UVA-induced damage to DNA and proteins: direct versus indirect photochemical processes

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Abstract. UVA has long been known for generating an oxidative stress in cells. In this paper we review the different types of DNA damage induced by UVA, i.e. strand breaks, bipyrimidine photoproducts, and oxidatively damaged bases. Emphasis is given to the mechanism of formation that is further illustrated by the presentation of new in vitro data. Examples of oxidation of proteins involved in DNA metabolism are also given.

1. Introduction

Even though there is a continuum of energies in the electromagnetic spectrum of radiations, the UV portion of sunlight has been divided into UVC (below 280 nm), UVB (280-315 nm) and UVA (315-400 nm) for categorizing the effects of solar exposure to human skin. Wavelengths below 290 nm are blocked by the stratospheric ozone. These wavelengths are of the highest energies (> 4.4 eV), coincide with maximal absorption by DNA and other biomolecules, and would be extremely damaging to genetic material and biological tissues in general. Therefore the stratospheric ozone layer protects life from UVC radiation. Longer wavelength UVB (λ > approximately 290 nm) penetrates the ozone layer and constitutes about 5-10% of the terrestrial solar UV radiation. It is absorbed by nucleic acids and has the ability to directly cause genotoxic damage to DNA and ultimately skin cancer.

In contrast, wavelengths in the UVA range received little attention up to the nineties, although they are by far the most abundant solar UV radiation (above 90%) that reaches the surface of earth. UVA radiation also penetrates human skin more effectively than UVB. Unlike UVB, the UVA component of solar radiation is weakly absorbed by DNA [1], but rather excites other endogenous chromophores, generating various reactive oxygen species (ROS) in cells. Moreover, most UVA-mediated biological events are oxygen-dependent. These observations provided the basis for considering UVA as a strong generator of oxidative stress. Singlet oxygen is the major ROS involved in UVA-mediated cell inactivation [2] and gene activation [3], it is also a primary source of UVA-mediated signalling [4]. Once thought to be relatively innocuous, UVA is now known as a damaging agent for DNA, proteins, lipids, with harmful consequences such as skin ageing and carcinogenesis. Due to the popularity of the UVA tanning saloons and to the widespread use of efficient UVB-absorbing sunscreens blocking
erythema, while accompanied by prolonged periods of sunbathing, exposure of the human population to UVA has increased significantly in the last decades. As a consequence, the deleterious effect of UVA has recently emerged as a source of concern for public health. As an example, sunbeds have been included into the list of carcinogens to humans by the International Agency of Research on Cancer [5]. The cellular and molecular responses to UVA in relation to skin carcinogenesis have been recently reviewed [6]. Here, we focus on damage induced to DNA and protein by UVA radiation and discuss the possible mechanisms of formation.

2. DNA damage induced by UVA

As stated in the introduction, UVC and UVB radiation are directly absorbed by DNA, causing the formation of cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) and their Dewar valence isomers. These bipyrimidine photoproducts are not expected to be formed by UVA because of the inability of DNA to readily absorb in the UVA range. Meanwhile, a pioneer work of Tyrrell’s group reported the induction of CPDs by very pure narrow-band 365-nm UVA in bacteria, with a higher relative frequency of TT CPDs and a reduced excision of these CPDs, comparatively to 254-nm UVC [7,8]. The same group also reported the induction of single strand breaks (alkali-labile bonds) by UVA in bacteria and phage DNA [9]. It is well established that UVA promotes photosensitization of DNA, triggered by singlet oxygen (type II photosensitization), electron abstraction (type I photosensitization) or hydroxyl radicals. Therefore, the formation of oxidative DNA damage is expected. The induction of single strand breaks (SSB) and DNA-protein crosslinks has indeed been observed in mammalian cells upon UVA irradiation [10]. In the nineties, the availability of new and sensitive methods allowed the efficient measurement of various types of DNA lesions. These methods are comet assays, commercially available specific antibodies and DNA repair enzymes, HPLC coupled with electrochemical detection (HPLC-ED) to reveal 8-oxoguanine, and more recently HPLC coupled with tandem mass spectrometry (HPLC-MS/MS) to measure 8-oxoguanine and all the different bipyrimidine photoproducts. UVA-induced DNA damage has thus been timely revisited.

The *Escherichia coli* Fpg or Nth proteins have been used to reveal oxidation products of purine (mainly 8-oxoG) or pyrimidine, respectively, and Nfo protein to reveal abasic sites. The 8-oxoguanine (8-oxoG) has been more specifically measured by HPLC-ED. We and other have shown that UVA radiation induces single strand breaks (SSB), oxidized pyrimidines, oxidized purines in mammalian cells [11-14], and that the most abundant oxidative DNA damage produced is 8-oxoG [15-17].

By the use of sensitive methods, CPDs have been detected in mammalian cells, including in human skin upon UVA irradiation [11, 13, 15, 17, 18-22 and 23 for review]. It has been found that CPDs are produced about $10^5$ fold less efficiently by UVA than by 254-nm UVC [7,19]. 6-4 photoproducts were barely detected using specific antibodies [19]. In agreement with the original studies in bacteria [7], the distribution of CPDs induced by UVA drastically differs from that produced by UVC, UVB or simulated sunlight (SSL). UVA-induced CPDs predominantly form at TT sites, while those produced at CC sites are poorly represented [20]. HPLC-MS/MS also revealed predominance for TT CPDs over CT and TC CPDs, whereas CC CPDs and 6-4PPs were not detected [17,22]. As shown in Fig. 1, CPDs, 8-oxoG, oxidatively damaged pyrimidines, and SSB are formed in the ratio 10:3:1:1. Interestingly, CPDs are the major lesions induced by UVA in mammalian cells and human skin [17,22].

Moreover, it has been shown that UVA is able to photoisomerize 6-4 photoproducts produced by the UVB photons of simulated sunlight into Dewar photoproducts [17,19]. This sustains the observation that Dewar photoproducts are more frequently produced by natural and simulated sunlight, than by UVB [19]. DNA double strand breaks, revealed by neutral comet assay or γ-H2AX foci (phosphorylated H2AX variant histone), are not readily produced in human transformed fibroblasts or keratinocytes by direct UVA irradiation, but occurs at low extent during the process of DNA lesions [24,25].
Figure 1. Distribution of CPDs, 8oxoguanine (8oxoG), oxidatively damaged pyrimidines (oxPy), single strand breaks (SSB), revealed respectively by T4 phage DenV protein, bacterial Nth and Fpg proteins in combination with alkaline agarose gel, in Chinese hamster ovary cells exposed to UVA. The yields of formation were obtained by linear regression of the amount of lesions with respect to the dose (from [17]).

3. Processes involved in the formation of UVA-induced DNA damage

The induction of single strand breaks in bacterial DNA by narrow-band 365-nm radiation is totally dependent on the presence of oxygen [9]. The low amounts of SSB and oxidized pyrimidines, likely produced by hydroxyl radicals originated from Fenton reaction, and the predominance for 8-oxoG likely sign a major contribution of singlet oxygen in the formation of oxidative DNA damage in cells by UVA [23]. A type I mechanism by hydrogen or electron abstraction mediated by a photosensitizer cannot be totally excluded.

The photochemical process underlying the formation of UVA-induced CPDs has been a matter of debate for many years. The quantum yields were $7 \times 10^{-5}$ less at 365 nm than at 254 nm in bacteria [7] and $10^{5}$ less for broad-band UVA than at 254 nm [19]. Such yield of CPDs formation by UVA is in perfect agreement with the DNA absorption spectrum [1]. This led us to conclude that CPDs formation by UVA could be compatible with direct absorption by DNA [19]. However, in bacteria, the longer wavelength radiation induced predominantly thymine dimers (TT:CT in the ratio 5:1), whereas at the shorter wavelength CPDs were induced in the ratio 5:4:1 (TT:CT:CC) [7]. In mammalian cells, CPDs also predominately formed at TT sites at a gene level [20] and at a higher extent in the bulk DNA [17,21,22]. The principal induction of CPDs at T-containing bipyrimidine sites, and mostly at TT sites, is a reminiscence of what was observed by photosensitization reaction of DNA by aromatic ketones [26, 27]. This could suggest that, in contrast to what was stated above, most of CPDs are formed through triplet energy transfer from photosensitizer to excited thymine. The identification of an hypothetical UVA-absorbing chromophore tightly bound to DNA that allows energy transfer remains elusive.

Evidence recently emerged in favour of a direct absorption of UVA by DNA that mediates CPD formation. Firstly, the distribution of the different types of DNA damage induced by UVA is similar in plasmid or isolated DNA and in cells [28-31]. In particular, upon UVA irradiation, CPDs are formed in plasmid DNA by a mechanism that should not involve photosensitization reactions, due to the absence of photosensitizer in the irradiation mixture. Therefore, CPDs in plasmid or isolated DNA should be formed by direct absorption. Moreover, it has clearly been demonstrated that UVA radiation
is able to induce 6-4PPs in plasmid DNA [30], while 6-4PPs are produced in tiny amount in cells [19]. Importantly, 6-4PPs cannot be formed by photosensitization and are only produced by direct absorption. To highlight our point of view, we provide new evidence in favour of a direct mechanism. Figures 2 and 3 show that CPD distributions along the adenylphosphoribosyl transferase (APRT) gene are superimposable whether isolated cellular DNA or cells were UVA-irradiated, as also observed for UVB or UVC-induced CPDs which are formed exclusively by direct absorption.

**Figure 2.** Induction of CPDs by UVA, UVB, and simulated sunlight (SSL), at nucleotide resolution, in the aprt exon 2 gene of Chinese hamster ovary cells. Cells were either unirradiated (No-UV) or irradiated as indicated and CPD formation was analyzed by LMPCR along the non-transcribed strand of exon 2 of aprt gene. The first four lanes from the left of the autoradiogram exhibit LMPCR of DNA treated in standard Maxam-Gilbert cleavage reactions. For the next 18 lanes, each band in a lane corresponds to a site of CPD formation. Either cells (*in cellulo*) or DNA isolated from cells (*in vitro*) were irradiated with 4500, 3000, 1500 kJm⁻² of UVA, 3.75, 1.20, 0.60 kJm⁻² of UVB, or 7500, 5000, 2500 kJm⁻² of SSL, as indicated. The doses for UVB, SSL, and UVC induced approximately equal global CPD. The red dots indicate UVA-induced CPDs at TT sites. The figure reveals that the patterns of bands (sites of CPDs) produced by UVB, SSL and UVA are the same whether DNA isolated from cells or cells were exposed to radiation. Moreover, it shows that the pattern of band for UVA differs from that of UVB or SSL and reveals formation of CPDs at TT sites (compare with Maxam and Gilbert ladder). For further details in procedures see [20].
Figure 3. The sites and frequencies of CPDs formation in vitro and in vivo by UVA are similar. This figure represents the mean of quantified LM-PCR experiments such as those described in figure 2. For further details in quantification see [20]. The highest frequencies for UVA-induced CPDs are at TT sites.

Moreover, the frequencies of CPD formation in vitro and in cellulo are the same in these experiments, as well as in data presented in Table 1. Finally, an other argument is provided by the similarities in the yields of CPD formation within calf thymus DNA and keratinocytes exposed to UVA through filters exhibiting increasing cut-off wavelength (322-390 nm) [31]. Collectively, these observations strongly suggest that the photochemical process underlying the formation of CPDs by UVA is direct absorption of UVA photons by DNA. The low energy of UVA photons is sufficient to directly excite DNA with subsequent formation of bipyrimidine photoproducts, both CPDs and 6-4PPs, yet at low level. The predominance for CPD formation at TT sites may be explained by the fact that thymine has, in fact, the lowest triplet state energy with regard to other bases. In contrast, 8-oxoG is essentially formed by photosensitization reaction mediated by singlet oxygen.

|         | in vivo     | in vitro    |
|---------|-------------|-------------|
| UVC (254 nm) | 2.2 (+/-0.2) x10^{-2} | 1.5 (+/-0.3) x10^{-2} |
| UVB (λ>300 nm) | 2.0 (+/-0.2) x10^{-4} | 1.5 (+/-0.4) x10^{-4} |
| UVA     | 3.0 (+/-0.4) x10^{-7} | 1.4 (+/-0.2) x10^{-7} |

4. Protein oxidation induced by UVA
Proteins are major cellular targets for photooxidation. This is due to their abundance in cells and also to the fact that they carry chromophores, i.e. primarily side chains or bound photosensitizers. Proteins absorbs in the 250-320 nm region due to the aromatic amino acids. Some proteins bind prosthetic groups which absorb in the UVA and visible range, such as flavins, hemes. As a result, direct
photooxidation may occur and arises from absorption of radiation by the protein or by the bound chromophores, therefore generating excited states or radicals through type I processes. Indirect photooxidation of proteins via type II mechanism may also occur and involves singlet oxygen generated by energy transfer from either protein-bound or other endogenous chromophores to oxygen. All these processes have been detailed in [32-34]. All amino acid residues are susceptible to oxidation [35]. Anyhow, the thiol function in cystein residues is among the most susceptible oxidant-sensitive targets within proteins and can undergo various reversible and irreversible redox alterations in response to ROS.

UVA, as an oxidative agent, has been shown to produce various protein damage [36]. In fact, the observation that near UV wavelength is able to inactivate photoreactivating enzyme was made more than 30 years ago [37]. More recently, in human skin cells, UVA irradiation was shown to induce sulphhydryl oxidation in proteins that depends on iron, singlet oxygen and hydrogen peroxide [38]. UVA also caused changes in catalase charge in epidermis reconstituted with low phototype melanocytes, possibly due to oxidative modification of Trp and Met residues [39]. Photoaging has also been associated with protein oxidation in human skin, with accumulation of protein carbonyls, especially in the upper dermis. It was recently shown that the repair enzyme methionine-S-sulfoxide reductase (MSRA) was expressed in human epidermis and specifically upregulated by low doses of UVA radiation [40]. MSRA, by reducing the methionine sulfoxide (MetO) to methionine, thus reverses the inactivation of proteins caused by oxidation of critical methionine residues. Another example is given with the base excision repair protein, DNA N-glycosylase hOGG1 which removes 8-oxoguanine and has been shown to be sensitive to oxidation, e.g. cystein oxidation [41]. Interestingly, glutathione depletion of skin fibroblasts and melanoma cells, which increases oxidative stress, causes a strong repair retardation of 8-oxoguanine after exposure to non-toxic doses of visible light or UVA radiation [42]. Moreover, the cleavage activity at 8-oxoguanine residues measured in protein extracts from irradiated cells drops transiently after irradiation. A modification of hOGG1 is suspected, but it is not caused by critical oxidation of Cys.

We have recently shown that exposure of eucaryotic cells (i.e. fission yeast S. pombe and mammalian cells) to oxidative stress induced by UVA radiation led to inhibition of DNA replication by a yet unknown process, which does not require a functional DNA damage checkpoint response, despite ATM(p\text{inMec1}), ATR(p\text{Rad3)}-, p38(p\text{Sly1)}-dependent pathways activation [24,43]. Data led us to propose that inhibition of DNA replication is due to impaired replication fork progression, rather as a consequence of UVA-induced oxidative damage to protein than to DNA. To highlight this point, we carried investigations on two proteins involved in DNA metabolism, Proliferating Cell Nuclear Antigen (PCNA), a cofactor of DNA polymerases, and XRCC3 protein involved in repair of double strand breaks by homologous recombination.

PCNA is a homotrimeric protein which acts as sliding clamp on DNA, conferring high processivity to replicative DNA polymerases. It also plays a pivotal role in translesion synthesis. PCNA undergoes posttranslational modification such as mono- or poly- ubiquitylation upon cells treatment with UVC or hydroxyurea which block DNA replication forks. In the left part of figure 4, the bands migrating slower than monomeric PCNA likely reflect ubiquitillated PCNA. Such PCNA modification occurs in active PCNA, bound to chromatin. It is not induced by UVA. In contrast, right part of figure 4 shows that a small fraction of PCNA present in the soluble protein fraction migrates as a trimer (90 kDa protein) and is specifically induced by UVA. This UVA-induced PCNA modification increases in a dose-dependent manner, it occurs very rapidly and it is stable for more than 6 hours after irradiation (not shown). It is largely reduced when UVA irradiation is performed in the presence of the antioxidant N-acetylcysteine or NaN\text{3}, a quencher of singlet oxygen. Altogether, it appears that UVA-induced ROS lead to irreversible, oxidative, covalent cross-linking between the three subunits of PCNA. Such an irreversible PCNA crosslinking has been previously reported [44] upon treatment of human cells with azathioprine plus low doses UVA. Singlet oxygen thus produced by photosensitization was also responsible for this PCNA modification. The authors suspect an interstrand crosslink involving His153 and Lys77, since no covalent trimeric PCNA is observed in
cells carrying an amino acid change in PCNA at His153. The chemical process underlying this covalent reaction is still unknown.

**Figure 4.** MRC5Vi cells were either untreated or exposed to 1 mM hydroxyurea (HU) for 16 h, 16 J/m² UVC, 80 kJ/m² UVA, 100 mM H₂O₂ for 30 min or 10 Gy γ-rays. Immediately post treatment, cells were lysed on ice in lysis buffer and total soluble and insoluble protein were loaded on reducing 9 % SDS-PAGE. Proteins were then transferred onto nitrocellulose membrane and PCNA protein detected using mouse monoclonal anti-PCNA antibody (PC10, Santa Cruz Biotechnology). PCNA mono = PCNA monomeric. PCNA tri = PCNA trimeric.

In vertebrates, XRCC3 is one of the five RAD51 paralogs which play non-redundant essential functions in the repair of DNA double strand breaks by homologous recombination. XRCC3 deficiency results in impaired DNA damage-induced RAD51 foci formation, impaired homologous recombination and elevated chromosome aberrations. XRCC3 contains 8 cystein residues, and in normal growing conditions, XRCC3 is mainly reduced (thiol groups). Protein extracts from human cells HCT116 deficient (XRCC3−/−) or not in XRCC3 were prepared and migrated on SDS-PAGE. The comparison of the bands (see non-reducing conditions in figure 5) allowed to allocate the fast migrating band to XRCC3 protein (absent in XRCC3 deficient cells). When cells were UVA-irradiated and protein extracts migrated under non-reducing conditions, XRCC3 protein band decreases, to almost disappear at 160 kJ/m², while the protein is still present in the extract as revealed when extract migrated under reducing conditions. (figure 5, compare the two left western blots). This demonstrates that XRCC3 underwent modification upon UVA irradiation, which prevents antibodies to recognize the protein when migrating in non-reducing conditions. Since modified XRCC3 is detected by the antibodies when migrating under reducing conditions, it means that two or more cysteins were oxidized upon UVA irradiation and that it is reversible by the reducing agent β-mercaptoethanol. We also found that singlet oxygen produced by UVA radiation causes this reversible disulfide bound in XRCC3. Furthermore, we observed that this phenomenon is quickly reversed in cells.

While the consequences of irreversible (PCNA) or reversible (XRCC3) oxidation on the functionality of these proteins has not yet been fully investigated, our data emphasize that protein oxidation may play a role in the deleterious effect of UVA radiation.
Figure 5. XRCC3-proficient and deficient human HCT116 cells were exposed to increasing doses of UVA radiation. Immediately post radiation, cells were lysed on ice in lysis buffer containing 4 mM N-ethylmaleimide (NEM) an irreversible thiol alkylating agent that protects cysteine residues from oxidation during lysis. Equal amounts (30 mg) of soluble protein extracts in non reductive (without β-mercaptoethanol) and reductive (150 mM β-mercaptoethanol) conditions were loaded on 9 % SDS-PAGE and transferred onto nitrocellulose membrane. Human Xrcc3 protein was detected using rabbit polyclonal anti-Xrcc3 antibody (Novus Biological, clone 100-165). The star indicates non specific cross-reactivity of the antibody.

5. Conclusion
Excessive exposure to sunlight is a major cause of skin cancer but also contributes to premature skin aging. The two main components of sunlight that contribute to photocarcinogenesis and photoaging are UVB and UVA radiation, respectively. While it is known for long time that UVB is a major source of DNA damage, more recent data highlight also a key role of UVA radiation in the formation of CPDs, 8-oxoguanine and strand breaks. Efficient repair of such DNA damage is crucial for the maintenance of genomic stability. In other words, inability of cells to efficiently carry out repair could lead to mutations and consequently to genomic instability, a marker of cancer cells. Interestingly, UVA-induced ROS can also oxidize DNA repair proteins (i.e. PCNA, XRCC3, Ogg1) raising the possibility that during sunlight exposure, there is a narrow window during which DNA repair proteins could be oxidatively modified, and possibly inactivated, and DNA damages are produced. For a better understanding of the deleterious effects of UVA, it seems therefore essential, not only to measure the various type of DNA lesions induced by UVA (or simulated sunlight) but also to measure the extent of protein oxidation with particular emphasis on DNA repair enzymes.

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References

[1] Sutherland J C and K P Griffin 1981 *Rad. Res.* 86 399.
[2] Tyrrell R M and Pidoux M 1989 *Photochem. Photobiol.* 49 407.
[3] Basu-Modak S and Tyrrell R M 1993 *Cancer Res.* 53 4505.
[4] Ryter S and Tyrrell R M 1998 *Free Radical Biol. Med* 24 1520.
[5] El Ghissassi F, Baan R, Straif K, Grose Y, Secretan B, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L and Cogliano V 2009 *Lancet Oncol.* 10 751.
[6] Ridley A J, Whiteside J R, McMillan T J and Allison S L 2009 *Int. J. Rad. Biol.* 85 177.
[7] Tyrrell R M 1973 *Photochem. Photobiol.* 17 69.
[8] Tyrrell R M, Webb R B 1973 *Mutat Research* 19 361.
[9] Tyrrell R M, Ley R D and Webb R B 1974 *Photochem. Photobiol.* 20 395.
[10] Peak M J and Peak J G 1990 *Photochem. Photobiol.* 51 649.
[11] Zhang X S, Rosenstein B S, Wang Y, Lebwohl M, Mitchell D M and Wei H C 1997 *Photochem. Photobiol.* 65 119.
[12] Kvam E and Tyrrell R M 1997 *Carcinogenesis* 18 2379.
[13] Douki T, Perdiz D, Grof P, Kulucsics Z, Moustacchi E, Cadet J and Sage E 1999 *Photochem. Photobiol.* 70 184.
[14] Besaratinia A, Kim S and Pfeifer G P 2008 *FASEB J.* 22 2379.
[15] Kielbassa C, Roza L and Epe B 1997 *Carcinogenesis* 18 811.
[16] Pouget J P, Douki T, Richard M J and Cadet J 2000 *Chem.Res. Toxic.* 13 541.
[17] Douki T, Reynaud-Angelin A, Cadet J and Sage E 2003 *Biochem.* 42 9221.
[18] Young A R, Potten C S, Nikaido O, Parsons P G, Boenders J, Ramsden J M and Chadmick C A 1998 *J. Inves. Derm.* 111 936.
[19] Perdiz D, Grof P, Mezzina M, Nikaido O, Moustacchi E and Sage E 2000 *J.Biol. Chem.* 275 26732.
[20] Rochette P J, Therrien J P, Drouin R, Perdiz D, Bastien N, Drobtsky E A and Sage E 2003 *Nucl. Acids Res.* 31 2786.
[21] Courdavault S, Baudouin C, Charveron M, Favier A, Cadet J and Douki T 2004 *Mutation Res.* 556 135.
[22] Mouret S, Baudouin C, Charveron M, Favier A, Cadet J and Douki T 2006 *Proc. Nat. Acad. Sci. USA* 103 13765.
[23] Cadet J, Sage E and Douki T 2005 *Mutation Res.* 571 3.
[24] Girard P M, Pozzebon M, Delacôte F, Douki T, Smirnova V and Sage E 2008 *DNA Repair* 7 1500.
[25] Wischermann K, Popp S, Moshir S, Scharfetter-Kochanek K, Wlaschek M, de Gruijl F, Hartschuh W, Greinert R, Volkmer B, Faust A, Rapp A, Schmezer P and Boukamp P 2008 *Oncogene* 27 4269.
[26] Lamola A A 1968 *Photochem. Photobiol.* 7 619.
[27] Charlier M and Helene C 1972 *Photochem. Photobiol.* 15 527.
[28] Kulucsics Z, Perdiz D, Brulay E, Muel B and Sage E 1999 *J. Photochem. Photobiol B: Biol.* 49 71.
[29] Jiang Y, Rabbi M, Kim M, Ke C, Lee W, Clark R L, Mieczkowski P A and Marszalek P E 2009 *Biophys. J.* 96 1151.
[30] Schuch A P, da Silva Galhardo R, de Lima-Bessa K M, Schuch N J and Menck C F 2009 *Photochem. Photobiol. Sci.* 8 111.
[31] Mouret S, Philippe C, Gracia-Chantegrel J, Banyasz A, Karpati S, Markovitsi D and Douki T 2010 *Org. Biomol. Chem.* 8 1706.
[32] Bensasson R V, Land E J and Truscott T G 1993 Oxford University Press, Oxford (UK)
[33] Davies M J 2003 *Biochem.Biophys. Res. Comm.* 305 761.
[34] Pattison D I and Davies M J 2006 L.P. Bignold editor Birkhaüser verlag (Switzerland) 131-157
[35] Shacter E 2000 *Methods Enzymol.* 319 428.
[36] Tyrrell R M and Sage E 2011 *Handbook of Photochemistry and Photobiology* 3rd edition CRC Press in the press

[37] Tyrrell R M, Webb R B and Brown M S 1973 *Photochem. Photobiol.* 18 249.

[38] Vile G F and Tyrrell R M 1995 *Free Rad. Biol. Med.* 18 721.

[39] Maresca V, Flori E, Briganti S, Camera E, Cario-Andrè M, Taïeb A, and Picardo M *J. Invest. Dermat.* 126 182.

[40] Ogawa F, Sander C S, Hansel A, Oehrl W, Kasperczyk H, Elsner P, Shimizu K, Heinemann S H and Thiele J J 2006 *J. Invest. Dermat.* 126 1128.

[41] Bravard A, Vacher M, Gouget B, Coutant A, de Boisfuron F H, Marsin S, Chevillard S and Radicella J P 2006 *Mol. Cell. Biol.* 26 7430.

[42] Eiberger W, Volkmer B, Amouroux R, Dhérian C, Radicella J P and Epe B 2008 *DNA Repair* 7 912.

[43] Dardalhon D, Angelin A R, Baldacci G, Sage E and Francesconi S 2008 *Cell Cycle* 7 611.

[44] Montaner B, O'Donovan P, Reelfs O, Perrett C M, Zhang X, Xu Y Z, Ren X, Macpherson P, Frith D and Karran P 2007 *EMBO Rep.* 8 1074.