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Differential sensitivity of coral larvae to natural levels of ultraviolet radiation during the onset of larval competence

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Abstract

Scleractinian corals are the major builders of the complex structural framework of coral reefs. They live in tropical waters around the globe where they are frequently exposed to potentially harmful ultraviolet radiation (UVR). The eggs and early embryonic stages of some coral species are highly buoyant and remain near the sea surface for prolonged periods of time and may therefore be the most sensitive life stages with respect to UVR. Here, we analysed gene expression changes in five developmental stages of the Caribbean coral *Montastraea faveolata* to natural levels of UVR using high-density cDNA microarrays (10 930 clones). We found that larvae exhibit low sensitivity to natural levels of UVR during early development as reflected by comparatively few transcriptomic changes in response to UVR. However, we identified a time window of high UVR sensitivity that coincides with the motile planula stage and the onset of larval competence. These processes have been shown to be affected by UVR exposure, and the transcriptional changes we identified explain these observations well. Our analysis of differentially expressed genes indicates that UVR alters the expression of genes associated with stress response, the endoplasmic reticulum, Ca^{2+} homoeostasis, development and apoptosis during the motile planula stage and affects the expression of neurogenesis-related genes that are linked to swimming and settlement behaviour at later stages. Taken together, our study provides further data on the impact of natural levels of UVR on coral larvae. Furthermore, our results might allow a better prediction of settlement and recruitment rates after coral spawning events if UVR climate data are taken into account.

Keywords: development, gene expression, global climate change, neurogenesis, stress response, ultraviolet radiation

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Introduction

Scleractinian corals are keystone species of one of the most productive and diverse marine ecosystems: coral reefs. Yet, during the past decades, a dramatic increase in coral mortality and a worldwide decline in coral reefs have been observed (Hoegh-Guldberg 1999; Pandolfi *et al*. 2003). The main reasons for this global decline in reef cover are bleaching and disease (Glynn 1993; Hoegh-Guldberg *et al*. 1997; Harvell *et al*. 2007). Mass bleaching (i.e. the breakdown of the coral–algal symbiosis) events are principally brought about by increases in seawater temperature (Hoegh-Guldberg 1999). Ultraviolet radiation (UVR) has been shown to be an agent in coral bleaching directly (Gleason & Wellington 1993) or interactively with temperature (Lesser *et al*. 1990; Glynn *et al*. 1993; Lesser 2010). Hence, UVR may

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harm adult corals and contribute to the worldwide degradation of coral reef ecosystems. While studies on the effect of temperature on coral larvae have been conducted (Rodriguez-Lanetty *et al.* 2009; Voolstra *et al.* 2009a; Portune *et al.* 2010), research on the effect of UVR on scleractinian coral larvae has been scarce, although eggs and embryos are thought to be the most sensitive life stages with respect to UVR (Banaszak & Lesser 2009). Many broadcast spawning species release their eggs in shallow waters where they stay afloat near the surface for several days before they develop into motile larvae and settle. For example, Wellington & Fitt (2003) reported that larvae from *Acropora palmata*, *Montastraea annularis* and *M. franksi* contain high amounts of lipids and float in surface waters for 3–4 days. Studies on the coral *Porites astreoides* showed that planulae actively avoid areas of higher UVR as soon as they become motile, implying that coral larvae are able to sense UVR (Gleason *et al.* 2006). Moreover, experiments with larvae from different coral species, including *M. franksi* and *M. annularis,* showed a significant decrease in survivorship in response to ambient UVR levels for larvae originating from deeper colonies. This effect correlated with the concentration of UV-absorbing mycosporine-like amino acids (MAAs) in larval tissue (Gleason & Wellington 1995; Wellington & Fitt 2003). Furthermore, UVR has a negative effect on the settlement of *Pocillopora damicornis* larvae despite the presence of MAAs (Kuffner 2001). In sea urchin embryos, UVR induces cell death caused by the accumulation of DNA damage (Lesser *et al.* 2003). UVR-induced cellular and molecular damage has also been observed in the larvae of various fish species including the Atlantic cod and the Japanese flounder (Browman *et al*. 2003; Yabu *et al*. 2003) as well as crabs, molluscs and other marine invertebrates (Hovel & Morgan 1999; Helbling *et al.* 2002; Przeslawski *et al*. 2005; Dahms & Lee 2010). UVR directly damages DNA and induces oxidative stress through the generation of reactive oxygen species (ROS) that are a product of photochemical reactions. As a consequence, proteins and lipids are oxidized and damaged, leading to loss of function and membrane instability (Bose *et al.* 1989; Lesser & Farrell 2004; Lesser 2006). An increase in harmful UVR as brought about by anthropogenic action might further advance the impact of this stressor in the future (Crutzen 1992; Kerr & McElroy 1993; Bergmanson & Sheldon 1997; McKenzie *et al*. 2007; Hegglin & Shepherd 2009). More specifically, Hegglin & Shepherd (2009) predict an increase in UVR in the tropical regions owing to climate change–induced changes in the spatial distribution of stratospheric ozone.

In the light of global climate change, it is critical to gain a detailed understanding of the effects of UVR on coral larvae as their successful settlement provides the basis for the future of coral reefs. We present here the first study on the impact of UVR on larvae of the Caribbean coral *Montastraea faveolata* with high-density cDNA microarrays (10 930 clones). Briefly, we analysed the transcriptional response of coral larvae to natural levels of UVR at different time points during development (i.e. 12, 36, 60, 84 and 132 h postfertilization). Our results indicate that UVR sensitivity varies significantly during development. Whereas we observed only few transcriptional changes in response to UVR for most of the time points analysed, we identified a time point of high sensitivity. Namely, after 84 h we found that UVR affects the expression of genes associated with stress response, the endoplasmic reticulum (ER), Ca^{2+} homoeostasis, development and apoptosis. In particular, we observed differential expression of several factors associated with neurogenesis, suggesting a direct effect on the formation and/or function of the larval nervous system. In addition, we found differential expression of neuropeptides involved in larval behaviour, which might explain the effects on locomotion and larval settlement in response to UVR as observed in previous studies (Kuffner 2001; Wellington & Fitt 2003). Overall, our data provide a first insight into the molecular responses associated with exposure to natural levels of UVR and how this might impact developmental processes in coral larvae.

Materials and methods

Gamete capture and larval rearing

Montastraea faveolata gametes were captured and reared as described by Voolstra *et al.* (2009a). Briefly, gametes from 10 colonies were captured during a spawning event on the night of the 10 September 2009 at approximately 22:00 h using collecting nets attached to plastic enclosures at 'La Bocana Chica' reef (20º50′N, 86º52′W) located in the 'Parque Nacional Arrecife de Puerto Morelos'. Within 10 min, the gametes were brought to the research vessel 'Carybdea', where they were all mixed and placed in 5 μm filtered sea water (FSW). Large zooplankton were removed, and the egg–sperm mixture was mixed gently to enhance the process of fertilization during transportation to the research station. After 1 h, the egg–sperm mixture was repeatedly washed with 5 μm FSW to ensure that all unused sperm and any remaining zooplankton were removed. The embryos were placed in round, bottomless, cultivation bins fitted with 100-μm mesh, which were housed in coolers filled with abundant 5 μm FSW. Fertilization success, measured 6 h after the washing procedure, was estimated at 95% by counting the number of eggs

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undergoing division as a proportion of the total number of eggs (dividing and nondividing). The embryos were placed into nine cultivation bins (150 L volume), which served as the main embryo source (and will be referred to as such in the following experimental procedure), monitored frequently and kept under a 12-h light/dark cycle throughout the course of the experiment in order to mimic natural daylight cycles.

Experimental procedure

Embryos were taken from the main embryo source at the following developmental time points: (i) 12-h blastula stage, (ii) 36-h late gastrula stage, (iii) 60-h nonmotile, floating larvae, (iv) 84-h motile planulae and (v) 132-h planulae ready for settlement.

At the first developmental time point (12-h blastula stage), approximately 36 000 embryos were randomly pooled from the main embryo source and divided equally into 12 replicate 1-L containers filled with 5 μm FSW. All 12 containers were inserted into a floating rubber mat in an 800-L fibre glass aquarium filled with flowing sea water at 29 °C during the 6-h exposure (09:00–15:00 h) to ambient solar radiation. The mat had cut-outs to accommodate the 12 containers in order to maintain them horizontal and prevent them from sinking. Six replicates were covered with a sheet of 6-mm-thick Plexiglass G UVT that has a full width at half maximum at 282 nm and was therefore transparent to UVR (treatment), and six replicates were covered with a sheet of 4-mm-thick Plexiglass G UF-3 that has a full width at half maximum at 390 nm and was therefore opaque to most UVR wavelengths (control). At the end of the 6-h exposure period, all embryos were harvested and preserved in RNAlater (Ambion), placed at 4 °C for 24 h to ensure infiltration of the fixative and frozen at −80 °C until further processing.

At the second developmental time point (36-h late gastrula stage) and subsequent time points (60, 84 and 132 h), fresh, previously unexposed embryos or larvae from our main embryo source were sampled, exposed for 6 h to ambient solar radiation and fixed in exactly the same manner, thus ensuring that each developmental stage was only exposed once for 6 h (Fig. S1, Supporting information) and that each stage received approximately the same UV dose (Table 1).

We would like to note that coral larvae development is not synchronized, i.e. different developmental stages are present at any given time point. The larval stages denoted for the different time points are therefore based on the dominant larval stage present in the main embryo source at these time points. No experiment was performed at 108 h because of low UV levels (i.e. cloudy weather conditions) in the morning.

Light measurements and modelling

Solar radiation (400–1100 nm in per Wm^2) was measured with a type SZ terrestrial pyranometer sensor (LI-200SZ, LI-COR. Nebraska) mounted on the roof of the weather station at the pier at the Unidad Académica de Sistemas Arrecifales (Puerto Morelos). The data were converted to photometric units from 400 to 700 nm using calibration factors determined with a cosine-corrected sensor (LI-190) attached to a LI-COR 1100 data logger on the roof of the weather station. A model (Zenith A) was used to determine the intensities of UVA and UVB with respect to PAR for the study site. Ozone data for the dates of the experiments (11–16 September 2009) were obtained from http://jwocky.gsfc.nasa.gov, 5 and atmospheric data were obtained from http://www.wunderground.com. The proportions of UVA (320–400 nm) and UVB (280–320 nm) were applied to the PAR data obtained from the weather station to determine daily PAR exposures and approximate UVA and UVB daily exposures during the experiment (Table 1).

RNA, hybridization and microarrays

Microarray protocols were performed as described by Voolstra *et al.* (2009a) with slight modifications in order to account for the larger array platform (referred to as Mfav G2). This generation of microarrays contains 10 930 different PCR-amplified cDNA clones from *M. faveolata* that are spotted in duplicate on poly-L-lysine-coated slides, yielding a microarray with 21 860 total features. Please note that this is much larger than the array platform that was used in previous experiments (DeSalvo *et al.* 2008; Voolstra *et al.* 2009a,b). Spotted cDNAs were chosen from EST libraries partially described by Schwarz *et al*. (2008) and extensions thereof. The libraries were constructed from adult coral as well as different embryonic and larval stages, and clones were selected solely based on clone size for the construction of the array. Hence, the set of cDNAs should represent a random sample of the transcriptome. A survey on our spotted cDNAs shows that our array contains many genes that are related to UV stress (data now shown, but the full array platform is available on NCBI GEO GSE24949). Fifty-two per cent of the 10 930 cDNAs had similarity to known genes as determined by tBLASTx and BLASTx (E-value cut-off 1e-5) against the Swissprot, Uniprot and GenBank nonredundant DNA and protein databases (nr). The databases were queried successively in the order named, and the best hit was used for annotation. All clones are accessible at the SymBioSys database at http://sequoia.ucmerced.edu/SymBioSys/index.php (Sunagawa *et al.* 2009). For

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Table 1 Daily PAR exposure and approximate daily UVA and UVB exposures for the different time points/developmental stages as well as the number of differentially expressed genes as determined by BAGEL

Time	12 h	36 h	60 h	84 h	132 h
Stage	Late blastula	Late gastrula	Non-motile Planula	Motile Planula	Diving Planula
PAR daily dose (kJ)	6175	6120	5870	5446	6020
UVA daily dose (kJ)	719	712	683	633	699
UVB daily dose (kJ)	42	42	40	37	41
Total # of differentially expressed genes	37	23	37	707	52
Upregulated	22	10	19	256	39
Downregulated	15	13	18	451	13

each sample, total RNA of approximately 1000 coral embryos was isolated using Qiazol lysis reagent (Qiagen) according to the manufacturer's instructions. RNA quantity and integrity was assessed using a NanoDrop ND-1000 spectrophotometer and an Agilent 2100 Bioanalyzer, respectively. After analysis of RNA quantity and quality, three of the six replicates from every treatment and time point were chosen for subsequent RNA amplification and microarray analysis based on RNA quality and quantity. For each replicate, 1 μg of total RNA was amplified (aRNA) using the MessageAmp II aRNA kit according to the manufacturer's instruction (Ambion). Subsequent cDNA synthesis and dye labelling was performed using 3 μg aRNA per sample as described by Voolstra *et al.* (2009a). Cy3-labelled common reference and Cy5-labelled sample cDNAs were mixed together in a hybridization buffer containing 0.25% SDS, 25 mM HEPES and 3× SSC, resulting in a final volume of 70 μL. The hybridization mixtures were boiled for 2 min at 99 °C and allowed to cool at room temperature for 5 min. The cooled hybridization mixtures were pipetted under an mSeries Lifterslip (Erie Scientific), and hybridization took place in Corning hybridization chambers overnight at 63 °C. Microarrays were washed twice in 0.6× SSC, 0.01% SDS and were kept in 0.06× SSC until analysis. Slides were dried via centrifugation and scanned using an Axon 4000B scanner. The experimental set-up followed a reference design, i.e. all samples (three replicates of UV-treated and non-UV-treated for all five time points, yielding a total of 30 microarray hybridizations) were hybridized against the same pool made up of equal amounts of aRNA from all samples.

Microarray data analysis

Microarray slides were scanned as described by DeSalvo *et al.* (2008), and TIGR Spotfinder 2.2.3 was used to extract spot intensities and subtract the background (Saeed *et al.* 2003). The data were normalized using TIGR MIDAS 2.21 printtip-specific LOWESS followed by in-slide replicate analyses (Saeed *et al.* 2003). The data have been deposited in NCBI's Gene Expression Omnibus (Edgar *et al.* 2002) and are accessible through GEO Series accession number (GSE24949). Relative expression level estimates were calculated based on the ratio of fluorescence intensity of the two channels using Bayesian analysis of gene expression levels (Townsend & Hartl 2002) and yielded estimates for 8395 of 10 930 clones after quality filtering. Significantly differentially expressed genes were determined using the conservative gene-by-gene criterion of nonoverlapping 95% credible intervals as further detailed by Ranz *et al.* (2003). In order to determine the rate of false positives, we analysed random permutations of the data. Briefly, we resampled with replacement the original matrix of normalized ratios for the two channels and constructed a new matrix that was then used as a new input for the Bayesian analysis software (Townsend & Hartl 2002). This procedure yielded an estimated false-positive rate of 0.8%; hence, no adjustment was made for multiple tests. To visualize the temporal expression of differentially expressed genes from the 84-h time point, we used the TIGR's TMeV 4.0 software (Saeed *et al.* 2003) to perform a hierarchical cluster analysis of log_2-transformed ratios (i.e. treatment/control) of relative expression level estimates using Euclidean distance complete linkage (Fig. 1). Assessments of putative functions for significant genes were based on manual perusal of literature, GO annotation (Ashburner *et al.* 2000) and database searches (e.g. PFAM and InterPro) (Mulder & Apweiler 2008; Finn *et al.* 2010). The portion of GO-annotated differentially expressed genes was analysed using GOEAST (Zheng & Wang 2008) under a hypergeometric test statistic using FDR-corrected (Hochberg) *P*-values with a cut-off of ≤0.05 in order to identify processes and pathways affected by UVR. Briefly, 3528 of 8395 clones with GO annotations were compared against 247 GO-annotated genes from the 84-h time point.

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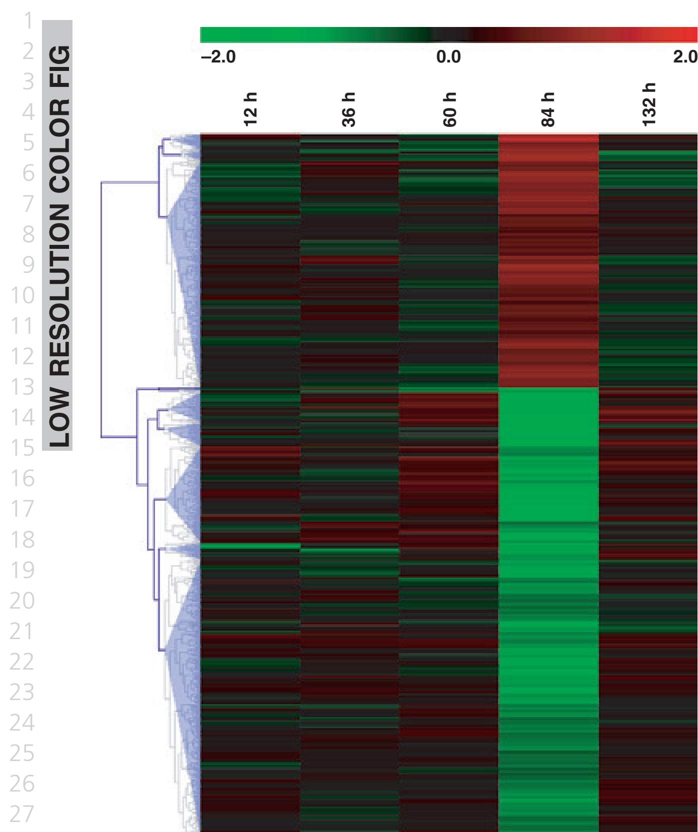

Fig. 1 Heat map depicting the temporal expression of genes differentially expressed at 84 h based on ratios of log_2-transformed relative expression level estimates.

Results

Differential sensitivity to UVR during coral larval development

We exposed embryos at five developmental time points (12, 36, 60, 84 and 132 h postfertilization) for 6 h to natural solar radiation with UVR and analysed changes in gene expression in comparison with embryos exposed to sunlight without UVR (as described in the Materials and methods). UVR-exposed and non-UVR-exposed samples were hybridized against a common reference consisting of equal amounts of RNA from all thirty samples. Analysis of expression profiles of 12-, 36-, 60- and 132-h-old larvae showed comparatively low numbers of differentially expressed genes in response to UVR exposure (Table 1). In contrast, in 84-h-old larvae, we identified a substantial increase in differentially expressed genes in UVR-treated samples (Table 1). This time point represents the motile planula stage. We found a total of 707 genes to be differentially expressed at this time point (~8% of all clones analysed), with fold changes ranging from 3.3-fold upregulation for UPF0538 protein C2orf76 homolog (CCHW15272) to fivefold downregulation for 78-kDa glucose-regulated protein (BiP) (CCHW13633) (Table S1, Supporting information). Of the 707 differentially expressed genes at 84 h, 311 (44%) were annotated, and of those 247 had GO annotations. No considerable overlap was found between differentially expressed genes at 84 h and other time points analysed (Fig. 1).

Molecular processes and genes affected by UVR

We performed an enrichment analysis of GO-annotated differentially expressed genes using GOEAST (Zheng & Wang 2008) to obtain an overview of the molecular processes affected by UVR. Briefly, we tested for overrepresentation of GO-annotated processes of differentially expressed genes in comparison with the GO annotation of all annotated clones assayed (Table S2, Supporting information). We identified 81 categories that were significantly enriched (FDR-corrected *P*-value <0.05). Those were manually sorted to higher-order processes. These processes were as follows: (i) stress response; (ii) endoplasmic reticulum; (iii) Ca^{2+} binding, signalling and transport; (iv) development; (v) apoptosis and (vi) transcription (Table 2). We identified a substantial number of differentially expressed genes that could be assigned or sorted to these higher-order processes that did not show up in this GO-based enrichment analysis because of incomplete GO annotations. In the following, we provide a detailed analysis of the processes and genes affected at 84 h, where we have added those additional genes (Table 3).

Stress response

We found several stress-related genes to be differentially expressed in UVR-exposed larvae. These included UV excision repair protein RAD23 homolog A (CCHW9908), cytochrome c oxidase subunit 1 (CCHW10819, CAGI2311), CCAAT/enhancer binding protein gamma (C/EBP-γ) (CAGI2441) and GFP-like fluorescent chromoprotein cFP484 (AOSF1131) among others (Table 3).

UV excision repair protein RAD23 homolog A was found to be significantly upregulated. This protein is a central component of the nucleotide-excision repair (NER) mechanism, which plays an important role in removing UV-induced DNA damage. It is involved in the recognition of DNA lesions and the recruitment of repair factors (Dantuma *et al.* 2009). Studies in yeast further suggest a role in the regulation of proteolysis, which might allow the stabilization of DNA repair and stress factors (Ortolan *et al.* 2000).

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Table 2 Significantly overrepresented categories as determined by GOEAST (Zheng & Wang 2008) (FDR-corrected *P*-value <0.05) manually grouped by higher-order processes

GO Term	Description	Pop. Set Tot. #3528	Study Set Tot. #247	*P*-value	log odds ratio
Stress response					
GO:0016579	Protein deubiquitination	5	3	0.0014	3.0993
GO:0003684	Damaged DNA binding	5	3	0.0014	3.0993
GO:0070646	Protein modification by small protein removal	5	3	0.0014	3.0993
GO:0051100	Negative regulation of binding	8	3	0.0140	2.4212
GO:0047987	Hydroperoxide dehydratase activity	5	2	0.0277	2.5143
GO:0046906	Tetrapyrrole binding	42	7	0.0407	1.2513
Endoplasmic reticulum					
GO:0033018	Sarcoplasmic reticulum lumen	7	4	0.0004	3.0289
GO:0005788	Endoplasmic reticulum lumen	38	10	0.0005	1.9103
GO:0016529	Sarcoplasmic reticulum	9	4	0.0020	2.6663
GO:0016528	Sarcoplasm	9	4	0.0020	2.6663
GO:0044432	Endoplasmic reticulum part	159	19	0.0365	0.7713
GO:0005783	Endoplasmic reticulum	217	24	0.0430	0.6597
Ca^{2+} binding, signaling and transport					
GO:0005262	Calcium channel activity	5	3	0.0014	3.0993
GO:0005261	Cation channel activity	17	5	0.0081	2.0707
GO:0006816	Calcium ion transport	11	3	0.0293	1.9618
GO:0015276	Ligand-gated ion channel activity	6	2	0.0317	2.2513
GO:0022834	Ligand-gated channel activity	6	2	0.0317	2.2513
Development					
GO:0045595	Regulation of cell differentiation	42	10	0.0013	1.7659
GO:2000027	Regulation of organ morphogenesis	6	3	0.0038	2.8363
GO:0030856	Regulation of epithelial cell differentiation	6	3	0.0038	2.8363
GO:0030858	Positive regulation of epithelial cell differentiation	6	3	0.0038	2.8363
GO:0050793	Regulation of developmental process	50	10	0.0059	1.5143
GO:0031016	Pancreas development	7	3	0.0080	2.6139
GO:0055123	Digestive system development	8	3	0.0140	2.4212
GO:2000026	Regulation of multicellular organismal development	41	8	0.0177	1.4787
GO:0045664	Regulation of neuron differentiation	14	4	0.0199	2.0289
GO:0030099	Myeloid cell differentiation	9	3	0.0231	2.2513
GO:0022603	Regulation of anatomical structure morphogenesis	16	4	0.0277	1.8363
GO:0048538	Thymus development	5	2	0.0277	2.5143
GO:0001708	Cell fate specification	5	2	0.0277	2.5143
GO:0021953	Central nervous system neuron differentiation	5	2	0.0277	2.5143
GO:0050767	Regulation of neurogenesis	17	4	0.0281	1.7488
GO:0045597	Positive regulation of cell differentiation	17	4	0.0281	1.7488
GO:0045596	Negative regulation of cell differentiation	24	5	0.0287	1.5732
GO:0010001	Glial cell differentiation	6	2	0.0317	2.2513
GO:0042063	Gliogenesis	6	2	0.0317	2.2513
GO:0051094	Positive regulation of developmental process	25	5	0.0346	1.5143
GO:0007417	Central nervous system development	41	7	0.0354	1.2861
GO:0051093	Negative regulation of developmental process	26	5	0.0417	1.4578
GO:0051239	Regulation of multicellular organismal process	79	11	0.0428	0.9919
GO:0051960	Regulation of nervous system development	19	4	0.0442	1.5883
GO:0016331	Morphogenesis of embryonic epithelium	19	4	0.0442	1.5883
GO:0048754	Branching morphogenesis of a tube	7	2	0.0495	2.0289
GO:0045665	Negative regulation of neuron differentiation	7	2	0.0495	2.0289
GO:0048592	Eye morphogenesis	7	2	0.0495	2.0289
Apoptosis					
GO:0042981	Regulation of apoptosis	96	16	0.0035	1.2513
GO:0010941	Regulation of cell death	98	16	0.0042	1.2216
GO:0043067	Regulation of programmed cell death	98	16	0.0042	1.2216
GO:0006915	Apoptosis	84	14	0.0067	1.2513

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Table 2 (*Continued*)

GO Term	Description	Pop. Set Tot. #3528	Study Set Tot. #247	*P*-value	log odds ratio
GO:0016265	Death	99	15	0.0133	1.1138
GO:0008219	Cell death	99	15	0.0133	1.1138
GO:0012501	Programmed cell death	90	14	0.0136	1.1518
GO:0043065	Positive regulation of apoptosis	48	9	0.0152	1.4212
GO:0010942	Positive regulation of cell death	49	9	0.0177	1.3915
GO:0043068	Positive regulation of programmed cell death	49	9	0.0177	1.3915
GO:0043028	Caspase regulator activity	5	2	0.0277	2.5143
GO:0001906	Cell killing	7	2	0.0495	2.0289
Transcription					
GO:0051090	Regulation of transcription factor activity	6	3	0.0038	2.8363
GO:0090046	Regulation of transcription regulator activity	6	3	0.0038	2.8363
GO:0001071	Nucleic acid binding transcription factor activity	109	15	0.0277	0.9750
GO:0003700	Sequence-specific DNA binding transcription factor activity	109	15	0.0277	0.9750
Miscellaneous					
GO:0003690	Double-stranded DNA binding	13	5	0.0016	2.4578
GO:0043566	Structure-specific DNA binding	17	5	0.0081	2.0707
GO:0051101	Regulation of DNA binding	8	3	0.0140	2.4212
GO:0006278	RNA-dependent DNA replication	5	2	0.0277	2.5143
GO:0051098	Regulation of binding	16	4	0.0277	1.8363
GO:0006555	Methionine metabolic process	5	2	0.0277	2.5143
GO:0050794	Regulation of cellular process	670	62	0.0303	0.4024
GO:0015267	Channel activity	32	6	0.0311	1.4212
GO:0022803	Passive transmembrane transporter activity	32	6	0.0311	1.4212
GO:0030278	Regulation of ossification	6	2	0.0317	2.2513
GO:0051052	Regulation of DNA metabolic process	12	3	0.0401	1.8363
GO:0004725	Protein tyrosine phosphatase activity	12	3	0.0401	1.8363
GO:0003682	Chromatin binding	34	6	0.0426	1.3338
GO:0046872	Metal ion binding	698	63	0.0480	0.3665
GO:0061134	Peptidase regulator activity	35	6	0.0493	1.2919
GO:0080090	Regulation of primary metabolic process	337	34	0.0495	0.5271
GO:0030218	Erythrocyte differentiation	7	2	0.0495	2.0289
GO:0034101	Erythrocyte homeostasis	7	2	0.0495	2.0289
GO:0031253	Cell projection membrane	7	2	0.0495	2.0289
GO:0030522	Intracellular receptor mediated signaling pathway	7	2	0.0495	2.0289

Cytochrome *c* oxidase subunit 1 is the main subunit of the cytochrome *c* oxidase complex, which is a component of the mitochondrial electron transfer chain and plays an important role in the induction of apoptosis upon stress. Cytochrome *c* oxidase also has a scavenging function and is activated in a ROS-dependent fashion (Atlante *et al*. 2000; Wang *et al*. 2003).

On the regulatory level, we found a homolog of the transcription factor CCAAT/enhancer binding protein gamma (C/EBP-γ) to be upregulated. C/EBP-γ is a member of the C/EBP transcription factor family, which includes six members involved in the regulation of various processes such as cell proliferation, differentiation and apoptosis (Nerlov 2007). Interestingly, studies on the stress response upon thermal and UV-induced stress in the sea anemone *Anthopleura elegantissima* showed differential expression of C/EBP-α and C/EBP-β as part of the stress response (Richier *et al*. 2008). C/EBP-β was also found to be upregulated in *Montastraea faveolata* during heat treatments (DeSalvo *et al*. 2008). While C/EBP-α and C/EBP-β are involved in the inhibition of proliferation and the regulation of cell cycle progression (Nerlov 2007), C/EBP-γ has been found to be a primary regulator of antioxidant and DNA repair genes like superoxide dismutase 1 and glutathione-transferase P1 in human lung epithelium (Mullins *et al*. 2005).

We found a GFP-like fluorescent chromoprotein cFP484 homolog to be significantly upregulated in coral larvae in response to UV exposure. Although the function of fluorescent proteins in cnidarians is not yet fully understood, they have been previously associated with photo-protection (Salih *et al*. 2000, 2006) and ROS scavenging (Bou-Abdallah *et al*. 2006; Palmer *et al*. 2009).

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Table 3 Differentially expressed genes of *Montastraea faveolata* larvae exposed to natural levels of UVR at 84 h postfertilization manually sorted to higher-order processes. Fold changes were determined in relation to the expression of the corresponding non-UVR-exposed replicates

CloneID	Δ Fold	Annotation	Description	Gene symbol
Stress response				
CCHW9908	1.90	UV excision repair protein RAD23 homolog A	Response to DNA damage, nucleotide-excision repair	RAD23A
CCHW9658	−1.39	DNA repair protein RAD51 homolog 1	Response to DNA damage, double-strand break repair	RAD51
CCHW9858	−1.51	DNA mismatch repair protein Msh3	Response to DNA damage, mismatch repair	MSH3
CCHW10819	1.87	Cytochrome c oxidase subunit 1 COX1	Response to oxidative stress, apoptosis	COX1
CAGI2311	1.76	Cytochrome c oxidase subunit 1 COX1	Response to oxidative stress, apoptosis	COX1
CCHW10702	−2.26	Microsomal glutathione S-transferase 3	Response to oxidative stress, glutathione transferase activity	MGST3
CCHW11534	−2.09	2-oxoglutarate dehydrogenase, mitochondrial	Response to oxidative stress, mitochondrial matrix	OGDH
CCHW2109	1.38	Tryptophan 2,3-dioxygenase	Response to oxidative stress, oxidoreductase activity	AGAP
CCHW8396	−2.05	Alkane 1-monooxygenase	Response to oxidative stress, oxidoreductase activity	ALKB
CCHW5348	2.07	Deoxyhypusine hydroxylase	Response to oxidative stress, oxidoreductase activity	DOHH
CCHW1902	1.55	Methylenetetrahydrofolate reductase	Response to oxidative stress, oxidoreductase activity	MTHFR
CCHW7513	−1.74	Dehydrogenase/reductase SDR family member 7	Response to oxidative stress, oxidoreductase activity	DHRS7
CCHW1389	1.67	Peroxisomal membrane protein PEX14	Response to oxidative stress, peroxisome organization	PEX14
CCHW8624	1.56	Zinc-binding alcohol dehydrogenase domain-containing protein 2	Response to oxidative stress, peroxisome, oxidoreductase activity	ZADH2
CCHW17353	−2.16	Peptidyl-glycine alpha-amidating monooxygenase	Response to oxidative stress, response to hypoxia	PAM
CCHW4492	−1.76	Estradiol 17-beta-dehydrogenase 12-B	Response to oxidative stress,endoplasmic reticulum	HSD17B12B
CCHW7313	1.46	Allene oxide synthase-lipoxygenase protein	Response to oxidative stress, hydroperoxide dehydratase activity	AOS
CAGI2441	1.48	CCAAT/enhancer-binding protein gamma	Response to stress, positive regulation of DNA repair	C/EBP-γ
CCHW14273	1.44	Serine/threonine-protein kinase Sgk1	Response to stress, apoptosis, endoplasmic reticulum	SGK1
CCHW2210	1.61	Allograft inflammatory factor 1	Response to stress, calcium binding, negative regulation of cell proliferation	AIF1
CCHW2639	1.86	Inorganic pyrophosphatase	Response to stress, hydrolase activity	IPP1
CCHW12281	−1.74	Cytochrome P450 74A,	Hydroperoxide dehydratase activity, oxidation reduction	CYP74A
CCHW6956	1.31	WD repeat-containing protein 48	Protein deubiquitination, modification-dependent protein catabolic process	WDR48
CCHW12006	−2.30	Probable ubiquitin carboxyl-terminal hydrolase FAF-X	Protein deubiquitination, ubiquitin-dependent protein catabolic process	FAFL
AOSC877	1.69	Ubiquitin carboxyl-terminal hydrolase 7	Protein deubiquitination, ubiquitin-specific protease activity	USP7
Endoplasmic reticulum				
CCHW13633	−5.04	78 kDa glucose-regulated protein	Endoplasmic reticulum lumen, stress response	GRP78

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Table 3 (*Continued*)

CloneID	Δ Fold	Annotation	Description	Gene symbol
CCHW11678	−2.95	Endoplasmin	Endoplasmic reticulum lumen, stress response	GRP94
CCHW7976	−2.24	Hypoxia up-regulated protein 1	Endoplasmic reticulum lumen, stress response	GRP170
CCHW8302	−3.12	Protein disulfide-isomerase 2	Endoplasmic reticulum lumen, embryonic development	PDI2
CCHW13505	−2.38	Protein disulfide-isomerase A3	Endoplasmic reticulum lumen, positive regulation of apoptosis	GRP58
CCHW8465	−1.54	Prolyl 4-hydroxylase subunit alpha-2	Endoplasmic reticulum lumen, response to oxidative stress	P4HA2
CCHW6838	−1.47	Endoplasmic reticulum lectin 1	Endoplasmic reticulum lumen, ER-associated protein catabolic process	ERLEC1
CCHW1488	−1.95	ERO1-like protein beta	Endoplasmic reticulum, oxidoreductase activity	ERO1LB
CCHW13835	−2.88	Protein PRY1	Endoplasmic reticulum, nuclear envelope	PRY1
CCHW12681	−1.46	Cubilin	Endoplasmic reticulum, calcium ion binding	CUBN
CCHW7209	−1.59	Vesicle-associated membrane protein 7B	Endoplasmic reticulum, endosome to lysosome transport	VAMP7B
CCHW16336	−2.02	Protein transport protein Sec24C	Endoplasmic reticulum, intracellular protein transport	SEC24C
CCHW16224	−2.23	Signal recognition particle receptor subunit alpha	Endoplasmic reticulum, receptor activity	SRPR
CCHW7389	1.26	N-acetylglucosaminyl-phosphatidylinositol biosynthetic protein	Endoplasmic reticulum, transferase activity	PIGA
CCHW10992	−2.08	Transmembrane emp24 domain-containing protein 7	Endoplasmic reticulum, transport	TMED7
CCHW10339	−3.03	Calumenin	Sarcoplasmic reticulum, calcium ion binding	CALU
CCHW2273	−2.02	Calumenin	Sarcoplasmic reticulum, calcium ion binding	CALU
CCHW4261	−2.65	Calumenin	Sarcoplasmic reticulum, calcium ion binding	CALU
CCHW5754	−2.12	Calumenin	Sarcoplasmic reticulum, calcium ion binding	CALU
Ca^{2+} binding and transport				
CCHW13855	−2.23	Calmodulin	Calcium ion binding, calcium-mediated signaling	CaM
CCHW9193	−1.97	Calmodulin	Calcium ion binding, calcium-mediated signaling	CaM
CCHW9667	2.64	Calcium/calmodulin-dependent protein kinase type 1D	Calcium ion binding, regulation of apoptosis	CAMK1D
CCHW2043	−2.35	Phospholipase A2	Calcium ion binding, endoplasmic reticulum	PLA2G1B
AOSF1395	1.46	Phospholipase A2, membrane associated	Calcium ion binding, regulation of cell proliferation	PLA2G2A
CCHW5833	−3.16	Sushi, von Willebrand factor type A	Calcium ion binding, chromatin binding	SVEP1
CCHW8064	−2.23	Sushi, von Willebrand factor type A	Calcium ion binding, chromatin binding	SVEP1
CCHW9506	−2.06	Cytosolic phospholipase A2	Calcium ion binding, hydrolase activity	PLA2G4A

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Table 3 (*Continued*)

CloneID	Δ Fold	Annotation	Description	Gene symbol
CCHW9765	−1.68	LETM1 and EF-hand domain-containing protein 1, mitochondrial	Calcium ion binding, mitochondrial inner membrane	LETM1
AOSF561	1.49	B4KTE1_DROMO;GI19538	Calcium ion binding, proteolysis	GI19538
CCHW16191	−2.11	Matrix metalloproteinase-24	Calcium ion binding, proteolysis	MMP24
CCHW12227	−1.65	Inositol 1,4,5-trisphosphate receptor	Calcium channel activity, response to oxidative stress, perception	IP3R
CCHW4878	−1.48	Predicted protein	Calcium channel activity, calcium ion transport	V1G210354
CCHW7245	−2.51	Predicted protein	Calcium channel activity, calcium ion transport	V1G210354
CCHW15413	−2.07	Multiple C2 and transmembrane domain-containing protein 1	Calcium ion binding, calcium-mediated signaling	MCTP1
CCHW1426	−1.70	EF-hand calcium-binding domain-containing protein 1	Calcium ion binding	EFCAB1
Development				
CCHW9023	−1.50	Neurogenic locus notch homolog protein 1	Cell fate specification, generation of neurons	Notch1A
CCHW8316	1.75	T-cell leukemia homeobox protein 3	Cell fate specification, neuron migration	Tlx3
CCHW5295	−2.33	D site-binding protein	Cell fate specification, regulation of cell proliferation	DBP
CCHW3279	−1.48	Catenin beta-1	Regulation of cell proliferation, cell fate specification, apoptosis	CTNNB1
AOSF1291	1.83	Homeobox protein SIX1	Cell differentiation, generation of neurons	SIX1
CCHW12906	−1.79	Homeobox protein Nkx-2.2	Multicellular organismal development, neurogenesis	Nkx-2.2
CCHW7017	−2.39	Insulin gene enhancer protein ISL-2	Multicellular organismal development,neuron fate commitment	ISL-2
CCHW14603	−2.95	UDP-glucuronic acid decarboxylase 1	Multicellular organismal development	UXS1
CCHW12112	−1.77	Ribonuclease ZC3H12A	Multicellular organismal development, apoptosis	MCPIP1
CCHW12140	−1.83	Poly(U)-binding-splicing factor half pint	Multicellular organismal development, cell differentiation	HFP
CCHW11845	−1.59	Sprouty	Multicellular organismal development, regulation of signal transduction	SPRY
CCHW2911	−2.05	LWamide neuropeptides	Neuropeptide signaling pathway, behavior	LWamide
CCHW9044	−1.78	LWamide neuropeptides	Neuropeptide signaling pathway, behavior	LWamide
CCHW3495	1.53	Pyroglutamylated RFamide peptide receptor	Neuropeptide Y receptor activity, behavior	QRFPR
CCHW8207	1.88	Neuropeptide FF receptor 2	Neuropeptide receptor activity,, behavior	NPFFR2
CCHW11268	1.44	Nucleolar GTP-binding protein 1	Negative regulation of cell proliferation, axon guidance	NOG1
CCHW17077	−1.94	ADP-ribosylation factor 6	Dendrite development, apoptosis	ARF6
CCHW2857	−1.51	Pancreas transcription factor 1 subunit alpha	Embryonic development, generation of neurons	PTF1A
CAOO655	−2.42	Neurofascin	Synapse organization, axon guidance	NFASC
CCHW6676	−1.49	Nuclear pore complex protein Nup133	Development, mRNA transport,transmembrane transport	NUP133
Apoptosis				
CCHW3246	−1.41	F-box/WD repeat-containing protein 7	Apoptosis, cell cycle	FBWX7

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Table 3 (*Continued*)

CloneID	Δ Fold	Annotation	Description	Gene symbol
CCHW14546	−2.17	Apoptotic chromatin condensation inducer in the nucleus	Apoptosis, cell differentiation	ACIN1
CCHW991	−1.73	Nucleolysin TIAR	Apoptosis, defense response	TIAL1
CCHW14241	1.45	Proto-oncogene tyrosine-protein kinase receptor Ret	Apoptosis, MAPKKK cascade, nervous system development	RET
CCHW10174	2.06	Amyloid beta A4 protein	Apoptosis, neurogenesis	APP
AOSF1247	−1.46	Cullin-1	Apoptosis, protein degradation, cell proliferation	CUL1
CCHW4108	−1.62	Apoptosis-inducing factor 3	Apoptosis, response to oxidative stress	AIFM3
CCHW8262	−2.01	Major facilitator superfamily domain-containing protein 10	Apoptosis, transmembrane transport	MFSD10
CCHW9367	1.94	Nuclear protein 1	Positive regulation of apoptosis	NUPR1
CCHW7910	−1.68	Cysteine/serine-rich nuclear protein 3	Positive regulation of apoptosis, positive regulation of transcription	CSRNP3
CCHW11728	−1.52	Cysteine/serine-rich nuclear protein 3	Positive regulation of apoptosis, positive regulation of transcription	CSRNP3
CCHW10371	−2.35	Ephrin type-A receptor 7	Positive regulation of apoptosis, positive regulation of transcription	EPHA7
CCHW1476	−2.06	FAS-associated factor 1	Positive regulation of apoptosis, regulation of cell adhesion	FAF1
CCHW16170	−1.61	Protein BTG1	Regulation of apoptosis, endothelial cell differentiation	BTG1
CCHW9599	−2.58	Protein BTG1	Regulation of apoptosis, endothelial cell differentiation	BTG1
CCHW14258	2.07	Interferon-induced helicase C domain-containing protein 1	Regulation of apoptosis, innate immune response	MDA5
CCHW13419	−1.73	TNF receptor-associated factor 1	Regulation of apoptosis, signal transduction	TRAF1

Endoplasmic reticulum and Ca^{2+} homoeostasis

We found several genes associated with Ca^{2+} channel activity, Ca^{2+} binding and Ca^{2+} ion transport to be differentially expressed at 84 h (Table 3). A detailed analysis of the genes within these groups revealed many key proteins with important functions in protein folding and the maintenance of ER Ca^{2+} and redox homoeostasis to be significantly downregulated in response to UVR exposure. Please note that we also identified many genes associated with the ER and ER lumen in our analysis (Table 3). Hence, we conclude that the differential expression of Ca^{2+} homoeostasis and ER genes might be linked processes. The identified proteins included the ER luminal chaperones, 78-kDa glucose-regulated protein (BiP, GRP78), endoplasmin (GRP94) and hypoxia-upregulated protein 1 as well as protein disulphide isomerase 2 (PDI) (CCHW8302), protein disulphide isomerase A3 (GRP58) (CCHW13505) and ERO1-like protein beta (CCHW1488). These genes encode for proteins that are highly important for Ca^{2+}-dependent folding and maturation of secretory proteins and/or the maintenance of Ca^{2+} and redox homoeostasis in the ER lumen (Groenendyk *et al.* 2006; Ni & Lee 2007).

Consistent with previous experiments, we also observed downregulation of calmodulin (CaM) (CCHW13855, CCHW9193). CaM is a key protein that transduces a signal in response to increases in intracellular Ca^{2+} (Chin & Means 2000). Furthermore, it is downregulated in response to UVR in human cell lines and upon heat stress in embryos and adults of *M. faveolata* (DeSalvo *et al.* 2008; Voolstra *et al.* 2009a). Taken together, we found indications for a disturbance of Ca^{2+} homoeostasis and signalling in response to UVR.

Development

Our analysis revealed several differentially expressed genes involved in development including a Notch 1 homolog (CCHW9023), catenin beta-1 (CCHW3279), amyloid beta A4 protein (CCHW10174) and the homeobox genes T-cell leukaemia homeobox protein 3 (Tlx3) (CCHW8316), Nkx-2.2 (CCHW12906), SIX1 (AOSF1291) and the LIM hox gene insulin gene enhancer protein ISL-2 (CCHW7017) among others (Table 3).

The Notch signalling pathway is a highly conserved cell–cell signalling system, which is involved in different developmental processes such as the specification of

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the body axis and neurogenesis in various organisms including the cnidarians *Hydra* and *Nematostella* (Käsbauer *et al.* 2007; Marlow *et al.* 2009). Consistent with an expression profiling study in human keratinocytes, where downregulation of members of the Notch family following UVB irradiation was observed (Murakami *et al.* 2001), we observe a downregulation of a Notch 1 homolog upon UV exposure. We also observed a downregulation of β-catenin, which is a transcriptional coactivator that controls key developmental gene expression programs and integrates the Notch and Wnt signalling pathways (Yu & Malenka 2003; Hayward *et al.* 2005; Barker 2008; Gulacsi & Anderson 2008). We also observed differential expression of several homeobox genes. The homeobox gene superfamily encodes transcription factors that act as master regulators of developmental processes. Examples include Tlx, Nkx, SIX and LIM homeobox genes, which are key neurogenic regulators in vertebrates and invertebrates (Hobert & Ruvkun 1998; Briscoe *et al.* 1999; Galliot *et al.* 2009). Furthermore, we found several neuropeptides and receptors to be differentially expressed such as LWamide neuropeptides (CCHW2911, CCHW9044), a pyroglutamylated RFamide peptide receptor (CCHW3495) and a neuropeptide FF receptor 2 (CCHW8207).

Apoptosis

UV irradiation has been shown to induce cell cycle arrest and apoptosis (Kulms & Schwarz 2002; Gentile *et al.* 2003). Here, we observed differential expression of various apoptosis-associated genes such as F-box/WD repeat-containing protein 7 (CCHW3246), proto-oncogene tyrosine protein kinase receptor Ret (RET) (CCHW14241), nuclear protein 1 (NUPR1) (CCHW9367) and the interferon-induced helicase C domain–containing protein 1 (MDA-5) (CCHW14258) among others.

F-box/WD repeat-containing protein 7 mediates the ubiquitinylation and consequent degradation of proteins that regulate cell cycle progression, including cyclin E, c-Myc, c-Jun and Notch (Ishikawa *et al.* 2008), and thereby links developmental processes such as neurogenesis to apoptosis. F-box/WD repeat-containing protein 7, proto-oncogene tyrosine protein kinase receptor Ret and amyloid beta A4 protein are also involved in the regulation of developmental processes and might serve to integrate apoptotic pathways to developmental processes (Bordeaux *et al.* 2000; Lee *et al.* 2003; Arighi *et al.* 2005; Ishikawa *et al.* 2008).

Discussion

While several studies on the impact of UVR on coral larvae have been conducted (Gleason & Wellington 1995; Wellington & Fitt 2003; Gleason *et al.* 2006; Reef *et al.* 2009), this is the first transcriptome analysis of the effects of UVR on development by means of high-density cDNA microarrays (Mfav-G2, 10 930 unique clones). An important aspect of our study is that we analysed the sensitivity of each of five different developmental time points rather than analysing the effects of cumulative UV exposure over time. This allowed us to detect variation in UV sensitivity between the different developmental stages and to identify potential processes that are affected by UVR in a time/developmental stage-specific manner. Moreover, we used natural levels of UVR in order to assess its impact under biologically relevant conditions.

We found that UVR sensitivity varies greatly over the course of early development. While larvae appear to exhibit comparably low sensitivity to ambient levels of UVR during early developmental time points, based on the few transcriptional changes observed, we found that the number of differentially expressed genes increased drastically at 84 h (planula stage). The comparably low number of differentially expressed genes observed for most time points might be attributable to the presence of UV-screening substances such as MAAs and fluorescent proteins. Moreover, it might reflect evolutionary adaptations to a life stage that is characterized by floating on the water surface and, hence, being exposed to a high UVR environment. In contrast, the large number of differentially expressed genes specifically at 84 h (planula stage) indicates that processes or genes that play a role at this time point might be particularly sensitive to UVR, despite the presence of MAAs. This has also been observed in previous studies analysing the effects of UVR on coral larvae that showed that the concentration of MAA did not seem to be significantly correlated with the reduction in settlement observed, i.e. larvae with a significantly higher MAA content (two-fold) did not show significantly less reduction in settlement rates (Kuffner 2001). Levels of UVR were slightly lower at 84 h compared with the other four time points (Table 1), so that it is unlikely that the observed pattern is an artefact stemming from UVR levels at that time point. In the following, we provide a detailed discussion of the factors and pathways that might be affected at the 84 h time point and how they potentially interact.

Stress response

In general, high UVR induces cellular damage at various levels including DNA damage as well as protein and lipid peroxidation through the production of ROS (Bose *et al.* 1989; Lesser & Farrell 2004; Lesser 2006). Although we chose to assay transcriptomic responses upon ambient levels of UVR, we did find differential

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expression of genes associated with different stress response pathways such as DNA repair (RAD23), ROS scavenging (COX1), protein degradation (USP7) and peroxidized lipid degradation (AOS) (Table 3). Some of these pathways might be regulated via C/EBP-γ, which is known to control expression of DNA repair and antioxidant genes in response to stress (Mullins *et al*. 2005).

Interestingly, we observed upregulation of fluorescent protein expression, which is in line with previous findings that fluorescent protein expression can be positively regulated by light in scleractinian corals (D'Angelo *et al.* 2008; Roth *et al.* 2010). Although the exact role of fluorescent proteins is currently unclear, studies showed that fluorescent proteins might play a role in UVR screening, ROS scavenging and photo-protection (Salih *et al.* 2000, 2006; Bou-Abdallah *et al.* 2006; Leutenegger *et al.* 2007; Bay *et al.* 2009; Palmer *et al.* 2009; Roth *et al.* 2010). With regard to these putative functions, our results may support a role in UV screening. We did not find signs of a strong antioxidant response; therefore, we conclude that the production of additional screening proteins would be a more suitable reaction of the organism to confer protection from other UVR-induced effects.

Taken together, the transcriptional changes observed for stress-related genes suggest that coral larvae do not exhibit high levels of stress in response to ambient UVR levels, which is likely to reflect an evolutionary adaptation to the tropical high light environment.

Endoplasmic reticulum and Ca^{2+} homoeostasis

Endoplasmic reticulum-associated genes were highly affected in our experiment, and many of them are associated with ER stress, Ca^{2+} homoeostasis and signalling. The ER is the central organelle of the cell where protein folding and modification take place. Therefore, changes in the expression of ER-associated genes can affect a variety of cellular processes such as Ca^{2+} signalling, apoptosis or the expression of developmental genes (Verkhratsky & Solovyova 2002; Groenendyk *et al.* 2006). From our data, we conclude that the transcriptomic changes observed for ER-related genes might be linked to the differential expression of apoptotic genes and developmental processes. However, the downregulation of several proteins with chaperone function might also hint towards a general downregulation of protein expression in response to UVR. This would be in line with the notion that we find a higher number of downregulated genes in our experiment (256 upregulated vs. 451 downregulated clones). Interestingly, experiments in human keratinocytes and in *Escherichia coli* have shown a similar effect on gene expression, suggesting that transcriptional downregulation might be a general response to UVR (Gentile *et al.* 2003; Thomassen *et al.* 2010).

Development

We found a differentially expressed Notch 1 homolog. This hints towards changes in developmental processes, because Notch 1 is one of the key developmental genes (Artavanis-Tsakonas *et al.* 1999). Interestingly, it has been found that 4-hydroxynonenal, an aldehyde produced during UV-induced lipid peroxidation, mediates the downregulation of Notch 1 in the Hl-60 cell line, thereby linking Notch expression to UVR-induced damage (Yang *et al.* 2003; Pizzimenti *et al.* 2008). We also found evidence for changes in expression of genes belonging to the Wnt signalling pathway. This pathway is involved in body axis specification and neurogenesis in hydroids (Plickert *et al.* 2006; Muller *et al.* 2007). The Notch and Wnt signalling pathways are essential for many processes that involve cell fate decisions in developing organisms (Hayward *et al.* 2008). One of the nodal points that integrate the Notch and the Wnt pathway is β-catenin, a nuclear effector of Wnt signalling (Hayward *et al.* 2005). The observed downregulation of Notch and β-catenin therefore suggests that two major developmental cell-signalling pathways might be affected in response to UVR. Also, we found further indication for an alteration in β-catenin/Wnt and Notch signalling, e.g. PRY-1, a negative regulator of Wnt signalling, and amyloid beta A4 protein, a negative regulator of Notch signalling, were upregulated in UV-exposed embryos (Kimberly *et al.* 2001; Korswagen *et al.* 2002; He & Shen 2009). Hence, we conclude that UVR might influence developmental processes, probably at the level of cell fate specification and consequently in downstream processes.

It is interesting to note that many of the differentially expressed genes associated with development are involved in neurogenesis, e.g. it has been shown that Notch and β-catenin as well as Tlx3, NK and SIX homeobox genes are highly important for neurogenesis in different organisms, including cnidarians (Beatus & Lendahl 1998; Yu & Malenka 2003; Cheng *et al.* 2004; Gulacsi & Anderson 2008; Galliot *et al.* 2009). A possible effect of UVR on neurogenesis is further emphasized by the observed differential expression of the RFamide receptor and the LWamide neuropeptides. These molecules are known to regulate several aspects of larval behaviour in the cnidarian *Hydractinia echinata* such as locomotion, phototaxis, settlement and metamorphosis (Katsukura *et al.* 2004; Plickert & Schneider 2004; Watanabe *et al.* 2009). The search for a settlement site is not a continuous process, but instead consists of cycles of active migration and inactive resting periods.

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These cycles of activity are controlled by the LWamide and RFamide neuropeptides. Whereas LWamide stimulate migration by prolonging the periods of activity, RFamide blocks the initiation of migration periods and prolongs resting periods (Katsukura *et al.* 2004). We identified LWamide neuropeptides to be downregulated and RFamide peptide receptors to be upregulated. Thus, the expression signature identified here coincides with a pattern of decreased migration and increased resting periods. UVR might therefore affect neurogenesis and, as a consequence, the formation of the larval neural system as well as neuropeptide and neuropeptide receptor expression. This would explain why larvae that were exposed to UVR exhibit impaired locomotion and settlement regardless of their MAA content as shown in previous studies (Kuffner 2001; Wellington & Fitt 2003). As both LWamide and RFamide neuropeptides have similar roles in phototaxis and the initiation of metamorphosis, we assume that these processes might be similarly affected (Iwao *et al.* 2002; Katsukura *et al.* 2003; Plickert & Schneider 2004; Watanabe *et al.* 2009).

Apoptosis

Apoptosis is induced when cells become damaged beyond repair, but apoptosis is also an integral part of many developmental processes (Meier *et al.* 2000). Notch and β-catenin/Wnt signalling are involved in the induction of apoptosis during neurogenesis (Ishikawa *et al.* 2008; Raab *et al.* 2009). We identified members of these pathways that were differentially expressed. Furthermore, some of our differentially expressed genes with known functions during apoptosis, such as F-box/WD repeat-containing protein 7 and amyloid beta protein, have been shown to interact with these pathways during neurogenesis (Kimberly *et al.* 2001; Haughey *et al.* 2002; Ishikawa *et al.* 2008; Pizzimenti *et al.* 2008; Almeida *et al.* 2010; Hoeck *et al.* 2010). Hence, differential expression of apoptosis genes might be linked to the differential expression of developmental processes rather than a direct consequence of UVR. However, we did not observe a simple induction of apoptotic genes, but a more complex response that combines up- and downregulation of apoptosis-inducing factors as well as inhibitors. Although coral larvae have been shown to tolerate natural UVR levels for a short period of time (Reef *et al.* 2009), we found differential expression of apoptotic genes under these conditions. Our observations could therefore imply that even ambient levels of UVR are able to evoke a response of the cellular stress management system. Further extending this reasoning, longer periods of irradiation might ultimately exceed the capacity of the cellular stress management and might explain increased mortality in response to UVR as observed in previous experiments (Gleason & Wellington 1995; Wellington & Fitt 2003). At this point, it is not possible to assess whether the observed transcriptional changes of apoptosis-associated genes are mainly driven by induction of stress or changes in developmental processes. Yet, the moderate changes observed in the expression of stress-related genes suggest that the observed changes in apoptosis-associated genes are mainly driven by changes in the developmental processes.

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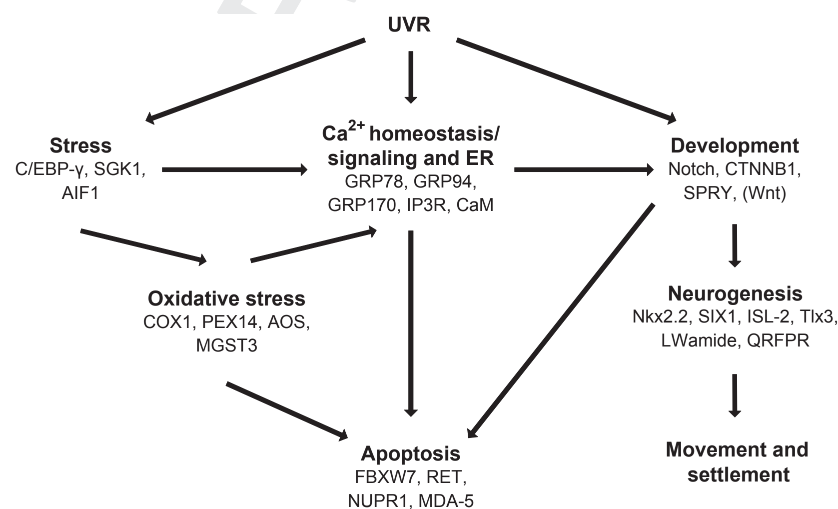

Fig. 2 Model of UVR response in coral embryos. Upon UVR exposure, motile larvae respond with differential expression of genes playing a role in stress response, calcium homoeostasis, calcium signalling, endoplasmic reticulum, development and apoptosis. Differentially expressed genes within these functional groups are interconnected.

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Towards a model of the molecular response of coral larvae to UVR

Based on the results of our analyses, we propose a model of the molecular response of coral larvae to UVR at 84 h (planula stage) of development (Fig. 2). Upon UVR exposure, cells respond with the expression of genes associated with stress including members of the oxidative stress response genes related to protein degradation. Oxidative stress and DNA damage in turn might trigger apoptosis (Buttke & Sandstrom 1994; Hayward *et al.* 2005; Roos & Kaina 2006). Furthermore, UVR has previously been shown to interfere with Ca^{2+} signalling and Ca^{2+} homoeostasis (Hightower *et al.* 1999; Watson *et al.* 2000; Sun *et al.* 2006), which in turn might affect the expression of ER-associated Ca^{2+}-binding proteins among others. Changes in the expression of important ER proteins have been shown to trigger the unfolded protein response (UPR), which ultimately might lead to the initiation of apoptosis (Groenendyk *et al.* 2006; Ni & Lee 2007). Changes in the ER as well as UVR-induced damage are also known to interfere with developmental pathways such as the Notch and Wnt signalling pathways (Yang *et al.* 2003; Pizzimenti *et al.* 2008; Zoltewicz *et al.* 2009). This in turn might result in the aberrant activation of developmentally related apoptosis as well as misexpression of neurogenic genes. Compromised neurogenesis could consequently impair larval behaviour such as locomotion and settlement (Katsukura *et al.* 2004; Plickert & Schneider 2004; Watanabe *et al.* 2009), resulting in reduced motility and decreased settlement rates at later stages. We would like to note that this model incorporates known functions and interactions of the set of differentially expressed genes identified. However, the results presented are based on incomplete transcriptome data, and therefore alternative models based on so far unknown regulatory interactions might also exist.

Conclusion

We present here the first transcriptome analysis of the effects of UVR exposure on scleractinian coral larvae. We have analysed gene expression changes in response to natural levels of UVR in order to assess the sensitivity of different developmental stages. Our results indicate that coral larvae appear to be well adapted to a high light environment, which is reflected in few transcriptional changes during early developmental as well as very moderate expression of stress-responsive genes during the most sensitive time point analysed. However, we identified significant changes in key developmental genes that suggest an influence of UVR on developmental processes, in particular neurogenesis. Our findings are in line with results from previous studies and contribute to the understanding of the molecular and developmental processes affected by this stressor. Furthermore, our observations suggest that coral larvae are particularly susceptible to UVR during the motile planula stage. This stage coincides with the onset of swimming and the search for settlement sites, and we find molecular evidence that these behaviours might be affected. Although our results suggest that many of the effects observed here might result from a susceptibility of certain processes to UVR, we cannot exclude the possibility that the gene expression changes we observe are because of phenotypic plasticity. More specifically, larval development might have evolved towards postponement of certain (UV-sensitive) processes until UVR levels decrease. Repeating this experiment with a focus on neurogenic genes and transcriptional regulation with finer-scale temporal resolution might provide more insight into the nature of these effects. Overall, the use of UVR intensity data to predict larval settlement and recruitment rates after mass spawning events may be a worthwhile endeavour. This approach could be important given the key role coral larvae play in the persistence of coral populations, especially under the current threat of climate change that is predicted to further reduce coral cover.

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Data accessibility

Microarray data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE24949.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Differentially expressed genes at 84 h as determined by BAGEL.

Table S2 Listed are all significantly overrepresented categories as determined by GOEAST (cut-off corrected *P*-value >0.05).

Fig. S1 Experimental workflow diagram.

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