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UV-induced DNA damage and repair: a review

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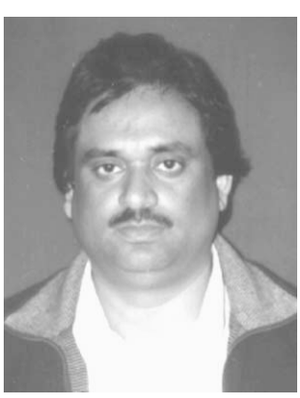
Increases in ultraviolet radiation at the Earth's surface due to the depletion of the stratospheric ozone layer have recently fuelled interest in the mechanisms of various effects it might have on organisms. DNA is certainly one of the key targets for UV-induced damage in a variety of organisms ranging from bacteria to humans. UV radiation induces two of the most abundant mutagenic and cytotoxic DNA lesions such as cyclobutane–pyrimidine dimers (CPDs) and 6–4 photoproducts (6–4PPs) and their Dewar valence isomers. However, cells have developed a number of repair or tolerance mechanisms to counteract the DNA damage caused by UV or any other stressors. Photoreactivation with the help of the enzyme photolyase is one of the most important and frequently occurring repair mechanisms in a variety of organisms. Excision repair, which can be distinguished into base excision repair (BER) and nucleotide excision repair (NER), also plays an important role in DNA repair in several organisms with the help of a number of glycosylases and polymerases, respectively. In addition, mechanisms such as mutagenic repair or dimer bypass, recombinational repair, cell-cycle checkpoints, apoptosis and certain alternative repair pathways are also operative in various organisms. This review deals with UV-induced DNA damage and the associated repair mechanisms as well as methods of detecting DNA damage and its future perspectives.

1 Introduction

There is conclusive evidence that the stratospheric ozone layer, which shields the Earth from the biologically most hazardous short-wavelength solar radiation, is currently experiencing continuous depletion, catalyzed by anthropogenically released atmospheric pollutants such as chlorofluorocarbons (CFCs), chlorocarbons (CCs) and organo-bromides (OBs) and the consequent increase in solar ultraviolet-B (UV-B; 280–315 nm) radiation reaching the Earth's surface.[1–4] Ozone depletion has been reported in the Antarctic as well as in the Arctic and subarctic regions,[5] but it is most pronounced over the Antarctic, where ozone levels have declined by more than 70% during late winter and early spring during the last few decades. This decline in ozone level is commonly attributed to a unique combination of extreme cold and stratospheric circulation (the polar vortex) which results in conditions that are favorable for the CFC–ozone reactions. Polar stratospheric clouds play important roles in the formation of the springtime Antarctic ozone hole by activating chlorine and denitrifying the stratosphere. Recent TOMS (total ozone mapping spectrometer) data indicate an Antarctic ozone hole that is three times larger than the entire land mass of the United States. The hole had expanded to a record size of approximately 28.3 million square kilometers in the year 2000.[6] Moreover, ozone depletion and the associated increased UV radiation have been predicted to continue throughout most of this century.[7–9] Recent reports indicate that widespread severe denitrification could enhance future Arctic ozone loss by up to 30%.[9]

The xenotoxic effects of solar UV radiation are thought to have precluded the development of terrestrial life for two or possibly three billion years, before the stratospheric ozone layer developed. Ultraviolet radiation induces deleterious effects in all living organisms ranging from prokaryotic bacteria to eukaryotic lower and higher plants, animals and humans. While UV-C (<280 nm) radiation is ecologically not relevant since it is quantitatively absorbed by oxygen and ozone in the Earth's atmosphere, the longer wavelength UV-B (280–315 nm) and UV-A (315–400 nm) radiation can have significant effects on the biota, even though the majority of the extraterrestrial UV-B is absorbed by stratospheric ozone.[10] The adverse effects of solar radiation on living systems are mostly attributed to the small amount of UV-B that is absorbed by cellular DNA. UV-A wavelengths are less efficient in inducing DNA damage because they are not absorbed by native DNA but they can still produce secondary photoreactions of existing DNA photoproducts or damage DNA *via* indirect photosensitizing reactions. Some of the biological effects of solar UV radiation include reduction in growth and survival, protein destruction, pigment bleaching and photoinhibition of photosynthesis in



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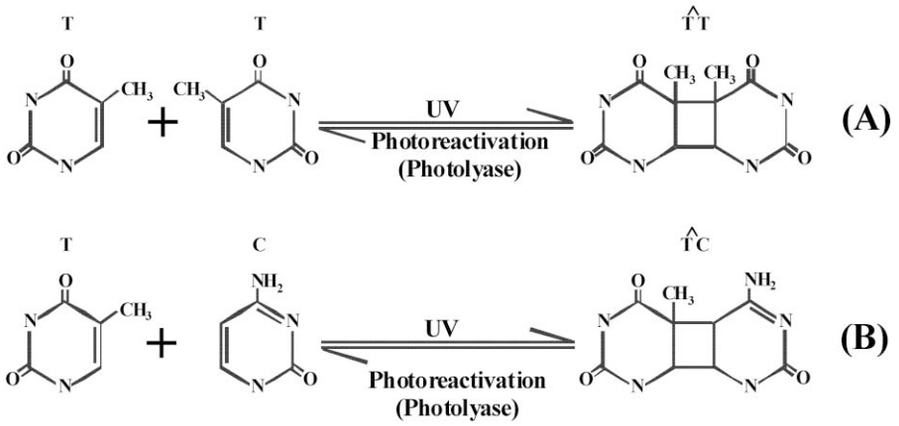

Fig. 1 Formation of the most toxic and mutagenic DNA lesion, cyclobutane–pyrimidine dimers by UV radiation. Dimers can form between two adjacent pyrimidines. Shown here is (A) thymine–thymine cyclobutane–pyrimidine dimer, and (B) thymine–cytosine dimer and their photoreactivation by the enzyme photolyase in the presence of light. For details, see text.

several organisms.[11–17] Photodynamic reactions are potential mechanisms by which ultraviolet radiation damages living cells.[18] The high energy short-wavelength photons absorbed by chromophoric molecules can lead to the formation of singlet oxygen or free radicals known to destroy membranes and other cellular components. In fact, photons from UV-A radiation and visible light up to 670 nm are also able to generate $^1O_2$ through type II photosensitization reactions.[19,20]

DNA is obviously one of the key targets for UV-induced damage in a variety of organisms such as bacteria,[21,22] cyanobacteria,[23] phytoplankton,[24,25] macroalgae,[26] plants,[27] animals and humans.[28,29] All biological cells are rich in UV-absorbing agents such as nucleic acids and proteins. A number of organisms produce additional UV-absorbing pigments such as scytonemin (exclusively in some cyanobacteria) mycosporine-like amino acids (MAAs; in many cyanobacteria, phytoplankton and macroalgae), parietin (in some lichens), flavanoids (in higher plants) and melanin (in animals and humans); however, they cannot completely avoid UV radiation from reaching DNA in superficial tissue.[30,31] DNA damage has both cytotoxic and genotoxic effects. Radiation damage to DNA is potentially dangerous to cells, since a single photon hit may have a carcinogenic or even lethal effect. This review summarizes the current status of UV-induced DNA damage and associated repair mechanisms as well as methods for detecting DNA damage and its future perspectives.

2 UV-Induced DNA damage

Even under the best circumstances, DNA is constantly subject to chemical modification. Several different types of DNA damage have been identified that result from (i) alkylating agents (essential for a number of biosynthetic processes), that can turn a legitimate base into either a mutagenic, miscoding deviant, or a lethal, noncoding lesion, (ii) hydrolytic deamination that can directly change one base into another and (iii) free radicals and reactive oxygen species formed by various photochemical processes.[32–35] However, the two major classes of mutagenic DNA lesions induced by UV radiation are cyclobutane–pyrimidine dimers (CPDs) (Fig. 1) and 6–4 photoproducts (6–4PPs, which are pyrimidine adducts), and their Dewar valence isomers (Fig. 2).[36–41] After UV irradiation the CPDs are the most abundant and probably most cytotoxic lesions but the 6–4PPs may have more serious, potentially lethal, mutagenic effects. Dewar isomers are formed by the photoisomerization of 6–4PPs by wavelengths longer than 290 nm.[42,43] UV sources containing a higher proportion of radiation bordering between UV-B and UV-A, such as solar UV radiation, should produce a higher proportion of Dewar isomers since the photoisomerization is most efficient around 320 nm, which corresponds to the UV absorption maximum of 6–4PPs. Consequently it has been suggested that all 6–4PPs should be converted into Dewar isomers upon exposure to sunlight.[44] The CPDs and the 6–4PPs make up around 75 and 25%, respectively, of the UV-induced DNA damage products. Both classes of lesions distort the DNA helix. CPDs and 6–4PPs induce a bend or kink of 7–9° and 44°, respectively.[45,46] The ability of UV radiation to damage a given base is determined by the flexibility of the DNA; the nature of the bases plays a major role since the distribution of the dimeric photoproducts strongly depends on the pyrimidine bases involved. Sequences that facilitate bending and unwinding are favorable sites for damage formation, *e.g.*, CPDs form at higher yields in single-stranded DNA and at the flexible ends of poly(dA)-(dT) tracts, but not in their rigid centre.[47,48] CPD formation is less frequent when there is bending of the DNA towards the minor groove.[49] One of the transcription factors having a direct effect on DNA damage formation and repair is the TATA-box binding protein (TBP). TBP induces the selective formation of 6–4PPs in the TATA-box, where the DNA is bent, but CPDs are formed at the edge of the TATA-box and outside, where the DNA is not bent.[50] CPDs have been reported to be formed preferentially at the major p53 mutational hotspot in UV-B induced mouse skin tumors.[37] The biological effects of CPDs have been extensively studied in microbes and mammals. CPDs have been reported to inhibit the progress of DNA polymerases. Mammalian RNA polymerase II has been reported to stall at both CPDs and 6–4PPs.[51,52] If unrepaired, a single CPD is sufficient to completely eliminate expression of a transcriptional unit. There is evidence that the stalled RNA polymerase II remains bound to the site of the obstruction.[53] Persisting lesions may thus not only reduce the overall concentration of free RNA polymerase but also eliminate transcription of the gene in which they are located. Every CPD acts as a block to transcription and replication, and only a small fraction of dimers results in a mutation.[31,33] Therefore, these DNA lesions, if unrepaired, may interfere with DNA transcription and replication and can lead to misreading of the genetic code and cause mutations and death.

3 DNA repair mechanisms

The accurate transmission of genetic information from one cell to its daughters is the key for the survival of organisms. Such faithful transmission requires (i) extreme accuracy in replication of DNA and precision in chromosome distribution, and (ii) the ability to survive spontaneous and induced DNA damage while minimizing the number of heritable mutations.[54] To achieve this goal organisms have developed efficient DNA repair mechanisms in order to counteract the lethal effects of DNA lesions. Specialized repair proteins scan the genome

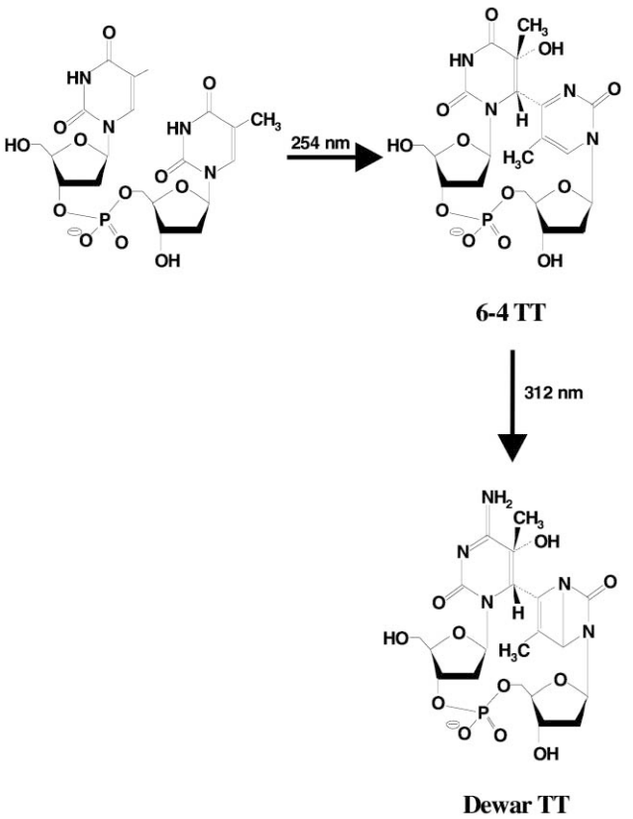

Fig. 2 Formation of the UV-induced second most frequently occurring 6–4 photoproducts and their Dewar valence isomers. 6–4 photoproducts are formed at 5′-T–C-3′, 5′-C–C-3′, 5′-T–T-3′ but not at 5′-C–T-3′ sites in DNA. These DNA lesions are also toxic and mutagenic.

continuously for the presence of DNA lesions. Once a lesion recognition protein encounters a mismatched base, an apurinic or apyrimidinic site, or structurally altered bases, it triggers an efficient DNA repair, which ultimately leads to the restoration of the genetic information.[55] The following sections describe some of the important DNA repair mechanisms.

3.1 Photoreactivation

This is perhaps one of the simplest and oldest repair systems consisting of a single enzyme: photolyase. To remove DNA lesions formed by UV, many organisms contain the photolyase enzyme that specifically binds to CPDs (CPD photolyase) or 6–4PPs (6–4 photolyase) and reverses the damage using the energy of light (Fig. 1), a process known as photoreactivation.[56–60] CPD photolyases have been reported in bacteria, fungi, plants, invertebrates and many vertebrates, while 6–4 photolyases have been identified in *Drosophila*, silkworm, *Xenopus laevis*, and rattlesnakes but not in *E. coli* or yeast.[33,56] Photolyases seem to be absent or non-functional in humans.[59,61,62] Since DNA photolyases are found in a number of archaebacteria, they are considered to be ancient repair proteins, which may have helped in the evolution of the earliest organisms on primordial Earth.[55] DNA photolyases are monomeric flavin-dependent repair enzymes with a molecular weight of between 50 and 65 kDa. Ten to twenty enzyme molecules are believed to scan the genome for UV lesions in every cell nucleus. Each one binds tightly to an encountered CPD with a $K_d = 10^{-8}$ M (thymine dimer). DNA photolyases have two chromophores. One of the chromophores (which can be either 5,10-methenyltetrahydrofolate or 8-hydroxy-5-deazariboflavin, with absorption maxima of ~380 and ~440 nm, respectively) is a light-harvesting antenna that absorbs the blue-light photon and transfers excitation energy to the active (catalytic) cofactor, which is invariably a two-electron-reduced flavin–adenine dinucleotide ($FADH^-$). Flavin in the excited state then donates an electron to the CPD, splitting the cyclobutane ring, and the electron is transferred back to the flavin concomitantly with the generation of the two canonical bases.[56,59] The crystal structures of CPD photolyase of *E. coli* and *A. nidulans* suggest that upon binding to DNA, the enzymes flip the pyrimidine dimer out of the duplex into a hole that contains the catalytic cofactor.[63,64] CPD photolyases recognize CPDs with a selectivity similar to that of sequence-specific DNA-binding proteins, which suggests that they could compete with histones for DNA accessibility in a manner similar to transcription factors.[65] Once photolyase has bound to a CPD, the efficiency of photoreactivation is extremely high: approximately one dimer split for every blue-light photon absorbed.[33] Photolyase genes have been cloned from a number of bacteria and fungi and their sequences display obvious homologies.[66] In *E. coli* the *phr* gene codes for deoxyribodipyrimidine photolyase that, with the cofactor folic acid, binds in the dark to the thymine dimer. When the cell is then exposed to light, folic acid absorbs a photon and uses the energy to break the cyclobutane ring of the thymine dimer; the photolyase then leaves the DNA. The major photoreactivating factor, *phrA*, in the cyanobacterium *Synechocystis* sp. strain PCC 6803 codes for a CPD specific DNA photolyase.[67]

In a landmark discovery Todo *et al.*[68] detected a photolyase (6–4 photolyase) specific for the 6–4 photoproduct in *Drosophila* cell-free extracts. They demonstrated that the 6–4 photolyase restores biological activity to UV-irradiated DNA. Kim *et al.*[58] obtained direct evidence that the 6–4 photolyase converted this photoproduct to unmodified bases. A chemical mechanism was proposed that involves an oxetane intermediate and an electron transfer reaction for repair by 6–4 photolyase.[58] In contrast, two articles published shortly after this report showed that the apoproteins of the blue-light photoreceptors (cryptochrome) of *Arabidopsis thaliana*[69] and mustard (*Sinapis alba*)[70] are very similar to the microbial CPD photolyase and are structurally related, although the photoreceptors function in signal transduction and not in DNA repair. The purified photoreceptors contained the folate and flavin chromophores characteristic of the folate class of photolyases.[71] These findings suggested a previously unknown mechanism of signal transduction, *i.e.*, photo-induced electron transfer that does not involve a net redox reaction. In an another landmark work, Todo *et al.*[72] have shown that the three systems, the CPD photolyase, the 6–4 photolyase and the plant blue-light photoreceptors, are evolutionary and mechanistically related. They cloned and sequenced the 6–4 photolyase gene of *Drosophila* and found that the photolyase polypeptide exhibits a sequence similar to CPD photolyases of microbial origin and to the blue-light photoreceptors. In fact, the 6–4 photolyase of *Drosophila* (which contains both CPD photolyase and 6–4 photolyase) is more similar to blue-light photoreceptors than to *Drosophila* CPD photolyase and other animal photolyases. A number of workers have also identified a human homolog with an amazing 40% sequence identity to the *Drosophila* 6–4 photolyase, but whether this protein behaves as a photolyase or as a photoreceptor remains to be elucidated.[72–74]

The unpredictable distribution of the photolyase enzyme among species and among the different tissues of a given species has led to speculations that it may carry out an alternative function more consistent with its distribution.[75] An important clue for an alternative function of photolyase came from the observation that, in the absence of photoreactivating light, the enzyme binds to Pyr< >Pyr and stimulates the removal of UV damage by stimulating the nucleotide excision repair system *in vivo*[76,77] and *in vitro*[77,78] and therefore is capable of contributing to cellular defence against DNA damage even in the absence of light. Fox *et al.*[79] have reported that yeast photolyase binds to other lesions in DNA and in particular the binding to cisplatin-damaged DNA was highly specific. Özer *et al.*[80] have demonstrated that *E. coli* DNA photolyase binds specifically to

the cisplatin 1,2-d(GpG) intrastrand cross-link and stimulates the removal of the lesion by *E. coli* UVrABC exonuclease and thereby making cells more resistant to cisplatin killing.

Although the general light-driven splitting mechanism of photoreactivation is well understood, a number of aspects of the repair process remain obscure such as (i) how repair enzymes recognize single DNA lesions with high precision in a structurally heterogeneous megabase prokaryotic or even chromatin-containing eukaryotic genome,[81–83] (ii) how the initial reduction of FAD to $FADH^-$ takes place. Although based on site-directed mutagenesis[84,85] and EPR investigations[86] it was suggested that the formation of $FADH^-$ results from a temporary photo-reduction and requires an electron transfer from a distant tryptophan to the light-excited FAD radical quartet state. In addition, by using time-resolved absorption spectroscopy it has been shown recently in *E. coli* DNA photolyase that the excited FAD radical abstracts an electron from a nearby tryptophan in 30 ps. After subsequent electron transfer along a chain of three tryptophans, the most remote tryptophan (as a cation radical) releases a proton to the solvent in about 300 ns, showing that electron transfer occurs before proton dissociation.[87] A similar process may take place in photolyase-like blue-light receptors, (iii) how the enzymes mediate the energy and electron transfer processes in order to achieve repair with almost maximal efficiency (quantum yield $\phi = 0.7–0.9$), and (iv) in view of the lack of any knowledge of how photolyases recognize their substrate, the different cleavage rates observed for dimers possessing different configurations and constitutions remain obscure.[88]

3.2 Excision repair

In contrast to photoreactivation, dark repair pathways are much more complex and do not directly reverse DNA damage but instead replace the damaged DNA with new, undamaged nucleotides.[33,41,89–92] There are two major categories of excision repair pathways: base excision repair (BER) and nucleotide excision repair (NER).

3.2.1 Base excision repair (BER). The base excision pathway has evolved to protect cells from the deleterious effects of endogenous DNA damage induced by hydrolysis, reactive oxygen species or other intracellular metabolites that modify the DNA base structure. In addition, BER is also important for withstanding lesions produced by ionizing radiation and strong alkylating agents, which are similar to those induced by endogenous factors.[92]

The pivotal enzymes involved in BER are DNA glycosylases, which remove different types of modified or damaged bases by cleavage of the *N*-glycosidic bond between the base and the 2-deoxyribose moieties of the nucleotide residues. Different DNA glycosylases remove different kinds of damage, and the specificity of the repair pathway is determined by the type of glycosylase involved.[92] Once the base is removed, the apurinic/apyrimidinic (AP) site is removed by an AP endonuclease or an AP lyase, which nicks the DNA strand 5′ or 3′ to the AP site, respectively. The remaining deoxyribose phosphate residue is excised by a phosphodiesterase; the resulting gap is filled by a repair DNA polymerase, and the strand is sealed by DNA ligase.[92,93] It has been suggested that the repair polymerase (pol β) itself possesses the ability to excise the 5′ deoxyribose phosphate residue that is generated by the combined actions of DNA glycosylases and class II AP endonucleases.[94] The polβ enzyme achieves the incorporation of a single nucleotide after excision of the damaged base (short patch). A long patch repair pathway may also be involved in the BER. This implies the removal of a short oligonucleotide and the participation of the FEN 1 protein. Probably the most frequent type of endogenous DNA damage is the AP site which arises spontaneously by hydrolytic loss of purine bases at a frequency approaching 10000 per human cell per day.[32] Another frequently occurring hydrolysis reaction is the deamination of cytosine to uracil and, at much lower frequency, adenine to hypoxanthine.

3.2.1.1 Uracil glycosylase. Seven different genes for DNA glycosylases have been identified in *E. coli* (Table 1). Comparatively less is known about glycosylases in eukaryotes and only two cDNAs for such enzymes have been detected from mammalian cells.[92] Enzymatic base excision was first demonstrated for uracil.[95] Uracil accumulates in the genome at a rate of around 100 lesions per cell per day (for a genome size of 3×10^9 bp).[33] Since this lesion is directly mutagenic, all living organisms probably produce a uracil glycosylase. The uracil DNA glycosylase is specifically involved in the repair of uracil-containing DNA, but it was recently shown that the enzyme can also remove 5-hydroxyuracil.[96] Cloning of the human cDNA was attained by purification of the enzyme to homogeneity followed by peptide sequencing and cDNA screening using a corresponding oligonucleotide pro.[97] The enzyme is extremely well conserved from bacteria to humans (56% identity), indicating the essential nature of this type of function in preventing mutations arising from deaminated cytosine residues in DNA. The crystal structures of the uracil DNA glycosylases from humans and the herpes simplex virus have recently been established and suggest that the active site is composed of a narrow pocket that accommodates uracil, but no other base, within a single-stranded DNA context,[98–100] thereby explaining the high degree of specificity displayed by this enzyme. Although a gene corresponding to this enzyme has not yet been reported in plants, the activity has been shown in several plant materials.[101,102] Some reports indicate that this activity is down-regulated (by as much as 20-fold) in fully differentiated cells.[103]

3.2.1.2 3-Methyladenine glycosylase. This enzyme has been reported in bacteria, yeast, mammals and *Arabidopsis* and shows a varying degree of substrate specificity. 3-Methyladenine is a non-coding lesion which like uracil occurs spontaneously at a significant rate.[33] *E. coli* has two 3-methyladenine glycosylases for repair of alkylation damage (Table 1). The product of the *tag* gene is highly specific for 3-methyladenine, the major cytotoxic alkylation product in DNA[104] while the product of the *alkA* gene comprises only about 10% of the glycosylase activity in cells growing under normal conditions but may be induced 10-fold when cells are exposed to sublethal doses of alkylation.[105] The *alkA* gene has a broad substrate specificity, cleaving quantitatively the important alkylation product 7-methylguanine, in addition to several minor but important products (see Table 1).[92,106] It also has some ability to remove the deamination product hypoxanthine.[107] It seems that the mammalian enzyme does not share significant sequence homology with either Tag or AlkA, which, in turn, are different from each other.[108–110] The *Arabidopsis thaliana* glycosylase Amag is similar to the mammalian enzyme,[111] whereas the *Saccharomyces cerevisiae* enzyme MAG is similar to AlkA.[112,113] Recent GenBank and EMBL databases of plant-expressed sequence Tags of *Arabidopsis thaliana* and rice shoot show strong homology to *E.coli* Tag, indicating that this type of gene is not restricted to prokaryotes.[92]

3.2.1.3 UV-endonucleases. In addition to several DNA glycosylases, certain organisms contain enzymes popularly known as UV-endonucleases because they produce strand breaks at the site of the pyrimidine dimers. UV-endonucleases cleave the *N*-glycosidic bond of the 5′-pyrimidine of the dimer followed by AP-lyase-mediated strand cleavage. The structure of this enzyme has been illustrated by X-ray crystallographic analysis and the reaction mechanism has been demonstrated from the structure[114] and site-directed mutagenesis experiments.[115] These enzymes are normally present only in UV-resistant organisms, such as *Micrococcus luteus*.[116] However, a similar enzyme has also been coded by the *denV* gene of the bacteriophage T4 and such activity has been detected in *S. cerevisiae*.[117] True eukaryotic UV-endonucleases have been

Table 1 DNA glycosylases and their probable substrates in *E. coli*, *S. cerevisiae* and *H. sapiens*[a]

Glycosylases	Organisms	Genes	Substrate(s)
Uracil DNA glycosylase	*E. coli*	*ung*	(i)
	S. cerevisiae	*UNG*	(i)
	H. sapiens	*UDG*	(i)
3-Methyladenine DNA glycosylase	*E. coli*	*tag*	(x)
	E. coli	*alkA*	(x), (xi), (xii), (viii), [(ix), (ii)]
	S. cerevisiae	*MAG*	(x), (xi), [(ii)]
	H. sapiens	*MPG*	(x), [(v)]
UV-Endonuclease	T4	?	pyrimidine dimer
	M. luteus	?	pyrimidine dimer
Endonuclease III/thymine glycol DNA glycosylase	*E. coli*	*nth*	(vi), (vii), (iii)
	S. cerevisiae	?	?
	H. sapiens	?	?
Endonuclease VIII	*E. coli*	*nei*	(vi), (vii), (iii)
fapy/8-oxoguanine DNA glycosylase	*E. coli*	*fpg/mutM*	(iv), (v), (iii)
	S. cerevisiae	?	(iv) and/or (v)
	H. sapiens	?	(iv) and/or (v)
A-G-mismatch DNA glycosylase	*E. coli*	*mutY*	Adenine/(c)
	H. sapiens	?	Adenine/(c)
G-T-mismatch DNA glycosylase	*H. sapiens*	?	T-G, (U-G)
Formyluracil DNA glycosylase	*H. sapiens*	?	(vii)
Hydroxymethyl uracil DNA glycosylase	*H. sapiens*	?	(viii)

[a] (i), uracil; (ii), hypoxanthine; (iii), 5-hydroxycytosine; (iv), 2,6-diamino-5-formamidopyrimidine; (v), 8-oxo-7,8-dihydroguanine; (vi), urea; (vii), thymine glycol; (viii), 5-formyluracil; (ix), 5-hydroxymethyluracil; (x), 3-methyladenine; (xi), 7-methylguanine; (xii), 2-methylcytosine. (i) and (ii) are deamination products formed by hydrolysis; (iii)–(ix) are lesions produced by oxygen radicals and (x)–(xii) are products of alkylation damage caused by chemical methylating agents.

identified in *S. pombe* and *N. crassa*, which recognize both CPDs and 6–4PPs and which generate an incision immediately 5′ to the lesion.[118,119] Partial characterization of endonuclease activities from plant extracts showing some specificity for UV-irradiated DNA has been achieved by several research groups.[120–123]

In addition to the enzymes summarized above, a number of other glycosylases and endonucleases have been identified, such as fapy/8-oxoguanine DNA glycosylase,[124,125] endonuclease III/thymine glycol DNA glycosylase,[126] endonuclease VIII,[127] A-G-mismatch DNA glycosylase,[128] 5-hydroxymethyl- and 5-formyl-uracil DNA glycosylases,[129–131] and their possible substrates, *etc.* are shown in Table 1. The three-dimensional structures and modes of action of several DNA glycosylases have been clarified and reviewed recently.[132–134]

3.2.2 Nucleotide excision repair (NER). NER removes a wide range of DNA distorting lesions, including CPDs and 6–4PPs. It is highly conserved in eukaryotes and present in most organisms. NER uses the product of around 30 genes to remove a damage-containing oligonucleotide from cellular DNA. Although NER is not essential for viability, defects in repair genes may result in three distinct sun-sensitive, cancer-prone genetic disorders such as Xeroderma pigmentosum (XP), Cockayne's syndrome (CS) and trichothiodystrophy (TTD) in humans.[38,41,56,91] NER defective individuals have 1000 times more risk of skin cancer in comparison to normal individuals.[41] NER is divided into two subpathways: (i) transcription-coupled repair (TC-NER)—the preferential repair of transcribed strands in active genes—and (ii) global genome repair (GG-NER)—repair in non-transcribed parts of the genome, including the non-transcribed strand of transcribed genes.[41,91]

The eukaryotic NER pathway has been demonstrated at the molecular level in yeast and human cells. The principal reactions have been reconstituted (*in vitro*) from purified components using damaged DNA as a template.[41,56,90,135–137] A schematic representation of the NER pathway is illustrated in Fig. 3. Table 2 shows the DNA repair enzymes and their probable functions in eukaryotes such as humans and yeast. Six core factors, comprising 15 to 18 polypeptides, are required for dual incision of damage, and around another dozen polypeptides are needed for the repair synthesis step. In the first step, the XPC-hHR23B (Rad4–Rad23 complex in yeast) act as damage

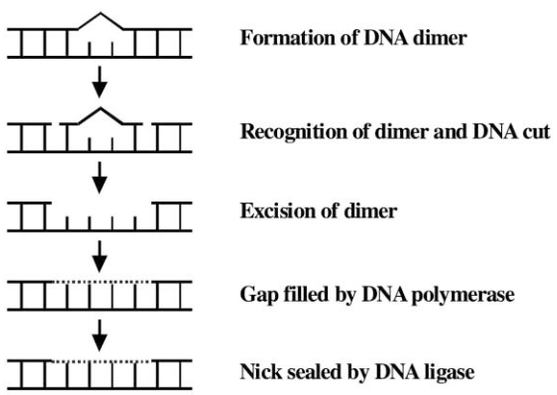

Fig. 3 Schematic representation of the nucleotide excision repair pathway. For details see text.

detectors that bind to the DNA distortion and initiate NER.[138] In a second step, XPA (Rad14), RPA (Rfa) and the general transcription factor TFIIH enter the reaction to form an open complex. Thereafter, the DNA helicase activities of XPB (Rad25/Ss12) and XPD (Rad3) in TFIIH help in unwinding the DNA. The facts that XPA and Rad14 are preferentially used for binding damaged DNA[139,140] and that Rad3 helicase activity is inhibited by DNA damage[141] indicate that these proteins are involved in verifying the damage site in the open complex.[138] In a further step, nuclease activity is required. The 3′ incision is performed by XPG (Rad2), whereas the 5′ incision is made by a complex of XPF-ERCC1 (Rad1–Rad10). Ultimately, the gap is filled in by DNA synthesis (polymerase; pol δ or ε holoenzyme) and closed by a DNA ligase, probably LIG1.[56,142] Recently, an alternative order of assembly of the excision complex for the first steps in damage recognition has been suggested in humans,[143] showing that XPA and RPA are the initial damage-sensing factors that bind damaged DNA and then use TFIIH, XPC and hHR23B to form an open complex known as a pre-incision complex. In yeast, damage recognition is supported by Rad7 and Rad16 that form a complex that, together with the Rad4–Rad 23 complex, binds to UV-damaged DNA synergistically in an ATP-dependent reaction.[137,144]

In plants, the rate of dark repair of CPDs has been reported to vary widely between species. High rates of repair have been

Table 2 DNA repair enzymes and their probable function in eukaryotes

Enzymes		
S. cerevisiae	*H. sapiens*	Probable functions
Ccl1	CycH	Cyclin
Kin28	CdK7	CDK, C-terminal domain kinase; CAK
Rad1	XPF	Part of endonuclease (5′ incision)
Rad2	XPG	Endonuclease (3′ incision); stabilizes full open complex
Rad3	XPD	5′ to 3′ helicase
Rad4	XPC	Binds damaged DNA; recruits other NER proteins; works with hHR23B
Rad10	ERCC1	Part of endonuclease (5′ incision)
Rad14	XPA	Binds damaged DNA after XPC or RNA pol II
Rad23	hHR23B	Cooperates with XPC; contains ubiquitin domain
Rpa1,2,3	RPAp70,p32,p14	Stabilizes open complex (with Rad14/XPA); positions nucleases
Ss11	p44	DNA binding?
Ss12 (Rad25)	XPB	3′ to 5′ helicase
Tfb1	p62	?
Tfb2	p52	?
Tfb3/Rig2	MAT1	CDK assembly factor
Tfb4	p34	DNA binding?

shown for carrot suspension cultures[145] and protoplasts of carrot, *Haplopappus*, *Petunia* and tobacco,[146] whereas excision repair of CPDs was undetectable in cultured soybean cells.[147]

In comparison to eukaryotic NER, prokaryotic NER involves only 3 proteins, UvrA, UvrB and UvrC, which carry out the complete process of damage recognition and excision. It is increasingly evident that the overall strategy for NER in eukaryotes has many similarities to the process initiated by the UvrABC nuclease in prokaryotes, even though the latter uses many fewer enzyme subunits. In both humans and *E. coli*, there is an energy-independent distortion recognition factor (XPC in humans and UvrA in *E. coli*), followed by energy-dependent recognition of DNA damage using DNA helicase (TFIIH in humans and UvrB in *E. coli*). In both cases, the helicases create an open preincision complex that is cleaved by structure-specific nucleases, and an oligonucleotide is then released by dual incision.[41,90,148,149]

3.3 Other DNA polymerases

Pol V is a member of a superfamily of newly discovered polymerases having at least four subfamilies: (i) Pol V, present only in *E. coli* and some other bacteria, (ii) DinB, present in both pro- and eukaryotes, (iii) Rev1, and (iv) Rad30/Pol η, are found exclusively in eukaryotes.[150–155] These polymerases are unrelated in sequence to the classical DNA polymerases. The three previously characterized DNA polymerases in *E. coli*, pol I–III and the five in eukaryotes, Pol α–ε, all have a common architecture.[156] The new polymerases seem to be quite different, but have several sequence domains in common with each other.[152,157,158]

3.4 Mutagenic repair or lesion bypass

In a situation where repair cannot occur for reasons unknown, the only recourse is a lesion bypass if the cell is to survive. Most mutagenesis resulting from damage by UV radiation, ionizing radiation or various chemicals seems to be due to a process of translesion synthesis, in which a polymerase or replicative assembly encounters a noncoding or miscoding lesion, inserts an incorrect nucleotide opposite the lesion and then continues elongation (Fig. 4).[38,159] In *E. coli*, *umuC,D* gene products are thought to bind to DNA polymerase and relax its normally stringent requirements for the stable insertion of a new base, thereby enabling it to perform translesion synthesis.[160] Recent studies suggest that translesion synthesis past a CPD is facilitated by pol η. This process seems to be quite accurate and efficient, with adenines being inserted opposite both bases of a T⩘T CPD.[161,162] However, this is not the case for 6–4 photoproducts since translesional synthesis of 6–4 TT may lead to a G insertion. In addition to T⩘T CPD, pol η may also replicate across certain other types of DNA damage, such as abasic sites, AAF (acetyl aminofluorene), guanine adducts and cis-platinated guanines.[161] In the yeast *Saccharomyces cerevisiae*, efficient bypass of a T⩘T CPD by yeast DNA polymerase, pol η has been reported.[163] In the same organisms, pol ζ (consisting of Rev3 and Rev7 proteins) has been reported to replicate across a T⩘T CPD.[164] Human homologues of Rev3 and Rev7 have been documented showing that human pol ζ plays a vital role in error-prone translesion replication in normal cells.[165–169] Humans contain two Rad30 paralogs, pol η and pol ι.[158] Recently, it has been shown that pol ι replicates undamaged DNA with very low fidelity and possesses the ability to insert nucleotides opposite UV photoproducts as well as to perform unassisted translesion replication.[170]

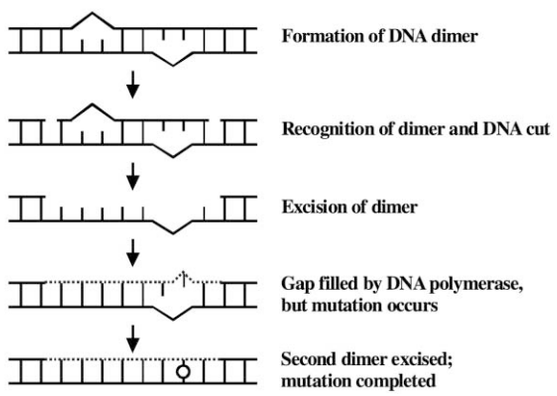

Fig. 4 Schematic representation of the mutagenic repair pathway. For details, see text.

3.5 Recombinational repair

Recombination is one of the most important processes involved in DNA repair, ensuring the transmission of correct genetic information from parents to offspring. Double-strand breaks (DSBs) and single-strand gaps in damaged DNA are efficiently repaired by mechanisms associated with recombination (for a review see ref. 171). Recombination is a series of complex biochemical reactions involving at least 20 gene products in *E. coli*. Genes homologous to bacterial and yeast recombination genes have been cloned in higher eukaryotes, indicating that there might be a common fundamental mechanism of recombination

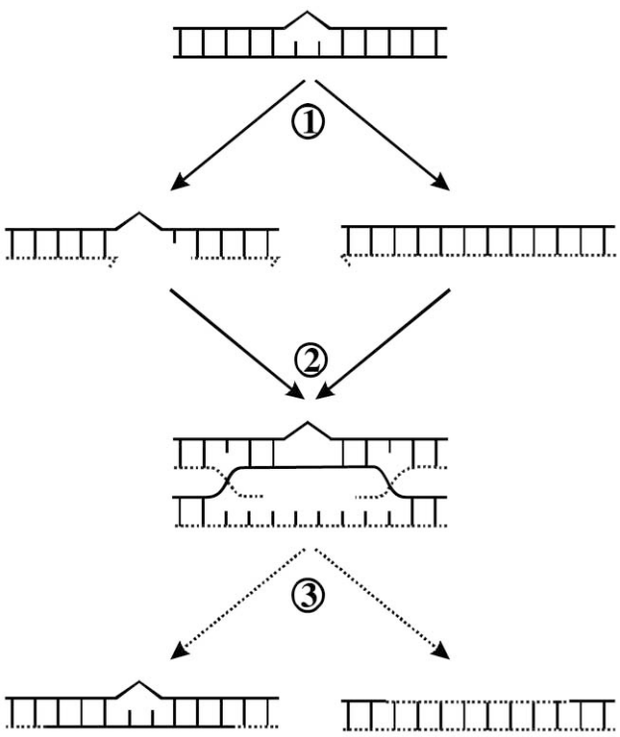

Fig. 5 Schematic representation of the recombinational repair pathway. For details, see text.

among a wide variety of species.[171] In contrast to lesion bypass, recombinational repair (Fig. 5) fills the daughter strand gap by transferring a preexisting complementary strand from a homologous region of DNA to the site opposite the damage. Similar to the lesion bypass mechanism, the lesion is left unrepaired, but the cell manages to get through another round of replication, and the damaged base is now available as a substrate for excision repair. When the complementary strand is obtained from the newly replicated sister chromatid, the resulting repair is error free (Fig. 5).[33]

The RecA protein forms a right-handed helical nucleoprotein filament on the DNA and carries out a homology search followed by a strand-exchange reaction that is essential for recombination.[38,172] In *S. cerevisiae*, mutants in the *RAD52* epistatic genes are sensitive to X-ray (but not UV) radiation and are defective in mitotic and/or meiotic recombination. At least 8 genes such as *RAD50*, *-51*, *-52*, *-54*, *-55*, *-57*, MRE11 and *XRS2* belong to the *RAD52* group.[173,174] Homologues of the *RAD51* and *RAD52* genes are present in a wide variety of organisms. There is 70% identity between the yeast and human proteins in the case of RAD51,[171] whereas yeast and human RAD52 homologues are 60% identical, the homology being only in the amino-terminal half of the proteins.[175] Although UV-induced chromosomal rearrangements including homologous intrachromosomal recombination events have been shown in plants,[176] it remains to be elucidated whether the filling of daughter-strand gaps *via* homologous recombination is a significant UV tolerance mechanism in plants.

3.6 Alternative repair pathways

Recent studies have shown that the fission yeast *Schizosaccharomyces pombe* and the filamentous fungus *Neurospora crassa* contain an alternative DNA excision repair pathway, distinct from the well characterized BER and NER, and which is initiated by an ATP-independent, direct-acting 5′-endonuclease, to remove UV-induced DNA lesions (for reviews, see refs. 177 and 178). Like *S. cerevisiae*, *S. pombe* has an NER pathway, but unlike *S. cerevisiae*, *S. pombe* NER deletion mutants still show a considerable capacity to remove CPDs and 6–4PPs from their DNA[179] suggesting that *S. pombe* possesses an additional pathway for the removal of UV-induced DNA lesions. The observation that *S. pombe* contains an ATP-independent but UV-damage-dependent supercoiled-plasmid-nicking activity,[180] led to the discovery of a new type of DNA repair endonuclease, SPDE.[118] It is a divalent-cation-requiring enzyme that catalyses a single ATP-independent incision immediately 5′ to both CPDs and 6–4PPs in duplex DNA and generates terminal containing 3′-hydroxy and 5′-phosphoryl groups.[177,181] Similarly, a DNA repair gene from *N. crassa* has recently been isolated *via* complementation of UV-sensitive *E. coli* cells.[119] The gene, which is defective in the UV-sensitive *N. crassa* mutant *mus-18* encodes a 74 kDa protein that shares no sequence similarities with any known DNA repair enzymes and is functionally identical to SPDE.

In addition to the above repair mechanisms, cells may also protect themselves against the damaging effects by triggering cell-cycle checkpoints (arrest of the cell cycle so that the cells do not progress from one phase of the cycle to the next with damage in their genomes) and apoptotic (programmed) cell death, thereby, protecting the organism at the expense of the individual cell (for a review, see ref. 182).

4 Methods for detecting DNA damage

A number of methods are in use to determine DNA damage in a variety of organisms.[23–25,27,183–198] Here we present a brief account of the methods used by various workers. UV-Induced DNA degradation has been reported in a cyanobacterium *Synechocystis* by using radioactive methods[183] and showing percentage radioactivity loss from DNA as a measure of DNA degradation. An alkaline agarose gel method for quantitating single-strand breaks in nanogram quantities of nonradioactive DNA was developed by Freeman *et al.*[184] Another method for cyclobutane dimer detection was presented by Mitchell *et al.*[185] They first labelled the DNA with radioactive substances, followed by agarose gel electrophoresis and densitometric analysis and finally digesting with endo III and endo V before analyzing on sequencing gels. For the detection of UV-induced DNA damage, lesion-specific antibodies[186,187] and T4 endonuclease/alkaline sucrose gradient assay was used.[188] Nucleotide level detection of CPD was done by using oligonucleotides and magnetic beads, which facilitates labelling of DNA fragments incised at the dimers, and chemical sequencing reference ladders.[189] An exquisitely sensitive gel electrophoresis based method involving the extraction of intact DNA, followed by the cleavage of the DNA at CPDs and the quantitative assay of various size classes of single-stranded DNA size to arrive at an average frequency of dimers was developed by Quaite *et al.*[27] Buma *et al.*[24,25] developed an immunofluorescent thymine dimer detection method by labelling dimers with antibody followed by a secondary antibody (fluorescein isothiocyanate) staining and finally visualization of DNA damage with flow cytometry or fluorescence microscopy. More or less, the same method was used in subsequent publications by Pakker *et al.*,[26] Sommaruga and Buma[190] and van de Poll *et al.*[191] Alkaline biased sinusoidal field gel electrophoresis followed by electronic imaging system was used to monitor the changes in CPDs in rice.[192] The immuno-dot-blot assay technique was used to detect the CPDs, 6–4PPs and their Dewar valence isomers in Chinese hamster ovary cells irradiated with UV radiation.[193] A simple and efficient quantitative method for determining the frequency of thymine dimers (Fig. 6) in a variety of organisms such as cyanobacteria, phytoplankton and macroalgae was developed by using thymine dimer specific antibodies followed by blotting and chemiluminescence methods.[23] Yet another method for measurement of thymine dimeric lesions/photoproducts by using an electrospray-mass spectrometer[194] and high performance liquid chromatography–tandem mass spectrometry (HPLC-MS) was presented recently.[195,196] Analytical methods

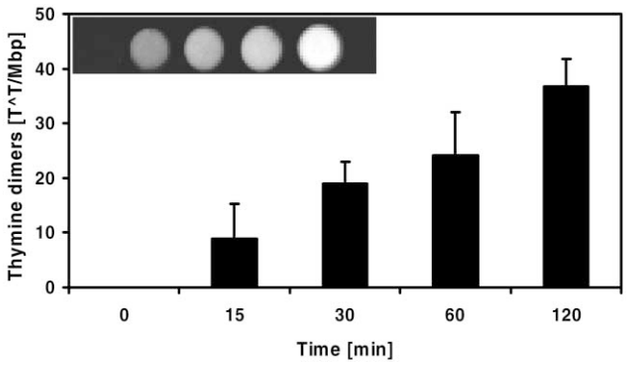

Fig. 6 DNA dimer formation in a cyanobacterium, *Nostoc* sp. by UV irradiation as detected by the dot-blot and chemiluminescence method using thymine dimer specific antibody. There was a gradual increase in the number of dimers with increasing UV exposure time. Insert shows the dot-blot of thymine dimers after increasing UV exposure time (from left to right) as shown for the figure.

(chromatography coupled with mass spectrometry) were developed to measure the main photooxidation products of 2′-deoxyguanosine (dGuo), arising from either the type I (electron transfer) or the type II (singlet oxygen) photosensitization mechanism.[197] Immunological approaches combined with chromatography and comet assay are being used to analyse the UV-induced DNA damage in a number of organisms.[198–200] Comet assay (an electrophoretic technique) has been found to be more sensitive to UV damage when used in conjunction with T4 endonuclease V. This technique can be used as an alternative method of detecting DNA damage in single cells caused by UV radiation.[200]

5 Future perspectives

The prominent function of the NER pathway is to remove UV-induced lesions from DNA. Defects in this pathway result in the serious cancer-prone inherited disease Xeroderma pigmentosum (XP). It is remarkable that humans do not have any backup pathway for this important cellular defense mechanism. Therefore, NER-defective individuals are more or less unable to excise pyrimidine dimers from DNA. This situation is unique to placental mammals since lower eukaryotes, plants and bacteria all have additional defense systems against UV radiation such as DNA photolyases which monomerize dimers or DNA glycosylases or nucleases to specifically incise DNA at pyrimidine dimers.[41] Photoreactivation is apparently a rapid repair mechanism that can be extremely important for organisms living in strong sunlight. Although photolyase homologues have been identified in humans,[72–74], as stated earlier, photolyase activity has not yet been confirmed in placental mammalian cells. The ultimate goal should be the development of artificial DNA photolyases, which could be able to simulate the efficient recognition and repair of UV-induced DNA lesions *in vivo* and could bring relief to individuals suffering from Xeroderma pigmentosum (XP) or trichothiodystrophy (TTD).[55]

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