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Interaction of UV radiation with DNA

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It has long been known that the incidence of ultraviolet radiation on microorganisms can lead to cell death[1] and mutation.[2] Ultraviolet radiation is also harmful to higher organisms and, in humans, it causes sunburn, skin aging, corneal damage and skin cancer. As early as 1943, Hollaender discovered[3] that the killing of bacteria by ultraviolet radiation is wavelength dependent and proposed that below 300 nm the lethal effect results from a direct photochemical process, *i.e.* photon absorption by nucleic acids. Thirty years later Setlow postulated that "ultraviolet light-induced skin cancer probably arises from photochemical changes in DNA, the shorter wavelengths being much more effective than the longer ones in damaging this polymer", and he pointed to "overwhelming evidence that changes in DNA, such as formation of pyrimidine dimers and other photochemical products, have important biological consequences".[4] Based on an accurate measurement of the absorption spectrum of DNA above 300 nm and on its properties, Sutherland and Griffin suggested that the photophysical processes following absorption of long-wavelength photons differ on average from those induced by shorter-wavelength photons.[5] Since then, a large scientific community worldwide has investigated the formation and detection of DNA photolesions induced by direct UV absorption or *via* photosensitization reactions, as well as their repair and their roles in mutation induction and carcinogenesis, with particular emphasis on the major types of skin cancer, including melanoma.

In parallel, the knowledge that base pairing induces an important decrease in the intensity of the absorption spectra (hypochromism) triggered the first studies on the excited states of DNA. Already in the 1960s, Tinoco and Rhodes had performed theoretical calculations with the aim of explaining the nature of the absorption spectrum of DNA.[6,7] The detection, for the first time in 1971, of DNA fluorescence at room temperature by Daniels & Hauswirth and Vigny[8,9] allowed discussion of excited state relaxation under physiological conditions. Yet, for several decades such studies remained quite limited because of the extremely low fluorescence quantum yield of DNA (of the order of 10^{-4}) and the very short fluorescence lifetime of its building blocks. At the turn of this century, the development of femtosecond spectroscopy and the improvement of computational techniques allowed the study of primary processes preceding photochemical reactions and revealed the complexity of the excited state relaxation processes. Thus, the oversimplified terms "singlet" or "triplet" excited states, hitherto used to explain the UV-induced reactivity of DNA, gave way to a variety of excited states: $\pi\pi^*$, $n\pi^*$, $\sigma\pi^*$, excitons, and charge transfer states. Charge transfer states involving neighbouring bases proved to be responsible for the hypochromism of DNA and to play a key role in its direct damage induced by UVA radiation. The overall picture was further complicated by the demonstration that charge and energy transfer take place among the DNA bases.

It is thus understandable that, despite the intense research activity of the past fifty years, a large number of questions remain to be answered. In the present issue, dedicated to the interaction of UV radiation with DNA, salient new insights are featured. They include biological effects and photochemical mechanisms as well as characterization of the electronic excited states and their relaxation.

Their major role as premutagenic lesions in DNA has engendered new studies on the formation and properties of cyclobutane photodimers containing cytosine and its derivatives. Owing to the chemical instability of cytosine dimers, it has hitherto proved difficult to prepare them at the dinucleotide level for molecular and biological studies. Peyranea and Clivio have been exploring a novel synthetic route and succeeded in synthesizing in good yield a stable precursor to the unstable cytosine cyclobutane dimer, which should allow introduction of this lesion into oligonucleotides. Pfeifer *et al.* have investigated the propensity of the rare DNA base 5-hydroxymethylcytosine to undergo photodimerization with neighbouring pyrimidines in synthetic oligonucleotides and have also demonstrated that dimers containing 5-hydroxymethylcytosine are present in the DNA of UV-irradiated human cells. By using ligation mediated PCR, Drouin *et al.* have mapped and quantified the formation of pyrimidine photodimers in six human gene sequences subjected to UVB irradiation *in vitro* and *in cellulo*. Statistical analysis of the results has revealed that there is a distinct tendency

for the damage hotspots to occur at pyrimidine doublets containing cytosine, which have higher mutagenic potential than TT dipyrimidine sites. Indeed, many of these sites map to frequently mutated sites. Ikehata *et al.* have examined sites of DNA damage formation through the window of mutation spectra induced in mouse skin by solar UV radiation, following exposure to UVB, UVA2 (320–340 nm), UVA1 (340–400 nm), 364 nm laser, and natural sunlight. They find that the solar UV-signature mutation, *viz.* C to T base substitution mutation at methylated CpG-associated dipyrimidine (Py-mCpG) sites, is observed with wavelengths throughout the UVB and UVA ranges, being preferentially induced by UVA1 rather than by shorter wavelengths. Interestingly, this finding suggests that the cyclobutane pyrimidine dimer formation that is responsible for such mutations is mediated by excited states of the pyrimidine bases that can be generated even by low energy UVA1 photons.

Menck *et al.* have investigated the relative role of DNA damage induced by UVA irradiation in human cells. Using a sensitive immunological method, these authors report for the first time the detection of (6-4) photoproducts in *Xeroderma pigmentosum* group A (XP-A) cells upon UVA irradiation and show that, in the absence of repair (in XP-A cells), both cyclobutane pyrimidine dimers and (6-4) photoproducts trigger cell death by apoptosis and necrosis. UVA radiation also causes prolonged oxidative stress, an accumulation of DNA strand breaks and oxidized bases, and protein carbonylation, all of which are more specifically related to the induction of necrosis. Even though UV light is a proven risk factor in the development of numerous ocular pathologies, including cataract and possibly age-related macular degeneration, little is known about the production of DNA photolesions in the various ocular structures. Mallet and Rochette have examined cyclobutane pyrimidine dimer induction in the human cornea by UVC, UVB and UVA radiation in order to establish the penetrance of these different UV components into the structures of the eye. They show that UVC and UVB do not penetrate deeper than the corneal epithelium, whereas UVA can induce cyclobutane pyrimidine dimers throughout the entire cornea and in the first layers of the iris. While DNA photolesions can have deleterious outcomes such as cell death or mutation induction leading to skin cancer, they can also serve as a biological sensor for environmental sunlight exposure, as proposed by Schuch *et al.* Indeed, the DNA damage profile largely depends on the ratio of UVA/UVB in sunlight and thus varies with latitude, the season, time of day, cloud cover, and air pollution. In principle, DNA-based biological dosimeters can also be employed for the evaluation of sunscreen efficacy.

The use of highly sensitive analytical tools to detect UV-induced reaction products is a key factor for understanding the photoreactivity of DNA; the impact of chromatographic techniques to this end is emphasized in a review by Douki. Focusing on photochemical reactions in nucleosomes, the contributions by Rokita *et al.* and Kohler *et al.* examine factors that may affect thymine dimerization. Their experiments were carried out *in vitro* under continuous irradiation, where the accumulation of cyclobutane dimers reflects both their formation and their reversal. The former study could not detect any periodicity effect due to the specific architecture of nucleosomes, while the latter indicated that the yield of the photoreaction depends strongly on the nature of the bases adjacent to the reacting thymines, as also emphasized by Drouin.

Individual UV-induced photoproducts are also the subject of current investigations. In relation to the secondary photochemistry of the (6-4) photoproduct, Gilch *et al.* report a photophysical study of 1-methyl-2(1*H*)-pyrimidinone in various solvents carried out by femtosecond spectroscopy. The conversion of the oxetane reaction intermediate to (6-4) photoproduct is investigated theoretically by Eriksson *et al.* Finally, a theoretical study by Kumara and Sevilla rationalizes proton-coupled electron transfer in Watson–Crick pairs between 8-oxo-guanine and adenine or cytosine, a mechanism put forward previously to explain experimental results pertaining to the repair of TT dimers.

Proton-coupled electron transfer is also suggested by Lewis and Markovitsi *et al.* in order to explain the quenching of $\pi\pi^*$ fluorescence in hairpins and duplexes with alternating guanine–cytosine sequence; unexpectedly, such systems emit long-lived fluorescence at higher energy, associated with charge transfer states. Interconversion between charge transfer states and $\pi\pi^*$ excitons in adenine stacks is described theoretically by Santoro and Improta *et al.*, while Lischka *et al.* examine the properties of charge transfer states in the adenine dinucleotide. Excitons populated directly upon photon absorption are discussed by Nielsen *et al.* A study by Voityuk deals with their degree of delocalization, showing that the $\pi\pi^*$ excited states of adenine–thymine homo-oligomers are extended over three bases.

One would think that by now the excited state relaxation of the DNA building blocks has no more secrets. But obviously this is not true. Kwok *et al.*, combining femtosecond fluorescence spectroscopy and transient absorption, report unprecedented emission from the guanine nucleoside and nucleotide, attributed to the $\sigma\pi^*$ state. In a joint experimental and theoretical study, Gustavsson and Improta *et al.* revisit the behaviour of adenosine with particular care paid to the role of solvent effects. In a theoretical work, Lia and Blancafort study the excited state tautomerization of cytosine.

In addition to natural bases, modified bases have been investigated by time-resolved spectroscopy and theoretical methods. These studies are useful in several respects. First they contribute to our understanding of the behaviour of natural bases. This is the case with the study on the ultrafast electronic deactivation of inosine dimers reported by Temps *et al.* Secondly, they serve as probes of local dynamics and energy transfer in oligonucleotides. 2-Aminopurine is a typical example: Crespo-Hernandez *et al.* present a complete photophysical characterization using transient absorption from the femtosecond to

the microsecond time-scale. Matsika *et al.* report a theoretical study on the excited state relaxation mechanisms of stacked dimers formed between 2-amonopurine and adenine or guanine, while similar effects were also examined by Nachtigalova, Lischka *et al.* in the case of xanthines *via* various computational methods. Strongly fluorescent tricyclic cytosines are used by Wilhelmsson *et al.* to trigger light-induced changes in duplex stability. The photochemistry of sulphur-containing nucleobases is of considerable interest owing to their ability to sensitize DNA towards UV radiation, with potential applications in photochemotherapy. In this context, Miranda *et al.* have made a detailed experimental and theoretical study of the excited state properties of 2-thiouracil and how these are altered by substituents at the C(5)-position. Depending on their modes of interaction with DNA, complexes of ruthenium(II) with polypyridyl ligands can sensitize photodamage by different mechanisms. In this respect, Vicendo *et al.* have investigated how intercalation and redox potential influence the nature of the DNA lesions resulting from photosensitization by individual ruthenium complexes.

The diverse and important biological effects resulting from the interaction of UV radiation with DNA have inspired numerous photophysical and photochemical studies. Although such studies are performed *in vitro* or *in silico*, their conclusions may nonetheless be relevant *in cellulo*. Yet, their inclusion in discussions of photobiological phenomena requires improved communication between communities who use a different "language". We hope that the present themed issue constitutes a step in this direction.

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References

1 F. L. Gates, A study of the bactericidal action of ultraviolet light. III. The absorption of ultra-violet light by bacteria, *J. Gen. Physiol.*, 1930, **14**, 31–42.
2 A. Hollaender and C. W. Emmons, Wavelength dependence of mutation production in the ultra-violet with special emphasis on fungi, *Cold Spring Harbor Symp. Quant. Biol.*, 1941, **9**, 179–186.
3 A. Hollaender, Effect of long ultraviolet and short visible radiation (3500 to 4900 Å) on *Escherichia coli*, *J. Bacteriol.*, 1943, **46**, 531–541.
4 R. Setlow, The wavelengths in sunlight effective in producing skin cancer: A theoretical analysis, *Proc. Natl. Acad. Sci. U. S. A.*, 1974, **71**, 3363–3366.
5 J. C. Sutherland and K. P. Griffin, Absorption spectrum of DNA for wavelengths greater than 300 nm, *Radiat. Res.*, 1981, **86**, 399–409.
6 I. Tinoco Jr., Hypochromism in polynucleotides, *J. Am. Chem. Soc.*, 1960, **82**, 4785–4790.
7 W. Rhodes, Hypochromism and other spectral properties of helical polynucleotides, *J. Am. Chem. Soc.*, 1961, **83**, 3609–3617.
8 M. Daniels and W. Hauswirth, Fluorescence of the purine and pyrimidine bases of nucleic acids in neutral aqueous solution at 300 K, *Science*, 1971, **171**, 675–677.
9 P. Vigny, Fluorescence des nucléosides et nucléotides à température ambiante, *C. R. Acad. Sci. Paris*, 1971, **272D**, 3206–3209.

