

Review

Mutations induced by ultraviolet light

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Abstract

The different ultraviolet (UV) wavelength components, UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm), have distinct mutagenic properties. A hallmark of UVC and UVB mutagenesis is the high frequency of transition mutations at dipyrimidine sequences containing cytosine. In human skin cancers, about 35% of all mutations in the *p53* gene are transitions at dipyrimidines within the sequence 5'-TCG and 5'-CCG, and these are localized at several mutational hotspots. Since 5'-CG sequences are methylated along the *p53* coding sequence in human cells, these mutations may be derived from sunlight-induced pyrimidine dimers forming at sequences that contain 5-methylcytosine. Cyclobutane pyrimidine dimers (CPDs) form preferentially at dipyrimidines containing 5-methylcytosine when cells are irradiated with UVB or sunlight. In order to define the contribution of 5-methylcytosine to sunlight-induced mutations, the *lacI* and *cII* transgenes in mouse fibroblasts were used as mutational targets. After 254 nm UVC irradiation, only 6–9% of the base substitutions were at dipyrimidines containing 5-methylcytosine. However, 24–32% of the solar light-induced mutations were at dipyrimidines that contain 5-methylcytosine and most of these mutations were transitions. Thus, CPDs forming preferentially at dipyrimidines with 5-methylcytosine are responsible for a considerable fraction of the mutations induced by sunlight in mammalian cells. Using mouse cell lines harboring photoproduct-specific photolyases and mutational reporter genes, we showed that CPDs (rather than 6-4 photoproducts or other lesions) are responsible for the great majority of UVB-induced mutations. An important component of UVB mutagenesis is the deamination of cytosine and 5-methylcytosine within CPDs. The mutational specificity of long-wave UVA (340–400 nm) is distinct from that of the shorter wavelength UV and is characterized mainly by G to T transversions presumably arising through mechanisms involving oxidized DNA bases. We also discuss the role of DNA damage-tolerant DNA polymerases in UV lesion bypass and mutagenesis.

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1. UV-induced DNA photoproducts

Irradiation of DNA or cells with ultraviolet light (UV) induces the formation of several types of mutagenic DNA lesions. The most frequent lesions induced by UVB or UVC radiation are the *cis-syn* cyclobutane pyrimidine dimers (CPDs) and the pyrimidine (6-4) pyrimidone photoproducts [(6-4) photoproducts; (6-4)PPs]. Several minor photoproducts such as purine dimers and pyrimidine mono-adducts are also formed [1]. CPDs are formed between the 5,6 bonds of any two adjacent pyrimidine bases. (6-4)PPs are characterized by a stable bond between positions 6 and 4 of two neighboring pyrimidines and appear to form preferentially at 5'-TC and 5'-CC sequences. They are formed at levels considerably lower than those of CPDs [2,3]. UVA irradiation may induce CPDs in DNA [4,5] and also, through an indirect mechanism, can promote the formation of oxidized DNA bases [6–10].

2. UV and skin cancer mutations

Skin cancer is the most common tumor diagnosed in the United States and the numbers of both non-melanoma and melanoma skin cancers have increased dramatically over the last few decades [11,12]. The available epidemiological evidence indicates clearly that solar UV irradiation is associated with skin cancer [13].

Mutations in cancer-relevant genes are produced by those UV photoproducts that are not repaired before DNA replication. Thus, DNA excision repair systems play an important role in preventing UV mutagenesis. Several human genetic disorders including xeroderma pigmentosum (XP) and Cockayne syndrome (CS) are characterized by a defect in DNA repair. Cells from patients suffering from XP or CS are hypersensitive to UV light. XP is a genetically heterogeneous disease characterized by eight different complementation

groups [14]. The incidence of skin cancer of all types is increased by several orders of magnitude in XP patients [13,15]. Although repair of (6-4)PPs in normal cells is generally faster than that of CPDs [2], repair of both types of photoproducts is absent in many of the skin cancer-prone XP patients. XP-V is a unique complementation group in which DNA excision repair is normal but a specific UV lesion tolerant DNA polymerase (DNA polymerase eta) is defective.

In our work over the recent years, we have attempted to relate UV-induced DNA damage to the specific genetic changes that are observed in skin cancer. Using the ligation-mediated polymerase chain reaction (LMPCR) technique developed in our laboratory for mapping of UV damage [16,17], we have analyzed the distribution and repair of UV-photoproducts along several human genes at the DNA sequence level [18–21]. In some of our studies, we focused on the human tumor suppressor gene *p53*, which contains mutations in a high percentage of human skin cancers [22–25]. We found that sites of mutation hotspots coincide with sites where the DNA repair process, as measured by removal of UV-induced CPDs, is particularly slow [19]. In addition, we showed that the sequence distribution of CPDs induced by sunlight is very different from that induced by UVC at 254 nm at positions containing 5-methylcytosine bases and the sunlight-induced CPD distribution provides a much better match with skin cancer mutations [26]. The results suggest that sunlight-induced formation of CPDs and their DNA repair efficiency may strongly contribute to the mutation spectrum in the *p53* gene.

3. UV and melanoma

The causative relationship between UVB exposure and non-melanoma skin cancer is well documented [27,28]. The link between sunlight exposure and melanoma is somewhat less compelling but still apparent. Melanoma, the most lethal form of skin cancer, shows a dramatic rate of increase worldwide and causes over 7000 deaths each year in the United States [29]. There is epidemiological evidence suggesting that a history of sunburn and intermittent exposure to strong sunlight, particularly during childhood, may promote the development of melanoma [29,30]. However, the specific molecular targets in sunlight-induced

melanoma remain mysterious [12]. The *p16/INK4A* gene often carries germ line mutations in melanoma kindreds [31]. Sporadic melanomas also carry somatic missense mutations in the *p16* gene (reviewed in [32]). Although the finding of common UV-signature C to T transition mutations in the *p16* gene of melanomas suggested targeting of this gene by UVB irradiation [33], the observation of similar C to T mutations in some other tumors [32,34] has called this association into question. Recently developed mouse models have been useful in finding a connection between UV irradiation and melanoma induction [35]. One mouse model has shown that a single neonatal dose of UV is sufficient to induce cutaneous malignant melanoma in mice transgenic for hepatocyte growth factor/scatter factor [36]. In another melanoma model driven by H-RAS activation, inactivation of the retinoblastoma/*p16* pathway was identified as a critical event in UV mutagenesis [37]. Together, the available data would suggest that *p16* could be an important target in melanoma development.

Various epidemiological studies have addressed the relative contributions of UVB wavelengths and UVA wavelengths to photocarcinogenesis, in particular with regards to the development of melanoma [38–42]. The main conclusions from these studies have been that UVB is clearly linked to non-melanoma skin cancers and that UVA may play a role in melanoma, although the latter assumption is somewhat controversial. UVB radiation induces squamous-cell carcinoma in mice [43]. UVA radiation is also a complete rodent skin carcinogen [44]. Although UVB dominates the carcinogenic effect of sunlight, UVA is estimated to contribute 10–20% to the carcinogenic dose [45]. Certain high-powered modern tanning lamps emit mainly long wave UVA (340–400 nm). Exposure to sunlamps or sunbeds has been linked to melanoma with a two-fold risk increase [46]. UVA radiation is far more abundant in sunlight than is UVB (>20 times radiant energy). UVA causes oxidative DNA damage that is potentially mutagenic [7,9,13]. In contrast to UVB, UVA radiation is not significantly absorbed by DNA itself. Hence, the absorption by other molecules (endogenous photosensitizers) becomes more relevant. Radicals, in particular reactive oxygen species, can be generated by UVA irradiation that can damage DNA. UVA radiation is also thought to contribute substantially to the immunosuppression that, at least

in mice, prevents immunological rejection of nascent ultraviolet-radiation-induced skin cancers [47]. UVA is capable of inducing melanoma-like lesions in opossums [48] and in certain fish species [49]. An additional concern regarding the possible carcinogenic effect of UVA is the widespread use of sunscreens that protect efficiently against UVB wavelengths yet do allow significant penetration of UVA, thus increasing the amount of time a person can spend in the sun.

4. The DNA damage spectrum produced by simulated sunlight

Experimental systems for determining the levels and sequence dependence of photoproduct formation in DNA have often used high doses of short wave (UVC) irradiation. We have re-assessed this issue by using DNA sequencing technologies and different doses of UVC as well as more physiologically relevant doses of solar irradiation emitted from a solar UV simulator [3]. We used UV damage endonuclease in combination with CPD photolyase for specific detection of (6-4) photoproducts and T4 endonuclease V for detection of CPDs. Consistent with previous results, (6-4)PPs were less abundant than CPDs, when UVC was used for irradiation. Detectable (6-4)PPs were formed at 5'-TpC and 5'-CpC sequences but very low levels were seen at all other dipyrimidines including 5'-TpT. Contrary to expectation, we found that (6-4) photoproducts form at almost undetectable levels under conditions of irradiation for up to 5 h with a solar UV simulator. The same treatment produced high levels of CPDs. From these data, we concluded that CPDs are formed at least 20–40 times more frequently than other DNA photoproducts when DNA or cells are irradiated with simulated sunlight [3].

5. UVB mutagenesis

UV mutagenesis is characterized by a high frequency of transition mutations at dipyrimidine sequences containing cytosine bases implicating dimeric pyrimidine photoproducts containing cytosine as the mutagenic lesions (reviewed in [1,50]). However, the precise mechanism of how UV light causes C to T transition mutations at cytosine-containing dipyrimi-

dine sites is still unknown. The CPD is believed to be the major mutagenic lesion in mammalian cells owing to its high levels of induction, slow repair, and probably high bypass tolerance [1,51,52]. The contribution of (6-4)PPs to UV mutagenesis is essentially unknown [1,2]. (6-4) Photoproducts appear to be much more mutagenic than CPDs in experiments with site-specific lesions. The mutation frequency obtained with a site-specific 5'-TT-CPD is generally low [53–56]. This is consistent with the infrequent recovery of mutations at 5'-TT sequences in UV-irradiated cells. Surprisingly, the mutagenicity of a site-specific CPD containing the 5'-TC sequence also is very low with >95% accurate lesion bypass [57]. The 5'-TC (6-4)PP was found to be less mutagenic than the 5'-TT (6-4) product but more mutagenic than a CPD at the sequence 5'-TT [58]. Eighty percent of the mutations were C to T transitions at the 3' base. Since a change from 5'-TC to 5'-TT is a type of mutation frequently observed after UV irradiation, the 5'-TC (6-4) photoproduct could be a candidate for a strongly premutagenic lesion. Using a set of mouse cell lines engineered to express foreign photolyase genes (either CPD photolyase or (6-4) photolyase), we have previously shown that the CPDs are responsible for >80% of the UVB-induced mutations in DNA repair-proficient mammalian cells [59] but a contribution of (6-4)PPs to the rest of the mutations is possible.

6. 5-Methylcytosine and UVB mutagenesis

It has long been known that dipyrimidines containing cytosines are the preferred targets for UV-induced mutagenesis [60,61]. More recently, it has been recognized that 5-methylcytosine plays an even more important role in UVB-induced mutations. 5-Methylcytosines are present almost exclusively at CpG dinucleotides in mammalian cells. These sequences are known mutational hotspots in cancer-relevant genes and are often mutated in genes associated with human genetic diseases. The increased mutability of CpG sequences usually is attributed to two different mechanistic pathways: the spontaneous deamination of 5-methylcytosine leading to thymine, and the preferential targeting of methylated CpG sequences by carcinogens [62,63]. In skin cancers, C to T transition mutations are particularly frequent at the trinucleotide sequence 5'-PyCG in the *p53* gene (Fig. 1). These sequences

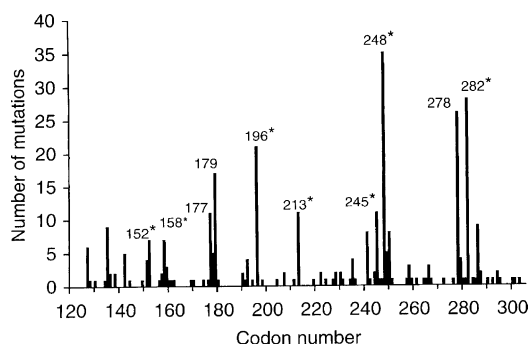


Fig. 1. Distribution of mutations along the *p53* coding sequence in non-melanoma skin cancers. The majority of these mutations are C to T transitions. Data are for non-XP and XP skin tumors combined and were collected from the IARC *p53* mutation database [113] and from the primary literature. Mutational hotspots containing a dipyrimidine sequence with 5-methylcytosine (PyCG sequence) are indicated by asterisks. Codon 278 does not contain a CG sequence but is embedded in the palindrome CCTGG, which may potentially be methylated in stem cells of the skin.

contain 5-methylcytosines in human keratinocytes [64] and are the preferential sites for CPD formation when sunlight is used for irradiation [26]. Several mutational hotspots are seen. Five of the eight most commonly mutated sites in *p53* (codons 196, 213, 245, 248, and 282) contain the mutated dipyrimidine in the sequence context 5'-CCG or 5'-TCG (Fig. 1). A review of publications on *p53* mutations in skin tumors (from non-xeroderma pigmentosum and xeroderma pigmentosum patients) shows that the relative contribution of transition mutations affecting dipyrimidines within these trincucleotide sequences is 134/377 (35%). However, 5'-CCG and 5'-TCG occur only 19 times (=5.7% of the total sequence) in the 1000 bp double-stranded target sequence for preferred mutagenesis of the *p53* gene between codons 120 and 290.

Mechanistically, the longer wavelength absorption of 5-methylcytosine relative to cytosine most likely explains the preferential susceptibility of dipyrimidines containing 5-methylcytosine for sunlight- or UVB-induced CPD formation [65]. In addition, 5-methyl-dCMP has a significantly lower excited singlet state energy than TMP and dCMP [66] and therefore could serve as a singlet energy trap in DNA. By analyzing different mutational reporter genes, we demonstrated that 5-methylcytosines contribute significantly to sunlight-induced mutational hotspots in mammalian cells [65,67]. One important mech-

anism for the selective UVB-induced mutagenesis at 5'-PymCG sites is the efficient deamination of 5-methylcytosines within CPDs, an event that can then be followed by "correct" polymerase bypass leading to a mutation [68]. The available evidence clearly indicates that 5-methylcytosine plays a major role in UVB mutagenesis in mammalian cells. In addition, these data indicated that the thymine–thymine dimer, a lesion that had been considered as almost equivalent to UV damage for decades, is in fact not the most efficiently produced dipyrimidine lesion after exposure of DNA to sunlight.

In order to define the contribution of 5-methylcytosine to sunlight-induced mutagenesis, we have used mouse fibroblasts containing the CpG-methylated *lacI* transgene as a mutational target. We sequenced 182 UVC (254 nm UV)-induced mutations and 170 mutations induced by a solar UV simulator, along with 75 mutations in untreated cells. More than 95% of the UVC- and solar irradiation-induced mutations were targeted to dipyrimidine sites, and the majority were transitions. After UVC irradiation, 6% of the base substitutions were at dipyrimidines containing 5-methylcytosine and only 2% of all mutations were transitions within this sequence context. However, 24% of the solar light-induced mutations were at dipyrimidines that contain 5-methylcytosine and most of them were transitions. Two sunlight-induced mutational hotspots at methylated CpGs coincided with sequences at which high levels of CPDs were formed after irradiation with sunlight but not with UVC [65]. In a related study, we have analyzed the *cII* transgene in mouse cells, a mutational target gene that we found also to be methylated at most CpG sequences. Also in this gene, there was a much higher involvement of dipyrimidines containing 5-methylcytosine in the solar UV mutational spectrum relative to that established for UVC (33% versus 11% of all mutations) [67]. A comparison of sunlight-induced mutational spectra of the *cII* and *lacI* transgenes, as well as the *p53* gene in skin tumors, showed that 5-methylcytosine is involved in 25–40% of all mutations in all three systems. The combined data make a strong case that CPDs forming preferentially at dipyrimidines with 5-methylcytosine are responsible for a considerable fraction of the mutations induced by sunlight in mammalian cells [65,67].

In mouse skin tumors induced by irradiation with UVB lamps or solar UV simulators, a major

mutational hotspot has been reported at codon 270 of the *p53* gene (Arg to Cys) involving a sequence change from 5'-TCGT to 5'-TTGT. This codon corresponds to codon 273 in the human *p53* gene but there is no dipyrimidine sequence in the human gene. Using human cells, we had shown earlier that CPD formation by UVB or sunlight is enhanced up to 10-fold at 5'-CCG and 5'-TCG sequences due to the presence of 5-methylcytosine bases [26]. Sequence analysis showed that the CpG at codon 270 is methylated in mouse epidermis. Irradiation of mouse cells with UVB produced the strongest CPD signal within exon 8 at the 5'-TCG sequence, which is part of codon 270. Time course experiments showed that CPDs at this particular sequence persist longer than those at several neighboring positions. The data suggest that formation of CPDs is responsible for induction of the major *p53* mutational hotspot in UV-induced mouse skin tumors [69].

7. Cyclobutane pyrimidine dimers are responsible for the majority of mutations induced by UVB irradiation in mammalian cells

The most prevalent DNA lesions induced by UVB are the CPDs and the (6-4)PPs. It has been a long-lasting debate as to which of these photoproducts is responsible for UV-induced mutations in mammalian cells. We have introduced photoproduct-specific DNA photolyases into a mouse cell line carrying the transgenic mutational reporter genes *lacI* and *cII*. Exposure of the photolyase-expressing cell lines to photoreactivating light resulted in almost complete repair of either CPDs or (6-4) photoproducts in less than 3 h [59]. The mutations produced by the remaining, non-repaired photoproducts were then quantitated. The mutant frequency in the *cII* gene after photoreactivation by CPD photolyase was reduced from 127×10^{-5} to 34×10^{-5} (background $(8-10) \times 10^{-5}$). Photoreactivation with (6-4) photolyase did not lower the mutant frequency to any significant extent. In the *lacI* gene, the mutant frequency after photoreactivation repair of CPDs was reduced from 148×10^{-5} to 28×10^{-5} (background $(6-10) \times 10^{-5}$). We concluded that CPDs are responsible for at least 80% of the UVB-induced mutations in this mammalian cell model [59].

8. Deamination of cytosine and 5-methylcytosines within cyclobutane pyrimidine dimers is an important component of UVB mutagenesis

UVB mutagenesis is characterized by an abundance of C to T and 5-methylcytosine to T transitions at dipyrimidine sequences. It is not however known how these mutations might arise mechanistically. One hypothesis is that UV-induced mutations occur only after deamination of the cytosine or the 5-methylcytosine within the pyrimidine dimer [25,68,70–76]. Deamination of cytosines within CPDs does occur at a significant rate in human cells [76]. It is of interest to know how methylation of cytosines at the 5-position influences deamination and how this affects mutagenesis. We have conducted experiments with a CpG methylated *supF* shuttle vector that was irradiated with UVB and then incubated at 37 °C to allow time for deamination to occur before passage of the vector through a human cell line to establish mutations [68]. This led to a significantly increased frequency of CC to TT tandem mutations and of transition mutations at 5'-PymCG3' sequences. The increase in tandem mutations may be due to double deamination events. A spectrum of deaminated CPDs in the *supF* gene was determined using the mismatch glycosylase activities of the MBD4 protein in combination with ligation-mediated PCR. Methylation at the C5 position promoted the deamination of cytosines within CPDs and these two events combined led to a significantly increased frequency of UVB-induced transition mutations at 5'-PymCG3' sequences. Under these conditions, the majority of all *supF* mutations were transition mutations at 5'-PymCG3' and they clustered at several mutational hotspots. The exact same types of mutations are frequently observed in the *p53* gene of non-melanoma skin tumors, and these are particularly common in XP skin tumors [25]. This mutagenic pathway may become prevalent under conditions of inefficient DNA repair and slow proliferation of cells in the human epidermis [68].

9. UV lesion bypass

The low frequency of mutations at 5'-TT sequences in UV-irradiated cells is likely due to the action of DNA polymerase η (POLH), which correctly bypasses

these lesions. DNA polymerase η is encoded by the *RAD30* gene in yeast and by the *POLH* (*XPV*) gene in humans [51,77,78]. Two models of UV mutagenesis are conceivable to explain the preponderance of C to T transition mutations at dipyrimidine sites (Fig. 2). One pathway involves direct lesion bypass by a DNA polymerase that incorporates adenines opposite a cytosine or 5-methylcytosine within the cyclobutane pyrimidine dimer. The nature of this error-prone polymerase is unknown. The second pathway is represented by a model in which the cytosine or 5-methylcytosine first deaminates within the CPD lesion, a reaction, which is then followed by ‘correct’ bypass during DNA replication. In this scenario, the deamination event is entirely responsible for the mutation. We have recently provided evidence showing that the second pathway, the deamination pathway, plays an important role in UVB mutagenesis in mammalian cells, in particular when 5-methylcytosine is involved [68,76].

In the cell, the UV-induced DNA lesion may be initially encountered by a replicative polymerase, which

may or may not be able to efficiently insert nucleotides opposite the damaged site. If it is not able to insert, the replicative polymerase may either dissociate from the primer/template or be detached by an unknown mechanism. This may clear the path for a specialized DNA damage bypass polymerase. Even if the replicative polymerase can insert a nucleotide opposite the UV photolesion, it may be unable to extend further, and may have to hand over the primer/template to another DNA polymerase. It is therefore conceivable that more than one polymerase may contribute to the mutational spectrum of a single UV-induced lesion. The bypass in vivo may require additional proteins, which have not yet been identified.

Recently, a new class of DNA polymerases has been described, called the Y family, which contains the *umuC*/*DinB*/*Rad30* superfamily of DNA damage bypass polymerases [79,80]. Unlike the replicative polymerases, these polymerases can synthesize past a variety of DNA lesions in vitro and have a very low fidelity and processivity on undamaged templates. One

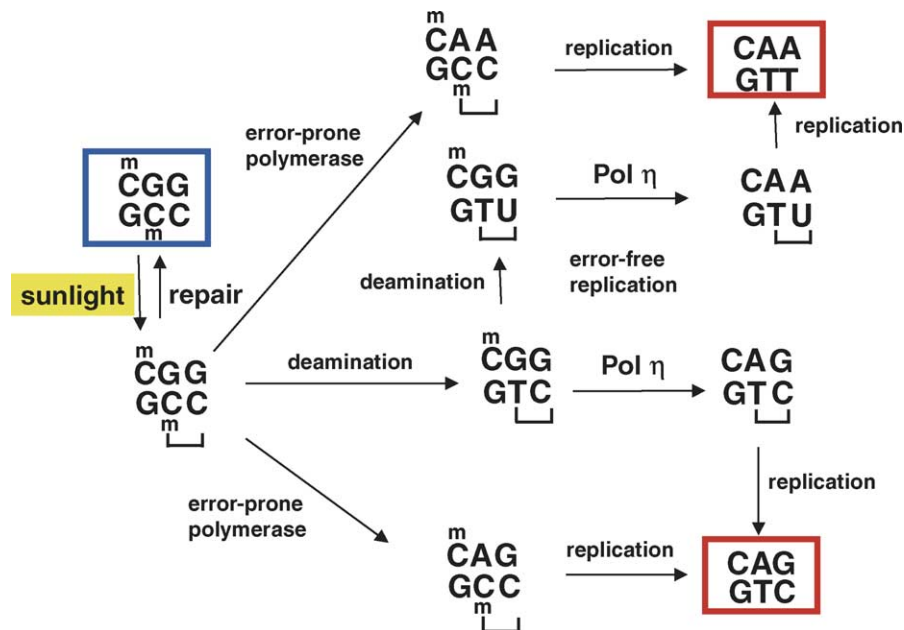


Fig. 2. Mechanisms of UV mutagenesis at a cyclobutane pyrimidine dimer (CPD). The sequence context shown, 5'-CmCG, is particularly prone to CPD formation by irradiation with sunlight due to the presence of the 5-methylcytosine base (mC). The CPD is indicated by a bracket. Bypass of the CPD by an error-prone DNA polymerase could produce a C to T or CC to TT mutation. Deamination may occur within the CPD and may affect the 5-methylcytosine alone or the 5-methylcytosine and cytosine (double deamination). If the deaminated CPD is bypassed mostly error-free by DNA polymerase η , a C to T transition or CC to TT tandem transition mutation will occur. The starting sequence is marked by a blue box. The two mutated sequences, which are eventually produced, are marked by red boxes.

of these enzymes, DNA polymerase η , is defective in XP-V cells, and strong evidence suggests that this polymerase carries out translesion synthesis (TLS) past CPDs in vivo [51,78,81]. In vitro, Pol η is able to replicate past a CPD and undamaged bases with about the same efficiency [82]. It can also carry out TLS past a variety of other DNA lesions such as 8-oxoguanine and O6-methylguanine, but cannot bypass the (6-4) photoproduct [83]. Pol η was able to elongate far more efficiently if two As were inserted opposite a 5'-TT CPD, so that the overall efficiency of correctly completing translesion synthesis opposite a CPD is quite high [82,84]. Studies using yeast strains proficient and deficient in Pol η (*RAD30*) showed that, in Pol η wild-type strains, the TT (6-4)PP is replicated either inaccurately or accurately 60 and 40% of the time, respectively. In the absence of Pol η , only the error-free mechanism was operative [85]. Thus, in contrast to the situation with the 5'-TT CPD, the bypass of the 5'-TT (6-4) photoproduct by Pol η seems to be error-prone.

The *REV3* gene encodes the catalytic subunit of an error-prone polymerase called DNA polymerase zeta (ζ). *REV3* is tightly associated with the *REV7* protein. This polymerase is required for UV-induced mutagenesis in *Saccharomyces cerevisiae* [86,87]. By using antisense constructs and transfection experiments, Gibbs et al. [88] demonstrated that *REV3* was required for UV mutagenesis in human cells. Pol ζ is able to extend primer templates opposite damaged sites, including CPDs, (6-4)PPs, and AAF-dG adducts [89,90]. It has been suggested that the principal role of Pol ζ is in the extension step of TLS following nucleotide insertion opposite the lesion by another polymerase. Recently, it has been shown that the DNA damage bypass polymerase iota (ι) is capable of inserting opposite the 3'-T of a TT (6-4) photoproduct but it does not insert opposite the 5'-T. In contrast, polymerase ζ is not capable of inserting opposite the 3'-T of the (6-4) photoproduct, but is capable of extending opposite the 5'-T. This has led to the concept of a two-polymerase bypass model. In this scenario, DNA damage bypass polymerases may be classified as either being inserters or extenders [90]. In the case of the (6-4)PP, TLS in vitro could be carried out by a combination of Pol iota and Pol ζ [90,91], Pol η and Pol ζ [92], or possibly by Pol ζ alone [89].

The *REV1* gene of *S. cerevisiae* is, like *REV3*, required for UV mutagenesis [87]. Rev1 is also a member of the Y-family polymerases. Rev1 has dCMP trans-

ferase activity and can incorporate dCMP opposite abasic sites [93]. However, Rev1 may also have a second function required for TLS, which is independent of its dCMP transferase activity [94]. A human homologue of Rev1 has been cloned [95,96]. Antisense and ribozyme studies have implicated REV1 in UV mutagenesis in human cells [96,97]. Another DNA polymerase that has been proposed by in vitro studies to be involved in the mutagenic bypass of UV photoproducts is Pol β [98], although this polymerase normally participates in the gap filling reaction during base excision repair.

We need to gain more knowledge as to which of these DNA polymerases is involved in handling different types of DNA damage in vivo. Except for a clearly known role of Pol η in TLS past TT CPDs, our understanding of the mechanisms of in vivo TLS is very limited. For example, we do not know how a specific polymerase is selected at the replication fork to carry out error-free or error-prone TLS of a specific UV lesion [CPD or (6-4)PP]. It is also unknown how the replicative DNA polymerase is removed from the template and hands over the synthesis to the TLS DNA polymerase, and whether lesions in the leading and lagging strands are bypassed differently. In addition, the exact mechanistic roles of REV1 and DNA polymerase ζ and potentially other unknown factors involved in translesion synthesis need to be clarified.

10. UVA mutagenesis

The genotoxicity of UVA has most commonly been ascribed to endogenous photosensitizers causing reactive oxygen species-mediated induction of DNA damage [6,7,9,10,28,99–102]. However, more recently, it has been reported that UVA can induce CPDs in rodent cells [4,5,103]. The poor absorbance of UVA by DNA itself favors the idea that type I (direct reaction) or type II (indirect through reaction of the excited photosensitizer molecule with oxygen) photosensitization reactions occurring within cells are primarily responsible for UVA-induced DNA damage. In addition, UVA may produce H_2O_2 through activation of flavin-containing oxidases [104] or through release of free iron in the irradiated cells from the iron storage protein ferritin [105]. UVA has been shown to induce 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG), a typical oxidative DNA lesion, as well as generate

DNA strand breaks in a variety of experimental systems [6,7,10,106–109]. UVA-induced mutagenesis has been assessed in reporter genes in bacteria and mammalian cells [99–101,108,110]. The results have not always been in agreement and a direct link between UVA-induced DNA damage and mutagenesis has not been clearly established. In one experimental system, Drobetsky et al. have identified the T to G transversion, an otherwise rare type of mutation, as a signature mutation for UVA [99]. These T to G transversions may be linked to CPDs at 5'-TT sequences that are inducible by UVA irradiation [4]. More recently, we have characterized UVA-induced mutational spectra in mouse fibroblasts carrying the *cII* transgene [111]. UVA irradiation at a dose of 18 J/cm² produced significant levels of 8-oxo-dG in DNA but did not yield detectable CPDs. The *cII* mutant frequency was increased almost five-fold over background. UVA-induced mutations were characterized by statistically significant increases in G to

T transversions and small tandem base deletions relative to mutations derived spontaneously. Compared to UVB, UVA did not significantly induce the formation of C to T transitions and CC to TT mutations, a specific signature of UVB mutagenesis, nor did it induce T to G transversions (Fig. 3). This mutational spectrum is not the same as those previously reported for UVA in other test systems. The reasons for the differences could be the experimental systems used, for example, different cell lines, irradiation in vitro versus in vivo, but most important could be the subtle differences in the exact wavelength output of the UVA lamps. If the lamps emit significant amounts of energy below 340 nm, small amounts of CPDs may be produced. The UVA mutation spectrum we obtained using a UVA source that emits negligible radiation at wavelengths below 340 nm corresponds well with the known spectrum of mutations established for the oxidative base lesion 8-oxo-dG. We concluded that the major mutagenic

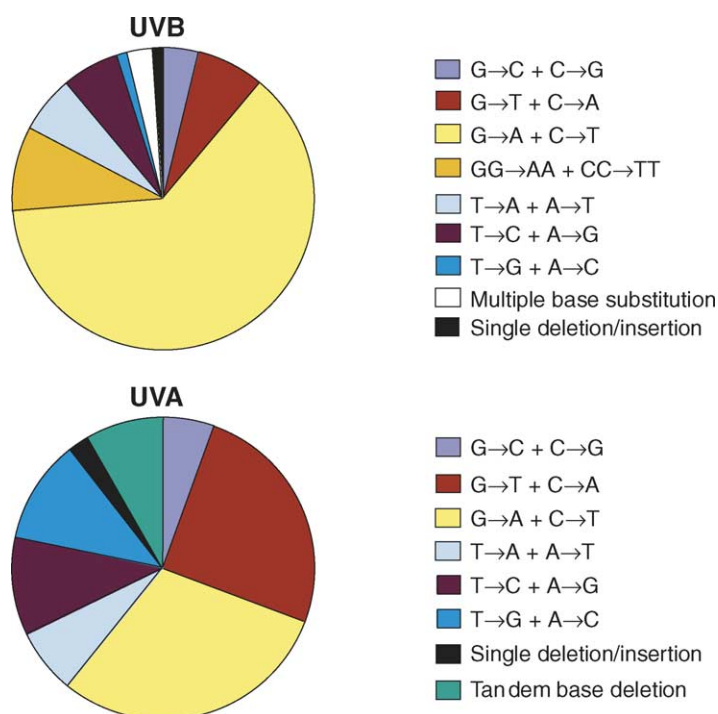


Fig. 3. The mutational spectra of UVA and UVB. Mutations were scored in the *cII* transgene of mouse embryo fibroblasts irradiated with 18 J/cm² of UVA or 0.05 J/cm² of UVB. Under these conditions, the *cII* mutant frequencies were increased 4.6-fold for UVA and 12-fold for UVB. The original data, including spectra in non-treated control cells, were reported in refs. [59,111]. The UVB spectrum is distinguished by a preponderance of C to T transitions including CC to TT tandem mutations. The tandem transition mutations are absent in the UVA spectrum. UVA substantially increases the proportion of G to T transversions and of small tandem base deletions.

effect of UVA is most likely mediated through oxidative DNA damage [111,112]. The established signatures of UVA-induced mutation are not easily detectable in the mutational spectrum of the *p53* gene in skin cancer patients. Consistent with studies using experimental animals for UV-wavelength-dependent skin cancer induction, it is likely that the majority of sunlight-induced skin tumors in the human population are due to the UVB portion of the solar spectrum. However, this situation may become different under conditions of intense UVA exposure such as those employed in modern UVA tanning salons.

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