¹¹⁵. A similar experiment with mice expressing CPD photolyase led to the conclusion that presence of CPDs is critical for skin cancer occurrence ¹¹⁶. These results were recently emphasized by the observation that the most frequent 64PP, TC 64PP, is also the most efficiently repaired ¹¹⁷.

Studies have shown that the four CPDs are not repaired with the same rate ^{102, 118}. TT CPD is the most slowly repaired while CT CPD is the fastest. TC and CC CPDs exhibit an intermediate rate. Interestingly, these results are in line with mutagenesis data since mutations at CT sites are almost never observed. HPLC-MS/MS analyses made 2h after UVB irradiation of cultured keratinocytes did not show differences in the repair rate of TT and TC 64PPs. Recent results using next sequencing generation at earlier time point suggest that TC 64PP is more efficiently repaired in transcribed regions ¹¹⁹. An important finding in the repair of UV-induced DNA damage is that UVA decreases the repair capacities of cells ^{112, 120}. This observation was recently explained, as least partly, by the oxidation of repair proteins such as PCNA and RPA ^{121, 122}. Other potential mechanisms such as modulation of regulation of specific pathways remain to be explored. Sunlight thus appears to exhibit two concomitant deleterious effects, namely the formation of mutagenic DNA damage and the decrease in the capacities of cells to remove them.

Formation of pyrimidine dimers in cells and skin

Because of the major role of bipyrimidine in the initiating step of skin cancer, a very large number of methods have been developed for their quantification in cellular DNA. These assays involve immunological detection, chromatographic techniques, [³²P]-postlabeling, LMPCR and more recently next generation sequencing of DNA repair lesions. The techniques involving spectrometric detection are usually excellent for the simultaneous and specific detection of the different photoproducts. However, they provide only average data on the overall genome. Sequencing techniques in contrast are less sensitive but provide information at the nucleotide level. LMPCR requires a preliminary treatment to convert CPDs into breaks. The recently developed approach ¹¹⁷ that involves sequencing of the oligonucleotides generated during the NER process is more flexible providing data on both CPDs and 64PPs depending on the antibody used for the

immunoprecipitation step. Antibody-based detection is also widely used in immunofluorescence and radioimmunoassays. These techniques often provide only relative values and do not permit the individual quantification of the four derivatives of a same class of photoproducts. However, their ease of use makes them very popular, especially in the field of DNA repair. Antibodies constitute also relevant tools for the immunohistochemical detection of both CPDs and 64PPs in skin.

In spite of some minor differences in the determination of the respective yields of the different bipyrimidine photoproducts, all techniques show that DNA reacts in a similar way following exposure to UVB and UVC either as an isolated form in aqueous solution or in cells. The absolute yields are obviously different between the two systems because absorption is impacted by the cellular context. In cultured cells, the reported yield of CPDs ranges between 0.1 and 1 dimer per 10⁵ bases per J/m² for UVC (254 nm) lamps ¹²³⁻¹²⁸ (Table 1). The mean value is approximately 0.45 CPDs per 10⁵ bases per J/m². With UVB sources, the yield is roughly one order of magnitude lower than at 254 nm with a mean yield of 0.05 CPDs per 10⁵ bases per J/m² ^{112, 120, 125, 126, 129, 130}. An interesting action spectrum has been published that shows the strong wavelength dependence on the formation of CPDs in the UVB range ¹⁸, with a ratio of roughly 60 between the yields determined at 290 and 310 nm. It was also observed that formation of CPDs in skin was 20-times less efficient in human skin explants than in the keratinocytes collected from the same donors, likely because of the protection afforded by melanin and the corneal layer ¹²⁰. It may be added that immunohistochemistry experiments have shown that the formation of CPDs is not uniform within the skin. For short wavelengths (<280 nm), the frequency of CPDs is lower in the deepest layer of the epidermis ¹³¹.

Pyrimidine dimers and in particular CPDs are also produced in cells and skin by UVA irradiation ^{18, 112, 126, 130, 132-138}. Formation of 64PPs has been reported using immunological approaches ^{126, 139} but not by more specific chromatographic techniques ^{120, 130, 140}. Quantitative data in the UVA range all point to a yield of CPDs at least 3 orders of magnitude lower in the UVA than the UVB range. A mean yield based on available quantitative data is approximately 0.3 CPDs per 10¹⁰ bases per J/m² ^{18, 112, 126, 130, 137, 138}. Contamination of the UVA sources by UVB as an explanation for these observations has been discarded by experiments involving either filters or the use of monochromatic laser pulses. Photosensitization has also been proposed. However, the observation that CPDs are

detected in UVA-irradiated isolated DNA and in a yield similar to that found in cells strongly suggests a direct mechanism triggered by the low but real UVA absorption of DNA^{46, 126, 141}. Interestingly, the fact that TT CPD is present in much larger proportion compared to other CPDs after UVA than after UVB or UVC exposure is in line with spectroscopic observations of different excited states in the different wavelength ranges⁴⁷. Because UVA is more than 20 times more intense than UVB in sunlight, UVA-induced CPDs represent a few percent of the overall load of CPDs in skin. In spite of this low yield formation in cells and skin, the role of UVA-induced CPDs in solar genotoxicity cannot be ruled out. The fact that mutations induced by UVA occur mostly at bipyrimidine sites^{142, 143} like with UVB further supports the biological relevance of UVA-induced CPDs¹⁴⁴. The likely biological relevance of UVA-induced CPDs is also shown by the observation that CPDs are produced in 3- to 5-fold larger amounts than 8-oxoGua^{18, 120, 130, 140}, the most frequent UVA-induced oxidatively generated lesion. One exception are melanocytes where the ratio between the yields of CPDs and 8-oxoGua is 1.4¹³⁸, showing that this cell type is more sensitive to oxidative stress¹⁴⁵. A study has shown that the formation of CPDs by UVA is favored in the basal layer as the result of scattering phenomena¹⁴⁶, where melanocytes are the most frequent. This observation raises some interesting question on the origin of melanoma that more significantly involves UVA than UVB as the critical UV domain¹⁴⁷.

In mammalian cells and skin, CPDs were found, using sequencing¹⁴⁸⁻¹⁵⁰ and chromatographic approaches^{16, 51}, to be produced in the following order of frequency: TT > TC > CT > CC. Recent techniques involving new sequencing techniques also showed that TT CPD was the most frequent photoproduct. Excision-seq confirmed that the decreasing CPD frequency was TT>TC>CT>CC⁶³. CPD-seq led to ratios between the frequencies of CPDs of 54:22:14:10 for TT, TC, CT and CC respectively¹⁵¹, which are very close to those determined by HPLC-MS/MS. The HS-damage-Seq assay also led to a distribution similar to HPLC-MS/MS. It should be stressed that some recent studies yielded distributions significantly different from all those mentioned above. Applying LMPCR to a large number of genes in UVB-irradiated cells, a more comparable yield of UVB-induced CPDs was determined at the four bipyrimidine sites¹⁵². These results may be explained by the fact that CC sites were more than twice as frequent as TT in the studied genes, and 1.4 times more than CT and TC. Using next generation sequencing, excision-seq and CPD-seq data show an inversion between the relative frequency of TC and CT CPDs

compared to the previously mentioned techniques¹¹⁹. This may be explained by the fact that excision-seq and CPD-seq are based on the sequencing of DNA excision repair products and that CT CPDs are repaired significantly faster than the other CPDs.

Ratio between CPDs and 64PPs in cells was found to range between 3 and 5 depending on the assay, a value similar to that determined in isolated DNA, at least for irradiations performed with UVB and UVC. In these wavelength ranges, the HPLC-MS/MS assay was able to detect TC and TT 64PPs in a 5:1 ratio, but could not detect CC and CT 64PPs in cells exposed to doses compatible with cell survival⁵¹. Next generation sequencing-based techniques confirmed the major formation of TC 64PP but also detected some 64PPs at CT and CC sites¹¹⁹. At biologically relevant doses of UVB and UVC, only tiny if any amounts of Dewars are detected^{51, 126, 130}. In contrast, Dewars have to be taken into consideration when simulated or real sunlight is used. Based on immunological approaches, Dewars have been detected in significant yield in SSL-exposed human fibroblasts¹⁵³, rodent cells¹²⁶ and mouse skin¹⁵⁴. HPLC-MS/MS measurements confirmed these results in cultured cells¹³⁰ and skin explants¹⁵⁵. In the latter samples 30% of the 64PPs were photo-isomerized at 3 MED of SSL. In contrast, Dewars were not detected by the same technique in various models at biologically relevant doses of UVB. Immunological detection yielded a positive signal with UVB but much lower than with SSL¹²⁶. Altogether, Dewars are undoubtedly biological relevant lesions for human exposure.

The relative yields of bipyrimidine photoproducts discussed above are average values for the whole genome. However, the distribution of CPDs, 64PPs and Dewars is not homogeneous in the genome. Important parameters that modify the yield of photoproducts are related to the location of the pyrimidine sites with respect to nucleosome and chromatin. It is well known that the yield of CPDs is lower in the nucleosome than in the linker region^{156, 157}. A recent work provided accurate information on the favored locations of CPDs within a rotationally phased nucleosome¹⁵⁸. The ratio between 64PPs and CPDs is also affected by the nucleosome structure¹⁵⁹. More recent work extended these *in vitro* data to yeast cells. A pattern of formation reflecting nucleosome position was observed¹⁵¹. Interestingly, a nucleosome has a strong impact on the deamination rate of cytosine dimers¹⁰⁸. Sequencing approaches have also unambiguously shown that formation of photoproducts is greatly reduced by DNA condensation in heterochromatin^{119, 160}. In agreement with previous works¹⁶¹, these techniques also showed that binding of transcription factors may modulate the

formation of photoproducts either positively or negatively^{119, 151}. Telomeres are another specific cellular DNA structure which were found to be more sensitive than the bulk of the genome to UV-induced damage¹⁶². More local sequence effects have also been observed. *In vivo*, CPDs are more frequent within pyrimidine tracks^{152, 163}. Both *in vivo* and *in vitro* data showed that flanking bases of a bipyrimidine site drastically modify its photoreactivity. For example formation of CPD at GTTG is much less efficient than in tetrads containing a 5'- or 3'-end C^{57, 148, 149}. The inhibitory effects of G could be explained by conformational factors and formation of charge transfer states leading to a fast deactivation of excited states^{57, 164}. Sequencing techniques showed a favored formation of 64PPs downstream C and upstream A^{63, 165}.

Preventing pyrimidine dimer formation

Because of their relevance to skin carcinogenesis, protection against pyrimidine dimers is necessary. As shown above, the formation of this class of damage involves direct absorption of UV photons by DNA. The most efficient way to protect cutaneous cells is thus to decrease the UV dose reaching the genome. This is achieved by natural pigments such as melanin and UV absorbing molecules present in sunscreens. Systemic photoprotection that has also been proposed mostly relies on supplementation by antioxidants. This protection will not be further discussed here because it mostly targets inflammatory and immunological responses. One worth noting exception is the nicotinamide supplementation aimed at restoring cellular energy and enhancing DNA repair¹⁶⁶.

Natural photoprotection

Epidemiological studies have shown that the risk for NMSC in black skins is approximately 70-time lower than in fair skins¹⁶⁷. The ratio is around 20 for MM^{167, 168}. This is explained by the high content in melanin of dark skins that behaves as a UV absorber, thereby preventing the formation of photoproducts in DNA and from other adverse effects of sunlight such as photoageing^{169, 170}. Yet, the yield of CPDs when the whole skin is considered only differs by a factor 10 between the two skin types. This does not completely explain the very low incidence of NMSC observed in dark skin. A relevant immunohistochemical observation was that while CPDs-containing keratinocytes are roughly homogeneously distributed within the different epidermal layers in fair skin upon exposure to sunlight, DNA damage is almost absent from the basal layer in black skin^{171, 172}. The same observation was reported for melanocytes¹⁷³ which are present only in

the basal layer. Since skin cancer involves mostly keratinocytes and melanocytes present in the basal layer, this targeted protection accounts for the drastically reduced carcinogenesis in dark skin.

Evidence was also provided for a protection afforded by pigmentation in Caucasian skins. The yield of formation of SSL or UVB-induced CPDs is larger in fair (phototypes I and II) than in darker skin (phototype IV)^{171, 173-176}. On the average, a factor of approximately 2 is observed. Comparative studies on this issue are made difficult because some authors apply the same physical dose to all volunteers while others use multiples of the individual minimal erythemal dose. As a general trend, a better correlation between phototype and yield of CPDs is observed for the physical dose. Only limited information is available on the link between phototype and UVA-induced CPDs. However one work showed that the protection afforded by skin pigmentation is similar to that in the UVB range¹⁷⁵. A last question on the protection against pyrimidine dimers in Caucasian skin is the effect of tanning. Several studies have shown that only a modest protection, by a factor 2 on average, is provided by tanning, irrespectively of the skin type¹⁷⁷⁻¹⁷⁹. Furthermore, the UVA-induced tanning, which involves pigment darkening rather than *de novo* melanogenesis, does not afford protection against DNA photoproducts formation¹⁸⁰.

Sunscreens

When natural photoprotection afforded by skin pigmentation is not sufficient, like in fair skin or in case of extended exposure during occupational or recreational activities, use of artificial photoprotection is required. Although adequate clothing is the most efficient strategy, it is not always possible or wished. In this case, application of sunscreens is a suitable alternative. By absorbing incident UV photons, filters present in sunscreens prevent the formation of CPDs and 64PPs, as shown *in vivo*¹⁸¹⁻¹⁸³ and *ex-vivo* in skin explants^{137, 155, 184, 185}. Photoprotection of DNA was also shown in several *in vitro* assays^{139, 186}. The protection properties of sunscreens are provided by UV mineral and organic UV-filters, used in combination in order to cover a wide spectrum including UVA and to provide photostability to the mixture. Modern sunscreens also often contain antioxidants¹⁸⁷. A few studies have shown a resulting decrease in the formation of pyrimidine dimers^{188, 189}. The underlying mechanisms remain unclear but possibly involves absorption of UVA by sunscreen components¹⁹⁰.

The question of the extent of actual DNA protection afforded by sunscreens is still open, especially when compared to the Sun Protection Factor (SPF) used to assess the efficiency with which commercial products

at prevent erythema induction. Although the yield of DNA photoproducts and the onset of erythema depend on the applied UV dose, they differ in terms of mechanism. Formation of CPDs and 64PPs are formed by direct photochemical reactions whereas erythema is a complex inflammatory response which exhibits a threshold dose. Data on the comparison between SPF and DNA protection factor are sparse and slightly contradictory. While very similar values between the two endpoints were reported by some authors^{181, 183}, other works suggested that DNA protection is slightly lower than the SPF^{137, 155, 184, 185}. Further investigation is required but it should be emphasized that, in spite of lower values reported in the latter studies, sunscreens (especially those with a high SPF) confer very efficient DNA protection¹⁹¹. The resulting decrease in skin cancer is shown by interventional studies where the amount of sunscreen is controlled and applied on a regular basis^{192, 193}. Yet, results of epidemiological works on sunscreen-based photoprotection are sometimes disappointing^{194, 195}. The possible explanations are that i) sunscreen users increase their overall recreational exposure^{196, 197} and ii) that the amount of sunscreen applied is much lower than the 2 mg/cm² used for the determination of SPF¹⁹⁸.

Oxidatively generated damage to DNA

The search for the formation of photo-induced oxidatively damage in cellular DNA has been hampered by the lack of accurate methods of measurement until the advent of high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD)¹⁹⁹. This has allowed as the two first relevant applications the detection of 8-oxoGua in the DNA of mammalian cells upon exposure to UVA radiation in the presence of riboflavin^{200, 201}. Since the mid 80's mechanistic studies involving mostly nucleosides have provided detailed information on the oxidative pathway of pyrimidine and purine bases triggered by UVA excited photosensitizers^{26, 202}.

Photo-induced oxidative reactions to isolated DNA and model compounds

Several mechanisms including photo-ionization, singlet oxygen (¹O₂) oxidation and hydroxyl radical-mediated degradation of isolated DNA and its constituents²⁰³⁻²⁰⁹ have been deciphered in relation with their potential implication in the direct and photosensitized effects of UVB and UVA components of solar radiation.

Photo-ionization

Earlier investigations have shown that the base moiety of 2'-deoxyguanosine (dG) could be subject to oxidative degradation in aerated aqueous solution upon UVC irradiation²¹⁰. The main resulting non identified decomposition product²¹¹ was suggested to arise from ionization of the guanine moiety²⁶, the most susceptible base to one-electron oxidation in agreement with its lowest oxidation potential among nucleic acid components²¹²⁻²¹⁴. Monophotonic photoionization of double-stranded oligonucleotides by UVC photons was also confirmed by the detection of ejected electrons²¹⁵. Photoionization of guanine in DNA received recent support from a detailed chemical study showing that guanine bases in isolated DNA could be converted into 8-oxoGua and also involved in a cross-link reaction with polyamines upon initial UVB-mediated one-electron oxidation reaction²¹⁶. Further confirmation was provided by a detailed photophysical study that demonstrated that the radical cation of guanine is generated in telomere G-quadruplexes by direct low-energy UVB radiation and subsequent monophotonic ionization²¹⁷. The reported quantum yield of 8-oxoGua formation, $(3.2 \pm 0.3) \times 10^{-4}$, that is 1 order of magnitude higher than that reported for calf thymus DNA²¹⁶ remains however lower, by about 4-fold, than that of CPD induction²¹⁷. A large body of information is available on the chemical reactions of the guanine radical cation (Gua^{•+}) that is able in aqueous solutions to undergo two main competitive conversion pathways^{204, 218, 219}. Deprotonation gives rise to a highly oxidizing guanine radical (Gua-H)[•]²²⁰ that is able to react with superoxide anion radical (O₂^{•-}) and not with molecular oxygen (O₂).²²¹ These reactions include one-electron reduction and addition leading to chemical repair²²¹ and formation of 2,2,4-triamino-5-(2H)-oxazolone (Z)²²² respectively. Hydration of Gua^{•+} that has been shown to be an efficient reaction in duplex DNA^{219, 223} generates 8-hydroxy-7,8-dihydroguanyl radical that through one-electron oxidation triggered by O₂ is converted into 8-oxoGua whereas competitive one-electron reduction gives rise to 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) (Fig. 6). Gua^{•+} may be also involved in other nucleophilic additions mediated by the free amino group of lysine and cytosine leading to the formation of DNA-protein adducts^{224, 225} and interstrand cross-links respectively^{226, 227}. The formation of DNA intrastrand cross-links between the C8 of Gua^{•+} and N3 of thymine has been also reported^{228, 229}. Evidence has been also provided showing that exposure of DNA to high intensity 266 nm nanosecond laser irradiation is able to ionize the four main DNA bases with similar efficiency according to a bi-photon ionization process^{230, 231}. Interestingly 8-oxoGua has been identified as the predominant final

oxidation damage in double-stranded DNA ^{229, 232, 233}. This has been rationalized in terms of redistribution of initial radical damage through efficient charge transfer involving a multi-step hopping process along the oligonucleotide chain ^{234, 235}. Preferential trapping by guanine bases that act as sinks of positive holes gives rise to stable degradation products in a sequence dependent manner with the 5'-guanine base at GG doublets being a preferential target ²³⁶.

Type I and Type II photosensitization reactions

Photodynamic agents comprise various endogenous and exogenous sensitizers that absorb in the UVA and/or visible ranges ^{15, 237, 238}. Once excited they operate in aerated solutions by either reacting directly with a substrate or oxygen according to two mechanisms classified as Type I and Type II respectively ^{239, 240}. The Type I photosensitization mechanism involves predominantly a charge transfer reaction involving the nucleobases, guanine being the preferential target of the photosensitizer in its generally long-lived triplet excited state. The resulting unstable base radical cations triggered by UVA-excited riboflavin^{218, 237} and 2-methyl-1,4-naphthoquinone ²⁴¹, two typical Type I photosensitizers, generate in naked DNA a similar spectrum of degradation products than those induced by either mono- or bi-photon ionization. These mostly include 8-oxoGua, Z, FapyGua in the decreasing order of their formation importance together with small amounts of several oxidized pyrimidine bases ²¹⁸. An alternative mechanism consists in hydrogen atom abstraction, involving in particular the methyl group of thymine as reported for benzophenone ⁸³. This explains the relative increase with respect to guanine of degradation lesions of 5-formyluracil ²¹⁸, a typical methyl oxidation product of thymine that arises from the initial formation of 5-(uracil)methyl radical ^{26, 204}. In addition superoxide anion radical ($O_2^{\cdot-}$) or its corresponding conjugated acid (HO_2^{\cdot}) is also generated as a side reaction through the O_2 -mediated oxidation of the radical anion or neutral H-atom adduct of the photosensitizer ^{239, 240}. $O_2^{\cdot-}$ which is predominant in neutral aqueous solution due to its low pK_a value shows a very low reactivity towards DNA components at the exception of oxidizing (Gua-H) $^{\circ}$ ²⁴². $O_2^{\cdot-}$ is able to undergo dismutation leading to the formation of hydrogen peroxide (H_2O_2) another poorly reactive ROS in the absence of transition metals ²⁴². However the situation is likely to be different in cells particularly upon UVA irradiation that has been shown to enhance the release of free ferrous ion ^{243, 244}. This is likely to favor the formation of highly reactive hydroxyl radical ($^{\circ}OH$) through the reduction of H_2O_2 in a Fenton-type reaction ²⁴⁵. Comprehensive degradation

pathways are available on the reactions initiated by $^{\circ}OH$ with both the bases ^{202, 204, 206, 209} and the 2-deoxyribose ^{207, 208} of isolated DNA. Oxidatively generated damage to pyrimidine bases that includes among the main decomposition products 5,6-dihydroxy-5,6-dihydrothymine, 5-formyluracil, 5-hydroxymethyluracil and 5-hydroxycytosine mostly arises from initial $^{\circ}OH$ addition to 5,6-double bond of the pyrimidine moiety and hydrogen abstraction from the methyl group of thymine and 5-methylcytosine ²⁴⁶⁻²⁴⁸. The situation is different for guanine since the formation of its main degradation products including 8-oxoGua and FapyGua is rationalized in terms of predominant implication of $^{\circ}OH$ -mediated pyrimidine peroxy radicals with vicinal guanine ²⁴⁹⁻²⁵². The sugar moiety of DNA is also an excellent target for $^{\circ}OH$ reactions. Hydrogen abstraction at C3 and C5 gives rise to single strand breaks whereas oxidized abasic sites are mostly generated subsequent to the initial formation of carbon centered 2-deoxyribose radical at C1 and C4 ^{207, 208}. An interstrand cross-link that involves cytosine is initiated by C4 hydrogen atom abstraction from the opposite sugar moiety and subsequent cascade of reactions of the peroxy radical thus formed by O_2 addition ^{253, 254}.

A second reaction of the triplet excited state of photodynamic agents such as rose bengal, methylene blue ^{26, 237} and polar R]-1-[(10-chloro-4-oxo-3-phenyl-4H-benzo[a]quinolizin-1-yl) carbonyl]-2-pyrrolidine-methanol (Ro19-8022) ²⁵⁵ in aqueous aerated solutions consists in energy transfer to triplet molecular oxygen. This ensures the generation of singlet oxygen (1O_2) in the $^1\Delta_g$ delta excited state ($E = 22.4 \text{ kcal mol}^{-1}$)²⁵⁶ according to the Type II photosensitization mechanism. Evidence has been provided from the determination of the rate quenching of 1O_2 by DNA components that only guanine is an efficient target of 1O_2 ²⁵⁷⁻²⁶⁰. This received confirmation from reactivity investigations on the main DNA bases that were based on HPLC-MS/MS analysis ²⁶¹ and theoretical studies ²⁶²⁻²⁶⁴. It was also found that 1O_2 readily reacts with 4-thiouracil, a component of RNA, giving rise to uracil and uracil-6-sulfonate ²⁶⁵. Detailed structural and mechanistic information is available on the main guanine oxidation products formed upon exposure of dG and isolated DNA to 1O_2 ²⁶⁵⁻²⁶⁹. This has been facilitated by the use of thermolabile *N,N'*-di(2,3-dihydroxypropyl)-1,4-naphthalenedipropanamide (DHPN)²⁷⁰ as a chemical source of 1O_2 that may be [^{18}O]-labeled ²⁷¹. The selective reaction of 1O_2 with the guanine moiety has been rationalized in terms of Diels-Alder [2 + 4] cycloaddition giving rise to an unstable 4,8-endoperoxide. Evidence for the occurrence of the latter reaction was provided by the ^{13}C NMR characterization of related endoperoxide upon Type II

photosensitization of a lipophilic derivative of 8-methylguanosine derivative in CD_2Cl_2 at low temperature²⁷². This recently received further support for computational studies indicating occurrence of a two step-pathway with an initial *syn* addition of 1O_2 at C8 of the guanine moiety that is followed by a cyclization reaction²⁷³. In the case of duplex DNA the resulting endoperoxide undergoes an exclusive degradation pathway that is initiated by the loss of H8 proton with the subsequent formation of 8-hydroperoxyguanine (8-OOHGua). Mild reduction of the hydroperoxide generates 8-hydroxyguanine that is in dynamic equilibrium with predominant 8-oxoGua, the 6,8-diketo tautomer (Fig. 6)²⁷⁴. The situation is more complex for isolated guanine nucleoside since a second major decomposition pathway of the endoperoxide that leads to a highly quinonoid intermediate^{275, 276} through dehydration has been identified. It may be pointed out that 1O_2 is unable to react with the 2-deoxyribose moiety and therefore to induce the formation of direct single strand breaks (SSBs) in isolated DNA²⁶¹⁻²⁶³. The previously reported generation of SSBs in isolated DNA exposed to 1O_2 ²⁷⁷ may be explained in terms of over-oxidation of 8-oxoGua that may lead to relatively unstable guanine oxidation products such as Z^{278, 279} and subsequent formation of labile abasic sites.

UVC and UVB radiations are poor oxidizing agents of cellular DNA

It was reported that UVB photons generate 8-oxoGua in the DNA of hairless mouse epidermis²⁸⁰ and several mammalian cells including rat epithelium cells²⁸¹, mouse keratinocytes²⁸², Chinese ovary (CHO) cells²⁸³, HeLa cells²⁸⁴ and human keratinocytes²⁸⁵⁻²⁸⁷. However UVB-mediated DNA oxidation is inefficient since 8-oxoGua is present at only 1% of the level of the CPDs that are the major photoproduct. This is even more pronounced for UVC irradiation that gives rise to CPDs with an about 800-fold higher efficiency than 8-oxoGua^{18, 283}. Another relevant indication of the poorly oxidizing ability of UVB radiation was provided by the low yield of strand breaks and/or alkali-labile sites (0.19 lesions per 10^6 nucleobases and per kJ/m^2) that was measured in the DNA of AS52 CHO cells¹⁸ using the sensitive alkaline elution technique²⁸⁶. This has to be compared with the frequency of CPDs (77), and also Fpg- (0.31) and endo III-sensitive sites (0.18) that represent mostly 8-oxoGua and several oxidized pyrimidine bases respectively. As already discussed in the two previous sub-sections, the formation of 8-oxoGua may involve 3 main mechanisms. The implication of 1O_2 that has been suggested to be generated in model studies^{288, 289} by the quenching of

the purine and pyrimidine bases in their triplet excited states by O_2 could be only a minor process since 1O_2 is not able in contrast to UVB radiation to generate SSBs in cellular DNA²⁹⁰. This remark applies as well to the putative contribution of ionization reaction of the guanine base as shown in model studies that also does not trigger the cleavage of the DNA backbone. Therefore the most important contributor appears to be $^{\bullet}OH$ as further supported by the generation of endonuclease III-sensitive sites, likely indicators of oxidized pyrimidine bases that are produced in similar amounts than DNA nicks. The UVB-induced formation of $^{\bullet}OH$ may be accounted for by initial generation of $O_2^{\bullet-}$ from UVB excitation of catalase²⁹¹, cyclooxygenase and NADPH^{292, 293} before undergoing dismutation into H_2O_2 that is then involved in the Fenton reactions. It has also been shown that UVB photons has a stimulating effect on the expression of nitric oxide synthase that is accompanied by enhanced release of nitrite oxide ($^{\bullet}NO$) in however a delayed process²⁹⁴. The fast reaction of $^{\bullet}NO$ with $O_2^{\bullet-}$ gives rise to peroxynitrite²⁹⁵ that in presence of CO_2 is converted into nitrosoperoxycarbonate with subsequent release of carbonate radical anion ($CO_3^{\bullet-}$), an efficient one-electron oxidant of guanine²⁹⁶. There is a need of further information on the nature and distribution of oxidized purine and pyrimidine bases generated either immediately after the UVB-generated or during the delayed post-irradiation process.

UVA-sensitized oxidatively generated DNA damage in cells and skin

UVA radiation is expected to oxidize DNA mainly through photosensitized reactions that would involve still not identified photodynamic agents^{17, 297-299}. In addition $O_2^{\bullet-}$ and $^{\bullet}NO$ have been shown to be generated in cultured cells in response to UVA exposure in a way similar to UVB irradiation^{300, 301}. It has been also reported that bystander effect through a signal response is able to induce the generation of ROS^{302, 303}. Furthermore UVA-induced inflammation processes in tissues could be involved in the delayed formation of both oxidizing and nitrating species^{300, 304}. However the role of the contribution of these secondary radicals to UVA-mediated cellular DNA oxidation reactions remains to be assessed. Therefore in the present review emphasis is placed on the identification and mechanism of immediate formation of the main oxidatively generated DNA damage upon UVA irradiation.

UVA sensitized DNA oxidatively generated DNA damage

Two main approaches, DNA repair-based and HPLC-based methods, have been used to investigate the

formation of the main DNA oxidation products. The data that were obtained using either gas chromatography coupled to mass spectrometry (GC-MS) or various forms of immunoassays for measuring oxidized bases including 8-oxoGua are not discussed due to a lack of reliability of the measurements³⁰⁵.

The UVA-induced formation of 8-oxoGua in the DNA in various mammalian cells has been clearly demonstrated on the basis of HPLC-ECD measurements by several groups^{283, 306-309} at the end of the 1990s following the initial observation made by Rosen et al.²⁸¹. This was further supported using a similar analytical approach by the detection of 8-oxoGua in the DNA of THP-1 human monocytes²⁰, human fibroblasts and keratinocytes¹⁴⁰, *Drosophila* larvae³¹⁰ and human skin explants¹²⁰ following UVA irradiation. HPLC-MS/MS has been shown to be a relevant alternative method for assessing the UVA-induced generation of 8-oxoGua in UVL9 CHO cells¹³⁰, human fibroblasts and human melanocytes¹³⁸. It was reported that the formation of 8-oxoGua in the DNA of both human keratinocytes and human fibroblasts was linear with the applied dose of UVA within the dose range (0 - 20 kJ m⁻²)¹⁴⁰ in agreement with measurements of Fpg-sensitive-sites in the DNA of CHO cells¹³⁰, human keratinocytes and human melanocytes¹³⁸. Furthermore, the presence of Fpg-sensitive sites was detected at the nucleotide level in the genome of UVA-irradiated mouse fibroblasts using the LMPCR assay¹³². Another relevant finding concerns the higher susceptibility of the DNA of fibroblasts with respect to keratinocytes and human skin explants to be oxidized by UVA irradiation^{120, 140}. This is illustrated by the yields of 8-oxoGua that were assessed as 2.58 ± 0.58, 1.51 ± 0.58 and 0.71 ± 0.25 per 10⁶ normal bases and per kJ m⁻² respectively. The comparison was extended to the UVA-induced formation of TT-CPDs that shows a strong predominance of the latter bipyrimidine photoproducts over 8-oxoGua with the ratio TT-CPDs/8-oxoGua decreasing in the following order: human skin (9.4) > fibroblasts (6.3) > keratinocytes (3.2)^{120, 140}.

Relevant information on other UVA-induced oxidatively generated lesions in cellular DNA was gained from the measurement of DNA strand nicks that include direct strand breaks and alkali-labile sites using the alkaline elution technique²³⁸, the alkaline comet assay²⁰ and alkaline gel electrophoresis²⁸³. In addition the same methods were used to detect oxidized pyrimidine bases that were revealed as newly generated strand breaks upon incubation with endonuclease III repair enzyme. In all cases the predominance of 8-oxoGua over strand breaks and oxidized pyrimidine bases was observed showing

however some differences in the relative distribution of the three main classes of oxidatively generated damage. The relative yields of the SSBs together with those of the sites sensitive to Fpg and endo III in UVA-irradiated CHO cells¹³⁰ and TPH-1 human monocytes²⁰ are reported in Table 3. No information is available so far on the nature of the lesions recognized by endo III despite an earlier HPLC-MS/MS attempt¹³⁰ that was unsuccessful likely due to a lack of sufficient sensitivity for detecting low amounts of lesions.

It has also been reported that double strand breaks (DSBs) were generated in UVA-irradiated cells on the basis of several measurements including detection of γ -H2AX foci, and neutral comet assay analysis³¹¹⁻³¹³. However it appears quite unlikely that DSBs, considered as hallmarks of molecular effects of ionizing radiation³¹⁴, may be generated by low energy UVA photons through a direct event that would require two simultaneous $^{\circ}\text{OH}$ hits^{315, 316}. As a reasonable alternative it has been suggested than unrepaired CPDs could be converted into DSBs during replication³¹⁷.

Mechanisms of DNA oxidative degradation by UVA radiation.

8-OxoGua, a ubiquitous DNA oxidation product, has been shown to be predominantly generated in cellular DNA over oxidized pyrimidine bases and SSBs upon UVA irradiation (Table 3). This pattern is different qualitatively and/or quantitatively from the DNA damage distribution that is induced upon exposure of cells to ionizing radiation, biphotonic ionization and ¹O₂. It may be reminded that ¹O₂ oxidation of cellular DNA has been shown to exclusively generate 8-oxoGua³¹⁸ at the exclusion of strand breaks and oxidized pyrimidine bases²⁹⁰. The presence of SSBs in UVA-irradiated DNA is strongly indicative of the implication of $^{\circ}\text{OH}$ that is the only ROS capable of cleaving DNA strands through initial hydrogen atom abstraction. However the contribution of $^{\circ}\text{OH}$ to the oxidation reactions of DNA is much higher in cells exposed to gamma-rays by comparison to UVA radiation²⁰. Thus the ratio SSBs/oxidized purine bases is 2.7 in γ -irradiated cells that is mostly due to the major involvement of $^{\circ}\text{OH}$. On the other hand the ratio is 0.47 in cells exposed to UVA radiation. This may be rationalized in terms of a relative contribution of 80% for ¹O₂ and 20% for $^{\circ}\text{OH}$ in the overall UVA sensitized oxidation of DNA²⁰. The occurrence of one-electron oxidation of nucleobase through Type I photosensitization appears to be unlikely since a very close proximity between the photosensitizers and DNA is required in order to allow efficient charge transfer reaction. This condition has not to be fulfilled by Type II photosensitizers since the intracellular diffusion of released ¹O₂ has been

estimated to be comprised between 150 and 220 nm^{319,320}. The minor °OH contribution to the overall effects of UVA radiation is an indirect process that involves the initial generation of low reactive O₂^{•-} as a side reaction product of Type I photosensitizers through O₂-mediated oxidation of related radical anions. Thus, O₂^{•-} and H₂O₂, its dismutation product, are able to diffuse in cells before reaching the nucleus where the presence of reduced transition metal ions such as Fe²⁺ would be able to locally initiate the formation of highly reactive °OH. It may be reminded that UVA radiation has been shown to favor the intracellular release of labile Fe²⁺^{238,239} that is essential for the reduction of H₂O₂. The observation of G → T transversion in the basal epithelial layer of skin biopsies of human squamous cell carcinoma³²¹ would suggest that this is related to the formation of 8-oxoGua as a critical oxidatively generated damage³²². The endogenous chromophores that are implicated in both Type I and Type II photosensitization reactions remain to be determined at the exception however of melanin as discussed in the next section. As a relevant piece of information it has been shown that the action spectrum for the UVA-visible light sensitized formation of Fpg-sensitive sites as predominant class of oxidatively generated DNA damage in AS52 CHO cells shows a maximum around 430 nm¹⁸. The corresponding value was reported to be 365 nm for the formation of 8-oxoGua in human keratinocytes³⁰⁶.

Photosensitizing features of melanin

The first evidence for the oxidative effect of UVA-excited melanin was provided by the measurement of SSBs in cultured human melanocytes derived from skin phototype I and phototype IV cells. The latter melanocytes whose melanin content is 10-fold higher than in the skin type I cells were much more susceptible to SSB formation³²³. Subsequently it was shown independently that the yield of 8-oxoGua in the DNA of human melanoma cells increased with the pigment content of the cell³⁰⁷. This received further confirmation with the UVA-mediated 2.2-fold enhancement of 8-oxoGua formation in the DNA of melanocytes with respect to keratinocytes from the same human donors¹³⁸. Another relevant data was provided by the measurement of SSBs together with alkali-labile sites which allowed the assessment of a 0.57 ratio between DNA nicks and Fpg-sensitive sites that has is much lower (0.99) in keratinocytes¹³⁸. This again is suggestive of a predominant involvement of ¹O₂ in the sensitized oxidation reactions triggered by UVA radiation on melanocytes with however a higher relative contribution of °OH than that observed in human monocytes²⁰. The significant increase in the

levels of oxidatively generated damage to DNA in UVA-irradiated melanocytes may be rationalized in terms of photosensitizing ability for excited melanin components to release both O₂^{•-} and ¹O₂³²⁴⁻³²⁷ despite the well-documented ability for the pigments to scavenge the two ROS^{325,328}. It is worth noting that the presence of melanin increases the contribution of oxidatively DNA damage with respect of CPDs¹³⁸ in agreement with previous similar observations¹⁴⁵. Another interesting feature of the generation of O₂^{•-} and also °NO is the delayed formation in post-UVA irradiation in melanocytes of CPDs⁸⁸. This was suggested to involve the formation of transient dioxetane through the reaction of peroxyxynitrite with an eumelanin monomer, 5,6-dihydroindole-2-carboxylic acid (DHICA)³²⁹ a degradation product of dopaquinone³³⁰. Interestingly it was recently proposed that ¹O₂ oxidation of DHICA could generate an endoperoxide from the transient formation of indole-5,6-quinone-2-carboxylic acid (IQCA).

The photosensitized role for melanin to promote DNA oxidation could be extended to visible light³³¹. The significant increase in the frequency of Fpg- and endo III-sensitive sites in epithelial cells upon visible light irradiation was rationalized in terms of melanin-mediated formation of ROS including ¹O₂.

Repair of photosensitized 8-oxoGua

Information on the rate of repair of 8-oxoGua, the main UVA induced oxidatively generated damage to DNA that is predominantly removed in human cells by 8-oxoguanine DNA N-glycosylase (hOGG1)³³² is rather scarce. This may be explained by the still challenging measurement of low amounts of 8-oxoGua in cellular DNA what is usually the case when UVA is used as the oxidant. Occurrence of 8-oxoGua repair within a few hours was demonstrated by HPLC-ECD measurements in mouse FM3A cells sensitized by riboflavin to visible light²⁰⁰ and in the epidermis of hairless mice upon exposure to near-UV light³³³. Further support for a fast repair of oxidized guanine bases was gained from the observed decrease in the frequency of Fpg-sensitive sites in UVA-exposed mouse embryonic fibroblast cells³³⁴ and SV40-transformed normal human fibroblasts²⁹⁹ during the post-irradiation period. The lack of repair of 8-oxoGua in UVA-irradiated OGG1 deficient *Saccharomyces cerevisiae* strains has been shown to be highly mutagenic with the induction of G to T transversions³³⁵ that usually are not observed in humans cells exposed to UVA radiation. One may note however one major exception for which it was reported that G → T transversions represent only 6% of the total of UVA-induced mutations¹⁴². Interestingly the *ogg1*

mutated cells were found to be highly sensitive to UVA radiation but not to other damaging agents including UVC radiation, H₂O₂ and gamma-rays³³⁵.

UVA radiation has been shown to induce the relocalization of hOGG1 from a soluble nucleoplasmic site to nuclear speckles in human cells through a signaling event implicating ROS³³⁶. It was demonstrated that the activity of hOGG1 was lower together with a lower gene expression in basal layer of human keratinocytes compared to the superficial layer in engineered human skin³³⁷.

Conclusions

The present review shows that abundant information is available on the chemical mechanisms leading to UV-induced DNA damage (Figure 7). The global picture of an overwhelming contribution of UVB-induced pyrimidine dimers is still valid. Yet, growing evidence shows the importance of UVA not only because of its well-known photo-oxidizing properties but also for its ability to induce CPDs either directly^{120, 126} or even in the dark⁸⁸. There is still a lack of both qualitative and quantitative information on the UVA oxidatively generated damage to cellular DNA, in particular on pyrimidine oxidation products. In addition, relevance of delayed oxidation reactions should be better investigated, for example in melanocytes where chemiexcitation is known to generate ¹O₂. The impact of UVA on DNA repair is another deleterious genotoxic pathway to consider^{112, 338}. The DNA damaging properties of the whole solar UV spectrum has thus to be considered for the development of efficient photoprotection strategies. In the case of sunscreens, the trend to increase in the absorbed wavelength range and SPF is positive^{339, 340}. A better education of the public on the proper use of these products is yet still necessary. Application of sunscreens has been sometime challenged since the screening of UVB photons could affect the production of vitamin D. However, works showing that short exposure to sunlight leading to limited and well repaired DNA damage^{174, 341} are compatible with sufficient vitamin D production. It seems thus most unlikely that sunscreen use completely inhibits the formation vitamin D. A few questions remain opened on the genotoxicity of sunlight. For example, the damaging properties of visible light are not completely assessed¹⁸. Possible implication of infra-red has been suggested but this issue has never been thoroughly studied^{340, 342}. Last, it should be kept in mind that solar light is a mixture of radiations and their combined action could be different for the sum of each individual

ones^{71, 343}. These few points illustrate that the research is still necessary to understand and prevent the genotoxicity of solar radiation and its consequences in terms of skin cancer.

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TABLES

Table 1 yields of formation of CPDs in cellular DNA following exposure to UVC and UVB radiations

UV source	Wavelengths	yield ^a	Sample type	Technique	reference
UVC	254 nm	0.60	SV 40 in <i>E. coli</i>	Endonuclease+electrophoresis	125
UVC	254 nm	0.20	<i>Chlamydomonas reinhardtii</i> (algae)	Radioactive labeling	127
UVC	254 nm	0.36	CHO cells	Radioactive labeling	123
UVC	254 nm	0.33	Human fibroblasts, HPRT gene	Endonuclease+electrophoresis	128
UVC	254 nm	0.14	Human fibroblasts, HPRT gene	Endonuclease+electrophoresis	124
UVC	254 nm	0.48	THP1 monocytes	HPLC-MS/MS	344
UVC	254 nm	1.10	CHO cells	Immuno-dot-blot	126
UVB	Broadband	0.060	SV 40 in <i>E. coli</i>	endonuclease+electrophoresis	125
UVB	290 nm	0.025	CHO cells	endonuclease+ alkaline elution	18
UVB	310 nm	0.0004	CHO cells	endonuclease+ alkaline elution	18
UVB	Broadband	0.010	CHO cells	Immuno-dot-blot	126
UVB	Broadband	0.005	CHO cells	HPLC-MS/MS	130
UVB	Broadband	0.040	Human fibroblasts	HPLC-MS/MS	129
UVB	Broadband	0.044	Human keratinocytes	HPLC-MS/MS	112
UVB	Broadband	0.224	Human keratinocytes	HPLC-MS/MS	120
UVB	Broadband	0.009	Human skin explants	HPLC-MS/MS	120

a: expressed in CPD/10⁵ bases per J/m²

Table 2 yields of formation of CPDs in cellular DNA following exposure to UVA

UV source	Wavelengths	yield ^a	Sample type	technique	reference
UVA	365 nm	0.06	CHO cells	Endonuclease+ alkaline elution	18
UVA	Broadband	1.50	CHO cells	Immuno-dot-blot	126
UVA	Broadband	0.23	CHO cells	HPLC-MS/MS	130
UVA	Broadband	0.13	Human melanocytes	HPLC-MS/MS	138
UVA	Broadband	0.07	human keratinocytes	HPLC-MS/MS	112
UVA	Broadband	0.13	human keratinocytes	HPLC-MS/MS	120
UVA	Broadband	0.09	Human skin explants	HPLC-MS/MS	120
UVA	Broadband	0.13	Human skin explants phototype IV	HPLC-MS/MS	175
UVA	Broadband	0.21	Human skin explants phototype II	HPLC-MS/MS	175

a: expressed in CPD/10¹⁰ bases per J/m²

Table 3. Relative yields of the main classes of oxidatively induced damage to cellular induced by UVA radiation and gamma rays

	CHO cells (UVA) ^a	Human monocytes (UVA) ^b	Human monocytes (γ rays) ^b
SSBs and alkali-labile sites	0.45	0.47	1
Fpg-sensitive sites	1	1	0.37
Endo III-sensitive sites	0.4	0.16	0.4

a: From reference ¹⁸; b from reference ²⁰

LEGENDS TO FIGURES

Figure 1: Chemical structure of the two isomers of thymine-cytosine cyclobutane pyrimidine dimer (TC CPD) found in DNA. The *trans,syn* isomer is formed only in single-stranded and destabilized double-stranded DNA. dR: 2-deoxyribose, P: phosphate

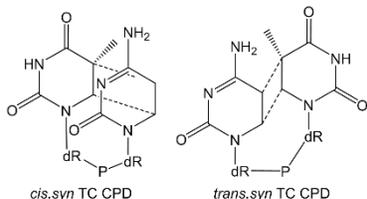


Figure 2: Formation of TT 64PP with involvement of an oxetane intermediate. In the case of TC or CC 64PPs the oxygen atom of the four-membered ring is replaced by a NH group and the intermediate is an azetidine. dR: 2-deoxyribose, P: phosphate

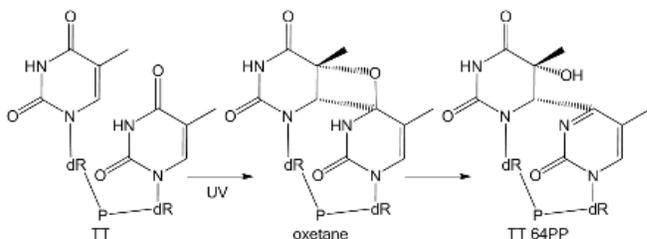


Figure 3: Fate of 64PPs upon exposure to UV radiation in the 320 nm region. Formation of Dewar valence isomer takes place both in model system and DNA. The photosensitizing properties of the pyrimidone moiety have not yet been demonstrated for 64PPs present within DNA but is an interesting hypothesis.

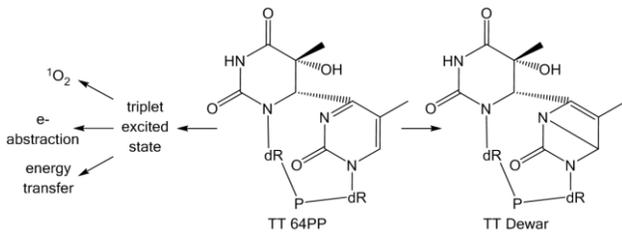


Figure 4: Proportion of the amounts of the four CPDs in isolated double-stranded DNA irradiated under different conditions. UVC: 254 nm lamp, UVA: 354 nm laser, TTET K: TTET by aromatic ketones + UVA, TTET F: TTET by fluoroquinolones + UVA. All yields were calculated in linear parts of the dose-dependent formation and normalized to that of TT CPD. Experiments were carried out in calf thymus DNA.

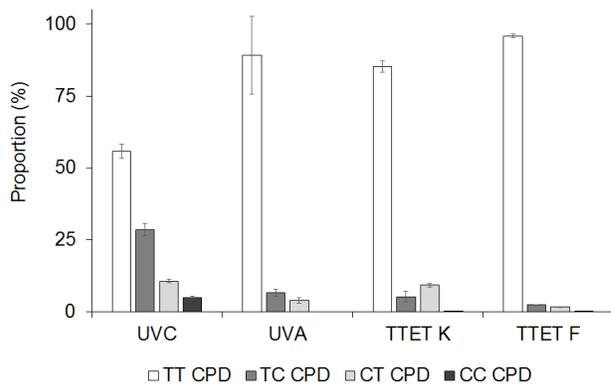


Figure 5: Deamination mechanism of TC CPD. In the first step, a water molecule, with the assistance of a second water molecule adds to the electrophilic C4 atom. The amino group then protonates and the ammonium ion is lost. The resulting cationic species deprotonates and gives rise to a uracil moiety. Note that for clarity, the stereochemistry of the dimer is not shown.

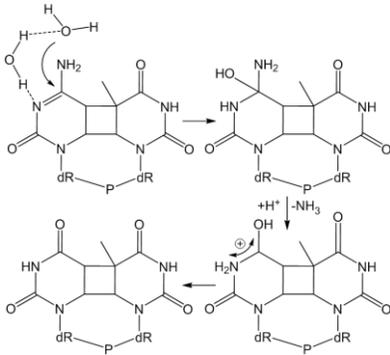


Figure 6: The main mechanisms leading to the formation of 8-oxoGua from Gua. From top to bottom: i) formation of an endoperoxide by addition of singlet oxygen, ii) formation of a reducing radical by addition of $\cdot\text{OH}$ radical, and iii) electron abstraction followed by hydration of the resulting radical cation.

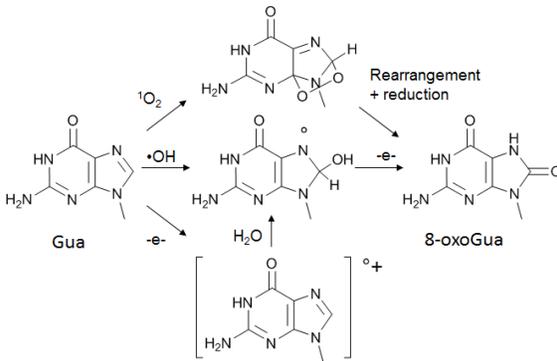


Figure 7: Main types of photoproducts induced by sunlight, UVB and UVA.

