ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

REVIEW ARTICLE MOLECULAR BIOLOGY

SOLAR ULTRAVIOLET RADIATION-INDUCED DNA DAMAGE AND PROTECTION/REPAIR STRATEGIES IN CYANOBACTERIA

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ABSTRACT

Ultraviolet radiation (UVR) coming from the Sun is an ever-present stress for living organisms including cyanobacteria that damages several biological processes either directly or indirectly through the formation of reactive oxygen species (ROS). DNA is one of most prominent target for UVR. A number of mutagenic and cytotoxic DNA lesions such as cyclobutane-pyrimidine dimers (CPDs), 6-4 photoproducts (6-4PPs), and their Dewar valence isomers as well as DNA strand breaks produced by means of solar UVR (particularly UV-B; 280-315 nm) can alter the genome integrity and affect the normal life process of all organisms. Cyanobacteria cope with UVR either through a combination of repair mechanisms such as photoreactivation, excision repair, recombinational repair, SOS response and apoptosis/programmed cell death (PCD) or by production of certain UV-absorbing/screening compounds such as mycosporine-like amino acids (MAAs) and scytonemin. Besides the above mentioned photoprotective mechanisms several other defence strategies such as avoidance and production of anti-oxidants are also operative in cyanobacteria against UVR. In the present review we discuss only the effects of UVR on DNA and protection/repair strategies applied by cyanobacteria against UVR.

KEY WORDS

UV radiation, Cyanobacteria, DNA damage, DNA repair, MAAs, Scytonemin

INTRODUCTION

Cyanobacteria are photosynthetic, oxygen evolving, Gram-negative bacteria found ubiquitously in nature even at extreme climatic conditions such as hypersaline pools, hot springs, deserts, alkali lakes, and polar areas[1-3]. They have diverse life forms such as unicellular to filamentous form and occur in colonies or live in symbiosis with eukaryotic organisms[4]. Recently, cyanobacteria have become an attractive source of several innovative classes of secondary metabolites that can be used in agriculture, industry, and especially in pharmaceuticals and biomedical research[5,6]. Many cyanobacteria can fix atmospheric N_2 into ammonia (NH_3) in the presence of nitrogenase enzyme and produce H_2 as a byproduct that can be used as a renewable and environmental friendly source of energy carrier[7].

Like all photoautotrophs cyanobacteria also depend on solar radiation as their primary source of energy. However, continuous increase in solar ultraviolet-B (UV-B: 280–315 nm) radiation reaching the Earth's surface due to anthropogenic depletion of the stratospheric ozone layer[8] may become detrimental to photosynthetic organisms including cyanobacteria. The ultraviolet radiation (UVR) may harm several key metabolic activities in cyanobacteria, such as photosynthesis, N_2 fixation, pigmentation, phycobiliprotein composition and $^{14}CO_2$ uptake[9-11]. One of the most prominent targets of solar UV-radiation is cellular DNA, which absorbs UV-B radiation and causes adverse effects on the normal life processes of all organisms ranging from prokaryotes to mammals[12,13]. UV-B radiation can directly damage cellular DNA or indirectly by oxidative damage from UV-induced reactive oxygen species (ROS)[14]. Recently, Rastogi et al (2010)[15] has detected the production of ROS induced by UVR. UV-B radiation may lead to the formation of two major classes of DNA lesions, such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine 6-4 pyrimidone photoproducts (6-4PPs)[12,13]. The 6-4PPs are readily converted into their Dewar valence isomers upon exposure to UV-B or UV-A (315-400 nm) radiation that may further undergo reversion to the 6-4PPs upon exposure to short wavelength UVR[16]. Two adjacent cytosines are considered as mutation hotspots of UV-B and UV-C (< 280 nm) radiations[17]. The exposure of UVR may also result in single as well as double DNA strand breaks. Among different types of damages, DNA double strand breaks (DSBs) are the most deleterious, since they affect both strands of DNA and can lead to the loss of genetic material.

In spite of adverse effects of solar UVR, cyanoacteria are not defenceless and have developed various strategies such as the formation of antioxidants or efficient DNA repair mechanisms to counteract the damaging effects of UVR[18]. A different but not less interesting property of these microorganisms is their capacity of overcoming the toxicity of UVR by means of UV-absorbing/screening compounds, such as mycosporine-like amino acids (MAAs) and scytonemin[6,19-21]. This review deals with the UV-induced DNA damage and subsequent protection/repair strategies adopted by cyanobacteria.

UVR-INDUCED DNA DAMAGE IN CYANOBACTERIA

UVR-induced DNA degradation has been demonstrated in several unicellular as well as filamentous cyanobacterial species[22-26]. Several studies have revealed that UV-B radiation induces a degradation of the physiological activity and an enhancement of the oxidative stress in higher plants and some cyanobacteria[14,27].

Kumar et al. (2004)[26] has investigated the impacts of UVR on the filamentous cyanobacterium, *Anabaena* strain BT2 by

adopting the PCR based assay and found that UVR drastically affects the genomic DNA. The harmful effects of UV-B radiation on DNA are mostly elucidated in terms of the formation of dimeric photoproducts involving two adjacent pyrimidine bases (Fig 1). Cyclobutanedipyrimidines (Pyr<>Pyr) constitute the major DNA photoproducts upon exposure to UV-B radiation. They arise from a cycloaddition of the C5–C6 double bonds of adjacent pyrimidine bases. It has been found that T-T and T-C sequences are more photoreactive than C-T and C-C sequences[28]. It has been demonstrated that the main photoproducts are *cis-syn*-configured CPD lesions, while *trans-syn*-configured CPD lesions are formed in much less quantity[29]. In double stranded B-DNA, where the dimer entails two adjoining pyrimidine bases on the same DNA strand, only the *syn*-isomers can be generated, whereas the *cis*-isomer is preferred over the *trans*-isomer to a great extent[17]. The incidence of *trans-syn* isomer in single-stranded or denatured DNA is more common because of the increased flexibility of the DNA backbone. The heterogeneous distribution of the UV-induced photolesions in the DNA depends on the sequences that facilitate DNA bending as well as the chromatin modulation through the binding of specific protein.

Even though dipyrimidine photoproducts are the preferential outcome of UV-B radiation, the biological importance of UVR-induced modifications of DNA purine bases has also been recognized[30]. These comprise the photoproducts that involve, at least, one adenine (A) residue that undergoes photocycloaddition reactions with contiguous adenine or thymine (T) upon exposure to UV-B radiation[31]. The extent of adenine-containing photoproduct (A-T) is very low but these lesions may contribute to the biological effects of UVR in view of the fact that the A-T adduct has been shown to be mutagenic[32.33]. UV-induced ROS acts as a powerful oxidant that may cause oxidative DNA damage. A number of oxidation products of purine bases such as 8-oxo-7,8-dihydroguanyl (8-oxoGua), 8-oxo-Ade, 2,6-diamino-4-hydroxy-5-formamidoguanine (FapyGua), FapyAde, and oxazolone have been reported to form upon exposure of DNA to UV radiation[34,35].

The formation of 6-4PPs involves a singlet excited state; represent the second class of pyrimidine photoproducts in terms of quantitative importance. They arise from a [2+2] cycloaddition involving the C5–C6 double bond of the 5'-end pyrimidine and the C4 carbonyl group of the 3'-end thymine.

UVR-induced DNA lesions

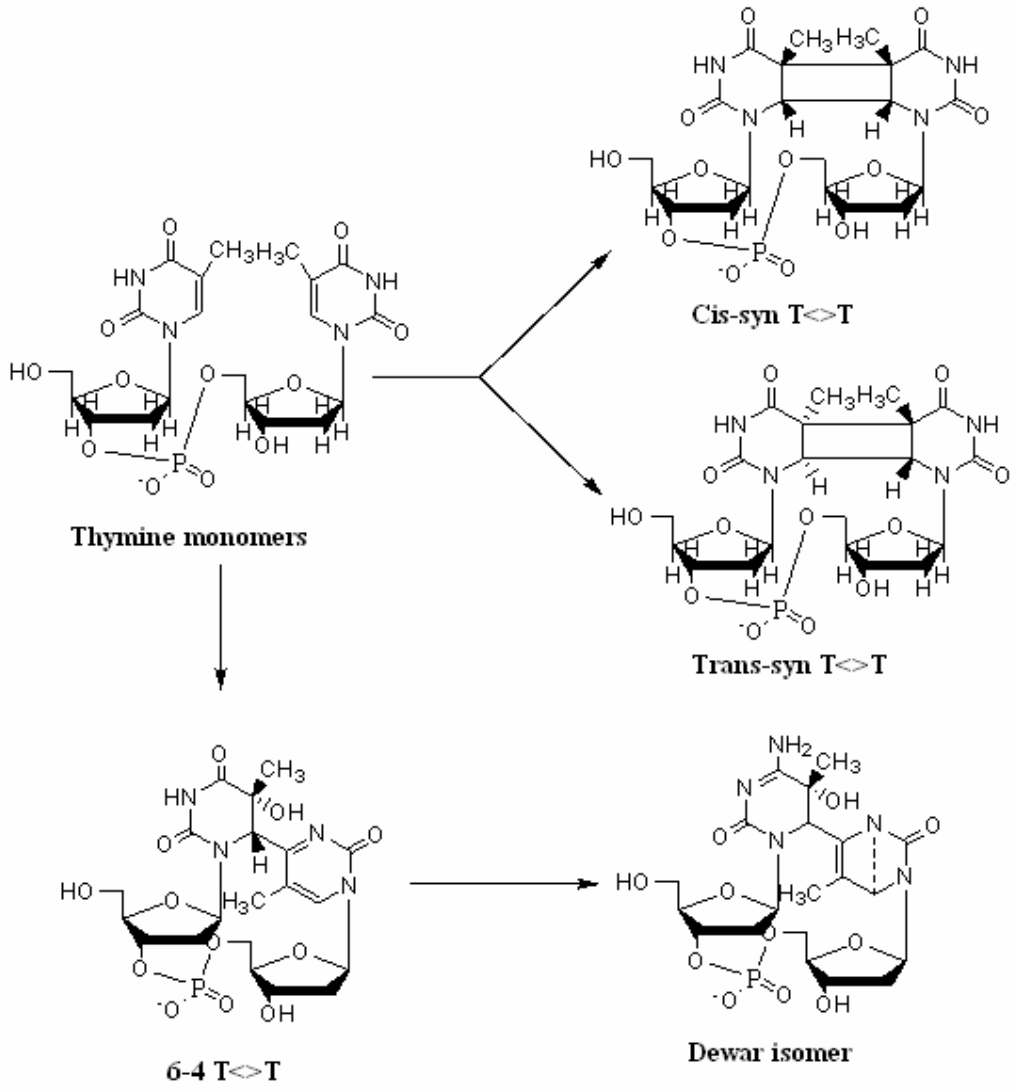

Figure 1
UVR-induced formation of thymine-thymine CPD, 6-4PP and their valence isomer.

A remarkable property of the 6-4PPs is their easy conversion into the related Dewar valence isomers upon exposure to UVB radiation[36]. The main conformational perturbations caused by the 6-4PPs and Dewar isomers are concerned with their effects on global DNA curvature. Besides the above lesions, DNA strand breaks are also observed extensively in cells under UV-B irradiation. UV-B-induced ROS as well as DNA lesions (CPDs and 6-4PPs) may cause primary as well as secondary breaks, respectively. It was assumed that initial photoproducts are converted into double strand breaks (DSBs) during DNA replication, due to not a distinct process, that is, “collapse of replication forks”. In general, it has been concluded that UV-induced DNA lesions such as CPDs, 6-4PPs and their Dewar isomers (Fig. 1) abasic site, strand breaks, and oxidative product are the predominant and most persistent lesions and if not repaired may alter the functional genome integrity and leading to mutagenesis and cell death[37].

ANALYSIS OF UV-INDUCED DNA DAMAGE IN CYANOBACTERIA

A number of biochemical and chemical assays have been developed to measure the different types of DNA lesions such as dimeric pyrimidine photoproducts, strand breaks, oxidative stress within DNA in a variety of organisms[13,38]. The majority of the available informationon regarding the distribution of photodamage in DNA was inferred from immunological detection involving the use of poly and monoclonal antibodies raised against CPD, 6-4PPs and related Dewar valence isomers[17,25,39]. Immunological detection of UV-induced photoproducts is a convenient approach which, however, may suffer from certain drawbacks such as the lack of calibration of the immunoassays may prevent accurate quantitative measurements. However, to overcome this difficulty, Sinha et al (2001)[25] has made an attempt to quantify the UV-induced thymine dimers in a cyanobacterium *Nostoc* sp. by calibrating the thymine dimer with blots of plasmid DNA (pBSK) with known sequence and DNA form *Nostoc* sp. (Fig. 2).

UV-induced DNA degradation has been reported in the cyanobacterium *Synechocystis* PCC 6308 by using radioactive methods and showing percentage radioactivity lost from DNA as a measure for DNA degradation[23].

Detection of thymine dimers by immunodot-blot assay

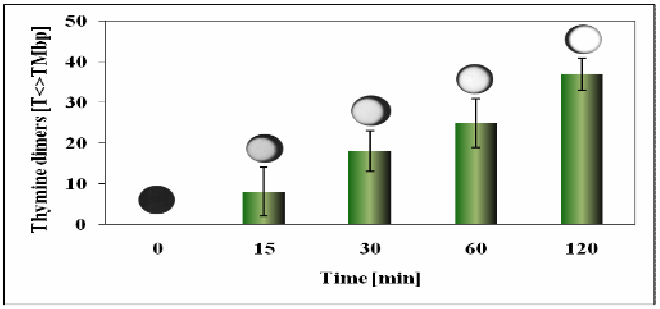

Figure 2

Detection of thymine dimers by immunodot-blot assay in Nostoc sp. after UV-B irradiation for different durations. Circles above each bar represent corresponding dot-blot of DNA indicating the intensity of chemiluminescence analogous to the thymine dimers. No thymine dimers were detected at 0 h (control) whereas intensity of chemiluminescence was found to increase after increasing irradiance time (modified from Sinha et al., 2001)[25].

UV-induced decrease in template activity of genomic DNA of cyanobacterium *Anabaena* strain BT2 was documented by Kumar et al. (2004)[26] using the PCR based assays such as random amplified polymorphic DNA (RAPD) and rDNA amplification. Similarly, UV-B-induced DNA damage was also detected in *Anabaena variabilis* PCC 7937 and *Rivularia* sp. HKAR-4 by PCR (Fig. 3). UV-induced DNA strand breaks such as single strand breaks (SSBs), or double strand breaks (DSBs) and alkali-labile sites can be detected by fluorometric analysis of DNA unwinding (FADU) assay[40]. The quantification of photoproducts (CPDs, 6-4PPs and Dewar valence isomers) within DNA can also be achieved by means of a suitable liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) system which operates in the electrospray ionization mode[41]. To get more information regarding the methods for analysis of DNA damage, readers are suggested to see the paper by Rastogi et al. (2010) [13].

Detection of UV-induced DNA damage by PCR

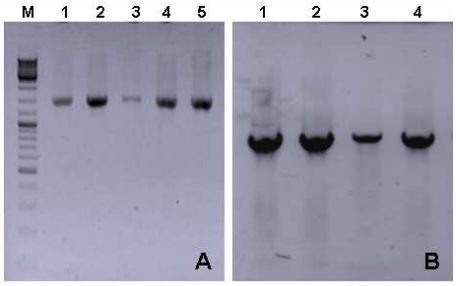

Figure 3

PCR based detection of DNA damage. Effects of UV-B irradiation on 16S rDNA amplification of genomic DNA of A. variabilis PCC 7937 (A) and Rivularia sp. HKAR-4 (B) after exposure to simulated solar radiation for 24 h. A: Lane M (DNA marker), lane 1 (dark control), lane 2 (light control), lane 3 (PAR + UV-A + UV-B), lane 4 (PAR + UV-A) and lane 5 (PAR only). B: Lane 1 (PAR), lane 2 (PAR + UV-A), lane 3 (PAR + UV-A + UV-B), lane 4 (light control).

PHOTOPROTECTION IN CYANOBACTERIA

Different species of cyanobacteria illustrate a wide variation in tolerance to UVR and acquire a variety of defence strategies that facilitate them to grow and survive in adverse environments with high UV-B fluxes. A number of photoprotective mechanisms such as avoidance of brightly lit habitats, production of UV-screening pigments/substances, quenching reactions for phototoxic products (such as ROS), and repair of UV-induced damage have been observed in various cyanobacteria[6,19,21,42-44] (Fig. 4). Synthesis of extracellular polysaccharides (EPS) may also help to limit UV damage in cyanobacteria. The EPS sheath of cyanobacteria forms a buffer zone between the environment and the cell. Recently, it has been reported that UV-B irradiation stimulates the extracellular glycan production of *N. commune*. The yield of EPS isolated from UV-B irradiated cultures was 3 times higher than that from control cultures[45,46]. It has been suggested that EPS synthesis is stimulated to provide a matrix for the UV-A/B-absorbing oligosaccharides, mycosporines - which are located in the sheath of *N. Commune*[45]. In the present review only the role of efficient repair mechanisms as well as sunscreening compounds in photoprotection of cyanobacteria against UVR have been discussed.

Defense strategies adopted by cyanobacteria against UVR

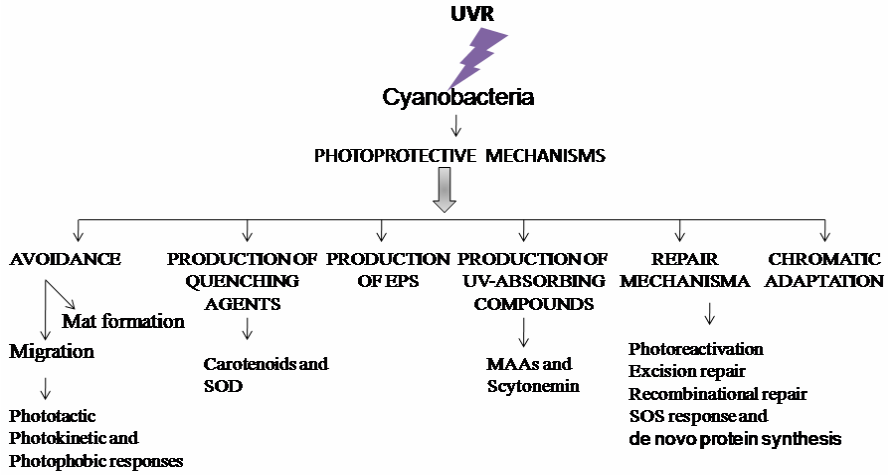

Figure 4

Defence strategies adopted by cyanobacteria to counteract the harmful effects of UV radiation.

DNA REPAIR MECHANISMS IN CYANOBACTERIA

The incidence of a particular repair pathway within the cell principally depends on the types and location of lesions in the genome[47]. The biochemical and molecular studies on repair pathways have been extensively investigated in some model organisms (such as *E. Coli*) where specialized repair enzymes scan the genome continuously and encounter the DNA lesions by triggering several distinct repair mechanisms such as photoreactivation, excision repair (BER and NER), mismatch repair (MMR), and some specialized forms of repair system such as SOS response, damage tolerance, and apoptosis[13]. Some of the important DNA repair mechanisms operated in cyanobacteria are listed below.

1. Photoreactivation

The removal of DNA lesions such as CPDs or 6-4PPs in presence of DNA photolyase enzyme CPD photolyase and 6-4 photolyase respectively using the energy of light (Long wavelength UV-A/blue light) is known as photoreactivation. The enzyme DNA photolyases are monomeric flavin-dependent repair enzymes having the molecular weight of about 45-66 kDa with 420–616 amino acid residues. It consists of two known cofactors i.e. a catalytic cofactor such as either 5,10-methenyltetrahydrofolate (MTHF)[48], 8-hydroxy-5-deaza-riboflavin (8-HDF)[49], and FMN[50] and a light-harvesting cofactor such as deprotonated reduced flavin adenine dinucleotide ($FADH^-$). Long wavelength UV-A or blue excitation energy is absorbed by either MTHF or 8-HDF (λ_{max} ~380 and ~450 nm) and transferred to the catalytic cofactor ($FADH^-$). The flavin in the excited state transfers an electron to the CPD, splitting the cyclobutane ring and the electron is transferred back to the flavin resulting in the generation of the two canonical bases[13](Fig. 5).

Process of photoreactivation

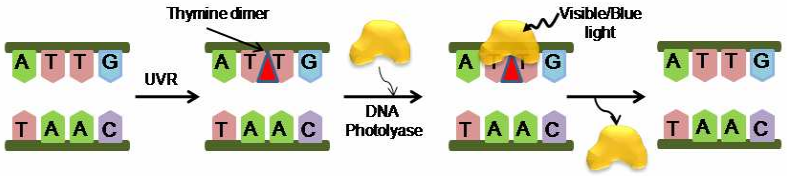

Figure 5

Diagrammatic representation of photoreactivation (for details see text).

Once the photolyase has been bound to a CPD, the efficiency of photoreactivation is extremely high with one dimer split for almost every blue light photon absorbed[51]. The major photoreactivating factor, *phrA*, in the cyanobacterium *Synechocystis* sp. strain PCC 6803 codes for a CPD-specific DNA photolyase[52]. Photoreactivation of UVR-damaged DNA has been reported in several strains of cyanobacteria such as *Agmenellum quadruplicatum*[53], *Anacystis nidulans*[22,54], *Gleocapsa alpicola* (*Synechocystis* sp. strain PCC 6308)[23,55], *Plectonema boryanum*[56,57], *Anabaena doliolum*[58] and *Anabaena* spp.[43,59].

2. Excision repair

In comparison to photoreactivation, excision repair (dark repair) is more complex pathways where damaged DNA is replaced by new undamaged nucleotides by means of complementary DNA strand. Excision repair can be distinguished into base excision repair (BER) and nucleotide excision repair (NER). The BER pathway has probably been evolved to protect cells from DNA damage induced by hydrolytic deamination, strong alkylating agents, ionizing radiation (IR), or by different intracellular metabolites and, indirectly, also by UV radiation via generation of ROS and proceeds through a series of repair complexes that act at the site of DNA damage (for details see Rastogi et al., 2010) [13]. The key enzymes involved in BER are DNA glycosylases, which remove different types of modified bases by

cleavage of the *N*-glycosidic bond between the base and the 2-deoxyribose moiety of the nucleotide residues. Depending on the biochemical modification there are different, highly specific DNA glycosylases such as uracil glycosylase, methyladenine glycosylase, UV-endonucleases, endonuclease III/thymine glycol DNA glycosylase, endonuclease VIII, A–G-mismatch DNA glycosylase, 5-hydroxymethyl- and 5-formyluracil DNA glycosylases as well as fapy/8-oxoguanine DNA glycosylase have been identified[12]. After base removal by DNA glycosylase, the apurinic/apyrimidinic (AP) site is excised by an AP endonuclease or an AP lyase, which nicks the DNA strand 5' or 3' to the AP site, respectively, followed by the excision of the remaining desoxyribose phosphate residue by a phosphodiesterase. Subsequently, the gap is filled by a repair DNA polymerase and the strand is linked by a DNA ligase[60,61] (Fig. 6A). The BER can be subdivided in to two forms i.e., short-patch BER (SP-BER) and long-patch BER (LP-BER). DNA having one nucleotide lesion is removed by short-patch BER (SP-BER) whereas two/more nucleotide lesion is repaired by long-patch BER (LP-BER) pathway[13,62,63].

In contrast to BER, NER is one of the most versatile and flexible repair systems that recover a wide range of DNA lesions, including CPDs and 6-4PPs caused by UVR, bulky chemical adducts, DNA-intrastrand crosslinks, and some forms of oxidative damage. This mechanism is present in most organisms and highly conserved in eukaryotes. Discovery of NER was first described in *E. Coli*[64] where about six proteins such as UvrA, B, and C (known as ABC-complex, which shows excinuclease activity), UvrD (helicase II), DNA polymerase I (pol. I), and DNA ligase are recruited to complete the repair[65,66] (Fig. 6B). Eukaryotic NER is known to be similar to prokaryotes regarding the biochemical strategy used but differs widely in the nature and number of proteins used[67]. NER can be subdivided into differentially regulated subpathways such as global genome NER (GG-NER) and transcription-coupled NER (TC-NER). Repair of DNA lesions over the entire genome, referred to as global genome repair (GGR), and repair of transcription-blocking lesions present in transcribed DNA strands, referred to as transcription coupled repair (TCR). Both repair systems removed a wide range of UV-induced DNA lesions in a sequential way that includes damage recognition, opening of DNA double helix at damage site, and dual incisions on both sides of the lesion followed by resynthesis and ligation[13,68].

Schematic representation of excision repair

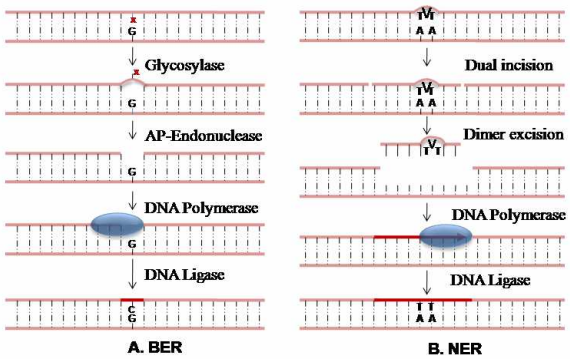

Figure 6

Schematic representation of the BER (A) and NER (B) pathways. Small alteration of bases caused by UVR, ionizing radiation, alkalyting agents, and oxygen radicals can be repaired by BER. DNA damage that distorts the normal architecture of the DNA helix is repaired through NER. This type of damage can be caused by UV radiaton, cisplatin, and other chemotherapeutic drugs (For details see Rastogi et al., 2010)[13].

There is certain positive evidence for excision repair in cyanobacteria. The gene for the DNA repair enzyme FAPY-DNA glycosylase (Fpg) was detected from a cyanobacterium *Synechococcus elongates*[69]. Likewise, UV-endonucleases were isolated from an unicellular cyanobacterium *Synechocystis* PCC 6308[70]. It has been found that DNA repair in the *Synechocystis* sp. PCC 6308 occurs under conditions that do not permit photoreactivation[71], and dimer excision takes place under both photoreactivating and nonphotoreactivating conditions[23]. In several cyanobacterial strains, sensitivity to UV-irradiation increases in the presence of caffeine or acriflavine (inhibitor of excision repair)[54,58,71] and gives indirect evidence for UV-inducible DNA repair in cyanobacteria. Exposure of *Synechocystis* sp. strain PCC 6308 to a sublethal dose of UVR results in decreased DNA degradation after a subsequent challenge dose, and this decrease is prevented by protein synthesis inhibitors administered between the exposures to UVR[55]. If the DNA degradation is attributable to excision repair, then the decrease in degradation implies that another repair pathway is operational and that it requires protein synthesis[55].

3. Recombinational repair

Recombination repair (Fig. 7) is one of the most prevalent mechanisms that efficiently repair double-strand breaks (DSBs) as well as single-strand breaks/gaps (SSBs) in damaged DNA by a series of complex biochemical reactions.

Schematic representation of the recombinatinal repair

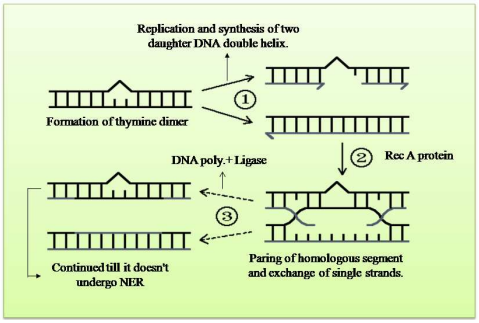

Figure 7

Schematic representation of the recombinatinal repair pathway (Modified from Sinha and Häder, 2002) [12].

Recombination repair fills the daughter strand gap by moving a complementary strand from a homologous region of DNA to the site opposite the damage. The lesion is left unrepaired and after the cell cycles through another replication the damaged base is available as a substrate for excision repair. If the complementary strand is derived from the newly synthesized sister chromatid, the repair is successful[12]. The lethal effects of DSBs can be conquered by the existence of two independent pathway, such as homologous recombination (HR) and non-homologous end joining (NHEJ)[13]. HR is an error free pathway, since; it requires an extensive region of sequence homology between the damaged and template strands, whereas NHEJ is an error prone, alternate pathway that essentially joins broken chromosomal ends independent of sequence homology.

4. SOS Response

The increase in substantial amount of DNA lesions within the cells under different physical as well as chemical agents such as UVR, mitomycin C (MC), methyl methane sulfonate (MMS), and many other chemicals that disrupt DNA, arrest DNA synthesis, and cell division, and lead to accumulation of single stranded (ss) DNA, may cause the occurrence of SOS response. The term “SOS repair” was given by Radman (1974)[72]. Each

of the SOS-induced damage-inducible (*din)* or *sos* genes has near its promoter/operator site a specific 20-nucleotide-long "SOS-box" (also named, LexA-box)[73]. SOS repair system is initiated by interaction of two crucial proteins the RecA (36 kDa) and the LexA (27 kDa) repressor. As a result of massive DNA damage and failure of all possible repair mechanisms, RecA proteins is expressed, which activate the auto breakdown of LexA proteins, allowing the induction of all SOS responding genes[13,74]. The pathway of SOS response is reversed when damages are repaired through the damage specific mechanisms. SOS response is highly mutagenic due to involvement of DNA polymerase V/IV. More recently, Li et al. (2010)[75] has analysed the LexA regulons in cyanobacteria and found that in most cyanobacterial genomes, LexA appears to function as the transcriptional regulator of the key SOS response genes.

In addition to the repair mechanisms discussed above, cells may also protect themselves against DNA damage by triggering cell-cycle checkpoints. Here, the cell cycle is arrested so that the cells do not progress from one phase of the cycle to the next, which causes significant damage in the genome and results in apoptotic or programmed cell death (PCD), thereby protecting the organism at the expense of the individual cell[76,77].

ROLE OF UV-ABSORBING COMPOUNDS IN PHOTOPROTECTION OF CYANOBACTERIA

Damaging effects of UVR can be screened by certain UV-absorbing compounds synthesized by several cyanobacteria as a competent defence mechanism[78]. MAAs and scytonemin are well known UV-absorbing/screening compounds that provide photoprotection against UV-B and/or UV-A radiation[21].

1. Synthesis of mycosporine-like amino acids (MAAs)

MAAs are small (<400 Da), colorless, water-soluble compounds composed of a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol[79]. Commonly, the ring system of MAAs contains a glycine subunit at the third carbon atom. Some MAAs also contain sulfate esters or glycosidic linkages through the imine substituent[80,81]. The absorption spectrum of different MAAs varies according to the presence of variations in the attached side groups and nitrogen substituents. High molar extinction coefficients (e = 28,100–50,000 M^{-1} cm^{-1}), strong UV absorption maxima (310 to 362 nm) (Fig. 8), high photostability and resistance against different physico-chemical stressors such as temperature, strong UVR, distilled to sea water and various solvents as well as pH[82] construct them a powerful photoprotectant in various habitats and organisms. MAAs protect the cells by absorbing highly energetic UVR and then dissipating this energy in the form of harmless heat radiation to their surroundings[83]. MAAs can also act as strong antioxidants to avoid the damaging effects of UVR[84].

Common MAAs in cyanobacteria

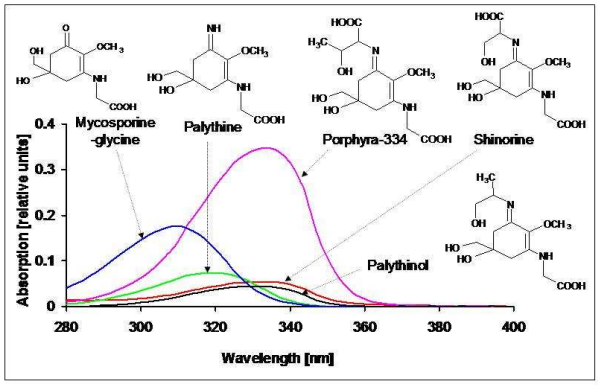

Figure 8

Absorption spectra of some common MAAs found in cyanobacteria (Modified from Rastogi et al., 2010) [6]***.***

It has been found that MAAs effectively block UVR-induced formation of thymine dimer *in vitro* however, *in vivo* experiments are indispensable to prove the role of MAAs in protecting DNA of organisms[85]. These compounds have been reported in various organisms[6,20,86] and can be used in several pharmaceutical industries as well as in biomedical research[5]. The photoprotective efficiency of MAAs depends on the position of these compounds in the cell. In various cyanobacteria, MAAs has been reported in the cytoplasm[87], however, in *Nostoc commune*, MAAs are actively excreted and accumulated extracellularly and hence are more effective against UVR[45].

The biosynthesis of MAAs has been suggested to occur via the first part of the shikimate pathway[6]. The synthesis of MAAs in cyanobacteria is also dependent on several abiotic factors[79,88]. The synthesis of MAAs has been reported to occur in bacteria, cyanobacteria, phytoplankton and macroalgae but not in animals, where these compounds are supposed to be accumulated either via the food chain or synthesized by their symbiotic algal partner due to the lack of the shikimate pathway, the predicted pathway for their biosynthesis[20,89]. However, recently, the gene for the shikimate pathway in the metazoan *Nematostella vectensis* has been disclosed by Starcevic et al. (2008)[90]. Recently, the initial steps in the biosynthesis of MAAs in *Anabaena variabilis* were elegantly elucidated[91]. Genome data mining subsequently identified this gene cluster in several cyanobacteria, fungi, dinoflagellates and even in an actinobacterium[91,92].

2. Synthesis of scytonemin

Scytonemin is a yellow-brown lipid soluble and inducible pigment, absolutely produced by certain cyanobacteria. It is located in the extracellular polysaccharide sheath of some cyanobacterial species[21] (Fig. 9). Scytonemin, first reported by Nägeli (1849)[93] is a dimer composed of indolic and phenolic subunits having a molecular mass of 544 Da and has an in vivo absorption maximum at 370 nm.

A purified scytonemin shows maximum absorption at 386 nm but it also absorbs significantly at 252, 278 and 300 nm (Fig. 10). Synthesis of scytonemin is primarily induced by UV-A radiation[94]. It has been observed that increase in temperature and oxidative stresses in combination with UV-A radiation have a synergistic effect on the synthesis of scytonemin[95].

Scytonemin in the extracellular polysaccharide sheath of Lyngbya sp.

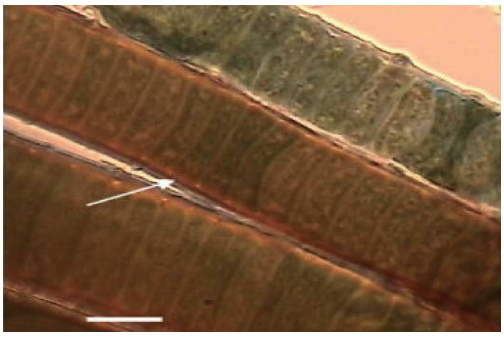

Figure 9

Filaments of the cyanobacterium Lyngbya sp. showing the yellow to red-brown UV protectant scytonemin in the extracellular polysaccharide sheath (see arrow).* [*bar = 10 mm*]. *(Adapted from Sinha and Häder, 2008)[21].

Molecular structure and absorption spectrum of scytonemin

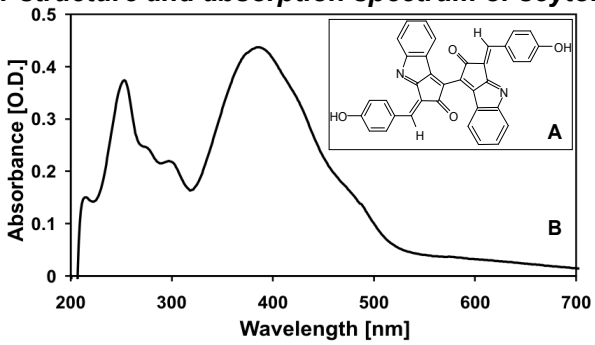

Figure 10
Molecular structure of scytonemin (A) and its absorption spectrum showing prominent peak at 386 nm (B).

Recently, Fleming and Castenholz (2008)[96] have found that a diazotrophically growing culture of *Nostoc punctiforme* PCC 73102 synthesizes 3 to 7 times higher amounts of scytonemin as compared to a non-diazotrophically growing culture; however, the exact mechanism for this is still unclear. Scytonemin is highly stable and performs its screening activity without any further metabolic investment even under prolonged physiological inactivity (e.g. desiccation). Thus, scytonemin can also protect cyanobacteria when other ultraviolet-protective mechanisms such as active repair of damaged cellular components are ineffective[97]. Three new pigments such as tetramethoxyscytonemin, dimethoxyscytonemin, and scytonine (derived from the scytoneman skeleton of the scytonemin) has been reported from the organic extracts of *Scytonema* sp.[98]. A gene cluster (NpR1276–NpR1259) responsible for scytonemin biosynthesis was identified in the genome of *N. punctiforme* ATCC 29133[99-102]. Recently, certain workers[6,100,103] have presented the possible biosynthetic route and a working model for the scytonemin biosynthesis.

CONCLUSION AND FUTURE PROSPECTS

Cyanobacteria are cosmopolitan in distribution and found in almost all photic habitats on the Earth, where they face biologically harmful UV-B radiation. However, these phototrophic organisms have developed several lines of defence mechanisms that maintain their successful growth and survival in diverse habitats receiving high solar UVR. The occurrence of photoprotective mechanisms in cyanobacteria has immense ecological importance, as it maintains the productivity and nitrogen economy of an ecosystem. Several defence mechanisms such as DNA repair as well as synthesis of certain UV-absorbing compounds (MAAs and scytonemin) has been reported to operate in the cyanobacteria to cope up the detrimental effects of UVR. However, identification of DNA repair mechanisms as well as signal pathways for these mechanisms is still a challenge. The process of PCD and related proteins has been reported in cyanobacteria to play an important role under oxidative stress, even so, the role of PCD under UVR stress will be a new field of research in future. The molecular structure and reaction mechanism of DNA repair enzymes such as photolyase and glycosylase from cyanobacteria is also not well known. The use of DNA microarray technology and 2-D electrophoresis will probably help in understanding the adaptation of cyanobacteria to UV stress both at genome and proteome level. With new developments in genomics, proteomics, metabolomics and analytical chemistry, new types of UV-absorbing/screening compounds will continue to be discovered and their biosynthetic and regulatory mechanisms elucidated. The molecular biology of UV-absorbing/screening compounds are in its developing stage and some of the gene products involved in their biosynthesis have recently been functionally

characterized. Much knowledge and efforts are needed to precede the research on these natural sunscreening compounds that will be a boon for cosmetic and other pharmaceutical industries.

ACKNOWLEDGEMENT

Rajesh P. Rastogi is thankful to University Grant Commission, New Delhi, India, for financial assistance in the form of a fellowship.

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