Photoreactivation is the main repair pathway for UV-induced DNA damage in coral planulae

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SUMMARY

The larvae of most coral species spend some time in the plankton, floating just below the surface and hence exposed to high levels of ultraviolet radiation (UVR). The high levels of UVR are potentially stressful and damaging to DNA and other cellular components, such as proteins, reducing survivorship. Consequently, mechanisms to either shade (prevent) or repair damage potentially play an important role. In this study, the role of photoreactivation in the survival of coral planulae was examined. Photoreactivation is a light-stimulated response to UV-damaged DNA in which photolyase proteins repair damaged DNA. Photoreactivation rates, as well as the localization of photolyase, were explored in planulae under conditions where photoreactivation was or was not inhibited. The results indicate that photoreactivation is the main DNA repair pathway in coral planulae, repairing UV-induced DNA damage swiftly (K=1.75 h^{-1} and a half-life of repair of 23 min), with no evidence of any light-independent DNA repair mechanisms, such as nucleotide excision repair (NER), at work. Photolyase mRNA was localized to both the ectoderm and endoderm of the larvae. The amount of cell death in the coral planulae increased significantly when photoreactivation was inhibited, by blocking photoreactivating light. We found that photoreactivation, along with additional UV shielding in the form of five mycosporine-like amino acids, are sufficient for survival in surface tropical waters and that planulae do not accumulate DNA damage despite being exposed to high UVR.

Key words: photolyase, *Acropora millepora*, CPD, MAAs, Phr.

INTRODUCTION

Reef building corals thrive in the clear warm waters of the tropical oceans where ultraviolet radiation (UVR) is higher than in any other marine environment (Baker et al., 1980). UVR has the potential to damage almost all cellular components; however, DNA damage can be considered to be the most significant relative to other cellular components such as proteins and lipids (Britt, 1996). The major form of UV-induced DNA damage is *via* the production of cyclobutane pyrimidine dimers (CPDs), which account for as much as 75% of observable UV-induced DNA lesions (Britt, 1996). UV also directly induces (6-4) photoproducts [(6-4)PPs] in DNA, the Dewar valence isomer of the (6-4)PP lesion, oxidized or hydrated bases and single-strand breaks (Sinha and Hader, 2002). CPDs are formed between the 5,6 bonds of two adjacent pyrimidine bases in DNA exposed to radiation. This lesion alters the shape of the DNA strand, blocking replication and transcription or, in some cases, causing point mutations. The stuttering of polymerases, unable to continue past the damaged site, induces cell cycle checkpoints, which can induce apoptosis (Vink and Roza, 2001).

Mass coral spawning on the Great Barrier Reef occurs a few days after the full moon in November (austral summer), with a smaller spawning in March (Harrison et al., 1984). During this time, corals from more than 36 genera release eggs and sperm into the water column. These gametes float to the surface where fertilization and subsequent development of larvae (planulae) take place (Babcock

et al., 1986; Hatta et al., 1999). In this study, we use the term 'planulae' to describe all stages of the free-living larvae prior to settlement and metamorphosis (Fadlallah, 1983). *Acropora millepora* (Ehrenberg 1834) is a common shallow reef hermaphrodite broadcast spawner that releases bundles of both eggs and sperm, which are externally fertilized (Fadlallah, 1983). The eggs and planulae normally lack symbionts, symbiosis with the dinoflagellates *Symbiodinium* sp. is established after the planulae settle and metamorphosis occurs (Babcock et al., 1986).

Almost 70% of the dry mass of *A. millepora* planulae is made up of lipids, most of which are wax esters (Arai et al., 1993). Wax esters, in addition to being an important energy store, function as buoyant substances in other floating organisms (Nevenzel, 1970; Phleger, 1998). Positive buoyancy also allows eggs and sperms to concentrate in a single layer at the surface, which increases the chance of encounters and hence fertilization. Greater buoyancy also enables greater dispersal ranges (Shanks et al., 2003). A negative consequence of accumulation at the surface, however, is the increased exposure to damaging UVR, which can be extremely high during the tropical summer. Furthermore, active DNA replication and mitosis within larval cells exacerbates potential damage to DNA. There is considerable evidence that exposure to natural levels of ultraviolet-B radiation (UVBR) reduces survivorship of coral planulae, as seen with *Agaricia agaricides* planulae where survivorship dramatically increased after UVBR was excluded

(Gleason and Wellington, 1995). *Porites astreoides* planulae were also shown to actively avoid high UV areas when settling (Gleason et al., 2006). It would consequently appear that avoiding UVR damage or repairing damage once it has occurred are likely to be important in the survival of planulae at the surface layers of the ocean (Epel et al., 1999).

Absorbing harmful UVR before it reaches sensitive cellular components represents a potential mechanism for mitigating the risk of UVR-induced damage. Many sessile marine organisms possess screening molecules that absorb or block UVR as it passes through their tissues (Shick and Dunlap, 2002). Mycosporine-like amino acids (MAAs), are a group of small, water-soluble compounds with high UVR absorption (Dunlap and Chalker, 1986; Dunlap and Shick, 1998). MAAs are biosynthesized through the shikimate acid pathway, which is absent from the animal host metabolism. In the coral–*Symbiodinium* symbiosis they are most probably derived from the symbiotic dinoflagellates, which may translocate them to the host tissues (Banaszak et al., 2000). The host tissue is where MAAs are predominantly found (Lesser et al., 1990; Yakovleva and Hidaka, 2004), and they can then be translocated to the oocyte before spawning occurs. The planulae of *A. millepora* contain high concentrations of MAAs (Dubinsky, 1990), which presumably shield them from the high levels of UVR in surface waters. In asymbiotic planulae, such as *A. millepora,* the source of MAAs is unclear. While planulae of the coral *Heteroxenia fuscescens* have recently been shown to acquire MAAs independently of the mother colony or the symbionts (Zeevi Ben-Yosef et al., 2008), most studies suggest parental provisioning of MAAs (Gleason and Wellington, 1995; Michalek-Wagner and Willis, 2001; Wellington and Fitt, 2003). If MAAs indeed originate from the parent colonies, then those planulae originating from deeper parent colonies with less MAAs (Wellington and Fitt, 2003) must uptake MAAs independently of the parent colony or rely on other pathways such as repair in order to survive the planktonic stage.

The two most significant strategies for the repair of UV-induced CPDs are photoreactivation and nucleotide excision repair (NER). Photoreactivation is a DNA repair pathway present in all organisms apart from placental mammals (Kanai et al., 1997). During photoreactivation, the enzyme photolyase uses visible light (violet/blue) as a source of energy (ATP independent) to repair UV-induced DNA damage in the form of CPDs or (6-4)PPs. DNA photolyase identifies UV-damaged DNA and breaks improperly formed covalent bonds within the CPD by a light-activated electron transfer reaction between the flavin adenine dinucleotide (FADH-), the electron donor, and CPD, the electron acceptor (Kao et al., 2005).

NER is the main dark (light-independent) repair pathway for UV lesions (Thoma, 1999) and, unlike photoreactivation, is an ATP-dependent process. NER is the predominant, and perhaps, universal mechanism for the maintenance of genomic integrity (Garfinkel and Bailis, 2002), involved in the removal of a variety of DNA lesions. During this process, regions of DNA containing the CPD are excised along with adjacent nucleotides and replaced by newly synthesized DNA. At least 20–30 proteins are involved in the pathway in a sequential manner (Garfinkel and Bailis, 2002).

Corals have been shown to be greatly impaired by UV exposure when placed in the dark following UV irradiation, implying a significant role for photoreactivation repair (Siebeck, 1981). UV-lesion repair mechanisms, however, have not been reported in simple organisms such as corals. In the present study, we identify a putative photolyase in the coral *A. millepora* for the first time and demonstrate that photoreactivation is the main repair pathway for UV lesions in coral planulae. We also show that this process is rapid and effective, conferring protection against the high UVR levels encountered during the planktonic stage of the common reef building coral *A. millepora*.

MATERIALS AND METHODS

This study was conducted during the mass coral-spawning event of November 2007 near Heron Island on the southern Great Barrier Reef (23°26′S 151°55′E). Gametes were collected from eight colonies of *A. millepora* and mixed together in large bins containing 5 μm filtered seawater. The combined gametes were mixed every hour. In addition, water changes were undertaken four times a day. Five days following fertilization, swimming planulae were placed in the experimental tanks and treated as detailed below.

UV irradiation and repair

Approximately 1500 asymbiotic *A. millepora* planulae were distributed between two 5-l glass beakers containing 25°C, 5 μm filtered, aerated seawater and irradiated for one hour by two 4 W Sankyo Denki UV-B lamps (G4T5E, Sankyo Denki, Kanagawa, Japan); UV-B irradiation at the surface was 4 W m^{-2}. The UV dosage was predetermined to obtain maximal DNA damage in *A. millepora* adult fragments in experimental trials. Immediately following the UV pulse, approximately 30 coral planulae were collected from each beaker and snap frozen in liquid N$_2$ (sample T0). Following the collection of T0 the remaining planulae were mixed and transferred in equal measures to eight 1-l beakers with 25°C, 5 μm filtered, aerated seawater. Three minutes after the UV pulse, four beakers were maintained in darkened conditions to prevent photoreactivation. Four beakers were left uncovered under two T8 Sylvania Aquastar 36 W lamps (Osram Sylvania, Danvers, MA, USA). Photosynthetically active radiation (PAR) intensity of 150 μE m^{-2} s^{-1} was measured at the beaker surface. The mixing caused by the air bubbler moved the planulae continually up and down in the beaker (water depth of 20 cm). Two samples (each containing approximately 30 planulae) were taken from each beaker for each time point: 10, 60, 205, 690, 1010 and 1155 min following the UV pulse and snap frozen in liquid N$_2$. Samples were kept at –80°C until further analysis.

Detection of cell death

DNA damage and fragmentation in larvae was detected by using terminal deoxynucleotidyl transferase (TdT) biotin-dUTP nick end labeling (TUNEL) technique, which incorporated labeled nucleotides into nicks in DNA. The TUNEL assay was used as a measure of DNA fragmentation, which is indicative of both apoptotic and necrotic cell death pathways. Coral planulae from the light and the dark treatments were collected after 19 h of repair following the UV pulse and fixed in 4% paraformaldehyde in phosphate buffer saline (PBS) overnight at 4°C and gradually dehydrated in a series of methanol rinses to 100% concentration. Samples were stored at 4°C.

Larvae were rehydrated under a series gradient of methanol rinses (100%, 95%, 85%, 70%, 50%, 40%, 25% in PBS, pH 8, for 3 min each). The larvae were transferred to clean 1.5 ml tubes and allowed to settle at the tube base prior to the rinse being removed and replaced. Larvae were not centrifuged in between rinses to preserve the integrity of the tissues. The remaining part of the protocol was adapted from a fluorometric TUNEL kit (DeadEnd^{TM}, Promega, Madison, WI, USA). Following two further PBS rinses for 5 min each, larvae were permeabilized with 0.2% Triton ×100 in PBS for 5 min and rinsed again in PBS for 5 min. The PBS was removed and replaced by 80 μl of equilibration buffer provided for 15 min.

The equilibration buffer was replaced by an rTdT enzyme/labeled nucleotides/buffer in accordance with the manufacturer's instructions and incubated in the dark at 37°C for 1 h. The TUNEL mix was later removed and larvae were rinsed in PBS and counterstained with Hoechst 33342 (Molecular Probes, Invitrogen Corporation, Carlsbad, CA, USA) for 1 h. As a positive control, a selection of larvae were treated with DNase I, and as a negative control, larvae were treated with the TUNEL mix without the rTdT enzyme as suggested by the manufacturer. The larvae were then rinsed in PBS prior to being mounted on glass slides with a 70% glycerol/PBS, pH 8, mountant solution. Slides were viewed on an LSM 510 metahead laser confocal microscope (Carl Zeiss AG, Oberkochen, Germany).

DNA extraction and CPD damage detection

DNA was isolated by homogenizing coral planulae in extraction buffer [100 mmol l^{-1} ethylenediaminetetraacetic acid (EDTA), 10 mmol l^{-1} Tris, 1% sodium dodecyl sulfate (SDS), pH 7.5] and incubating with 3% β-mercaptoethanol (BME) at 65°C for an hour. Proteinase K was added to a final concentration of 500 μg ml^{-1} and incubated at 37°C overnight. Samples were treated with RNase, extracted once with phenol:chloroform:isoamyl alcohol (25:24:1), once with chloroform and then ethanol precipitated twice and redissolved in TE (0.01 mol l^{-1} Tris-HCl, pH 7.5, 1 mmol l^{-1} EDTA). DNA quality was assessed by gel electrophoresis and quantified using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The samples were all brought to a final concentration of 0.02 ng μl^{-1} DNA in 1×PBS. CPD lesions were quantified by a colorimetric (492 nm) enzyme-linked immunosorbent assay (ELISA) using the primary antibody TDM-2 (Cosmo Bio, Tokyo, Japan) to bind to CPDs (Mori et al., 1991). The samples from T0 acted as a baseline to later time point samples and were defined as 100% damage with later time point samples normalized as percentages from initial T0 values of damage. The DNA repair curve was fitted with a one-phase decay model (Eqn 1) constrained so that initial CPD values were assigned 100%. K was defined as the rate of repair expressed in h^{-1}. Non-linear regression was performed using the *nls* function in R (R Development Core Team, 2005):

$$CPD(t) = 100e^{-Kt}, \qquad (1)$$

where *t* is time in hours.

Levels of CPD damage in planulae under natural UVR

We gauged levels of CPD damage in planulae exposed to natural solar radiation for a day at ambient reef-flat temperature (25°C). Five-day-old planulae were left in three uncovered glass beakers with a water depth of 25 cm. Control planulae were kept in three glass beakers covered by a UV opaque glass cover (cut-off 390 nm). Erythemally weighted UVR measured for the area was extremely high, 350 mW m^{-2} (Australian Bureau of Meteorology, Melbourne, VIC, Australia). Two hours after solar noon duplicate samples (approximately 30 planulae each) were taken from each beaker and snap frozen in liquid N$_2$. Samples remained at –80°C until further analysis. DNA extraction and CPD detection were performed as above.

UV absorbing compounds in planulae tissue

In order to determine the composition of UV absorbing MAAs in the planulae, approximately 300 five-day-old swimming coral planulae were snap frozen in liquid N$_2$ and stored at –80°C until further analysis. The frozen planulae samples were gradually transferred from seawater to freshwater in a series of gradient dilution washes over a period of 2 h to maintain the integrity of the planulae while desalting. Samples were then homogenized in microcentrifuge tubes using micro tissue grinders. Samples were resuspended in 500 μl of water, filtered (0.22 μm) and separated by high performance liquid chromatography (HPLC, Shimadzu LC-10AT VP liquid chromatograph, Kyoto, Japan). Samples (5 μl each) were eluted through a Develosil RPAQUEOUs column (Nomura Chemical, Seto, Japan) using a gradient from an aqueous mobile phase of 0.05% formic acid to 0.05% formic acid in 100% methanol with a flow rate of 0.25 ml min^{-1}. Individual peaks were identified by absorption profile derived from the photodiode array detector (Shimadzu SPD-M10A VP) and retention time (Carreto et al., 2005).

Isolating photolyase from *A. millepora* cDNA

Degenerate primers (*F*: GARYTBGCNGAYAAYTTYTG, *R*: YTTYTTNGCCCANTACAT) were designed according to a consensus sequence of the photolyase FAD-binding domain (Fig. 1) based on 1254 sequences from 625 species using pfam (Bateman et al., 2004). The FAD-binding domain is a highly conserved domain of photolyase. The degenerate primers were used on a cDNA library (λ-ZAP II vector) produced from *A. millepora*. The PCR reaction program consisted of 35 cycles of 94°C (1 min), 58°C (30 s) and 72°C (1 min), followed by 10 min at 72°C. The products were visualized by gel electrophoresis and ligated into a pGEM-T Easy vector (Promega), which was then amplified using Top10 *E. coli* cells (Invitrogen). Specific primers (*F*: TACCTGTACACCAGAG-AACAGCTTG, *R*: TTATCTTCCCAAACACTGGCCTTTCAG) were designed from the contiguous sequences of the potential photolyase. The amplified 530 bp product was visualized by gel electrophoresis and ligated into a pGEM-T Easy vector (Promega), which was then amplified using Top10 *E. coli* cells (Invitrogen).

In situ hybridization to photolyase

In order to identify and localise mRNA expression of photolyase within the coral planulae, *in situ* hybridization (ISH) was performed on tissue sections using sense (negative control) and anti-sense probes. Approximately 5 μg of plasmid DNA (pGEM-T Easy vector, Promega) including the cDNA insert (containing the 530 bp fragment, Fig. 1, GenBank accession number FJ805425.1) was linearized using SacII and *Sal*I (for the antisense and sense probes, respectively). The linearized DNA was purified using the Wizard SV Gel and polymerase chain reaction (PCR) Clean-up System (Promega). For transcription of the probes, we used SP6 (antisense) and T7 (sense) RNA polymerase, (20 U μl^{-1}; Roche Diagnostics AG, Basel, Switzerland), linearized plasmid DNA (1 μg), digoxigenin (DIG) RNA labeling mix, transcription buffer, RNaseOut (40 U μl^{-1}) and sterile water in a total volume of 20 μl. The reaction was incubated for 2 h at 37°C. *Acropora millepora* planulae from the
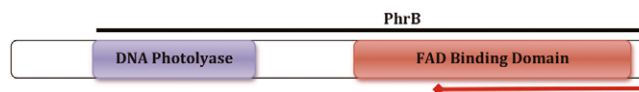

Fig. 1. A schematic of the cyclobutane pyrimidine dimers (CPD) photolyase protein showing the conserved domains (Marchler-Bauer et al., 2007). In blue, the conserved domain pfam00875 (DNA photolyase superfamily) and in red, pfam03441 (FAD-binding domain of DNA photolyase). The top black line represents protein family COG0415 (PhrB, deoxyribodipyrimidine photolyase). The bottom red line represents the sequence used for building the digoxigenin (DIG)-labeled *in situ* hybridization probe.

dark and light treatments at 10 min and 19 h following the UV pulse were fixed in 4% paraformaldehyde (w/v) in sterile PBS for 8 h at 4°C. Histological sections of the planulae were prepared after the paraformaldehyde-fixed larvae were dehydrated in a graded series of ethanol (100% to 50%), cleared in n-butyl alcohol and then embedded into paraffin (Paraplast Plus melting point 56°C, McCormick Scientific, St Louis, MO, USA). Histological sections 12 μm thick were cut with an M1R rotary microtome (Shandon Scientific, Cheshire, UK). Histological slides were prepared for each sample containing several replicates of the planulae and stained with Harris haematoxylin and eosin Y.

ISH was performed with the previously described DIG-labeled probes (Roche Diagnostics, Fig. 1) at a concentration of $1 ng μl^{-1}$ and was detected using alkaline phosphatase-conjugated anti-digoxigenin antibody. The sense and anti-sense probes were heat denatured at 80°C for 5 min and hybridized to the slides overnight at 65°C with gentle shaking. After hybridization, sections were rinsed twice to remove unbound probes in wash buffer 1 [50 ml formamide, 5 ml 20× SSC (saline–sodium citrate), 5 ml 3% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), 40 ml ddH₂O] for 30 min at 65°C, followed by an additional rinse in wash buffer 2 (10 ml 20× SSC, 10 ml 3% CHAPS, 80 ml ddH₂O) at 65°C for 15 min and two additional rinses in wash buffer 3 (1 ml 20× SSC, 10 ml 3% CHAPS, 89 ml ddH₂O) for 30 min at 65°C. A final 15 min rinse in maleic acid buffer (MAB) ($0.1 mol l^{-1}$ maleic acid pH 7.5, $150 mmol l^{-1}$ NaCl) was carried out at 25°C. The antibody was then hybridized to the remaining DIG-labeled probes for 3 h followed by six consecutive rinses in PBS containing 0.1% Tween 20 (PBTw) at 25°C. The antibody was then stained with BM purple overnight. Rinsing sections three times in PBTw stopped the staining reaction.

RESULTS

DNA repair following irradiation occurred in the light but not to the same extent in the dark (Fig. 2A,B).

A one-phase decay model was fitted to the photorepair data (Fig. 2A). The rate of photorepair (K) under a light intensity of $150 μE m^{-2} s^{-1}$ was $1.75 h^{-1}$, and the half-life of photoreactivation (the time to repair 50% of the DNA damage) was 0.39 h (~23 min). Light-independent repair (e.g. NER) did not seem to be an important pathway for CPD repair in coral larvae as repair under dark conditions (Fig. 2B) did not show a decay response (R^2=0.2). The rate of repair in the dark was extremely slow and the amount of CPDs remained unchanged at 60% once darkened.

A large number of the planulae had stopped swimming and were visibly decomposing, indicating mortality in the darkened beakers 19 h after the UV pulse. Our visual assessment of the beakers exposed to light was that larvae were swimming and intact. Onset of mortality was confirmed by an observed increase in TUNEL-positive stained nuclei in darkened planulae compared with those exposed to light (Fig. 2C,D). We ended the experiment at this point for fear that we might not be able to extract intact DNA from the planulae if they degraded any further.

The photolyase sequence from *A. millepora* showed a high similarity (e-value $3e^{-04}$) to the specific FAD-binding domain motif of DNA photolyase. ISH showed photolyase transcripts were ubiquitously localized throughout all tissues. Under the light treatment photolyase was localized in both the endoderm and the ectoderm (Fig. 3A) whereas in dark-treated planulae expression appears to be localized mainly in the ectoderm (Fig. 3B). Negative control sense labeling was not detected in any of the planulae (Fig. 3C).
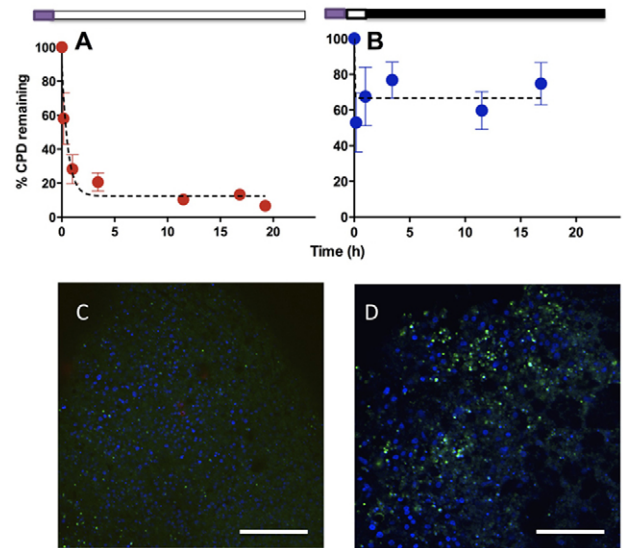

Fig. 2. Repair of cyclobutane pyrimidine dimers (CPD) damage in *Acropora millepora* planulae from Heron Island in the (A) light (photoreactivation, Phr) and (B) the dark (e.g. nucleotide excision repair, NER) over 19 h following the UV pulse. Values are in % compared with initial level of damage after UV pulse. Curves fitted are one-phase decay curves with decay rates for photoreactivation of K=1.75 h^{-1}. While repair *via* photoreactivation fits a one-phase decay model (R^2=0.9), dark repair does not (R^2=0.2). Error bars are ± s.e.m. The corresponding terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) stains of whole coral planulae tissue after 19 h of recovery from UV stress in the light (C) and dark (D) indicate higher mortality in the dark. Blue stain is a nucleic acid label (Hoechst 33348) as a counterstain, and green illustrates TUNEL-positive nuclei (Green=labeled DNA strand breaks), indicating cell death. Scale bars=20 μm.

HPLC analysis revealed the presence of at least five MAAs in quantifiable amounts in the five-day-old planulae tissues (Fig. 4). The MAAs were identified [based on retention time and absorption maxima ($λ_{max}$)] as mycosporine sulfate ester, $λ_{max}$=318 nm (Fig. 4, peak 2), shinorine $λ_{max}$=333 nm (Fig. 4, peak 3), mycosporine–glycine $λ_{max}$=311 nm (Fig. 4, peak 4), an unknown substance with $λ_{max}$ of 308 nm (Fig. 4, peak 5) and palythine $λ_{max}$=360 nm (Fig. 4, peak 6). Peak 1 was not well separated from peak 2 and was not regarded as an additional compound.

Under natural UVR levels, planulae exposed to UVR for one day did not display increased levels of CPD damage compared with larvae shielded from UVR [analysis of variance (ANOVA) $F_{1,10}$=0.499, P=0.495]. Although the mean amount of DNA damage in both the UV exposed and UV shielded treatments was significantly different from zero (P=0.0149 and P=0.03 for UV exposed and UV shielded treatments, respectively), the damage levels were very low (0.04% of maximal values detected for this species).

DISCUSSION

Exposure to light resulted in the photoreactivation of CPD damage within tissues of *A. millepora* planulae that was swift and effective. Planulae repaired half of the CPD DNA damage within 25 min of the UV pulse, with damage being no longer detectable after a few hours. When photoreactivation was inhibited, by placing the planulae in the dark, CPD damage remained, implying that light-independent repair pathways such as NER do not play a major role in the repair
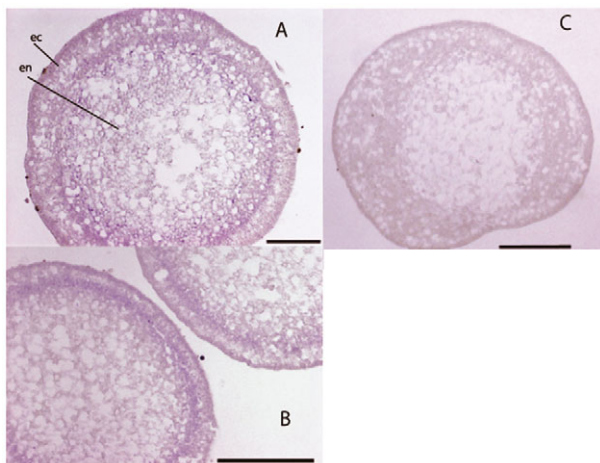
Fig. 3. BM purple stains of *in situ* hybridization to the mRNA of a putative cyclobutane pyrimidine dimers (CPD) photolyase in 12 μm thick sections of *Acropora millepora* planulae from light (A) and dark (B) treatments following a UV pulse. BM purple staining of sense probes (C) reveal no non-specific binding. Scale bars=50 μm. ec=ectoderm; en=endoderm.

of UV-induced DNA CPD damage. Initial reductions in CPD damage across all treatments can be explained by light exposure during sampling at T0 and prior to transfer to the dark (a process which took approximately 3 min).

The repair rate for *A. millepora* (K=1.75 h^{-1}) is higher than observed in other invertebrates. In similar experiments, repair rates in echinoid embryos ranged between K=0.58 h^{-1} and 1.25 h^{-1} (depending on temperature), with 50% of the damage repaired between 2.1 h and 0.6 h, respectively (Lamare et al., 2006). Repair rates in the Antarctic zooplankton *Euphasia superba* (Malloy et al., 1997) were also lower than reported in the present study, K=0.96 h^{-1}. Marsupial photolyase expressed in human cells showed repair rates of K=1.5 h^{-1} (Nakajima et al., 2004), 50% of the damage repaired in less than 30 min. Other vertebrates, such as the freshwater fish *Xiphophorous variatus* showed a repair rate of K=1.6 h^{-1} (Mitchell et al., 1993), and a repair rate of K=0.4 h^{-1} was measured for *Xenopus laevis* tadpoles (Pandelova et al., 2006). Malloy et al. compared
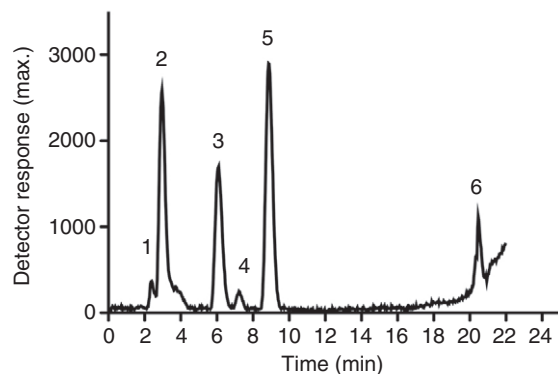

Fig. 4. A spectrum maxima chromatogram showing the detector response in relative units and the retention times of the different mycosporine-like amino acid (MAAs) from five-day-old *Acropora millepora* planulae separated through a Develosil RP-AQUEOUs column. Peaks are numbered and explained in the text. Peak 1 is ambiguous and does not clearly represent an additional MAA.

repair rates in Antarctic fish and found a positive relationship between levels of UVR exposure (depth where fish were found) and repair rates (Malloy et al., 1997). The high rates of repair in corals are not surprising given that they float at the surface of the water under tropical solar radiation that involves some the highest UVR exposure rates on planet Earth. The rate of CPD repair, however, is strongly correlated to the quantity of photoreactivating light present (Pandelova et al., 2006), making comparisons with other experiments where light levels might have been different, difficult. The conclusion that can be made from our present study and other studies cited here is that photoreactivation is rapid and effective. While we did not test the effect of temperature on repair rates in this study, Lamare et al. observed a strong temperature effect, with a temperature coefficient over a 10°C temperature range (Q_{10}) of 2.15 for the photoreactivation reaction in echinoid embryos (Lamare et al., 2006). The question whether coral planulae show a similar response will be the focus of future studies.

In a comparative study by Kuffner (Kuffner, 2001), larvae of the coral *Pocillopora damicornis* originating from deep-water colonies contained less than half the amount of MAAs than their shallow counterparts, and did not show differential mortality following exposure to UVR. One possible conclusion the author offers is that MAAs may not be as important as previously thought, even though UVR was an important physical parameter that affected *P. damicornis* larval ecology (Kuffner, 2001). The present study shows that MAAs are just part of the spectrum of UVR protective mechanisms, and that DNA damage repair mechanisms play a significant role in survival following UVR exposure even when MAAs are present. Photolyase repair of DNA may therefore explain why lower MAA content did not result in increased mortality in the Kuffner study (Kuffner, 2001).

UVR-induced CPDs are known to induce cell death through induction of apoptosis (Chigancas et al., 2000; Vink and Roza, 2001). In this study, the TUNEL assay indicated increased labeling, cell death and degradation among coral planulae when photoreactivation was inhibited by a lack of photoreactivating light. While it is highly likely the DNA damage triggered apoptosis in the planulae cells, as is commonly the case with CPD damage (Vink and Roza, 2001), Lo et al. demonstrated that CPD damage leads to cell cycle arrest and not necessarily to apoptosis (Lo et al., 2005). The TUNEL assay alone does not distinguish the pathways responsible for DNA fragmentation prior to cell death (Grasl-Kraupp et al., 1995). This aside, it is apparent that planulae were dying rapidly when deprived of the required light for photoreactivation.

Photolyase is the enzyme that facilitates photoreactivation of CPD damage. ISH to CPD photolyase mRNA showed that a putative CPD photolyase was localized in the *A. millepora* planulae, even when photoreactivating light was not available. While our measurements did not allow for the quantification of photolyase expression, we did find it expressed in both the endodermal and ectodermal layers of the planulae when exposed to light, probably indicating that photoreactivation machinery was repairing CPDs induced by the UVR. In zebrafish embryos the transcripts of a repair enzyme, (6-4) photolyase are also localized throughout the embryo (Tamai et al., 2004); however, while we found expression in both light and dark exposed planulae, Tamai et al. found that light was greatly responsible for transcriptional activation (Tamai et al., 2004). But as both the light- and the dark-treated planulae in our study were first exposed to UVR, a known inducer of photolyase expression (Pang and Hays, 1991), it is difficult to conclude what the role of light is in the regulation of photolyase gene expression in the planulae. Photolyase is found in organisms not regularly exposed

to light, such as the Enterobacteria *Escherichia coli*. Furthermore, photolyase expression levels are not always correlated to CPD formation (Ozer et al., 1995). This and the finding that photolyase is involved in the removal of non-UV-induced damage in yeast in the dark (Sancar and Smith, 1989), suggests that photolyase might have additional roles other than photoreactivation.

This study also explored other mechanisms of protection from UVR damage. At least five different MAAs were found in the *A. millepora* planulae, which were probably inherited from the mother colony through the egg (Michalek and Michalek-Wagner, 2001). MAA content and composition differs greatly between coral species, and the diversity of MAAs we found in *A. millepora* planulae is lower than the diversity of MAAs in other adult Acroporids. Teai et al. (Teai et al., 1997) found six MAAs in *Acropora* species collected in French Polynesia, and while there is some overlap between the compounds detected in *A. millepora* with the compounds detected in other *Acropora* species (Teai et al., 1997), some are different. We found one perhaps novel compound (λ_{max}=308 nm), which remains to be characterized. This is the first description of the MAA composition in *A. millepora* and provides a platform to compare with the MAA composition of adult colonies, which may then assist in adjudicating whether planulae MAA are derived from parent colonies (Michalek and Michalek-Wagner, 2001) or taken up independently from the parent colony.

The planulae in this study originated from shallow water colonies of *A. millepora*. Given the negative correlation found between depth and the amount of sunscreen molecules found in the planulae (Gleason and Wellington, 1995; Wellington and Fitt, 2003), future work should address the efficiency of photolyase in planulae originating from deeper coral colonies, which are not as well protected by sunscreens. The planulae of *A. millepora* can float in the water column for months before settlement (Ball et al., 2004) but the planulae in this study were tested for MAA content after five days. Although some evidence points to MAAs having a potentially long residence time of weeks to months in tissues [Shick et al. (Shick et al., 2005) and references therein], decreases in MAA concentration can occur over time. In asymbiotic planulae, unless MAAs are taken up through feeding, there is no new production of MAAs by *Symbiodinium* sp., and the protection offered by MAAs might reduce with time. This may suggest that the role of photoreactivation might be greater in older planulae.

This study indicates that coral planulae can respond effectively to UVR because of an efficient photorepair pathway and UV screening molecules. The photoreactivation pathway is energetically inexpensive and allows for swift and error-free repair of DNA damage. Planulae left exposed to naturally occurring UVR did not show evidence of accumulation of lethal DNA damage. This adaptation of coral planulae to high UVR can thus increase the competency period of the planulae and facilitate wider dispersal ranges, because it allows the planulae to remain at the surface waters for longer before settlement. Dispersal potential is tightly linked to many key ecological processes such as population connectivity and biogeography. Consequently, processes that influence the competency period, such as efficiency of UV damage repair, are likely to have far reaching ecological implications.

List of abbreviations

CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CPD	cyclobutane pyrimidine dimers
DIG	digoxigenin
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FADH-	flavin adenine dinucleotide (reduced)
HPLC	high performance liquid chromatography
ISH	*in situ* hybridization
K	rate of exponential decay
MAA	mycosporine-like amino acid
MAB	maleic acid buffer
NER	nucleotide excision repair
PAR	photosynthetically active radiation
PBS	phosphate buffer saline
PBTw	PBS containing 0.1% Tween 20
PCR	polymerase chain reaction
Q_{10}	temperature coefficient
SDS	sodium dodecyl sulfate
(6-4)PP	(6-4) photoproduct
SSC	saline–sodium citrate
TdT	terminal deoxynucleotidyl transferase
TUNEL	terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling
UV	ultraviolet
UVBR	ultraviolet-B radiation (280–320 nm)
UVR	ultraviolet radiation
λ_{max}	absorption maxima (nm)

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