Mechanistic considerations on the wavelength-dependent variations of UVR genotoxicity and mutagenesis in skin: Discrimination of UVA-signature from UV-signature mutation

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Abstract

Ultraviolet radiation (UVR) predominantly induces UV-signature mutations, C → T and CC → TT base substitutions at dipyrimidine sites, in the cellular and skin genome, although these UVR-specific mutations show a wavelength-dependent variation in their sequence-context preference, as evidenced by our *in vivo* mutation studies of mouse skin. The C → T mutation occurs most frequently in the 5'-TCG-3' context regardless of the UVR wavelength, but is recovered more preferentially there as the wavelength increases, resulting in prominent occurrences exclusively at the TCG context in the UVA wavelength range, which I will designate as a "UVA signature" in this review. The preference of the UVB-induced C → T mutation for the sequence contexts shows a mixed pattern of UVC- and UVA-induced mutations, and a preference pattern similar to the UVB-induced one is also observed for natural sunlight, in which UVB is the most genotoxic component. In addition, the CC → TT mutation hardly occurs at UVA1 wavelengths, although it is detected rarely but constantly in the UVC and UVB ranges. These wavelength-dependent, sequence-context preferences of the UVR-specific mutations could be explained by two different photochemical mechanisms of cyclobutane pyrimidine dimer (CPD) formation. The UV-signature mutations observed in the UVC and UVB ranges are known to occur mainly through error-free translesion DNA synthesis (TLS) by DNA polymerase η across deaminated cytosines in CPDs, which are produced through the conventional singlet/triplet excitation of pyrimidine bases by the direct absorption of UVC/UVB photon energy in those bases. On the other hand, a novel photochemical mechanism through the direct absorption of UVA energy to double-stranded DNA, which is called "collective excitation", has been proposed for the UVA-induced CPD formation. The UVA photons directly absorbed by DNA cause

CPD formation with a sequence context preference different from those caused by the UVC/UVB-mediated singlet/triplet excitation, producing CPDs preferentially at thymine-containing dipyrimidine sites, and probably also preferably at methyl CpG-associated dipyrimidine sites. Cytosine deamination in these CPDs, which is known to be accelerated for CPDs formed at the TCG context, can lead to the UVA-signature mutations through the DNA polymerase η-dependent, error-free TLS.

Introduction

Action spectrum analysis of the mouse skin cancer induction by ultraviolet radiation (UVR), which was performed mainly by Jan C. van der Leun's group, clearly demonstrated that the genotoxicity of UVR for mammalian skin depends on the wavelength, and suggested that, although the UVB component plays a major role in the genotoxicity, UVA, the longer wavelength components of UVR (320–400 nm), also makes a small but distinct contribution.[1] The genotoxicity of UVR induces mutation in the skin genome, which can result in the carcinogenesis as evidenced by *p53* mutations in skin cancers in sun-exposed areas of human skin[2–5] and those experimentally induced in mouse skin.[6–11] The mutagenicity of UVR is derived from its ability to produce DNA damage by direct or indirect photochemical reactions with DNA and/or by indirect oxidative DNA modifications through the formation of reactive oxygen species (ROS).[12] The former reactions produce UVR-specific base photolesions such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts (64PPs) at dipyrimidine sites in DNA.[13] The latter oxidative modifications include single strand DNA breaks and the formation of oxidative base damage such as 8-hydroxyguanine (8OH-G).[14] The contribution of the oxidative DNA modification to the

UVR genotoxicity has been noticed especially for UVA, where the efficiency of photolesion production by direct photochemical reactions is reduced by several orders of magnitude compared to the shorter wavelengths of UVR.[12,14–16] However, quantitative and mechanistic analyses of UVA-induced CPD formation in the last two decades have provoked a reconsideration on the origin of the UVA genotoxicity.[17–25]

I have studied UVR-induced mutation spectra in mouse skin using a transgenic mouse strain with λ-phage vector-based, bacterial *lacZ*-transgenes, which were developed for mutation analysis, and a variety of UVR sources emitting different wavelength components from UVC to UVA (Fig. 1A).[26–31] In this review, I provide an updated overview of the wavelength-dependent UVR genotoxicity mainly based on the mutation spectra obtained by *in vivo* analyses, and propose a model explaining the mechanism of wavelength-dependent variations in the mutation spectra by combining some recent findings in DNA repair, photochemistry and photobiology.

Wavelength dependence of UVR-induced mutation spectra

UVR induces specific types of mutation in DNA as reported for phages,[32–34] bacteria,[35,36] yeasts,[37–39] mammalian cultured cells[40–50], and mammalian skin.[26–31,51,52] These UVR-specific mutation types include the C → T transition at dipyrimidine sites and CC → TT tandem base substitution, which are called collectively "UV signature" as discriminative mutations indicating the trace of UVR genotoxic insults.[2] All UVR components, UVC (wavelengths <280 nm), UVB (280–320 nm), UVA2 (320–340 nm) and UVA1 (340–400 nm), can induce the UV-signature mutations as demonstrated in our studies,[26–31,53] summarized in Fig. 1B and C, although the wavelengths in the UVA1 region hardly induced CC → TT mutations.[29,30] Our studies revealed that the C → T

transition at dipyrimidine sites is the dominant type for all UVR components, comprising 59–84% of total mutations observed after irradiation, demonstrating that UVR genotoxicity results mostly from DNA photolesions specifically produced by UVR.[54] On the other hand, the influence of UVR-produced ROS is not remarkable or, if any, minor in the UVR-induced mutation spectra, judging from the contribution of G → T transversion, a mutation that can be caused by 8OH-G, one of the representative types of oxidative DNA damage.[55] Only in the sunlight-induced spectrum, the G → T mutation was significantly induced, although it was a minor component,[28] which might suggest some contribution of non-UVR wavelengths included in sunlight to the skin genotoxicity as also observed in other studies with yeast and phage.[38,39,56] Some photodynamic reactions might be relevant. Moreover, it should be noted that UVA1 sources, both the broadband UVA1 lamps and narrowband UVA1 laser, did not induce oxidative damage-related mutations such as G → T and G → C transversions[57] at a remarkable frequency,[29,30] although a dose-dependent formation of 8OH-G was observed in the skin after UVA1 irradiation,[29] as observed in cultured cells.[15] It is also known that 8OH-Gs are removed from cellular DNA much faster than CPDs.[58] These observations strongly support that UVR exerts its genotoxicity to the skin mainly through direct photochemical reactions with DNA, irrespective of its wavelength component. In addition, the ROS-mediated genotoxicity by UVR should be studied with caution, especially in *in vitro* studies, because artificial ingredients in the DNA solvent or cell/tissue culture media could cause or promote the production of ROS upon UVR irradiation.[17,59] To avoid these artifacts, analyses *in vivo* such as in the skin would be preferable. This is one of the reasons I have excluded the cell-based studies from my consideration of the UVR-induced mutation spectra in this review, although some

important, contradicting points shown in those studies are discussed below. A more detailed discussion on the disadvantages of the use of cell-based, *in vitro* mutation assays for the study of mammalian UVR-induced mutation spectra has already been made.[60] However, most of the studies with skin mentioned above examined only the UVR genotoxicity for normal skin after an acute single exposure. Multiple/chronic UVR exposures or exposures of the skin under pathological conditions could bring a ROS-mediated genotoxicity in addition to the genotoxicity mediated by direct photochemical reactions with DNA. Interestingly, it has been demonstrated that CPDs can be produced by ROS generated from melanin derivatives chemically excited long after UVR exposure, which suggests that ROS could also induce UV-signature mutations.[61] However, melanocytes usually reside in the dermal layer in mouse skin, and melanin is poor in the mouse epidermal layer, so that such mutations induced by ROS-produced CPDs would be difficult to detect with the current *in vivo* mutation assay system using transgenic mice.

Mutation induction mechanism by UVR-induced photolesions

The molecular mechanism of the mutagenesis by UVR-specific photolesions has been studied widely and elucidated fairly well for some aspects.[54] The mutation induction by UVR requires replicative DNA synthesis after irradiation.[62–64] CPD and 64PP are both replication-blocking DNA damage, so that they should be removed by DNA repair before a replication fork encounters them,[65] or should be overcome by some damage tolerance mechanisms so that the replicational DNA synthesis can be continued over the damage site, because a failure in replication can lead to cell death.[66,67] One of the damage tolerance pathways could be a recombinational bypass of these photolesions by

detouring the damage on the template strand using the genetic information of the other, newly replicated daughter strand.[66,67] This pathway would be error-free, but should be too elaborate to perform the over-damage replication efficiently. Delay in DNA replication, which leads to delay in cell proliferation, could cause a deficiency in the recovery of damaged tissues. Another tolerance pathway is translesion DNA synthesis (TLS), which can pass directly over the lesions on the template strand with the help of specialized DNA polymerases, TLS polymerases.[54,68,69] In the TLS mechanism, replicative DNA polymerases switch to TLS polymerases upon an encounter with replication-blocking DNA damage, and the TLS polymerases continue DNA synthesis opposite the DNA damage, usually ignoring the base-pairing rule of nucleic acids. After the replication fork has passed across the damage, replicative polymerases take over the DNA synthesis in place of TLS polymerases and continue DNA replication. Thus, in the mechanism of damage tolerance by TLS, the DNA replication could be continued efficiently at the damage site in a manner sufficient to assist the recovery of damaged tissues, although the DNA synthesis by TLS would usually be error-prone. Actually, it was suggested that sites with repair-resistant CPDs in the *p53* gene are also frequently mutated sites in human skin cancers.[70]

Among the TLS polymerases, however, DNA polymerase η (polη) is exceptional. Polη can synthesize a daughter strand error-free across a CPD on the template strand, probably by using the base pairing ability remaining in CPDs,[71,72] thus rather suppressing mutation induction by CPDs. This error-free TLS ability of polη appears CPD-specific because polη can hardly bypass 64PPs or bypass other types of base damage less error-free than CPDs.[73–76] However, this polη-dependent error-free TLS itself causes the UVR-specific mutations. It is known that cytosines in CPDs are highly

prone to deaminate at position 4 and change easily to uracils (or a thymine if the cytosine is methylated at position 5), which results in the conversion of cytosine-containing CPDs to uracil or thymine-containing ones.[77–80] If a replication fork encounters such deaminated CPDs, the error-free TLS by polη should insert adenine opposite the deaminated cytosine, namely uracil or thymine, thus resulting in the induction of UVR-specific C → T and CC → TT mutations (Fig. 2). Since CPD has been demonstrated to be the main mutagenic UVR photolesion in normal mammalian cells and skin,[81,82] the error-free TLS opposite deaminated CPDs by polη should be the major pathway in the induction of UVR mutations in repair-proficient cells and skin.

In the absence of polη, UVR can induce mutations in cells and skin at much higher frequencies than in the presence, although the mutation spectrum still shows the UV signature predominantly.[53,83–86] This polη-independent UVR mutagenesis has been explained by a mechanistic model called the "two-step model", in which inserter and extender DNA polymerases are involved in the TLS.[54,87–89] These DNA polymerases might include polymerase ι, κ, ζ, Rev1 as well as η,[54,71,90,91] which are TLS polymerases, and replicative DNA polymerases such as δ.[92] 64PPs and Dewar isomers, as well as CPDs, could induce UV-signature mutations by this "two-step" mechanism because the base insertions opposite photolesions by this mechanism is supposed to occur according to the "A-rule", in which an adenine is inserted with the base pairing rule ignored.[54,93,94] Although strongly supportive genetic studies have been reported,[87–89] the two-step model for the mutagenesis with UVR photolesions is, however, still presumptive, awaiting experimental demonstrations by biochemically reconstituted systems. Another UVR-specific mutation that could be explained by the two-step model is the triplet mutation, a mutation with multiple base substitutions or frameshifts within

a three-nucleotide sequence that includes a dipyrimidine sequence.[54] The triplet mutations were detected frequently in UVB-exposed mouse skin deficient in the nucleotide excision repair,[95–98] whereas the same mutations have also been detected in other systems including mammalian cultured cells and skin cancers, although their frequencies are variable depending on their repair abilities.[99] The multiple base substitutions and frameshifts occurring around a dipyrimidine site are easy to explain by multiple misincorporations by inserter and extender DNA polymerases in the two-step model.[54]

Variation of sequence context preference of the UVR-specific C → T mutation by wavelength

Although the mutation spectrum induced by each component of UVR shows a similar pattern of UV-signature mutations (Fig. 1B, C), we found that the sequence context preference of those UVR-specific mutations was remarkably different among UVR components, as reported in our studies with transgenic mice,[30,31] which are summarized in Fig. 3A. We focused on three-tandem-base sequences in which the UVR-specific C → T mutation occurs at the center base. There are 12 types of such triplet sequences, which possess a cytosine base at the center and also include one or two dipyrimidine(s). We found that UVR-specific C → T mutations occurred preferably at the 5'-TCG-3' (TCG) context in the *lacZ* transgene, [26–31] particularly with exposure to longer wavelength components of UVR.[27,29,30] Although the mutations at the TCG context were most frequent among all the triplet contexts regardless of the UVR source, their contribution to the mutation spectrum was moderate with the UVC source but prominent exclusively with the UVA sources (Fig. 3A).[31] Especially, with the UVA1

sources more than 80% of the UVR-specific C → T mutations occurred at the TCG context. In the UVB range, the mutations at the TCG context were fairly conspicuous but not as prominent as those by UVA, which were intermediate between UVC and UVA. The distribution of occurrences of the UVR-specific mutation by sunlight was relatively similar to that by UVB, reflecting the fact that UVB is the component in sunlight most genotoxic to the skin.[1,100–103] Thus, the occurrence of the UVR-specific C → T mutation at the TCG context becomes conspicuous gradually as the wavelength increases, finally overwhelming those at the other triplet contexts at UVA1 wavelengths (Fig. 3A). Based on these observations, I propose that the UVR-specific C → T mutation that occurs preferentially at the TCG context should be called the "UVA signature". Although we proposed previously to call this type of mutation the "solar-UV signature",[30,60] which we featured as a kind of the UVR-specific mutation that occurs preferably at methyl CpG-associated dipyrimidine sites, the context preferences of the sunlight- and UVB-induced mutations were rather a mixture of those of UVC and UVA, as shown in Fig. 3A. Thus, "UVA signature" is more appropriate as a designation for the TCG-preferential UVR-specific mutation.

Since, as mentioned above, UVR mutagenesis occurs in a polη-dependent manner in normal cells and skin (see Fig. 2), we examined how the defect in polη affects the TCG preference of the UVR-specific mutation.[53] We found that the polη deficiency made the mutation lose the TCG preference, as shown in Fig. 3B, clearly demonstrating that the sequence context preference of the mutation depends on the TLS by polη, and suggesting that the TCG preference of the mutation should reflect the preferable formation or deamination of CPDs at some specific sequence motifs, which should at least overlap with the TCG sequence.

The mechanisms inducing two types of UVR-specific mutation, UV signature and UVA signature

As shown in Fig. 1, the mutation spectra with UV-signature mutations can be induced by any components of UVR, whereas the mutation spectra with UVA-signature mutations, namely the exclusive occurrences at the TCG context of UVR-specific C → T mutations, are manifested specifically in the UVA range. Since both signature mutations are induced by TLS over deaminated CPDs by polη as mentioned above, then what causes the difference between them? UVA is known to induce CPDs significantly although not as efficiently as UVC,[17,18,21,104] but in a distribution pattern among dipyrimidine motifs different from those by UVC and UVB.[19,20,22] UVA, more specifically UVA1, produces CPDs of TT dipyrimidines (TT-CPDs) at much higher frequencies and CPDs of 5'-TC-3' (TC) and 5'-CT-3' (CT) dipyrimidines (TC- and CT-CPDs) at lower frequencies than the shorter UVR components, although it does not produce detectable amounts of CPDs of CC dipyrimidines (CC-CPDs).[20,22] Accordingly, CC → TT mutations were not detected in our UVA1-induced mutations in mouse skin.[29,30] It was supposed that the mechanism of CPD formation by UVA was different from that by the shorter UVR, and that a triplet energy transfer to DNA bases from some endogenous photosensitizers that can be activated by UVA energy would mediate the CPD formation in the UVA range, because the energy of UVA photons is not sufficient to directly activate pyrimidine bases to their excited singlet states, which is necessary to cause photochemical reactions.[20,105] However, such photosensitizers have not been identified *in vivo* so far, and direct CPD formations in DNA by UVA1 have been demonstrated in experiments with isolated DNA.[17,23,106,107] Recently, another

mechanism by which UVA directly produces CPDs was proposed,[108,109] in which the UVA energy is absorbed directly to double-stranded DNA through "collective excited states", which can be followed by redistribution of the energy to pyrimidine bases leading to CPD formation. On the other hand, UVC, and probably UVB, should produce CPDs principally through the conventional singlet/triplet excited states induced by direct absorption of the photon energy to pyrimidine bases,[110,111] although some minor contribution of the collective excitation would also be probable. Thus, UVA and UVC/UVB could both produce CPDs directly, but through different photochemical mechanisms (Fig. 4).[105]

It has been shown that UVB and/or solar UVR produce CPDs preferably at CpG-associated dipyrimidine sites.[112–114] The CpG motif is the target sequence of mammalian DNA methylation that modifies cytosine to 5-methylcytosine (mC).[115] This CpG preference of CPD formation requires CpG methylation,[113,114] and is not observed for UVC.[114,116,117] The CpG-associated dipyrimidine sites are 5'-TCG-3' and 5'-CCG-3', the former of which is also the target context of the UVA-signature mutation. As mentioned above, UVA produces predominantly TT-CPDs along with small amounts of TC- and CT-CPDs, in other words, preferentially induces thymine-containing CPDs. The molecular structure of 5-methylcytosine is similar to that of thymine, which would raise the possibility that UVA produces CPDs not only from thymine but also from 5-methylcytosine, probably in the order of dipyrimidine preferences of TT ≥ TmC > TC > CT ≥ CmC (Fig. 4). Although the preferable CPD formation at 5'-TmCG-3' and 5'-CmCG-3' contexts (TmCG and CmCG) has not been demonstrated for UVA so far, the methyl CpG (mCpG)-directed CPD formation was much more remarkable after exposure to sunlight than to UVB with 5 to 15-fold increases by sunlight and 1.7 to 1.8-

fold by UVB compared to UVC-induced formation,[113,114] suggesting some contribution of the UVA component. If we accept the hypothesis that UVA should produce CPDs preferably at mCpG-associated dipyrimidine sites, the TCG preference of the UVA-signature mutation can be easily explained. However, there is one perplexing matter. If UVA can also produce CPDs at the CmCG context, why don't the mutations at the same context contribute remarkably to the UVA-signature mutation?

It has been demonstrated that the propensity of cytosine deamination in CPDs depends on the sequence context in which the CPD resides.[118] CPDs in the CmCG context are 50-fold slower to deaminate than those in the TCG and TmCG contexts, which are most prone to deaminate with a half-life of around 6 hours in double-stranded DNA, as far as examined so far. This difference in the CPD deamination propensity can explain the poor recoveries of UVR-specific mutations in the CCG context after UVA exposure that was reported in our studies using mouse skin (Fig. 3A),[27,29,30] in which all the mutation-detected CpG sites in the mutational target *lacZ* transgene were confirmed to be fully methylated.[26,119] Consequently, the preferential mutation occurrences at the TCG context characteristic for the UVA-signature mutation can be rationalized by the preferable CPD formation at mCpG-associated dipyrimidine sites and the context-dependent propensity of CPD deamination under the mutation mechanism of the error-free TLS by polη (Fig. 4).

Moreover, the context-dependent CPD deamination affects not only the UVA-signature, but also the UV-signature mutations. As shown in Fig. 3A, the UVR-specific mutation was most frequent at the TCG context even in the UVC and UVB ranges, although their occurrence ratios were not as conspicuous as those in the UVA range. In these shorter UVR ranges, the mCpG-preferable CPD formation, which should be

mediated through the collective excitation mechanism, would be less remarkable than in the longer UVR ranges, probably overwhelmed by abundant CPDs produced by the singlet/triplet excitation mechanism, which functions dominantly in the UVC and UVB ranges but almost completely fades out in the UVA1 range. Thus, CPD formation at the TCG context would not be so prominent in the UVC and UVB ranges as in the UVA range. However, once CPDs are formed at the TCG context, they should deaminate efficiently and could cause mutations by the polη-dependent TLS mechanism, resulting in the distribution of mutation occurrences among the triplet contexts shown in Fig. 3A. The mutation occurrence distribution observed in the absence of polη (Fig. 3B) might reflect the distribution of CPD formations among the triplet contexts, if we suppose that the mutation induction occurs randomly through the TLS over UVR photolesions by other error-prone TLS polymerases with the mechanism of the two-step model. This, however, remains to be demonstrated.

The TCG preference of UVR-induced mutations was also demonstrated by an exome analysis of 74 cancer-related genes in human sun-exposed normal and three types of cancerous skin tissues, in which the most frequent and overwhelming mutation was C $\rightarrow$ T transitions, which occurred predominantly at the TCG context with fewer occurrences at the other dipyrimidine-containing triplet contexts, regardless of the skin tissue type.[120] This result corresponds well with our observations on the occurrence distribution of the sunlight-induced UVR-specific mutations among the triplet contexts shown in Fig. 3A.[28] Thus, the TCG preference of UVR-induced mutations is neither an experimental artifact nor an observation limited to the *lacZ* transgene in mouse. It occurs both in mouse and human, and would occur in other organisms with cytosine methylation in their genome as far as they possess polη-like TLS polymerases.

Although an exceptional case was reported for *p53* gene mutations in human skin cancers, which were detected rather more frequently in the CCG context than in TCG,[2–5,30,121] this discrepancy has not been observed for the *p53* mutations in mouse skin cancers,[6–11] and can be explained by the poverty of mutable TCG sites in the human *p53* gene on the transcribed strand, as discussed in detail previously.[30] The lack of mutable TCG sites in human *p53* gene further suggests that the human genome have evolved to prevent solar UVR from inducing malignant mutations by substituting genetically important but UVR-vulnerable TCG sites with other genetically equivalent and UVR-refractory sequences. This evolution would be promoted by the human features of hairless skin and diurnal activity under the threat of photochemically genotoxic UVR components in natural sunlight.

Studies inconsistent with the UVA-signature hypothesis

My proposal for the UVA-signature mutation is based on the CPD formation mechanism through the collective excited state-mediated photochemistry, with which CPDs should be produced preferably at thymine-containing dipyrimidine sites resulting in the paucity of CC-CPDs, which becomes evident after the exposure to UVA, especially UVA1. On the contrary, Rochette *et al.* reported the significant formation of CC-CPDs by UVA1 using a ligation-mediated PCR (LMPCR) method.[19] However, the LMPCR method seems to have a tendency to overestimate the amounts of cytosine-containing CPDs, especially that of CC-CPDs,[19,122] compared with other methods such as chromatographic analyses,[123] post-labeling CPD-specific enzymatic cleavage assays[124,125] and HPLC with tandem mass spectrometry (HPLC-MS/ MS).[126] Actually, little CC-CPD formation has been detected in DNA, cells and skin tissues exposed to

UVA1 with the HPLC-MS/MS, a far more sensitive, direct CPD detection method.[20,22,23] In addition, the LMPCR image (Fig. 1) given in the paper by Rochette *et al.* showed distribution patterns of UVA-induced CPD formation among dipyrimidine sites clearly different from those induced by other UVR sources such as UVC, UVB and simulated sunlight.[19] Although the bands corresponding to cytosine-containing dipyrimidine sites were easily discernible in the lanes for the shorter UVR sources, those were hardly distinguishable from the backgrounds in the UVA lanes, which would reduce the reliability of estimates of the amount of cytosine-containing CPDs for the UVA lanes.

The paucity of CC-CPDs should also suppress the CC → TT tandem mutations in the mutation spectrum induced by UVA, resulting in the lack of such tandem mutations in the UVA signature. Accordingly, in our studies, the tandem mutations were not observed in the mutations recovered from the mouse skin exposed to UVA1,[29,30] although they were detected after exposure to UVA2 (Fig.1),[27] which would indicate that the singlet/triplet excitation mechanism for CPD formation are still valid in this wavelength range. Drobetsky *et al.* studied a UVA-induced mutation spectrum in the *aprt* gene using Chinese hamster cells and reported the induction of a unique type of mutation, T → G transversion, which they named UVA fingerprint,[46] although the preferable induction of such mutations has not been confirmed in subsequent studies except for one.[127] In the same study, Drobetsky *et al.* also recovered a few CC → TT mutations.[46] Kappes *et al*. reported another UVA-induced mutation spectrum in the *hprt* gene using human primary fibroblasts, detecting again a few CC → TT mutations.[50] Since both studies used short-cut filter-equipped UVR sources emitting mainly UVA1, they suggest that UVA1 could induce the tandem base substitutions, in contradiction to

my consideration given above. The recoveries of the tandem mutation in these studies might result from the significant contribution of the UVA2 component to the irradiated UVA, especially for the former study because they used blacklight lamps,[46] which emit mainly UVA2 wavelengths that might leak through the short-cut filter. Moreover, both studies were performed with cultured cells, and irradiation to cultured cells often produces ROS depending on the ingredients of the cultured medium.[17,59] It is known that ROS could induce CC → TT mutations independently of UVR exposure,[128,129] especially in mononucleotidyl cytosine runs.[130] Although the mechanism of the ROS-mediated CC → TT mutation is unknown, it could have affected the mutation spectra observed in these cellular studies. In addition, the use of *aprt* and *hprt* genes as mutational markers was not appropriate for the study of UVR-induced mutations in mammalian cells because both genes are hypomethylated and poor in mutable dipyrimidine-associated CpG sites, whereas collective excited state-mediated, UVR-specific mutations are supposed to prefer mCpG sites as supported by our studies and *p53* gene mutations in human and mouse skin cancers. The short size of coding sequences of both genes (543 and 657 bp) is also disadvantageous for mutation spectrum studies because of their low variation in sequence contexts (the *lacZ* transgene is 3090-bp long). These points have already been discussed in detail in our previous review.[60] Reflecting these situations, the appearance of UV-signature mutations in the UVA-induced mutation spectra were much less remarkable in both the cellular studies (27–35%)[46,50] than those in our studies with skin (59–68%),[29,30] suggesting a much greater contribution of non-UVR-induced mutations to the spectra of the cellular studies.

Conclusion

In my studies with mice, UVC induced the UVR-specific C → T mutations most frequently at the TCG context but also at other cytosine-containing dipyrimidine contexts at comparable frequencies, whereas UVA induced the same mutations exclusively at the TCG context with rare mutations at the other contexts. The context preference of UVB-induced mutations showed a mixture between those of UVC and UVA. Based on the molecular mechanism of UVR mutagenesis that is mediated mainly through polη-dependent error-free TLS across deaminated CPDs, this wavelength-dependent context preference of the mutations can be explained by deamination tendencies of cytosine-containing CPDs and a recently identified/proposed photochemical mechanism of CPD formation, the collective excitation, which would justify the discrimination of UVA-induced UVR-specific mutations as the UVA signature from the UV-signature mutations induced by UVC/UVB, which would be caused mainly by CPDs formed through the conventional photochemical mechanism of singlet/triplet excitation.

Conflicts of interest

There are no conflicts of interest to declare.

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Figure legends

Fig. 1 Mutation spectra in mouse skin epidermis induced by various UVR sources. **(**A) Profiles of percent spectral energy outputs of UVR sources used for my studies on induced mutation spectra in mouse skin. UVC: germicidal lamps (GL15, Hitachi, Japan);[31] UVB: broadband UVB fluorescent lamps (FL20S.E, Toshiba, Japan);[26] sunlight: summer noon sunlight in Japan;[28] UVA2: blacklight fluorescent lamps (FL20S.BLB, Toshiba, Japan) with a Mylar filter (the cut-off output is indicated by a shaded area);[27] UVA1: Sellamed 2000 (Sellas, Germany);[30] and 364-nm laser (National Institute for Basic Biology, Japan).[29] (B)Mutation spectra induced in mouse epidermis by UVB, sunlight and UVA2.[26–28,53] (C) Mutation spectra induced in mouse epidermis by UVC, UVA1 and 364-nm laser.[29–31] Background is the mutation spectrum in the epidermis of unirradiated mice.[26] The tandem base substitutions are mostly CC → TT mutations, but those for the background and UVA1 do not include CC → TT.[26,30] PyPy, dipyrimidine.

Fig. 2 The mechanism of UVR mutagenesis by error-free TLS across deaminated CPDs by DNA polymerase η. UVR can produce CPDs at dipyrimidine sites (PyPy): 5'-TT-3', 5'-TC-3', 5'-CT-3' and 5'-CC-3' (TT, TC, CT and CC). DNA polymerase η (polη) can synthesize a DNA strand opposite a CPD on the template strand following the base pairing rule faithfully. Thus, translesion DNA synthesis (TLS) by polη can bypass CPDs error-free. However, cytosines in CPDs are unstable and easily deaminate to produce uracils, or thymines if the cytosine is methylated at position 5, converting a cytosine or 5-methylcytosine-containing CPD (C-CPD or mC-CPD) to an uracil or

thymine-containing CPD (U-CPD or T-CPD), which can induce the UV-signature mutations upon the "error-free" TLS by polη, although polη could bypass CPDs without inducing mutations if the deamination does not occur, as in the case of thymine dimer (TT-CPD).

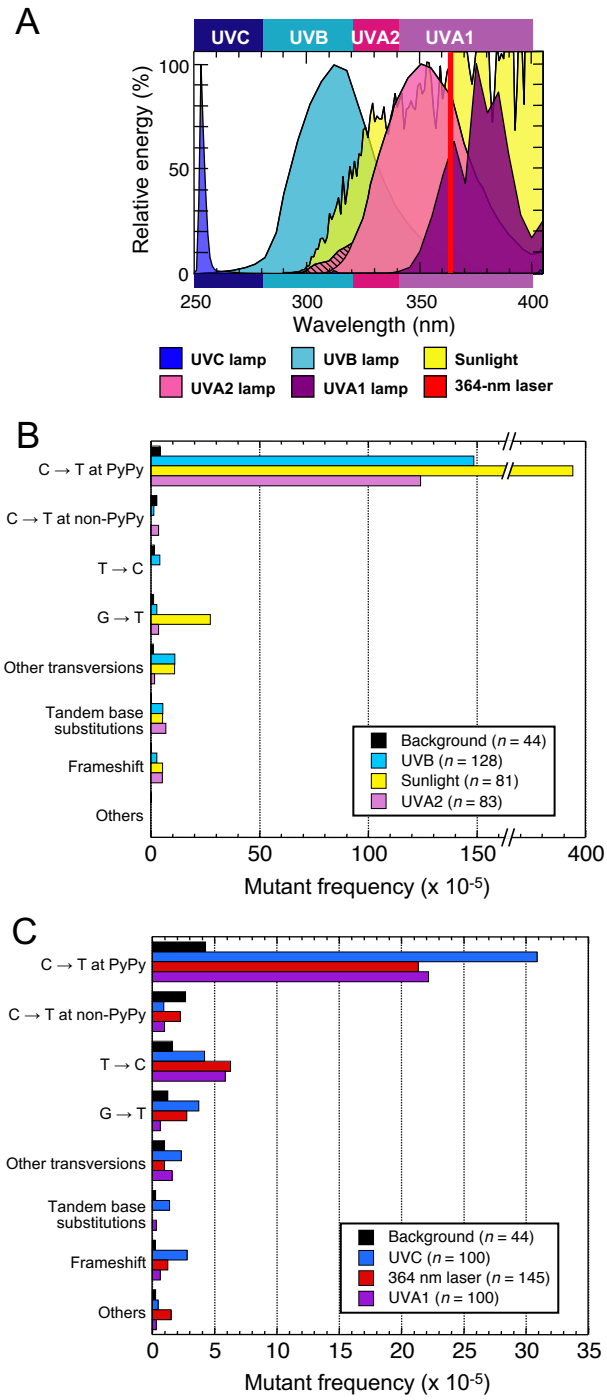
Fig. 3 Sequence context preference of the UVR-specific C → T mutation. (A) The distributions of the occurrence ratios (%occurrence) of the UVR-specific mutations among triplet sequence contexts were compared among UVR sources. The occurrence ratios were estimated as ratios of the occurrences of the UVR-specific C → T mutations at specific triplet contexts (shown in the box) to those at the total triplet contexts relevant to the mutation, which are cytosine-centered, dipyrimidine-containing three-base sequences. UVR sources are the same as those in Fig. 1. Pu, purine; Py, pyrimidine. (B) UVB-induced distributions of the UVR-specific mutation among triplet contexts were compared between mice with polη proficient (*Polh*+/+) and deficient (*Polh*-/-).

Fig. 4 A model of two independent, but overlapping mechanisms of the UVR-specific mutations of "UV signature" and "UVA signature". The shorter (UVC/UVB) and longer (UVA) UVR components produce CPDs differently through two distinct photochemical reactions mediated by the singlet/triplet excitation of pyrimidine bases, which would function at shorter wavelengths up to the UVA2 range, and the collective excitation of double-stranded DNA, which could work throughout the whole UVR ranges, with different distributions in dipyrimidine composition: TT > TC > CT > CC for the former and TT >> TC > CT with CC undetectable for the latter, respectively.

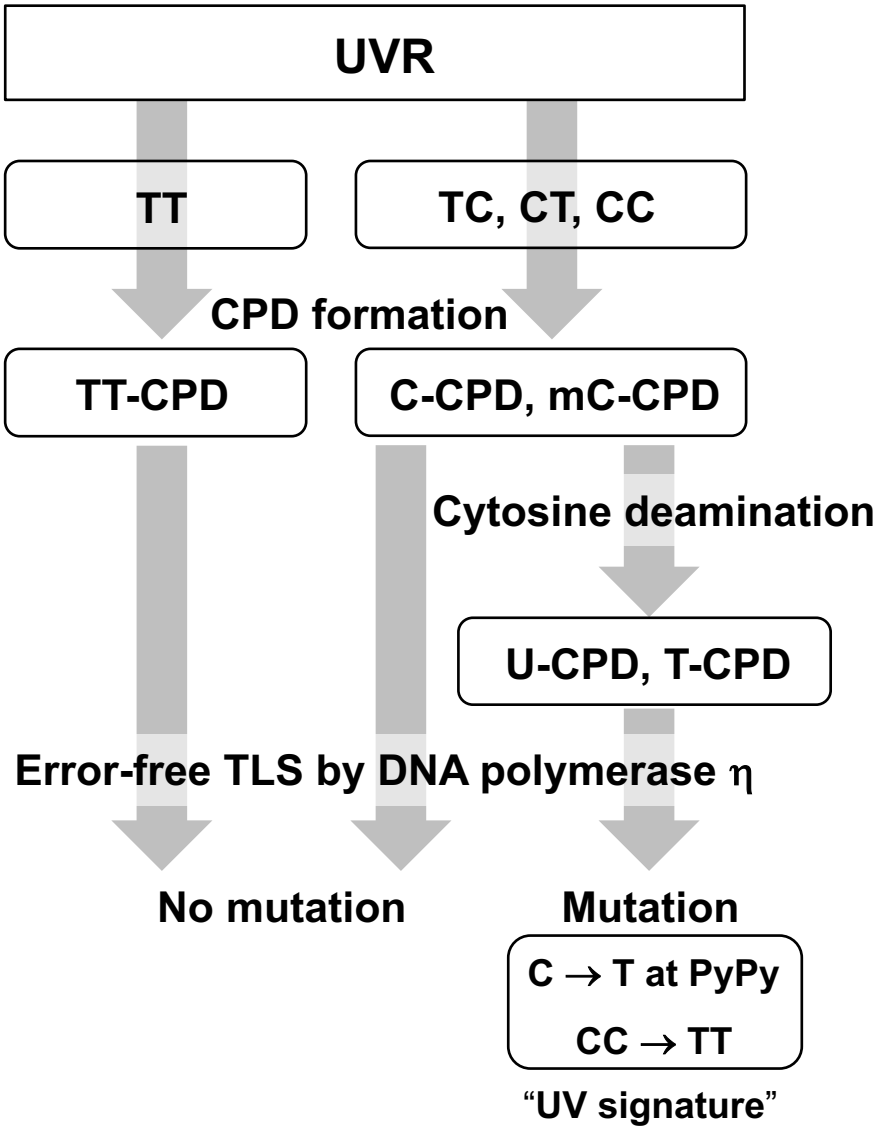
The CpG methylation can also enhance the CPD formation by the collective excitation mechanism. The formation of CPD enhances the deamination of its cytosine, although the propensity of CPD deamination depends on the sequence context it resides in, showing the most efficient deamination in the TCG context. The "error-free" TLS by polη across the deaminated CPDs results in the induction of the UVR-specific mutations of the "UV signature", C → T and CC → TT mutations at dipyrimidine sites, for the CPDs produced by the UVC/UVB-provoked singlet/triplet excitation and the "UVA signature", the preferential induction of C → T mutations at the TCG context, for the CPDs by the collective excitation, which becomes prominent in the UVA range.

Graphical abstract

Based on polη-dependent TLS across deaminated CPDs, the wavelength dependence of UVR mutations can be explained by different photochemistries of CPD formation, the singlet/triplet and collective excitations that cause UV-signature and UVA-signature mutations respectively.



H. Ikehata, Fig. 1



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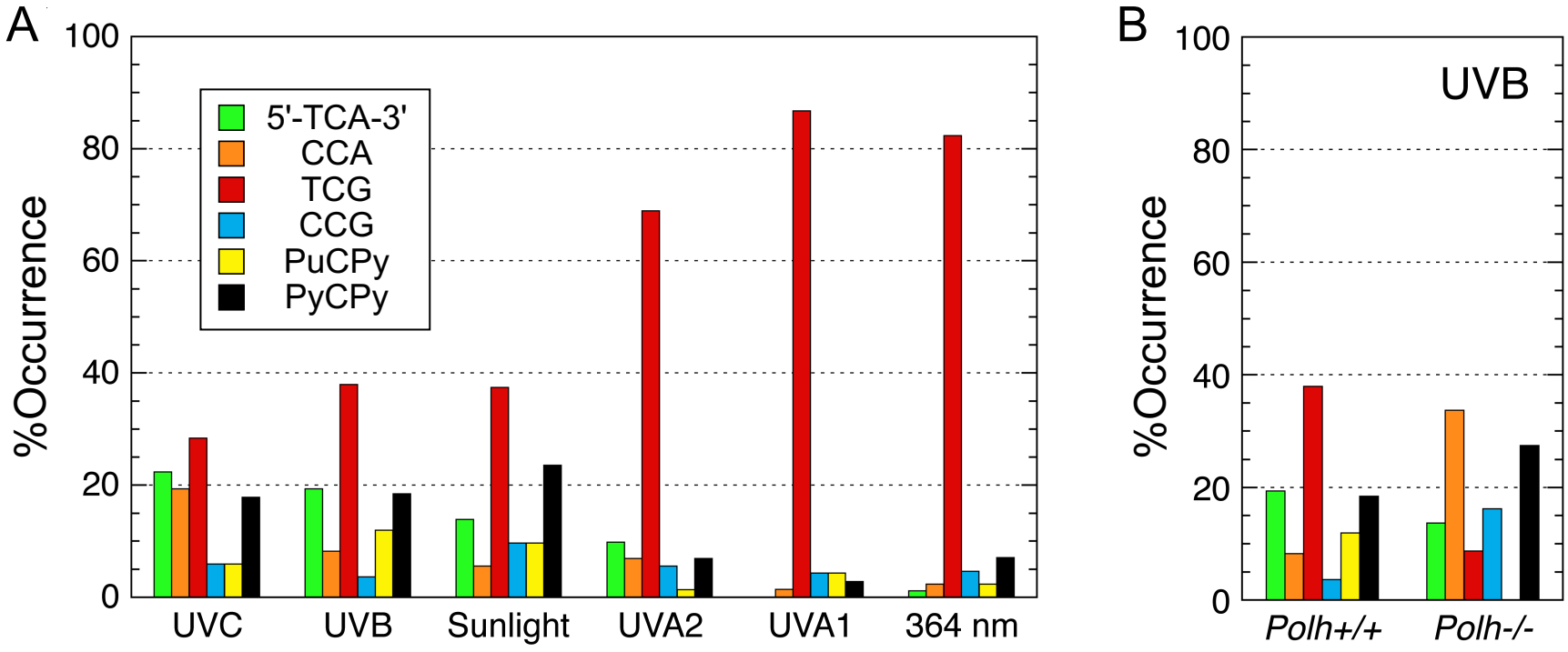
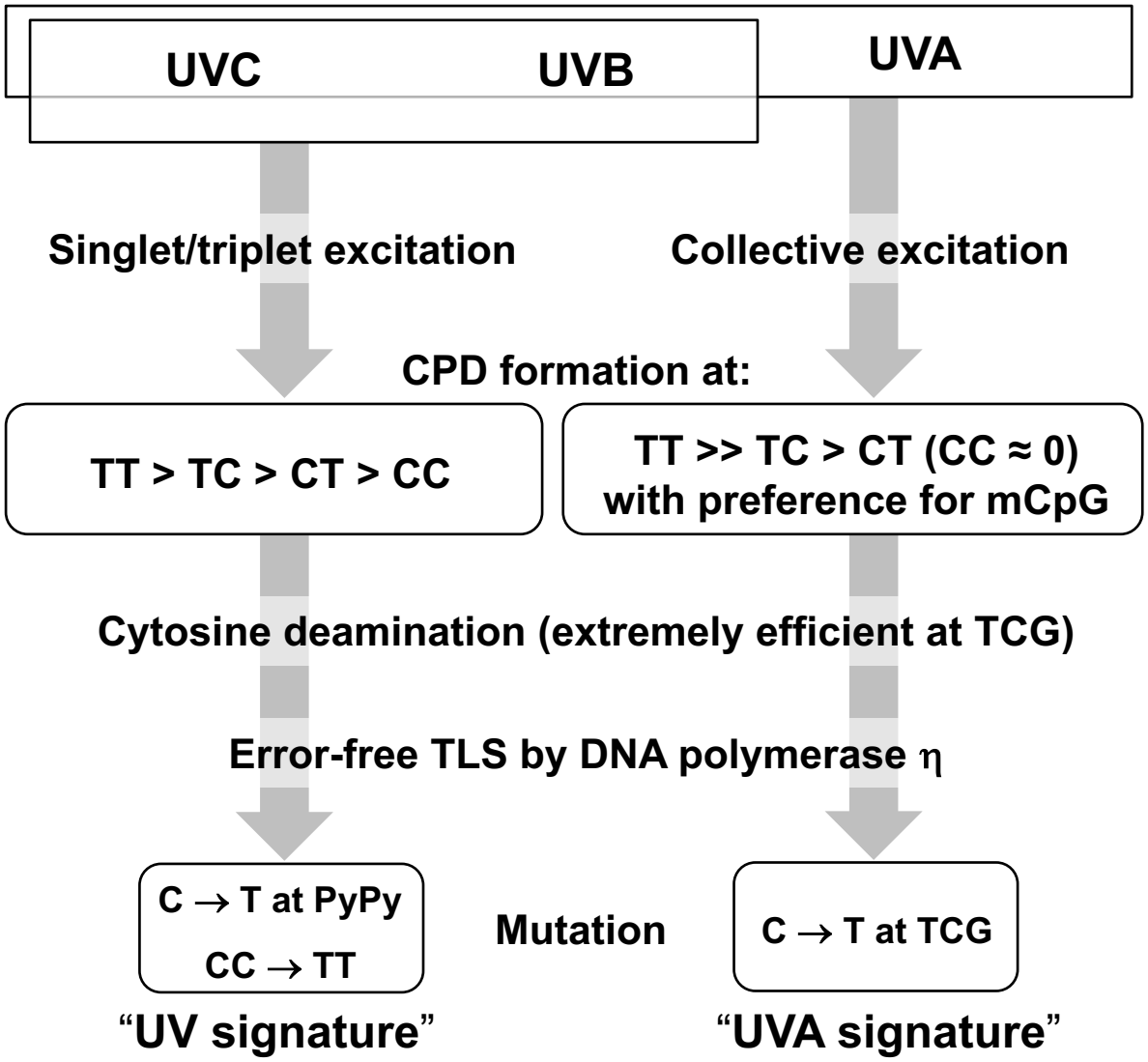
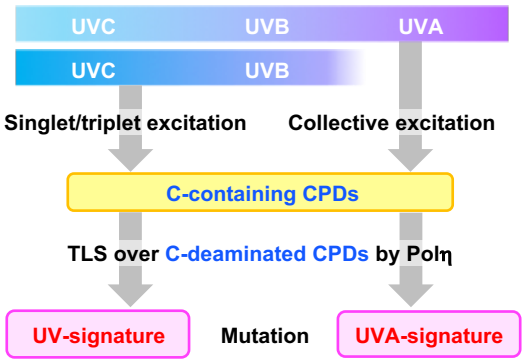


H. Ikehata, Fig. 3



H. Ikehata, Fig. 4



Based on polη-dependent TLS across deaminated CPDs, the wavelength dependence of UVR mutations can be explained by different photochemistries of CPD formation, the singlet/triplet and collective excitations that cause UV-signature and UVA-signature mutations respectively.

H. Ikehata, Graphical abstract