Ultraviolet Radiation Damage in DNA

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Introduction
The evolution of most forms of life has occurred in an environment exposed to the ultraviolet (UV) and visible radiation emanating from the sun. When molecules in living cells absorb photons in this region of the electromagnetic spectrum they are promoted to excited electronic states and this process can have profound biological consequences. In general, visible wavelengths (400–780 nm) are associated with effects which are beneficial to living organisms, such as photosynthesis and vision. However, ultraviolet photons, with wavelengths below 400 nm, have a capacity for damaging cells which becomes increasingly pronounced at shorter wavelengths and higher energies. Conventionally, the UV spectrum is sub-divided into three discrete sections: UVA (320–400 nm), UVB (280–320 nm) and UVC (wavelengths < 280 nm). Radiation at UVB and UVC wavelengths has genotoxic properties and is invariably harmful to living cells. By damaging the genetic material, DNA, it can induce such deleterious processes as mutagenesis, carcinogenesis and cell death [1,2].

This paper reviews the nature of the radiation damage produced in DNA by the direct absorption of UV photons. Special reference is made to the environmental impact of increased levels of UVB radiation in the biosphere resulting from depletion of the ozone layer. The main focus is on the structural identity and detection of the intramolecular photoproducts derived from the purine and pyrimidine nucleobases in DNA. The role of repair enzymes in removing these photoproducts from DNA is also discussed in connection with their use as probes for locating specific lesions by gel sequencing methods.

Historical background
Our current knowledge of ultraviolet radiation damage in DNA is based almost entirely on research that has been carried out during the past three decades. Nonetheless, the first pertinent scientific investigation dates from 1877, when Downes and Blunt [3] demonstrated that sunlight is capable of killing micro-organisms. By using a series of filters, they established that the 'actinic rays' in sun-
light (encompassing the UV region) were responsible for this lethal effect.

Despite its significance, this discovery did not stimulate further research at the time, and some 50 years were to elapse before Gates [4] measured action spectra for the killing of different bacteria. This work clearly established that UV light was responsible for the lethal biological effect. Moreover, Gates remarked that the shapes of the action spectra closely resembled the absorption spectrum of the nucleotide constituents of a cellular component then known simply as nucleic acid. This finding strongly implicated nucleic acid as being the main target for UV radiation damage within the cell but, in 1930, very little was known about its chemical nature. Soon thereafter, it was realized that UV light is also a powerful mutagenic agent, and once more the action spectrum for this effect correlated closely with the absorption spectrum of nucleic acid.

Further progress towards understanding the basis of the lethal and mutagenic properties of UV radiation was then very limited until it was recognized that one of the nucleic acids — namely DNA — acts as the carrier of genetic information in all cellular organisms. Following the determination of its structure by Watson and Crick [5], in 1953, it became possible to explain the action of a variety of cytotoxic and mutagenic chemicals in terms of their reactions with the purine and pyrimidine bases in DNA which encode the genetic information. Thus many common carcinogens and mutagens exert their effects by chemically modifying the structures of the DNA bases so that their coding properties are altered. It was natural that a similar explanation should be sought for the action of UV light, especially as the heterocyclic nucleobases constitute the chromophores in DNA that are responsible for its intense UV absorption in the region of 260 nm. The key breakthrough came in 1960 when Beukers and Berends [6] (in the Netherlands) and, independently, Wang [7] (in the United States) discovered that the pyrimidine base thymine forms a cyclobutane photodimer when it is irradiated at 254 nm in frozen aqueous solution. The same species of photodimer was isolated [8,9] from acid hydrolysates of UV-irradiated DNA, where it is produced by a photoaddition reaction between adjacent thymines on the same polynucleotide strand. The occurrence of UV radiation damage in DNA was thus firmly established and these studies initiated a great upsurge of research into the photochemistry and photobiology of nucleic acids which has continued unabated ever since.

**Mechanisms of DNA photodamage**

It is now known [10] that exposure of DNA to UV light can result in the formation of a range of different photoproducts whose distribution and relative yields depend on the wavelength and intensity of the incident radiation. Ultimately, the photoreactivity of DNA is a function of its absorption spectrum which is shown (with a logarithmic absorbance scale) in Figure 1. In essence, there is increasingly strong absorption at wavelengths below 300 nm with a peak at 260 nm; the small but finite absorption in the region of 300 nm tails off to zero at approx. 320 nm. This profile determines the mechanisms by which radiation damages DNA. Photons in the UVC region (below 280 nm), which are strongly absorbed by the purine and pyrimidine bases, generate excited-state species that undergo intramolecular photochemical reactions, such as thymine photodimerization. In contrast, photons with wavelengths in the UVA region (> 320 nm) are not absorbed at all. They can, however, damage DNA indirectly through the agency of photosensitizer molecules [11]. In this case, the photosensitizer absorbs the UVA radiation and initiates damage through processes such as triplet energy transfer, photoadduct formation or the generation of active oxygen species (e.g. singlet oxygen). Photons in the intermediate UVB range (280–320 nm), which are absorbed only weakly by DNA, can damage it by...
either or both mechanisms depending on the experimental conditions.

When cells are irradiated in their natural physiological environment, DNA damage induced by the direct absorption of photons is the most important source of mutations and cytotoxic effects. Consequently, this paper focuses primarily on the intramolecular photoproducts that are generated by direct absorption at UVB and UVC wavelengths. The discussion is confined to photoreactions induced by low intensity radiation. It does not deal with photolesions produced via two-photon excitation with high powered lasers, although this is a subject of considerable contemporary interest [12].

Solar UV radiation

At this point, it is pertinent to comment on the potential of natural sunlight to produce UV radiation damage in DNA, particularly as it relates to the seminal observations of Downes and Blunt [3] discussed above. The UV radiation present in sunlight has undoubtedly been a powerful influence affecting the evolution and development of the plant and animal kingdoms [13]. While its mutagenic action has provided a driving force for evolutionary change, solar radiation has, nevertheless, posed a constant threat to the actual survival of living organisms.

The energy spectrum of sunlight reaching the surface of the earth exhibits peak irradiance in the visible region, above 400 nm, and the intensity falls off sharply at ultraviolet wavelengths to a cut-off at around 290 nm. The exact position of this cut-off depends on such factors as latitude, altitude and the local weather conditions. The cell-damaging effects of sunlight are almost entirely attributable to the high-energy photons at UVB wavelengths below 315 nm that can be absorbed by DNA [13]. Collectively, these rays comprise only a very small proportion of terrestrial sunlight and amount to less than 1% of the energy incident at the surface of the earth. Although the sun emits throughout the UVB and UVC regions, wavelengths shorter than 290 nm are completely absorbed by the ozone layer in the upper atmosphere. Consequently, the ozone layer plays a crucial role in shielding the environment at the earth's surface from most of the biologically harmful radiation emanating from the sun.

There is, currently, mounting evidence and concern that chlorofluorocarbons (CFCs) and other man-made chemicals are gradually destroying the protective ozone layer [14]. The 'ozone hole' around the South Pole, which first came to notice [15] in the mid-1980s, was at its deepest [16] in the Antarctic spring of 1993. Also, satellite-based measurements [17] have shown that in 1992 the global average total ozone was 2-3% lower than for any other year on record. If erosion of the ozone layer continues there will be a reduction in the short wavelength cut-off of terrestrial sunlight and a concomitant increase in the intensity of UBV radiation in the environment. This may have serious repercussions throughout the biosphere. In human terms, an increase in the incidence of skin cancers can be expected [18], especially in fair-skinned people of Celtic descent. However, the ecological consequences for the vast populations of micro-organisms, animals and plants that experience prolonged exposure to sunlight in their natural habitat are largely unknown. For these reasons, it is vital to ensure that the current initiatives to regulate emissions of CFCs (and other ozone-destroying chemicals) on an international basis are successful.

Pyrimidine photoproducts in DNA

After the discovery of thymine photodimerization it was soon recognized that the other pyrimidine base, cytosine, can undergo a similar photoaddition reaction with adjacent cytosine or thymine bases on the same strand of DNA. All of the resulting photodimers contain a cyclobutane ring and have cis-syn stereochemistry; their structures are shown in Figure 2. While the thymine moieties of these dimers are chemically stable under physiological conditions, the cytosine moieties are liable to undergo deamination to give uracil or mixed
thymine–uracil photodimers. Collectively, the cyclobutane photodimers of thymine and cytosine are the most abundant photoproduc.ts found in UV-irradiated DNA and the overall quantum yield for their formation [10,19] is approx. $10^{-2}\text{mol} \cdot \text{einstein}^{-1}$. They exhibit the phenomenon of photoreversion, whereby they are split back into their constituent pyrimidine bases by UVC radiation; the efficiency of this process is greater at shorter wavelengths. Hence, at very high fluences, a state of photoequilibrium is reached in which the rate of production of cyclobutane dimers equals their rate of photoreversion. There is convincing evidence from biological studies that pyrimidine dimers can not only kill cells but also initiate carcinogenesis and cause mutations [1,20].

Adjacent pyrimidine bases in DNA can also become covalently linked in another way on exposure to UV light. This competing photoreaction produces bipyrimidine (6-4) photoadducts which contain a pyrimidin-2-one ring system [10,21]. The structures of the adducts formed by thymine at TC and TT doublets are shown in Figure 3. These photoproducts are generated by photoaddition of the 5,6-double bond of a 5'-pyrimidine base across either the exocyclic C(=O)-carbonyl group of a 3'-thymine, or the C(=N)-imino function of a 3'-cytosine. The oxetane or azetidine precursor thus formed then rearranges to give the stable (6-4) photoproduct. Typically, the yield of these photolesions in DNA is about 0.1–0.5× that of cyclobutane photodimers [10,21], and there is persuasive evidence [21] associating them with mutational events as well as cytotoxicity. There is, however, some uncertainty regarding the actual species responsible for the observed biological effects because the (6-4) photoproducts can undergo photoisomerization to Dewar valence isomers of the pyrimidin-2-one species. Accordingly, UV-irradiated DNA will normally contain a mixture of the individual (6-4) photoproduc.ts and their Dewar valence isomers.

An important monomeric pyrimidine photoproduc.ts arises by photochemical addition of the elements of water across the 5,6-double bond of cytosine. This yields 6-hydroxy-5,6-dihydrocytosine (Figure 5), which is usually known as cytosine photohydrate. Its formation occurs much less readily in native than in single-stranded DNA and, with a quantum yield of approx. $10^{-3}\text{mol} \cdot \text{einstein}^{-1}$, it is a minor photoproduc.ts in comparison with cyclobutane photodimers and (6-4) photoproducts [22]. Cytosine photohydrate has the potential to cause transition mutations through its deamination and subsequent dehydration to uracil [23].

When DNA is irradiated in aqueous solution, or a normal cellular environment, photomodification of the pyrimidine bases is confined almost entirely to the above photoproducts. In contrast, UV irradiation of bacterial spores, where the DNA is essentially in a dehydrated state, gives 5,6-dihydro-5-(α-thymyl)thymine (Figure 5) as the main pyri-

**Figure 4.** The isomerization is photochemically reversible, with UVB wavelengths favouring formation of the Dewar structure and UVC the conventional pyrimidin-2-one species. Accordingly, UV-irradiated DNA will normally contain a mixture of the individual (6-4) photoproduc.ts and their Dewar valence isomers.

**Figure 5.** Structures of cytosine photohydrate (left) and the 'spore' photoproduc.t (right).
midine photoproduct [19]. This altered behaviour illustrates the important principle that the photoreactivity of DNA is highly dependent on its conformational state.

Purine photoproducts in DNA

For many years the purine bases in DNA were considered to be essentially inert towards UV radiation and their photochemistry received relatively little attention. A major factor influencing this view was that, in contrast to their pyrimidine counterparts, adenine and guanine and their monomeric nucleoside and nucleotide derivatives are very resistant to photochemical change when irradiated at UVC wavelengths in solution [19,24]. This lack of photoreactivity appeared to extend to adenine and guanine bases incorporated into DNA because, by the early 1980s, no convincing evidence had been obtained for the presence of any intramolecular purine photoproducts in UV-irradiated DNA [11]. At that time, our laboratory embarked on a systematic study of the photoreactivity of purine-containing deoxydinucleoside monophosphates as model compounds for DNA. This has led to the discovery of new photoproducts derived from adenine which are present in UV-irradiated DNA. Our investigations were inspired by Pörschke's finding [25], in 1973, that adjacent adenine bases in oligomers and polymers of deoxyadenylic acid can undergo photodimerization. Although Pörschke's work clearly indicated the potential photoreactivity of adenines on a DNA backbone, it did not address the chemical characterization of the photoproduct(s), and subsequent experiments by Rahn [26] failed to demonstrate their occurrence in natural DNA.

There are 12 deoxydinucleoside monophosphate molecules corresponding to the doublet sequences in DNA that contain one or two purine bases. All of them were screened [27,28] for photoreactivity induced by 254 nm radiation and, while most were relatively inert, the formation of specific photoproducts was detected in the case of d(TpA) and d(ApA). The individual photoproducts were isolated and then purified to homogeneity by HPLC. Their molecular structures (described below) were determined by a combination of spectroscopic and chemical methods, details of which can be found in the cited references. Extensive use was made of high-resolution mass spectrometry, one and two-dimensional NMR spectroscopy, chemical modification and chemical degradation studies.

With d(TpA), photoaddition occurs between its constituent thymine and adenine bases to give the only known example of a mixed purine-pyrimidine photoadduct [29]. The reaction is sequence-specific [i.e. it does not take place in the sequence isomer d(ApT)] and it has a quantum yield of $7 \times 10^{-4}$. The photoproduct, denoted TA*, bears some resemblance to a pyrimidine photodimer since it contains a cyclobutane ring linking C(s) and C(h) of thymine to C(h) and C(s) of adenine respectively [30]. The actual conformation of TA*, shown in Figure 6, was determined [31] by two-dimensional NMR spectroscopy in conjunction with distance geometry and molecular dynamics calculations. The photoadduct has trans-syn stereochemistry with the orientation about the glycosidic bonds being syn for the thymidine residue and anti for the deoxyadenosine moiety. This geometry is somewhat unexpected because the normal stacked conformation of the bases in d(TpA) should favour cis-syn stereochemistry of the cyclobutane ring.

Detection of the thymine–adenine photoproduct in UV-irradiated polynucleotides and DNA is greatly facilitated by the fact that acid hydrolysis (1 M HCl, 100°C, 4 h) converts it specifically into the intensely fluorescent heterocyclic base 6-methylimidazo[4,5-b]pyridin-2-one (6-MIP) shown in Figure 7. The structural identity of 6-MIP has been independently confirmed by the photorearrangement of 1-methyl-1-deazapurine $N(3)^\text{C}$ oxide, a reaction which forms the basis for a very sensitive fluorogenic actinometry system for nucleic acid photochemistry [32]. It is straightforward to measure the amount of 6-MIP present in acid hydrolysates of UV-irradiated DNA because it can be separated from the normal nucleobases by HPLC.

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Structure of the thymine–adenine photoadduct, TA*

Adapted from [31].
HPLC or high-voltage paper electrophoresis and quantified by spectrofluorimetry. Alternatively, the 6-MIP can be isolated and estimated by radiochemical assay if the DNA is labelled by incorporation of \([\text{methyl-}^{3}H]\)thymine. In this way, quantum yields have been determined for the formation of TA* in a variety of oligo- and polynucleotides and in native and denatured DNA [33]. From the values shown in Table 1, it can be seen that base pairing causes marked quenching of the photoreaction with an approx. 5-fold reduction in quantum yield. While TA* is certainly formed in DNA, its quantum yield is several hundred times smaller than the quantum yields associated with pyrimidine photodimerization. Consequently, it must be considered as a minor photoproduct in DNA.

In the case of d(ApA), there is a single photodimerization reaction between the adjacent adenine bases, but the primary photoproduct is chemically unstable and decomposes by two competing pathways to give two different photoadducts which can be separated by reversed-phase HPLC [34,35]. During the photochemical step, UV radiation induces a \([Z_{21}\) photoaddition reaction in which the N(i-C(R, double bond of the 5'-adenine adds across the C(h) and C(5) positions of the 3'-adenine \[351.

The initial (precursor) photodimer thus formed (see Figure 8) contains a highly strained azetidine ring which rapidly undergoes fission by two distinct mechanisms. One of these (shown in Figure 8) involves a sequence of rearrangement and oxidative steps leading to the photoproduct designated AA*. On heating with acid, AA* is degraded specifically to the unusual heterocyclic base 8-(5-aminomidazol-4-yl)adenine (8-AIA), in which an adenine nucleus is linked to an aminimidazole moiety. The identity of this hydrolysis product was confirmed by independent chemical synthesis, and its production can be used diagnostically to detect the parent photoproduc, AA*, in samples of UV-irradiated DNA.

The second stable photoproduct [34], denoted \(A = A\), is derived from the primary photodimer by a process involving hydrolytic fission of the azetidine ring and internal rearrangement (see Figure 9). It is also converted by acid (in ~ 50% yield) into a distinctive hydrolysis product, 4,6-diamino-5-guanidinopyrimidine (DGPY), which can be used as a marker for its presence in DNA [36].

To assess whether adenine photodimerization occurs in natural DNA, and to determine its quantum yield, it is necessary to measure individual quantum yields for the formation of both photoproducts, AA* and \(A = A\), and then add them together [36,37]. This can be done by measuring the amounts of their respective hydrolysis products, 8-AIA and DGPY, that are present in acid hydrolysates of UV-irradiated DNA. Both of these compounds can be separated from the usual nucleobases and from each other by reversed-phase HPLC, and this allows their individual collection and quantification. To attain sufficient sensitivity a radiochemical assay has been devised. In this, DNA is labelled with tritiated adenine by nick translation and then exposed to a known dose of 254 nm radiation. Following treatment with acid, known amounts of unlabelled synthetic 8-AIA and DGPY are added to the DNA hydrolysate as carrier material to monitor their subsequent purification. The peaks eluting as 8-AIA and DGPY on HPLC are collected and freed from contaminating tritium activity by further chromatography on Sephadex G-10. Finally, the tritium activity associated with each hydrolysis product is determined and used to calculate the percentage conversion of the adenine bases in the irradiated sample into the photoproducts AA* and \(A = A\). In this way, the quantum yields for their formation can be estimated.

Overall quantum yields for adenine photodimerization in single- and double-stranded DNA are shown in Table 2 together with data for
poly(dA). In agreement with Pörshcke [25], a quantum yield of around $3 \times 10^{-3}$ is observed for single-stranded poly(dA). However, this is greatly reduced (at least 10-fold) upon base pairing with poly(dT). The results for native and denatured DNA also imply that the photodimerization reaction is quenched by base pairing, though not as strongly. This may be due to increased conformational rigidity in double-stranded DNA preventing optimal alignment of the adjacent bases for photoaddition.
Table 2
Estimated quantum yields for adenine photodimerization

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantum yield (μmol·einstein⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(dA)</td>
<td>2600</td>
</tr>
<tr>
<td>Poly(dA)·poly(dT)</td>
<td>220</td>
</tr>
<tr>
<td>Denatured DNA (E. coli)</td>
<td>33</td>
</tr>
<tr>
<td>Native DNA (E. coli)</td>
<td>20</td>
</tr>
</tbody>
</table>

Samples were irradiated at 254 nm in pH 7.0, 0.1 M Na⁺ buffer at 4°C.

[38]. Although adenine photodimerization contributes to UV radiation damage in natural DNA, its quantum yield is very low, being in the range 10⁻⁴ to 10⁻⁵. The dimeric adenine photoproducts, AA* and A=A, must therefore be very rare lesions in UV-irradiated DNA in comparison with the predominant pyrimidine photodimers and (6-4) photoproducts. This has consequences for the quantum yield measurements which inevitably refer to heavily irradiated DNA that is effectively saturated with pyrimidine photoproducts. Also, of course, the data in Table 2 pertain to ‘naked’ DNA irradiated in vitro, and the situation might be somewhat different for DNA associated with histones and other proteins in vivo.

The guanine bases in DNA are extremely resistant to direct photochemical modification. There is, as yet, no definitive evidence for the formation of any intramolecular photoproducts derived from guanine when DNA absorbs UV photons with wavelengths exceeding 240 nm. Guanine is, however, highly susceptible to photooxidation mediated by sensitizer molecules [10,11] and, like adenine, can undergo photoalkylation reactions [24] via a free radical mechanism. We have demonstrated [39] that UVB irradiation of 2'-deoxyguanosine, in the presence of acetone as photosensitizer, can convert it into 8-(2,3,4-trihydroxybutyl)guanine, but this reaction is not observed in DNA.

A number of important DNA repair enzymes act either specifically or in a general way on UV radiation damage. DNA photolyases have the remarkable property of using visible light photons at blue wavelengths to reverse the photodimerization of pyrimidines and thus regenerate the original intact bases [41]. A related activity has recently been characterized [43] in extracts from Drosophila melanogaster which is capable of using visible light to reverse the formation of (6-4) photoproducts. The members of a major class of DNA repair enzymes function by combining N-glycosylase and endonuclease activities, i.e. they cleave photochemically damaged bases from the DNA backbone and then cut it at that point [40]. Endonuclease V from bacteriophage T4, and the equivalent enzymes from prokaryotic and eukaryotic sources, are specific for pyrimidine photodimers [41], while endonuclease III (from Escherichia coli) deals with cytosine photohydrates and other oxidized pyrimidines [44]. The formamidopyrimidine-DNA glycosylase (Fpg protein) from E. coli incises DNA at the sites of photooxidized purines produced by photosensitization [45].

A ubiquitous system of excision repair is mediated by the uvr ABC complex in E. coli [46]. This is capable of dealing with a diverse range of chemical and photochemical lesions in DNA which
distort the geometry of the double helix. It functions by cutting the damaged strand a few nucleotides either side of the modified base(s), the excised oligonucleotide containing 12 nucleotide residues in E. coli and 29 in humans. The exposed and undamaged DNA sequence on the complementary strand is then used as a template by DNA polymerase and ligase enzymes to reconstruct the intact double helical segment.

Mutant organisms lacking normal DNA repair capacity are extremely vulnerable to UV radiation damage. Thus, an E. coli K12 strain deficient in DNA repair (uvr rec) can be inactivated by a dose of 254 nm radiation 2000 times smaller than that required to kill the wild-type bacteria [2]. Recent analysis of a UVB-sensitive strain of the plant Arabidopsis thaliana has shown [47] that it is impaired in its ability to remove (6-4) photoproducts from its DNA. The rare human syndrome Xeroderma pigmentosum arises from mutations affecting a group of genes involved in the repair of DNA photodamage. Afflicted individuals are extremely sensitive to sunlight and minimal exposure can lead to severe skin lesions which tend to become malignant and cause early death.

**Gel sequencing detection of DNA photoproducts**

Apart from their physiological importance, some of the purified DNA repair enzymes can be used as powerful tools for detecting specific types of UV radiation damage in DNA at the level of individual base pairs. Together with certain chemical treatments, they will cut DNA strands selectively at particular photolocations. If 32P-end-labelled DNA molecules are used as substrates, the cleavage fragments thus produced can be resolved on DNA-sequencing-gel autoradiograms and the sites of incision determined by reference to a standard chemical sequencing ladder [48,49]. Not only can specific photoproducts be located in a given DNA molecule but their relative yields, in different sequence contexts, can be estimated from the intensities of the cleavage bands measured by densitometry.

It is now routine practice to detect pyrimidine photoproducts in segments of UV-irradiated DNA through analysis of the cleavage patterns generated by T4 endonuclease V (or equivalent UV endonucleases) in the case of pyrimidine photodimers [49], treatment with hot piperidine for (6-4) photoproducts [50], and use of E. coli endonuclease III for cytosine photohydrates [44]. A good example of this approach can be found in reference [51]. Our studies have focused on possible probes for locating purine photoproducts in DNA. A feature of this work has been the introduction of appropriately designed synthetic oligonucleotides as irradiation targets in place of restriction endonuclease cleavage fragments.

The E. coli Fpg protein can nick DNA at ring-opened purines produced by photosensitization or ionizing radiation [45], but these entities are not significant photoproducts under conditions of direct excitation. However, as shown by Gallagher and Duker [52,53], UVC-irradiated DNA can be cleaved at sites corresponding to pyrimidine bases by an enzyme activity that is present in partially purified preparations of endonuclease V from T4-infected E. coli. The same authors noted [48,52] that crude extracts of Micrococcus luteus contain a similar activity, and we have sought to characterize this further. By a combination of ion-exchange and size-exclusion chromatography, the purine-incising activity of M. luteus has been separated from the UV endonuclease which is specific for pyrimidine photodimers. Experiments in our laboratory with a 32P-end-labelled 44-mer have confirmed [54] the observations of Gallagher and Duker. While essentially inactive towards the unirradiated double-stranded oligonucleotide, the enzyme preparation cleaves the UV-irradiated (254 nm) duplex at a number of sites mapping to pyrimidine bases in the chemical sequencing ladder. This behaviour is consistent with an endonuclease recognizing pyrimidine photolesions. However, the use of this enzyme fraction as a probe for purine photoproducts requires further validation because it is also active as an endonuclease against unirradiated single-stranded DNA. Its action on UV-irradiated duplex DNA might therefore be accounted for by incisions at unpaired purine bases in denatured regions opposite to pyrimidine photoproducts on the complementary strand.

In a photofootprinting technique, devised by Becker and Wang [55], cleavage of the DNA backbone at the sites of photoproducts is accomplished by successive reduction with sodium borohydride and heating with acidic aniline. This chemical treatment is assumed to be selective for 'non-aromatic' photolesions in which the conjugated \( \pi \)-electron systems of the parent nucleobases have been disrupted. When photofootprinting was used [56] to examine the distribution of photoproducts in 5'-end-labelled single- and double-stranded DNA molecules it revealed that, as expected, adjacent pyrimidine bases are major targets for photomodification. However, additional cleavage sites, indicative of photoproduct formation, mapped to purine bases.
flanked on their 5'-side by two or more pyrimidines. Accordingly, it was inferred [56] that purine bases in 5'-PyPyPu.3' triplets possess enhanced photoreactivity. We have subsequently shown [57], through experiments with 3'-end-labelled and 5-methylcytosine-substituted oligonucleotides, that this is not the case. The apparent cleavage at purine residues, which can also be induced by treatment with hot piperidine, is an artefact arising from the anomalous electrophoretic mobility of 5'-end-labelled fragments produced by chemical cleavage of the DNA at adjacent (6-4) photoproducts.

Besides (6-4) photoproducts, several other types of DNA damage caused by photomodification or oxidation are known to behave as alkali-labile lesions; these include abasic sites [48], 2'-deoxyribonolactone residues at ACA triplets [58], thymine glycols [59] and 8-hydroxyguanine [60]. Through experiments with model oligonucleotides, we have demonstrated that thymine-adenine photoaducts and dimeric adenine photoproducts also fall into this category. This is illustrated, in the latter case, by the densitometry profiles in Figure 10. These represent the cleavage patterns observed on sequencing gel autoradiograms when 5'- or 3'-end-labelled d(A,GA,GA,GA,CA,G) is heated with piperidine following UVC irradiation. There is strong cleavage at adenines located next to the unreactive guanines on the 3'-side but minimal cleavage at those on the 5'-side. This result implies that fragments are produced (via abasic sites) by selective fission of the glycosidic bonds to the 5'-base moieties of one or both types of dimeric adenine photoproduct.

Despite their alkaline lability, the intramolecular adenine photoproducts cannot normally be detected in UV-irradiated DNA by standard sequencing protocols. Owing to their very low quantum yields of formation, there is a strong probability that when a given DNA molecule contains a photoproduct at a particular AA or TA site, it will also contain additional (6-4) photoproducts as they are formed in much higher yield. If one of these (6-4) photoproducts is located nearer to the labelled terminus the associated chain cleavage induced by hot piperidine will prevent detection of the adenine photoproduct.

**Concluding remarks**

This paper has focused primarily on the photomodification of the pyrimidine and purine nucleobases ensuing from the direct absorption of UV photons by DNA. This process is ultimately responsible for the mutagenic, carcinogenic and cytotoxic properties of short-wavelength UV radiation. It also has a direct bearing on current concerns about the disappearance of the ozone layer which protects the environment from the harmful UVB rays in sunlight.

Reflecting my research interests, particular emphasis has been placed on the detection and characterization of purine photoproducts in DNA. Under conditions of direct excitation, they probably account for less than 1% of the total photodamage, which is dominated by pyrimidine photodimers and (6-4) photoproducts. Nonetheless, purine photolesions may contribute to the mutagenic and cytotoxic effects of UV light; for example, a mean lethal fluence (at 254 nm) for wild-type *E. coli* bacteria, which induces around 3000 pyrimidine dimers per genome [2], should also be sufficient to produce in the region of 10 adenine photodimers or thymine-adenine photoadducts. Clearly, the ability of cells to repair purine photolesions is a key factor determining their biological importance, and this subject needs to be addressed in future research. Such studies should be facilitated by continuing advances in the synthetic chemistry of nucleic acids allied to genetic manipulation techniques. There is now scope for investigating the biological properties of individual photoproducts by incorporating them into DNA constructs [61] which can be introduced specifically into target cells via suitable vectors [62]. This powerful approach is exemplified [63] by a
recent investigation of the mutagenic potential of a site-specific thymine–uracil photodimer in the M13 bacteriophage genome and could soon be extended [64] to the thymine–adenine photoadduct.

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38 Friedberg, E. C. (1985) DNA Repair, W. H. Freeman, San Francisco
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