

The Mechanisms of UV Mutagenesis

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UV-induced mutation/UV damage/UVB/UVA/TLS.

Ultraviolet (UV) light induces specific mutations in the cellular and skin genome such as UV-signature and triplet mutations, the mechanism of which has been thought to involve translesion DNA synthesis (TLS) over UV-induced DNA base damage. Two models have been proposed: “error-free” bypass of deaminated cytosine-containing cyclobutane pyrimidine dimers (CPDs) by DNA polymerase η , and error-prone bypass of CPDs and other UV-induced photolesions by combinations of TLS and replicative DNA polymerases—the latter model has also been known as the two-step model, in which the cooperation of two (or more) DNA polymerases as misinserters and (mis)extenders is assumed. Daylight UV induces a characteristic UV-specific mutation, a UV-signature mutation occurring preferentially at methyl-CpG sites, which is also observed frequently after exposure to either UVB or UVA, but not to UVC. The wavelengths relevant to the mutation are so consistent with the composition of daylight UV that the mutation is called solar-UV signature, highlighting the importance of this type of mutation for creatures with the cytosine-methylated genome that are exposed to the sun in the natural environment. UVA has also been suggested to induce oxidative types of mutation, which would be caused by oxidative DNA damage produced through the oxidative stress after the irradiation. Indeed, UVA produces oxidative DNA damage not only in cells but also in skin, which, however, does not seem sufficient to induce mutations in the normal skin genome. In contrast, it has been demonstrated that UVA exclusively induces the solar-UV signature mutations *in vivo* through CPD formation.

INTRODUCTION

Ultraviolet (UV) light has strong genotoxic effects to produce DNA damage, induce mutations, and, in the worst case, cause the development of tumors. The major natural source of UV is the sun, and solar UV is known to be one of the main causes of human skin cancers.^{1,2)} UV has been classified into three bands by the wavelength: UVA (320–400 nm), UVB (290–320 nm) and UVC (< 290 nm). UVA is also subclassified into UVA1 (340–400 nm) and UVA2 (320–340 nm). The UV component of the sunlight reaching the earth surface (daylight UV) consists of UVA and UVB (290–400 nm), the UVB portion of which shows a strong carcinogenic effect on the skin,^{1,3)} whereas some involvement of the UVA portion in carcinogenesis of the skin has also been suggested.^{4–6)} In this review, we characterize UV-induced mutations in terms of the causative DNA damage and UV wavelengths,

and show the latest overview of the mechanism of UV mutagenesis based on recent progress in the molecular knowledge of DNA damage bypassing.

UV-INDUCED DNA LESIONS

UV produces specific DNA damage such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts (64PPs) at dipyrimidine sites, where two pyrimidine (Py) bases are juxtaposed in tandem in the nucleotide sequence of DNA. These UV lesions are formed through a photochemical reaction, whose efficiency depends on the wavelength, following direct UV energy absorption by DNA bases.⁷⁾ The yields of CPD and 64PP are highest at around 260 nm, and the action spectra of their formation are in parallel to the absorption spectrum of DNA. It is also known that 64PP can change into an isomeric secondary product, Dewar valence isomer, after subsequent absorption of UVA wavelengths around 325 nm.^{8–10)} These photolesions, CPD, 64PP and Dewar, are assumed to cause UV-specific mutations.

UV also induces oxidative stress in irradiated cells through the production of reactive oxygen species (ROS) by activating some small molecules such as riboflavin, tryptophan and porphyrin,^{11–14)} which can then activate cellular

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oxygen.^{15,16} ROS attack DNA and can produce oxidative base damage such as 8-hydroxyguanine (8OH-G) and thymine glycol in DNA or can make strand breaks.^{17–19} ROS also attack cellular nucleotide pools, producing oxidized nucleotides such as 8-hydroxydeoxyguanosine-triphosphate (8OH-dGTP), which can still be used as nucleotide precursors for DNA synthesis.²⁰ Some of these types of oxidative DNA and nucleotide damage are known to be mutagenic.^{19,21} Thus, UV can induce oxidative stress-mediated mutations in the cellular genome through an indirect mechanism.

UV-INDUCED MUTATIONS (UVC-UVB-UVA2)

UV signature

UV induces specific types of mutation: base substitutions of cytosine (C) → thymine (T) at dipyrimidine sites and CC → TT tandem base substitutions, although the latter rarely occur. These two types of mutation are also called UV signature,²⁾ and their detection suggests past exposure to UV (Table 1). In one of the mechanisms of formation of these UV-specific mutations, deamination of cytosine bases in a CPD is thought to be involved.^{22,23)}

“Error-free” bypass of deaminated CPD

The cytosine base in a CPD is unstable and easily deaminates resulting in a uracil base (half life 2–100 hrs),^{24–27)} whereas an intact cytosine base is quite stable (half life 30,000 yrs).²⁸⁾ Thus, CPDs including cytosine residues are prone to change to uracil (U)-containing CPDs by deamination. Especially, CC dipyrimidines are thought to deaminate synergistically at both cytosines once they have changed to a CPD form, resulting in UU-CPDs at a high frequency.^{25,27)} These uracil-containing CPDs are presumed to represent the causative DNA damage for UV-specific mutations.

CPDs, 64PPs and Dewar isomers block DNA synthesis by preventing the replicative DNA polymerases from passing through them when they reside on a template strand during DNA replication.^{29–31)} Although cells try to excise these pho-

tolesions by a DNA repair mechanism called nucleotide excision repair (NER), failure in the repair before replication fork passing would lead to a stall and collapse of the fork at the damaged site, which could cause a DNA double strand break and result in cell death. To avoid such an unfavorable outcome, cells have several mechanisms to tolerate, one of which is translesion DNA synthesis (TLS), in which specialized DNA polymerases called TLS polymerases work to overcome the replication blocks by restarting the DNA synthesis stalled at obstructive, damaged bases on a template strand. It is generally supposed that TLS polymerases could perform TLS by relaxing or ignoring the conventional Watson-Crick base-pairing rule between an incoming dNTP and the template base of DNA. In other words, DNA synthesis with TLS should be error-prone and introduce mutations to the cellular genome at a high frequency. However, DNA polymerase (pol) η , which shows a function highly specific to CPD,^{32–35)} suppresses efficiently the induction of mutations after UV irradiation by performing an error-free TLS opposite CPDs³⁶⁾ using the base-pairing ability still remaining for CPDs.³⁷⁾ Actually, patients of a variant form of xeroderma pigmentosum (XP-V), which has deficiencies in the gene *POLH* encoding pol η , show high photocarcinogenic sensitivities in skin regions exposed to sunlight. Cells from such patients are also sensitive to UV-induced mutations. Thus, pol η seems to suppress CPD-mediated mutations through its “error-free” TLS activity for CPD. With this very activity, however, it could induce C → T and CC → TT mutations unintentionally at the sites of U-containing CPDs produced by deamination (Fig. 1a, b; Table 1, the mechanism A). The induction of CPD-mediated UV-specific mutations depends on a balance between cytosine deamination and pol η -mediated TLS as well as that between DNA replication and NER.

Solar-UV signature

It has been known that UVB and daylight UV induce CPDs preferentially at 5-methylcytosine (mC)-containing

Table 1. Types and characteristics of UV-induced mutations

Type	Mutation pattern	Causative damage	Mechanism	Relevant wavelength
UV signature	C → T at Py-Py CC → TT	CPD	A, B	UVC–UVA
		64PP?, Dewar?	B	
Solar-UV signature	C → T at Py-mCpG	CPD	A	UVB–UVA (solar UV)
Non-UV-signature	Triplet mutation	64PP, Dewar, CPD?	B	UVC–UVA?
Oxidative type	G → T	8OH-G	Mispair with dATP	UVA?
UVA fingerprint	T → G	8OH-dGTP?	Mispair with template adenine?	UVA?

A: “error-free” TLS opposite deaminated C in CPD by pol η (deamination model, see Fig. 3).

B: error-prone TLS by TLS and replicative polymerases (two-step model, see Fig. 3).

Py: pyrimidine, CPD: cyclobutane pyrimidine dimer, 64PP: pyrimidine(6-4)pyrimidone photoproduct, 8OH-G: 8-hydroxyguanine.

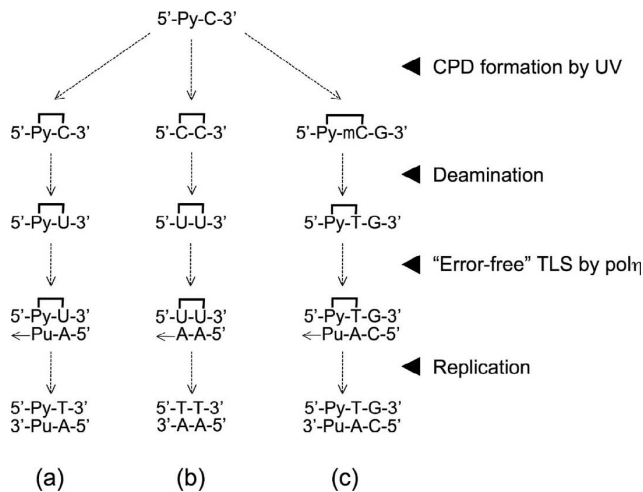


Fig. 1. Mechanistic model of UV-signature mutation formation through deamination-mediated "error-free" TLS of CPDs by polη. (a) The general pathway for PyC → PyT mutations by the deamination-mediated "error-free" TLS. (b) A specific pathway inducing a tandem base substitution of CC → TT. (c) The pathway for the induction of solar-UV signature mutations. Pyrimidine base pairs connected by a bracket indicate CPD dimers. Py, pyrimidine; Pu, purine; mC, methylcytosine.

dipyrimidine sites.^{38,39} This site preference of CPD formation requires the methylation of cytosines³⁹ and is not observed for UVC.^{40,41} In the mammalian genome, cytosines are methylated at a high ratio in 5'-CG-3' (CpG) sequences,⁴² providing plenty of mC-containing dipyrimidine sites. The abundance in mC suggests a high sensitivity of the mammalian genome at CpG sites to longer wavelength UV such as UVB and UVA. Actually, mutations in the *p53* gene found in human skin cancers, which are most likely to have been induced by solar UV, show the UV signature and occur frequently at the dipyrimidine sites associated with methylated CpG (mCpG).^{2,43–45} In mutagenic and carcinogenic studies using mouse skin exposed to UVB, UVA2 or solar UV, similar mutation patterns with frequent recovery of C → T transitions at mCpG-associated dipyrimidine sites have been reported.^{46–50} Thus, the UV wavelength range between UVB and UVA2, which coincides with the carcinogenic daylight UV wavelengths, seems to preferentially induce UV-signature mutations at mCpG-associated dipyrimidine (Py-mCpG) sites. Based on these observations, we have proposed calling such UV-specific mutations "solar-UV signature" (Table 1).⁴⁵

In the mechanism by which solar-UV-signature mutations are formed, the "error-free" bypass of deaminated CPDs mentioned above appears to be involved (Fig. 1c). The 3'-mC of CPDs formed at Py-mCpG sequences deaminates more rapidly than the cytosines of CPDs formed in the methylation-free same sequence contexts, resulting in a high frequency of PyT-CPDs,⁵¹ which could be bypassed "error-

free" by Polη, finally leading to the solar-UV signature, a C → T transition at the Py-mCpG site.⁵²

Error-prone bypass of CPD—two-step model

However, the mutagenesis mechanism of polη-mediated "error-free" TLS opposite deaminated CPDs cannot provide a full explanation for all of the UV-specific mutations. First, the UV-signature mutations were detected in organisms such as *Escherichia coli* that lack polη or its homologues.^{53,54} Secondly, polη-defective cells such as XP-V cells, in which UV induces mutations at much higher frequencies than in wild-type cells, still show the UV signature as the main types of UV-induced mutations.^{55–58} It should therefore be supposed that the UV-signature mutations, at least a part of them, also result from polη-independent pathways (Table 1, the mechanism B). It is known that *E. coli* pol V (UmuD'2C) and eukaryotic polζ, whose deficiency remarkably suppresses UV-induced mutagenesis,^{59,60} work in error-prone TLS. Pol V is believed to most frequently insert an adenine opposite damaged sites on a template strand,^{61,62} giving mechanical support for the "A-rule" model of bypassing non-instructional DNA lesions.^{63,64} Polζ has been shown to specifically extend newly synthesized DNA strands most efficiently from a 3'-terminal adenine residue inserted opposite a damaged site.⁶⁵ Consequently, these error-prone TLS polymerases could result in producing the UV-signature mutation of C → T for the UV damage of dipyrimidines. In addition to these polymerases, polt, polk and REV1 are also thought to be the error-prone TLS polymerases which could be involved in UV mutagenesis. Polt was reported to have a structural similarity to⁶⁶ and a physical interaction with⁶⁷ polη, which suggested some relevance of this polymerase to UV mutagenesis. Deficiencies of polt activity, however, cause little change in the UV-induced mutation frequency and spectrum in cells,^{36,58,68} or in the phenotypes and UV carcinogenicity in mice,^{69,70} though sensitize slightly but significantly polη-knockout mice to UV-induced skin cancer.^{57,70} Actually, polt is inefficient in inserting nucleotides opposite the 3'-bases of CPDs.^{71,72} On the other hand, defects in REV1 suppress UV-induced mutagenesis in both yeast and mammal.^{60,73,74} Repression of polk activities also diminishes the survival and mutagenic response of cells to UV.^{36,75,76} REV1 is known to have a deoxycytidyl transferase activity, incorporating cytosines opposite damaged and undamaged sites,⁷⁷ which, however, seems inconsistent with its expected role in the induction of UV-specific C → T mutations. Actually, this activity is not indispensable for TLS and tolerance to the DNA damage induced by UV.^{78,79} It has been demonstrated that REV1 can interact with various TLS and replicative polymerases and their accessory factors through its C-terminus and N-terminal BRCT domain,^{79–86} which suggests that REV1 plays a regulatory and/or structural role in TLS such as switching between replicative and TLS polymerases, and/or between different TLS polymerases.^{65,86–89}

Polk has been suggested to function in the extension step of TLS like pol ζ .⁹⁰ Both polk and pol ζ show poor abilities to bypass CPDs and 64PPs by themselves, and rarely or poorly insert nucleotides opposite the 3'-base position of these lesions.^{71,91-93} Instead, these polymerases can extend newly synthesized strands efficiently from mismatched terminal nucleotides inserted opposite the 3' base (and 5' base, of course) of CPDs.^{71,90} The role as an extender of mispaired primer termini assigned to these polymerases represents a part of the substantial molecular basis for the classical two-step model of UV mutagenesis,⁹⁴⁻⁹⁶ in which TLS bypass is assumed to proceed through the two steps of insertion and extension of mismatched nucleotides opposite UV lesions. The DNA polymerases responsible for the first misinsertion step, however, have still not been identified clearly, although the involvement of some replicative and/or TLS polymerases such as pol δ , pol η , polt and REV1 has been suggested for UV mutagenesis.^{36,76,71,97}

In conclusion, bypass of CPD seems to be performed through two different TLS pathways: an error-free pathway dependent exclusively on a single DNA polymerase pol η , and an error-prone two-step pathway involving several members of inserter and extender DNA polymerases (the mechanisms A and B in Table 1, respectively).

Mutagenicity of 64PP

The mutagenicity of 64PP still remains to be clarified. Although genetic studies have established the significant involvement of 64PP in UV mutagenesis,⁹⁸⁻¹⁰⁰ its contribution to the UV-signature mutation has not been determined yet. It is known that, in mammalian cells proficient in DNA repair, the vast majority of UVB-induced mutations are caused by CPD, not by 64PP,¹⁰¹ which would have precluded the identification of 64PP-mediated mutations. The minor contribution of 64PP to UV mutagenesis would be partly because 64PPs are excised from the mammalian genome much more efficiently than CPDs, resulting in much lower amounts of 64PP than CPD that could induce mutations.

Since no efficient error-free TLS polymerase working specifically on 64PP, such as pol η on CPD, has been found, some error-prone TLS polymerases should be involved in the TLS of such lesions. Pol ζ is known to be essential to the tolerance for 64PP¹⁰² and necessary for efficient TLS opposite 64PPs.^{88,103,104} However, as mentioned above, pol ζ itself has little ability to insert nucleotides opposite the 3'-base of 64PPs, though it can extend efficiently from the inserted terminal nucleotide opposite the 3'-base of the lesion. Thus, the TLS of 64PP by pol ζ should require some inserter polymerases, for which several replicative and TLS polymerases such as pol δ , pol η and polt have been suggested as candidates,^{33,34,71,72,97,103-105} while these inserters are poor at extending from the mismatched termini opposite 64PPs. On the other hand, another extender polymerase for TLS, polk, shows the inability to extend from the terminal nucleotides

inserted opposite the 3'-base of 64PPs⁹⁰ suggesting that polk makes little contribution to the TLS of 64PP, which was confirmed by a genetic study.¹⁰⁴

The contribution of another TLS polymerase REV1 is unique. REV1 plays a significant role in bypassing 64PPs,^{89,97,103} whereas its polymerase activity seems dispensable for the TLS reaction.^{78,79} It was reported that REV1 deficiency induced frameshift mutations with insertion or deletion of a nucleotide or two at 64PP sites, suggesting a role of REV1 in maintaining the frame fidelity of the TLS reaction on UV-damaged templates.¹⁰³ REV1 is known to interact with a variety of DNA replication and TLS factors through its several specific domains such as the N-terminal BRCT domain,⁸⁴ polymerase-associated domain (PAD),^{83,85,86} ubiquitin-binding motif (UBM),^{106,107} and conserved C-terminal domain (CTD),^{81,108} and each domain has been shown to be involved in DNA damage tolerance and mutagenesis after UV exposure.^{78,79,109,110} It was also shown that REV1 works for TLS in two different steps of replication of damaged DNA: one is in the replication fork stalling at damaged template DNA (replicative TLS) and the other is in single-strand gaps left at damaged sites behind the replication fork progression (gap-filling or postreplicative TLS).⁸⁹ The N-terminal BRCT domain of REV1 seems to be more involved in the replicative TLS,⁸⁹ and has been shown to interact physically with PCNA irrespective of UV irradiation,⁸⁴ whereas the CTD has been suggested to be necessary to the postreplicative or both the modes of TLS.¹⁰⁹ Pol ζ is known to interact with REV1 through this CTD,^{81,109} and has been shown to work preferentially in the postreplicative TLS.¹¹¹ 64PPs seem to be bypassed mainly through the pol ζ -dependent postreplicative TLS with the help of REV1,^{88,89,111} whereas most of CPDs are bypassed through the replicative TLS, which should be performed by pol η alone or by combinations of replicative and TLS polymerases, probably, with the help of REV1,^{88,89} as already mentioned above. These same polymerase combinations could work more effectively in the postreplicative TLS of CPDs that remain to be bypassed after DNA replication, because Rev1 protein is expressed at levels 50-fold higher in G2 and M phases than in G1 and most S phases of cell cycle in yeast.¹¹² Actually, the yeast RAD6-dependent DNA damage tolerance pathway, which includes pol ζ /Rev1-mediated error-prone TLS, has been shown to operate effectively in the G2/M phase uncoupled from replication forks.^{113,114}

Mutagenicity of Dewar isomer

Daylight UV has a broad wavelength range from UVB to UVA (290–400 nm). The shorter wavelength portion could produce 64PPs in the cellular genome, most of which, however, should be transformed subsequently into Dewar isomers by longer wavelength components (~325 nm) of the same daylight UV resulting in a large amount of Dewar isomers and residual 64PPs after solar UV irradiation.^{115,116}

Thus, the Dewar isomer is likely to be one of the important types of DNA damage involved in the solar UV genotoxicity. However, the genotoxic properties of Dewar isomers have been less explored compared with CPD and 64PP. Dewar isomers destabilize DNA duplexes as much as 64PPs, which distort DNA more severely than CPDs.¹¹⁷⁾ Accordingly, repair of Dewar isomers is as rapid as 64PP and in contrast to the slow removal of CPD from the cellular genome, as evidenced in a study with human keratinocytes using HPLC together with tandem mass spectrometry.¹¹⁸⁾

Reverse-genetic studies in *E. coli* with reporter DNA constructs possessing a site-specific UV lesion showed that 64PPs and Dewar isomers formed at TT sequences in DNA could frequently induce T → C transitions at the 3' side of TT dipyrimidines,^{29,119,120)} at which, however, only small numbers of mutations were observed in mammalian genetic studies *in vivo* after UVB irradiation,^{46–48)} which produces both 64PPs and Dewar isomers in the skin genome. Moreover, most of those *in vivo* mutations induced at TT sites were T → A transversions,^{47,48)} which would be more likely induced by CPDs formed at TT sites, as shown before.³⁰⁾ In addition, a duplex DNA containing a TT-Dewar isomer is more stable when the Dewar isomer pairs with AA than with 5'-GA-3' sequences, supporting the low recovery of T → C mutations at TT sites after UV irradiation.¹²¹⁾ Another reverse-genetic study also showed that 64PPs and Dewar isomers formed at 5'-TC-3' sequences in DNA could induce C → T transitions most frequently at the 3' side of the dipyrimidine,³¹⁾ which seems consistent not only with the UV signature but also with spectra of the *in vivo* UVB-induced mutation.^{46–48)} A yeast genetic study using a solar simulator, which should induce more Dewar isomers than 64PPs in DNA, suggested that the mutagenic bypass of Dewar isomers depends not on polη but on polζ.¹²²⁾

Non-UV-signature mutation—triplet mutation

We reported that a class of UV-specific mutations, the triplet mutation, which is separate from the UV signature, is induced in mouse skin after UVB irradiation especially remarkably in the genetic backgrounds of NER deficiency.^{123–126)} Triplet mutations were also detected in a retrospective search among previous reports on studies of the UV-induced mutation spectra in mammalian cells and *p53* mutations in skin cancers.¹²⁷⁾ The triplet mutation is defined as a multiple base substitution or frameshift within a three-nucleotide (triplet) sequence which includes a dipyrimidine sequence.^{123,127)} Most of the triplet mutations detected in those studies occurred at triplet sequences with a dipyrimidine at their 3'-side two bases.¹²⁷⁾ Triplet mutations appeared notably in genetic backgrounds and physiological conditions leading to NER deficiency¹²⁷⁾ suggesting that UV-induced DNA damage that causes triplet mutations should be repairable by NER. Since CPD is a relatively poor substrate for NER in mammalian cells,¹²⁸⁾ it would be excluded, at least,

from candidates of major causative damage, although a minor contribution could not be ruled out. On the other hand, it is highly probable that 64PPs and/or Dewar isomers are the main causative DNA damage since these photolisions are removed rapidly from damaged DNA in mammalian cells¹¹⁸⁾ (Table 1). We have proposed a model for the induction mechanism of the triplet mutation based on error-prone TLS opposite UV photolisions (Fig. 2).¹²⁷⁾ This model supposes that misinsertions by the TLS could occur not only opposite the photolisions but also opposite the undamaged template base one-nucleotide downstream from the lesions, and predicts that errors could occur at the extension step as well as at the insertion step in the two-step model of UV mutagenesis mentioned above,^{94–96)} which is consistent with the error-prone character of TLS polymerases specializing in the extension, polζ and polκ.^{71,90)} In addition, the pattern of base changes observed in triplet mutations suggests that the error-prone TLS causing those mutations largely follows the A-rule,^{63,64,127)} which is also consistent with the preference of polζ for a terminal mismatched nucleotide to extend from.⁶⁵⁾

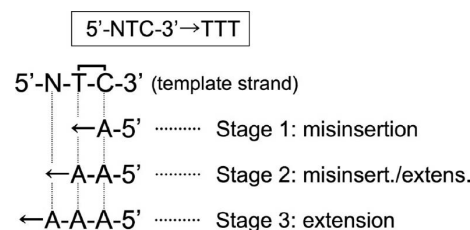


Fig. 2. Error-prone TLS model for triplet mutation formation. A mutation of 5'-NTC-3' to TTT is shown as an example of triplet mutations (N: any nucleotide). The 5'-NTC-3' triplet is on the template strand and a UV photolision resides at the TC dipyrimidine (bracket). A daughter strand is synthesized opposite the UV lesion by TLS polymerases from the right to the left in the figure, and the lesion is bypassed in the two steps of misinsertion and extension (stage 1 to 3). As shown, errors could happen not only at the insertion steps opposite the lesion (stages 1 and 2) but also at the extension step opposite the undamaged base directly downstream of the lesion (stage 3).

UVA-INDUCED MUTATIONS

UVA-induced CPD

It has been believed that the contribution of ROS, which could be produced by UV-induced oxidative stress as already mentioned,^{15,16)} to UVA-induced mutations should be considerable, because the direct absorption of the photon energy by DNA is extremely low at this wavelength range.⁶⁾ However, the assumption has been challenged since CPD formation by UVA, even if it is the longer low-energy component of UVA1, was demonstrated clearly for cellular and skin

DNA in several independent studies.^{129–134)} UVA1 induces remarkable amounts of CPDs and 8OH-Gs, but no detectable amounts of 64PPs or Dewar isomers.^{131–134)} UVA1 forms CPDs most preferably at TT dipyrimidine sites and also at TC or CT sites with lower efficiencies, but scarcely at CC sites.^{131,133)}

UVA-induced mutation in vitro

UVA-induced mutations in mammalian cells have been studied using cultured cells by several groups,^{135–138)} which, however, reported mutation spectra different from one another, bringing about controversies. Three different types of base substitution characterized the UVA-induced mutation spectrum that each group insisted on. One of those base substitutions is T → G transversion, which was detected most frequently together with the less frequent UV-signature mutation after UVA irradiation and designated as “UVA fingerprint” because it was so unique as to be rarely induced by other mutagenic agents and conditions.¹³⁵⁾ Detection of the UVA fingerprint mutations was also reported in a study with human skin tumors.¹³⁹⁾ However, T → G transversions were hardly detected in the UVA-induced mutation spectra by other groups, and the mechanism by which they are induced remains unclear, although 8OH-dGTP, one of the oxidative forms of cellular nucleotides, might have induced such mutations (Table 1).^{20,21)} Another type of the characteristic UVA-induced base substitution was G → T transversion, whose induction was observed at a similar frequency as the UV signature mutations after UVA1 exposure.¹³⁶⁾ This result strongly supported a major contribution of ROS to the UVA-mediated mutagenesis because G → T substitution is a representative ROS-specific mutation known to be induced by one of the most mutagenic types of oxidative DNA base damage, 8OH-G.²¹⁾ However, the third group of the UVA-induced mutation spectra was characterized exclusively by C → T base substitutions at dipyrimidine sites, namely UV signature mutations,^{137,138)} supporting the major contribution of CPD, the only known UV-specific DNA photolesion induced remarkably in the UVA range,^{131,133)} to the UVA-induced mutation.

These discrepancies in the UVA-induced mutation spectrum among the research groups might result from differences in the UVA sources used or in the cell lines used, but more probably could originate from differences in the conditions of the cell cultures such as the ingredients of culture media. Actually, an increase in the concentration of riboflavin, one of the representative endogenous UV photosensitizers, in the media elevated the formation of 8OH-G, the frequency of mutation, and the ratio of G → T transversion in the mutation spectrum in cells after UVA1 irradiation, all of which were counteracted by the addition of a strong antioxidant, vitamin C.^{140,141)} This observation indicates that the UVA-induced mutation spectrum could be easily changed by variations in the contents of the medium ingredients and sug-

gests that the controversies concerning the UVA-induced mutations will not be resolved as long as cultured cells are used for the study, relying on culture media composed of artificial ingredients.

UVA-induced mutation in vivo

To avoid the improper effects of artificial photosensitizers and antioxidants, *in vivo* evaluations of the UVA-induced mutation spectrum directly in the animal skin were conducted. We studied UVA-induced mutations in mouse skin epidermis using black light lamps at first, and reported a mutation spectrum overwhelmed by the solar-UV signature mutations.⁴⁹⁾ However, the black light lamps emit not only UVA but also a small amount of UVB, although we cut off most of the UVB components with a filter. The possibility that a small amount of the residual UVB affected the mutation spectrum could not be excluded. Therefore, we replaced the UVA source with a UVA1 laser, which produces a pure wavelength output of 364 nm without contamination by any other wavelengths, and showed that UVA1 induces the UV-specific mutations, especially the solar-UV signature, but not any ROS-mediated mutations, although the formations of both CPD and 8OH-G were induced in the skin genome after irradiation.¹³⁴⁾ This study indicated that UVA indeed induces oxidative stress *in vivo* and makes ROS-mediated DNA damage in the skin, which, however, fails to induce mutations, probably because of the rapid removal of this oxidative base damage from the affected genome by DNA repair, as shown before.¹⁴²⁾ On the other hand, CPDs are known to be relatively hard to remove from the genome overall by the mammalian DNA repair system,¹⁴³⁾ which probably led to the notable induction of UV-signature mutations in the skin genome after UVA1 irradiation in our study. Thus, UVA, even if it is the longer wavelength UVA1, induces solar-UV signature mutations, but not the oxidative types, *in vivo* in mammalian skin (Table 1), which, however, should be taken with caution because extremely high doses were needed in the case of UVA1 to induce sufficient mutations to evaluate¹³⁴⁾ suggesting that UVA1 is a weak environmental mutagen.

Whereas the acute mutagenic effects of UVA have been widely studied, the influence of chronic exposure, which might enhance oxidative stress, should be also evaluated. A mutation spectrum of the *p53* gene in skin cancers induced experimentally by chronic exposure to UVA1 was studied using albino hairless mice, and was found to show exclusively a single hotspot mutation of a solar-UV signature.¹⁴⁴⁾ It was reported previously that UVA induced malignant melanomas in fish skin,⁴⁾ in which photosensitized melanin radicals, which would not be produced in albino mice, were produced by the irradiation suggesting a major role of the UVA-induced oxidative stress in the carcinogenesis of melanoma.¹⁴⁵⁾ Another group, however, provided recently persuasive evidence that UVA makes no significant contribution to

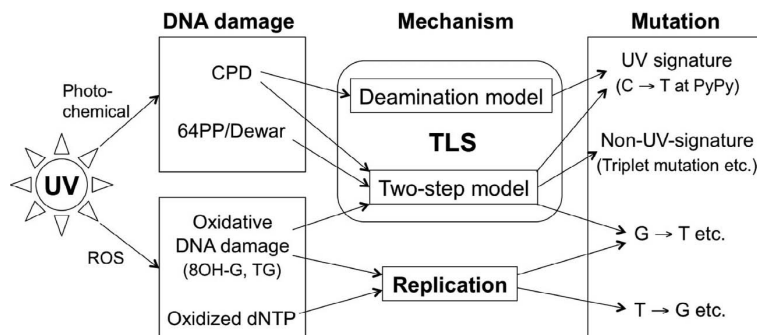


Fig. 3. Schematic overview of the mechanism of UV mutagenesis. DNA lesions UV is thought to produce are linked to the mutations detected after UV exposure in previous studies, mediated by possible intervening molecular mechanisms. The details of the deamination and two-step models are given in Fig. 1 and Fig. 2, respectively. The mutations mediated through replication could occur by incorporating incorrect nucleotides opposite damaged bases or damaged nucleotides opposite intact but mismatched template bases because of an altered base-pairing ability with the damaged molecules. TG, thymine glycol; PyPy, dipyrimidine.

the melanogenesis in pigmented fish skin.¹⁴⁶⁾ These results may suggest again a major contribution of CPD to chronic UVA genotoxicity.

CONCLUSION

It has been known that UV could exert its genotoxicity through direct photochemical and indirect ROS-mediated pathways, which produce mutagenic DNA damage such as CPD and 8OH-G, respectively. However, we have now learned that the former pathway seems to dominate all over the UV wavelengths at least in normal cells and healthy skin. Mutagenesis with such directly produced DNA damage is mainly mediated through mechanisms based on TLS, for which two models have been proposed: the model of “error-free” bypass of a deaminated cytosine-containing CPD by polη, and the model of error-prone two-step bypass, which consists of misinsertion and (mis)extension steps, by multiple TLS/replicative polymerases. Although the former model could explain the UV-signature mutation, the latter two-step model should also contribute because polη is not necessary to induce the mutation. Moreover, the two-step model should be relevant to the mutagenicity of 64PP and Dewar isomer, and could explain the triplet mutation, one of the UV-specific non-UV-signature mutations, although the contributing polymerases and causative DNA damage remain to be clearly identified. Wavelength influences the spectrum of UV-induced mutation as evidenced by the specifically frequent recovery of the solar-UV signature after exposure to UVB and UVA. These wavelengths comprising the solar UV induce CPDs preferentially at methylated cytosine-associated dipyrimidine sites as well as at TT dipyrimidine sites, resulting in the solar signature. However, the mechanism of the wavelength-dependent formation of the

site-specific CPDs has not been solved yet. A schematic overview of the mechanism of UV mutagenesis is given in Fig. 3.

REFERENCES

1. Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proc Natl Acad Sci USA* **71**: 3363–3366.
2. Brash DE, *et al* (1991) A role for sunlight in skin cancer: UV-induced *p53* mutations in squamous cell carcinoma. *Proc Natl Acad Sci USA* **88**: 10124–10128.
3. De Gruijl FR and Van der Leun JC (1994) Estimate the wavelength dependency of ultraviolet carcinogenesis in humans and its relevance to the risk assessment of a stratospheric ozone depletion. *Health Phys* **67**: 319–325.
4. Setlow RB, *et al* (1993) Wavelengths effective in induction of malignant melanoma. *Proc Natl Acad Sci USA* **90**: 6666–6670.
5. De Gruijl FR (1999) Skin cancer and solar UV radiation. *Eur J Cancer* **35**: 2003–2009.
6. De Gruijl FR (2000) Photocarcinogenesis: UVA vs UVB. *Methods Enzymol* **319**: 359–366.
7. Markovitsi D, Gustavsson T and Banyasz A (2010) Absorption of UV radiation by DNA: spatial and temporal features. *Mutat Res* **704**: 21–28.
8. Johns HE, *et al* (1964) The ultraviolet photochemistry of thymidyl-(3'→5')-thymidine. *J Mol Biol* **9**: 503–524.
9. Taylor J-S and Cohrs MP (1987) DNA, light, and Dewar pyrimidones: the structure and biological significance of TpT3. *J Am Chem Soc* **109**: 2834–2835.
10. Matsunaga T, Kotaro H and Nikaido O (1991) Wavelength dependent formation of thymine dimers and (6-4)photoproducts in DNA by monochromatic ultraviolet light ranging from 150 to 365 nm. *Photochem Photobiol* **54**: 403–410.
11. Peak JG, Peak MJ and MacCoss M (1984) DNA breakage caused by 334-nm ultraviolet light is enhanced by naturally

- occurring nucleic acid components and nucleotide coenzymes. *Photochem Photobiol* **39**: 713–716.
12. Walrant P and Santus R (1974) *n*-Formyl-kynurenine, a tryptophan photooxidation product, as a photodynamic sensitizer. *Photochem Photobiol* **19**: 411–417.
 13. McCormick JP, *et al* (1976) Characterization of a cell-lethal product from the photooxidation of tryptophan: hydrogen peroxide. *Science* **191**: 468–469.
 14. Krasnovsky Jr AA (1979) Photoluminescence of singlet oxygen in pigment solutions. *Photochem Photobiol* **29**: 29–36.
 15. Peak MJ and Peak JG (1987) Photosensitized DNA damages. *Photochem Photobiol* **45**: 57S.
 16. Tyrrell RM (2000) Role for singlet oxygen in biological effects of ultraviolet A radiation. *Methods Enzymol* **319**: 290–296.
 17. Kielbassa C, Roza L and Epe B (1997) Wavelength dependence of oxidative DNA damage induced by UV and visible light. *Carcinogenesis* **18**: 811–816.
 18. Kvam E and Tyrell R (1997) Induction of oxidative DNA base damage in human skin cells by UV and near visible radiation. *Carcinogenesis* **18**: 2379–2384.
 19. Kino K and Sugiyama H (2005) UVR-induced G-C to C-G transversions from oxidative DNA damage. *Mutat Res* **571**: 33–42.
 20. Sekiguchi M and Tsuzuki T (2002) Oxidative nucleotide damage: consequences and prevention. *Oncogene* **21**: 8895–8904.
 21. Grollman AP and Moriya M (1993) Mutagenesis by 8-oxoguanine: an enemy within. *Trends Genet* **9**: 246–279.
 22. Tessman I and Kennedy MA (1991) The two-step model of UV mutagenesis reassessed: deamination of cytosine in cyclobutane dimers as the likely source of the mutations associated with photoreactivation. *Mol Gen Genet* **277**: 144–148.
 23. Tessman I, Liu S and Kennedy MA (1992) Mechanism of SOS mutagenesis of UV-irradiated DNA: mostly error-free processing of deaminated cytosine. *Proc Natl Acad Sci USA* **89**: 1159–1163.
 24. Barak Y, Cohen-Fix O and Livneh Z (1995) Deamination of cytosine-containing pyrimidine photodimers in UV-irradiated DNA. *J Biol Chem* **41**: 24174–24179.
 25. Peng W and Shaw BR (1996) Accelerated deamination of cytosine residues in UV-induced cyclobutane pyrimidine dimers leads to CC → TT transitions. *Biochemistry* **35**: 10172–10181.
 26. Tu Y, Dammann R and Pfeifer GP (1998) Sequence and time-dependent deamination of cytosine bases in UVB-induced cyclobutane pyrimidine dimers *in vivo*. *J Mol Biol* **284**: 297–311.
 27. Burger A, *et al* (2003) *In vivo* deamination of cytosine-containing cyclobutane dimers in *E. coli*: a feasible part of UV-mutagenesis. *Mutat Res* **522**: 145–156.
 28. Federico LA, Kunkel TA and Shaw BR (1990) A sensitive genetic assay for the detection of cytosine deamination: determination of rate constants and the activation energy. *Biochemistry* **29**: 2532–2537.
 29. LeClerc JE, Borden A and Lawrence CW (1991) The thymine-thymine pyrimidine-pyrimidone(6-4) ultraviolet light photoproduct is highly mutagenic and specifically induces 3' thymine-to-cytosine transitions in *Escherichia coli*. *Proc Natl Acad Sci USA* **88**: 9685–9689.
 30. Banerjee SK, *et al* (1988) Frequency and spectrum of mutations produced by a single cis-syn thymine-thymine cyclobutane dimer in a single-stranded vector. *Proc Natl Acad Sci USA* **85**: 8141–8145.
 31. Horsfall MJ and Lawrence CW (1994) Accuracy of Replication past the T-C (6-4) adduct. *J Mol Biol* **235**: 465–471.
 32. Johnson RE, Prakash S and Prakash L (1999) Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, pol η . *Science* **283**: 1001–1004.
 33. Masutani C, *et al* (1999) Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity. *EMBO J* **18**: 3491–3501.
 34. Masutani C, *et al* (2000) Mechanisms of accurate translesion synthesis by human DNA polymerase η . *EMBO J* **19**: 3100–3109.
 35. McCulloch SD, *et al* (2004) Preferential *cis-syn* thymine dimer bypass by DNA polymerase η occurs with biased fidelity. *Nature* **428**: 97–100.
 36. Yoon J, Prakash L and Prakash S (2009) Highly error-free role of DNA polymerase η in the replicative bypass of UV-induced pyrimidine dimers in mouse and human cells. *Proc Natl Acad Sci USA* **106**: 18219–18224.
 37. Kemmink J, *et al* (1987) ^1H NMR study of the exchangeable protons of the duplex d(GCGT $^{\wedge}$ TGCG).d(CGCAACGC) containing a thymine photodimer. *Nucleic Acids Res* **15**: 4645–4653.
 38. Drouin R and Therrien J-P (1997) UVB-induced cyclobutane pyrimidine dimer frequency correlates with skin cancer mutational hotspots in *p53*. *Photochem Photobiol* **66**: 719–726.
 39. Tommasi S, Denissenko MF and Pfeifer GP (1997) Sunlight induces pyrimidine dimers preferentially at 5-methylcytosine bases. *Cancer Res* **57**: 4727–4730.
 40. Tornaletti S, Rozek D and Pfeifer GP (1993) The distribution of UV photoproducts along the human *p53* gene and its relation to mutations in skin cancer. *Oncogene* **8**: 2051–2057.
 41. Monti P, *et al* (1999) 5-methylcytosine at *HpaII* sites in *p53* is not hypermutable after UVC irradiation. *Mutat Res* **431**: 93–103.
 42. Grünwald S and Pfeifer GP (1989) Enzymatic DNA methylation. *Prog Clin Biochem Med* **9**: 61–103.
 43. Ziegler A, *et al* (1993) Mutation hotspots due to sunlight in the *p53* gene of nonmelanoma skin cancers. *Proc Natl Acad Sci USA* **90**: 4216–4220.
 44. You Y, Li C and Pfeifer GP (1999) Involvement of 5-methylcytosine in sunlight-induced mutagenesis. *J Mol Biol* **293**: 493–503.
 45. Ikehata H and Ono T (2007) Significance of CpG methylation for solar UV-induced mutagenesis and carcinogenesis in skin. *Photochem Photobiol* **83**: 196–204.
 46. Frijhoff AFW, *et al* (1997) UVB-induced mutagenesis in hairless *lacZ*-transgenic mice. *Environ Mol Mutagen* **29**: 136–142.
 47. Horiguchi M, *et al* (1999) UVB-induced *gpt* mutations in the

- skin of *gpt* delta transgenic mice. *Environ Mol Mutagen* **34**: 72–79.
48. Ikehata H, *et al* (2003) Analysis of mutation spectra in UVB-exposed mouse skin epidermis and dermis: frequent occurrence of C → T transition at methylated CpG-associated dipyrimidine sites. *Environ Mol Mutagen* **41**: 280–292.
 49. Ikehata H, *et al* (2003) UVA induces C → T transitions at methyl CpG-associated dipyrimidine sites in mouse skin epidermis more frequently than UVB. *Mutagenesis* **18**: 511–519.
 50. Ikehata H, *et al* (2004) Mutation spectrum in sunlight-exposed skin epidermis: small but appreciable contribution of oxidative stress-induced mutagenesis. *Mutat Res* **556**: 11–24.
 51. Lee D and Pfeifer GP (2003) Deamination of 5-methylcytosines within cyclobutane pyrimidine dimers is an important component of UVB mutagenesis. *J Biol Chem* **278**: 10314–10321.
 52. You Y and Pfeifer GP (2001) Similarities in sunlight-induced mutational spectra of CpG-methylated transgenes and the *p53* gene in skin cancer point to an important role of 5-methylcytosine residues in solar UV mutagenesis. *J Mol Biol* **305**: 389–399.
 53. Miller JH (1985) Mutagenic specificity of ultraviolet light. *J Mol Biol* **182**: 45–68.
 54. Schaaper RM, Dunn RL and Glickman BW (1987) Mechanisms of ultraviolet-induced mutation: mutation spectra in the *Escherichia coli lacI* gene for a wild-type and an excision-repair-deficient strain. *J Mol Biol* **198**: 187–202.
 55. Stary A, *et al* (2003) Role of DNA polymerase η in the UV mutation spectrum in human cells. *J Biol Chem* **278**: 18767–18775.
 56. Choi J and Pfeifer GP (2005) The role of DNA polymerase η in UV mutational spectra. *DNA Repair* **4**: 211–220.
 57. Dumstorf CA, *et al* (2006) Participation of mouse DNA polymerase ι in strand-biased mutagenic bypass of UV photoproducts and suppression of skin cancer. *Proc Natl Acad Sci USA* **103**: 18083–18088.
 58. Gueranger Q, *et al* (2008) Role of DNA polymerases η , ι and ζ in UV resistance and UV-induced mutagenesis in a human cell line. *DNA Repair* **7**: 1551–1562.
 59. Kato T and Shinoura Y (1977) Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. *Molec Gen Genet* **156**: 121–131.
 60. Lawrence CW and Christensen R (1976) UV mutagenesis in radiation-sensitive strains of yeast. *Genetics* **82**: 207–232.
 61. Tang M, *et al* (1999) UmuD'2C is an error-prone DNA polymerase, *Escherichia coli* pol V. *Proc Natl Acad Sci USA* **96**: 8919–8924.
 62. Tang M, *et al* (2000) Roles of *E. coli* DNA polymerase IV and V in lesion-targeted and untargeted SOS mutagenesis. *Nature* **404**: 1014–1018.
 63. Strauss BS (1991) The 'A-rule' of mutagen specificity: a consequence of DNA polymerase bypass of non-instructional lesions? *Bioessays* **13**: 79–84.
 64. Taylor J-S (2002) New structural and mechanistic insight into the A-rule and the instructional and non-instructional behavior of DNA photoproducts and other lesions. *Mutat Res* **510**: 55–70.
 65. Haracska L, *et al* (2001) Role of yeast DNA polymerases δ and ζ and of Rev1 in the bypass of abasic sites. *Genes Dev* **15**: 945–954.
 66. McDonald JP, *et al* (1999) Novel human and mouse homologs of *Saccharomyces cerevisiae* DNA polymerase η . *Genomics* **60**: 20–30.
 67. Kannouche P, *et al* (2003) Localization of DNA polymerase η and ι to the replication machinery is tightly coordinated in human cells. *EMBO J* **22**: 1223–1233.
 68. Choi J, *et al* (2006) The role of DNA polymerase ι in UV mutational spectra. *Mutat Res* **599**: 58–65.
 69. McDonald JP, *et al* (2003) 129-derived strains of mice are deficient in DNA polymerase ι and have normal immunoglobulin hypermutation. *J Exp Med* **198**: 635–653.
 70. Ohkumo T, *et al* (2006) UV-B radiation induces epidermal tumors in mice lacking DNA polymerase η and mesenchymal tumors in mice deficient for DNA polymerase ι . *Mol Cell Biol* **26**: 7696–7706.
 71. Johnson RE, *et al* (2000) Eukaryotic polymerase ι and ζ act sequentially to bypass DNA lesions. *Nature* **406**: 1015–1019.
 72. Tissier A, *et al* (2000) Misinsertion and bypass of thymine-thymine dimers by human DNA polymerase ι . *EMBO J* **19**: 5259–5266.
 73. Larimer FW, Perry JR and Hardigree AA (1989) The *REV1* gene of *Saccharomyces cerevisiae*: isolation, sequence, and functional analysis. *J Bacteriol* **171**: 230–237.
 74. Gibbs PEM, *et al* (2000) The function of the human homolog of *Saccharomyces cerevisiae* *REV1* is required for mutagenesis induced by UV light. *Proc Natl Acad Sci USA* **97**: 4186–4191.
 75. Okada T, *et al* (2002) Involvement of vertebrate Pol κ in Rad18-independent postreplication repair of UV damage. *J Biol Chem* **277**: 48690–48695.
 76. Ziv O, *et al* (2009) DNA polymerase ζ cooperates with polymerases κ and ι in translesion DNA synthesis across pyrimidine photodimers in cells from XPV patients. *Proc Natl Acad Sci USA* **106**: 11552–11557.
 77. Nelson JR, Lawrence CW and Hinkle DC (1996) Deoxycytidyl transferase activity of yeast *REV1* protein. *Nature* **382**: 729–731.
 78. Otsuka C, *et al* (2005) Roles of the polymerase and BRCT domains of Rev1 protein in translesion DNA synthesis in yeast *in vivo*. *Mutat Res* **578**: 79–87.
 79. Ross A-L, Simpson LJ and Sale JE (2005) Vertebrate DNA damage tolerance requires the C-terminus but not BRCT or transferase domains of REV1. *Nucleic Acids Res* **33**: 1280–1289.
 80. Masuda Y, *et al* (2003) Structural and enzymatic properties of a stable complex of the human REV1 and REV7 proteins. *J Biol Chem* **278**: 12356–12360.
 81. Guo C, *et al* (2003) Mouse Rev1 protein interacts with multiple DNA polymerases involved in translesion DNA synthesis. *EMBO J* **22**: 6621–6630.
 82. Tisser A, *et al* (2004) Co-localization foci and interaction of human Y-family members, DNA polymerase pol η and REV1 protein. *DNA Repair* **3**: 1503–1514.
 83. Acharya N, *et al* (2005) Complex formation of yeast Rev1

- and Rev7 proteins: a novel role for the polymerase-associated domain. *Mol Cell Biol* **25**: 9734–9740.
84. Guo C, *et al* (2006) REV1 protein interacts with PCNA: significance of the REV1 BRCT domain *in vitro* and *in vivo*. *Mol Cell* **23**: 265–271.
 85. Acharya N, *et al* (2007) Complex formation of yeast Rev1 with DNA polymerase η . *Mol Cell Biol* **27**: 8401–8408.
 86. Acharya N, *et al* (2009) Yeast Rev1 protein promotes complex formation of DNA polymerase ζ with Pol32 subunit of DNA polymerase δ . *Proc Natl Acad Sci USA* **106**: 9631–9636.
 87. Friedberg EC, Lehmann AR and Fuchs RPP (2005) Trading places: how do DNA polymerases switch during translesion DNA synthesis? *Mol Cell* **18**: 499–505.
 88. Shachar S, *et al* (2009) Two-polymerase mechanisms dictate error-free and error-prone translesion DNA synthesis in mammals. *EMBO J* **28**: 383–393.
 89. Jansen JG, *et al* (2009) Separate domains of Rev1 mediate two modes of DNA damage bypass in mammalian cells. *Mol Cell Biol* **29**: 3113–3123.
 90. Washington MT, *et al* (2002) Human *DINB1*-encoded DNA polymerase κ is a promiscuous extender of mispaired primer termini. *Proc Natl Acad Sci USA* **99**: 1910–1914.
 91. Johnson RE, Prakash S and Prakash L (2000) The human *DINB1* gene encodes the DNA polymerase Pol θ . *Proc Natl Acad Sci USA* **97**: 3838–3843.
 92. Ohashi E, *et al* (2000) Error-prone bypass of certain DNA lesions by the human DNA polymerase κ . *Genes Dev* **14**: 1589–1594.
 93. Guo D, *et al* (2001) Translesion synthesis by yeast DNA polymerase ζ from templates containing lesions of ultraviolet radiation and acetylaminofluorene. *Nucleic Acids Res* **29**: 2875–2883.
 94. Bridges B and Woodgate R (1985) The two-step model of bacterial UV mutagenesis. *Mutat Res* **150**: 133–139.
 95. Bridges B (2001) The two-step model for Translesion synthesis: then and now. *Mutat Res* **485**: 61–67.
 96. Woodgate R (2001) Evolution of the two-step model for UV-mutagenesis. *Mutat Res* **485**: 83–92.
 97. Gibbs PEM, *et al* (2005) The relative roles *in vivo* of *Saccharomyces cerevisiae* Pol η , Pol ζ , Rev1 protein and Pol23 in the bypass and mutation induction of an abasic site, T-T (6-4) photoproduct and T-T *cis-syn* cyclobutane dimer. *Genetics* **169**: 575–582.
 98. Wood RD (1985) Pyrimidine dimers are not the principal pre-mutagenic lesions induced in lambda phage DNA by ultraviolet light. *J Mol Biol* **184**: 577–585.
 99. Tanaka M, *et al* (2001) Effect of photoreactivation of cyclobutane pyrimidine dimers and pyrimidine(6-4)pyrimidone photoproducts on ultraviolet mutagenesis in SOS-induced repair-deficient *Escherichia coli*. *Mutagenesis* **16**: 1–6.
 100. Otsu E, *et al* (2000) Respective roles of cyclobutane pyrimidine dimers, (6-4)photoproducts, and minor photoproducts in ultraviolet mutagenesis of repair-deficient xeroderma pigmentosum A cells. *Cancer Res* **60**: 1729–1735.
 101. You Y, *et al* (2001) Cyclobutane pyrimidine dimers are responsible for the vast majority of mutations induced by UVB irradiation in mammalian cells. *J Biol Chem* **276**: 44688–44694.
 102. Nakajima S, *et al* (2004) UV light-induced DNA damage and tolerance for the survival of nucleotide excision repair-deficient human cells. *J Biol Chem* **279**: 46674–46677.
 103. Szüts D, *et al* (2008) REV1 restrains DNA polymerase ζ to ensure frame fidelity during translesion synthesis of UV photoproducts *in vivo*. *Nucl Acids Res* **36**: 6767–6780.
 104. Yoon J, Prakash L and Prakash S (2010) Error-free replicative bypass of (6-4) photoproducts by DNA polymerase ζ in mouse and human cells. *Genes Dev* **24**: 123–128.
 105. Johnson RE, *et al* (2001) Role of DNA polymerase η in the bypass of a (6-4) TT photoproduct. *Mol Cell Biol* **21**: 3558–3563.
 106. Guo C, *et al* (2006) Ubiquitin-binding motifs in REV1 protein are required for its role in the tolerance of DNA damage. *Mol Cell Biol* **26**: 8892–8900.
 107. Wood A, Garg P and Burgers PM (2007) A ubiquitin-binding motif in the translesion DNA polymerase Rev1 mediates its essential functional interaction with ubiquitinated proliferating cell nuclear antigen in response to DNA damage. *J Biol Chem* **282**: 20256–20263.
 108. D'Souza S and Walker GC (2006) Novel role for the C terminus of *Saccharomyces cerevisiae* Rev1 in mediating protein-protein interactions. *Mol Cell Biol* **26**: 8173–8182.
 109. D'Souza S, Waters LS and Walker GC (2008) Novel conserved motifs in Rev1 C-terminus are required for mutagenic DNA damage tolerance. *DNA Repair* **7**: 1455–1470.
 110. Jansen JG, *et al* (2005) The BRCT domain of mammalian Rev1 is involved in regulating DNA translesion synthesis. *Nucleic Acids Res* **33**: 356–365.
 111. Jansen JG, *et al* (2009) Mammalian polymerase ζ is essential for post-replication repair of UV-induced DNA lesions. *DNA Repair* **8**: 1444–1451.
 112. Waters LS and Walker GC (2006) The critical mutagenic translesion DNA polymerase Rev1 is highly expressed during G₂/M phase rather than S phase. *Proc Natl Acad Sci USA* **103**: 8971–8976.
 113. Kaarras GI and Jentsch S (2010) The *RAD6* DNA damage tolerance pathway operates uncoupled from the replication fork and is functional beyond S phase. *Cell* **141**: 255–267.
 114. Daigaku Y, Davies AA and Ulrich HD (2010) Ubiquitin-dependent DNA damage bypass is separable from genome replication. *Nature* **465**: 951–955.
 115. Clingen PH, *et al* (1995) Induction of cyclobutane pyrimidine dimers, pyrimidine(6-4)pyrimidone photoproducts, and Dewar valence isomers by natural sunlight in normal human mononuclear cells. *Cancer Res* **55**: 2245–2248.
 116. Douki T, *et al* (2003) Bipyrimidine photoproducts rather than oxidative lesions are the main type DNA damage involved in the genotoxic effect of solar UVA radiation. *Biochemistry* **42**: 9221–9226.
 117. Jing Y, Kao JFL and Taylor J-S (1998) Thermodynamic and base-pairing studies of matched and mismatched DNA dodecamer duplexes containing *cis-syn*, (6-4) and Dewar photoproducts of TT. *Nucleic Acids Res* **26**: 3845–3853.
 118. Courdavault S, *et al* (2005) Repair of the three main types of bipyrimidine DNA photoproducts in human keratinocytes exposed to UVB and UVA radiations. *DNA Repair* **4**: 836–

- 844.
119. Smith CA, *et al* (1996) Mutation spectra of M13 vectors containing site-specific *cis-syn*, *trans-syn*-I, (6-4), and Dewar pyrimidone photoproducts of thymidyl(3'5')-thymidine in *Escherichia coli* under SOS conditions. *Biochemistry* **35**: 4146–4154.
120. Kamiya H, Iwai S and Kasai H (1998) The (6-4) photoproduct of thymine-thymine induces targeted substitution mutations in mammalian cells. *Nucleic Acids Res* **26**: 2611–2617.
121. Lee J, Bae S and Choi B (2000) The Dewar photoproduct of thymidyl(3'5')thymidine (Dewar product) exhibits mutagenic behavior in accordance with the “A-rule”. *Proc Natl Acad Sci USA* **97**: 4591–4596.
122. Kozmin SG, *et al* (2003) Roles of *Saccharomyces cerevisiae* DNA polymerases pol η and pol ζ in response to irradiation by simulated sunlight. *Nucleic Acids Res* **31**: 4541–4552.
123. Wang F, *et al* (2006) Mutation spectrum in UVB-exposed skin epidermis of a mildly-affected *Xpg*-deficient mouse. *Environ Mol Mutagen* **47**: 107–116.
124. Ikehata H, *et al* (2007) Mutation spectrum in UVB-exposed skin epidermis of *Xpa*-knockout mice: frequent recovery of triplet mutations. *Environ Mol Mutagen* **48**: 1–13.
125. Ikehata H, *et al* (2007) Frequent recovery of triplet mutations in UVB-exposed skin epidermis of *Xpc*-knockout mice. *DNA Repair* **6**: 82–93.
126. Ikehata H, *et al* (2010) Influences of p53 deficiency on the apoptotic response, DNA damage removal and mutagenesis in UVB-exposed mouse skin. *Mutagenesis* **25**: 397–405.
127. Ikehata H, *et al* (2007) A model for triplet mutation formation based on error-prone translesional DNA synthesis opposite UV photolesions. *DNA Repair* **6**: 658–668.
128. Nairn RS, *et al* (1989) UV mutagenesis, cytotoxicity and split-dose recovery in a human-CHO cell hybrid having intermediate (64) photoproduct repair. *Mutat Res* **217**: 33–38.
129. Kuluncsics Z, *et al* (1999) Wavelength dependence of ultraviolet-induced DNA damage distribution: involvement of direct or indirect mechanisms and possible artefacts. *J Photochem Photobiol B: Biol* **49**: 71–80.
130. Rochette PJ, *et al* (2003) UVA-induced cyclobutane pyrimidine dimers form predominantly at thymine-thymine dipyrimidines and correlate with the mutation spectrum in rodent cells. *Nucleic Acids Res* **31**: 2786–2794.
131. Courdavault S, *et al* (2004) Larger yield of cyclobutane dimers than 8-oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells. *Mutat Res* **556**: 135–142.
132. Besaratinia A, *et al* (2005) DNA lesions induced by UV A1 and B radiation in human cells: comparative analyses in the overall genome and in the p53 tumor suppressor gene. *Proc Natl Acad Sci USA* **102**: 10058–10063.
133. Mouret S, *et al* (2006) Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proc Natl Acad Sci USA* **103**: 13765–13770.
134. Ikehata H, *et al* (2008) UVA1 genotoxicity is mediated not by oxidative damage but by cyclobutane pyrimidine dimers in normal mouse skin. *J Invest Dermatol* **128**: 2289–2296.
135. Drobetsky EA, Turcotte J and Châteauneuf A (1995) A role for ultraviolet A in solar mutagenesis. *Proc Natl Acad Sci USA* **92**: 2350–2354.
136. Besaratinia A, *et al* (2004) G-to-T transversions and small tandem base deletions are the hallmark of mutations induced by ultraviolet A radiation in mammalian cells. *Biochemistry* **43**: 8169–8177.
137. Robert C, *et al* (1996) Cell survival and shuttle vector mutagenesis induced by ultraviolet A and ultraviolet B radiation in a human cell line. *J Invest Dermatol* **106**: 721–728.
138. Kappes UP, *et al* (2006) Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cells. *J Invest Dermatol* **126**: 667–675.
139. Ager NS, *et al* (2004) The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc Natl Acad Sci USA* **101**: 4954–4959.
140. Besaratinia A, *et al* (2007) Riboflavin activated by ultraviolet A1 irradiation induces oxidative DNA damage-mediated mutations inhibited by vitamin C. *Proc Natl Acad Sci USA* **104**: 5953–5958.
141. Lutsenko EA, Cárcamo JM and Golde DW (2002) Vitamin C prevents DNA mutation induced by oxidative stress. *J Biol Chem* **277**: 16895–16899.
142. Besaratinia A, Kim S and Pfeifer GP (2008) Rapid repair of UVA-induced oxidized purines and persistence of UVB-induced dipyrimidine lesions determine the mutagenicity of sunlight in mouse cells. *FASEB J* **22**: 2379–2392.
143. Bohr WA, *et al* (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell* **40**: 359–369.
144. Van Kranen HJ, *et al* (1997) Low incidence of p53 mutations in UVA (365-nm)-induced skin tumors in hairless mice. *Cancer Res* **57**: 1238–1240.
145. Wood SR, *et al* (2006) UV causation of melanoma in *Xiphophorus* is dominated by melanin photosensitized oxidant production. *Proc Natl Acad Sci USA* **103**: 4111–4115.
146. Mitchell DL, *et al* (2010) Ultraviolet A does not induce melanomas in a *Xiphophorus* hybrid fish model. *Proc Natl Acad Sci USA* **107**: 9329–9334.

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