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Blue light regulation of host pigment in reef-building corals

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ABSTRACT: Reef-building corals harbor an astounding diversity of colorful GFP (green fluorescent protein)-like proteins. These pigments can easily be detected and thus may serve as intrinsic optical markers of physiological condition, provided that the determinants that control their expression are well understood. Here we have analyzed the effect of light on the regulation of major classes of GFP-like pigments in corals of the taxa Acroporidae, Merulinidae and Pocilloporidae. Pigment levels in the tissues of all studied species were observed to be tightly controlled by light. Two groups could be distinguished by their distinctly different light-dependent regulation. The low-threshold group contains mainly cyan fluorescent proteins; they are expressed in considerable amounts at very low light intensities, and their tissue content increases with light to a maximum at a photon flux of 400 µmol m^{-2} s^{-1}. The high-threshold group includes green and red fluorescent proteins as well as non-fluorescent chromoproteins. These pigments are essentially absent in corals grown under very low light, but their tissue content increases in proportion to photon flux densities >400 µmol m^{-2} s^{-1}. The enhancement of coral pigmentation is primarily dependent on the blue component of the spectrum and regulated at the transcriptional level. The specific regulation patterns suggest complex functions of GFP-like proteins related to the photobiology of reef corals. Moreover, the distinct response of coral coloration to light climate promises that the pigment complement can also be predicted in natural habitats. Our results stress the potential of GFP-like proteins as intrinsic markers of physiological processes, as well as overall health, in corals.

KEY WORDS: Fluorescent protein · Coral · Light-induced protein expression · Fluorescence · GFP · Green fluorescent protein · RFP · Red fluorescent protein · Light sensing · Coral health

INTRODUCTION

Coral reefs cover an area of 123 million km^2 of tropical oceans and are among the most complex ecosystems in the world (Schuhmacher 1976). Hermatypic corals are primarily responsible for the formation of modern reefs. They owe their success in oligotrophic waters to the light-dependent symbiosis with unicellular dinoflagellate algae called zooxanthellae (Falkowski et al. 1984). Excessive light exposure, however, leads to photoinhibition of the symbionts and the generation of harmful reactive oxygen species, especially at elevated water temperatures (Gleason & Wellington 1993, Brown 1997, Coles & Brown 2003, Lesser 2006). Oxidative stress can trigger

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expulsion of zooxanthellae by the host, which results in bleaching of the coral tissue (Brown 1997, Coles & Brown 2003, Smith et al. 2005, Lesser 2006). Mass mortality of bleached corals is frequently observed and contributes to the global degradation of coral reefs (Hughes et al. 2003, Donner et al. 2007).

Corals can acclimate to strong-light environments by controlling the type and/or number of harbored algal cells and their pigment content, and by adjusting the complement of UV-screening, mycosporin-like amino acids (MAAs) and antioxidant molecules (Falkowski & Dubinsky 1981, Hoegh-Guldberg & Smith 1989, Iglesias-Prieto & Trench 1994, Shick et al. 1995, Rowan et al. 1997, Richier et al. 2005). A photoprotective function has also been suggested for the host pigments that are mainly responsible for the intense bluish, green, or reddish hues of many anthozoans living in symbiosis with zooxanthellae (Kawaguti 1944, Kawaguti 1969, Wiedenmann et al. 1999, Salih et al. 2000). However, green fluorescent protein (GFP)-like proteins are not restricted to symbiotic anthozoans but can also be found in azooxanthellate species from dim-light habitats (Wiedenmann et al. 2004, Schnitzler et al. 2008). These pigments are proteins related to the GFP from *Aequorea victoria*. Fluorescent proteins (FPs) emit light in the color range from cyan to red (Wiedenmann 1997, Matz et al. 1999, Wiedenmann et al. 2000, 2002, 2004, Dove et al. 2001, Shagin et al. 2004, Wiedenmann & Nienhaus 2006, Oswald et al. 2007), whereas chromoproteins (CPs) display bright purple to blue colors but are non-fluorescent (Wiedenmann et al. 1999, 2000, Lukyanov et al. 2000, Dove et al. 2001, Shagin et al. 2004). In contrast to GFP, its anthozoan homologues usually form homotetramers (Nienhaus et al. 2003, 2005, 2006a,b). In recent years, proteins from the GFP family have become powerful tools in biomedical research. They have been employed as reporters of gene expression, variations of intracellular conditions (Griesbeck 2004), developmental processes, as protein labels in living cells (Wiedenmann & Nienhaus 2006) and, most recently, in super-resolution imaging beyond the diffraction barrier (Shaner et al. 2007).

The high expression levels of GFP-like proteins in coral tissue (Oswald et al. 2007) and their advantageous photophysical properties make these pigments extremely promising as easily accessible indicators of the health of threatened coral reefs in a globally changing environment. To this end, a deep understanding of the regulation of the pigment levels is of utmost importance. As yet, only very few reports have focused on the influence of habitat conditions on the expression levels of GFP-like proteins. *Montastrea annularis* and *M. faveolata* from shallow water habitats did not contain more FPs than specimens from greater depths (Mazel et al. 2003). In *Montastrea* spp. and *Anemonia* spp., expression levels of fluorescent and non-fluorescent GFP-like proteins were found to be genetically determined rather than modulated by the light environment (Kelmanson & Matz 2003, Leutenegger et al. 2007b, Oswald et al. 2007). Down-regulation of GFP-like proteins was most recently reported in response to stressful environmental conditions including elevated water temperatures (Leutenegger et al. 2007b, Smith-Keune & Dove 2007).

In contrast, we recently noticed that the green fluorescence of *Acropora nobilis* from the Heron Island reef flat was more intense in light-exposed parts of the branches, pointing to a light-dependent regulation of the pigment content. Likewise, a pink morph of *Pocillopora damicornis* showed an increase in pigmentation upon exposure to high light intensity (Takabayashi & Hoegh-Guldberg 1995). The latter findings motivated us to systematically explore the expression of GFP-like proteins in reef corals upon exposure to light of varying intensity and color, using 5 different species of hermatypic corals (*Acropora millepora*, *A. pulchra, Montipora digitata*, *Hydnophora grandis,* and *Seriatopora hystrix*) from the taxa Acroporidae, Merulinidae and Pocilloporidae. Here we establish that tissue pigmentation is indeed modulated by light intensity in all these species, and that blue light triggers the regulation at the transcriptional level.

MATERIALS AND METHODS

Coral material. Coral specimens were acquired via the German aquarium trade and kept in artificial seawater at 25°C and a 12 h light:12 h dark cycle in the sea water facility at the University of Ulm. Replicate colonies were incubated for at least 6 wk under moderate light intensity before being subjected to experimentation. All regeneration of replicates and experiments were performed within the same tank, ensuring that the specimens experienced identical water conditions.

Light treatments and spectroscopic analysis. The exposure of replicate coral colonies to different light conditions was performed as described by Leutenegger et al. (2007a). Briefly, colonies were split into 4 groups consisting of 6 colonies each and exposed to different intensities of white light at 80, 100, 400 or 700 µmol photons m^{-2} s^{-1}, provided by a metal halide lamp (Aqua Light), for 6 wk. Afterwards, tissue fluorescence was excited by blue (450 nm) or green (530 nm) light; photographs were taken with a Camedia C-730 Ultra Zoom digital compact camera (Olympus) through a yellow long pass filter (Nightsea) or a 550 nm long pass glass filter (Schott). Fluorescence spectra of each specimen were measured using a Varian Cary Eclipse fluorescence spectrometer (Varian) equipped with a fiber optic probe.

Illumination with different colors was achieved using lighting filters (Lee Filters) transmitting at ~450 nm (band pass 'Zenith Blue', 80 nm full width at half maximum, FWHM), ~512 nm (band pass, 'Dark Green', 80 nm FWHM) and >580 nm (long pass, Primary Red (see Supplementary Material, Appendix 1, available at: www.int-res.com/articles/suppl/m364p97_app.pdf). The spectra were decomposed using MATLAB® (Mathworks). In all experiments using color filters, the specimens experienced a total photon flux of 200 µmol m^{-2} s^{-1} within the spectral range of 400–700 nm for 6 wk. The fluorescence of primary polyps of *Acropora millepora* was measured with a modified experimental setup as described in the Supplementary Material, Appendix 2.

Cleared coral tissue extracts were prepared from different individuals directly after spectroscopic characterization of the animal tissue as described by Leutenegger et al. (2007a) and Oswald et al. (2007). Total protein content was measured using the BCA method (Pierce). Proteins (10 µg total) were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinyldiene difluoride (PVDF) membranes (Amersham). The differential regulation of GFP-like protein content was further analyzed by western blotting, using an antibody raised against coral FPs (Oswald et al. 2007).

Tissue extraction, protein content and western blot analyses. Cleared coral tissue extracts were prepared as described by Leutenegger et al. (2007a) and Oswald et al. (2007). Total protein content was measured using the BCA method (Pierce). Proteins (10 µg total) were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinyldiene difluoride (PVDF) membranes (Amersham). The differential regulation of GFP-like protein content was further analyzed by western blotting, using an antibody raised against coral FPs (Oswald et al. 2007). Protein extracts were prepared from different individuals directly after spectroscopic characterization of the animal tissue.

RNA preparation and RT-PCR. RNA was prepared from tissues of *Acropora millepora* and *A. pulchra* after exposure to blue, green or red light for 6 wk. Using primers specific for amilFP484, amilF597, apulFP483 and apulCP584, fragments with the correct length of ~700 base pairs were amplified.

For transcript analyses, approximately 100 mg of coral tissue was quickly frozen in liquid N_2. Total RNA was extracted using RNAqueous® (Ambion). The RNA concentration of each sample was determined spectroscopically; the RNA quality was analyzed on an agarose gel. cDNA was produced from 1 µg of RNA using 100 units of Moloney murine leukemia virus (MMLV) reverse transcriptase (Promega) in the presence of 8 mM dNTPs, 16 U RNasin (Promega) and 500 ng of oligo-dT primer (Promega). Fragments encoding GFP-like proteins and the housekeeping genes *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) and *AmES* (*Acropora millepora* expressed sequence, GenBank accession number DY587236, Kortschak et al. 2003) were amplified from 100 ng of cDNA (primer sequences are given in Appendix 3). The number of PCR cycles was individually optimized to ensure that the samples were analyzed during exponential amplification. Equal amounts (10 µl) of each PCR reaction were visualized on ethidium bromide-stained agarose gels and photographed with the Bio-Rad Fluor-S MultiImager (Bio-Rad). The intensity of every transcript band was quantified using Adobe Photoshop 5.0 Software (Adobe Systems) and normalized to the 2 analyzed housekeeping genes. The amplified FP cDNAs (amilFP484/497/512//597, apulFP483 and apulCP584) were cloned and the encoded proteins were characterized as described by Wiedenmann et al. (2005).

To study the response of corals to a changed light stimulus, specimens were incubated for 6 wk under red light and subsequently transferred to blue light, after which samples were taken over a period of 8 h. Following this, 2 colonies were maintained in red light and analyzed as a negative control and 2 were maintained in blue light for another 4 wk and their transcript levels analyzed as a positive control.

Statistical analysis. The software ANALYSE IT for Microsoft Excel, Version 1.73 (Microsoft) was used for statistical analyses. Using Student's *t*-test (2 independent groups), 2-tailed p-values <0.05 were considered statistically significant. Highly significant differences were assumed for p-values <0.01. The probability values and sample numbers used for statistical analysis are listed in Appendix 4.

RESULTS AND DISCUSSION

Identification of GFP-like proteins in coral tissue

The 5 coral species studied contained a variety of pigments ranging from cyan to red, which showed elution profiles in size exclusion chromatography typical of GFP-like proteins (Wiedenmann et al. 2005). The protomers of the tetrameric assemblies have a molecular mass of ~26 kDa, as determined by SDS-PAGE and immunoblot analysis using an antiserum raised against coral fluorescent proteins (Oswald et al. 2007). Further evidence of the GFP-like nature of these pigments was provided by cloning and sequencing of 2 representatives of *Acropora pulchra* and 6 of *A. millepora* (Table 1). The proteins amilFP484, amilFP512 and amilFP597 displayed >97% amino acids identical to those of other, previously sequenced GFP-like proteins

Table 1. *Hydnophora grandis, Seriatopora hystrix, Montipora digitata, Acropora pulchra,* and *A. millepora.* Spectral properties of green fluorescent protein (GFP)-like protein. $\lambda_{Abs/Ex}/\lambda_{Em}$: position of the absorption (Abs) or excitation (Ex) maximum given as wavelength λ (nm)/Position of the emission (Em) maximum given as wavelength (nm); protein name: proteins were named according to Cox et al. (2007); Chromophore structure: basic structural features were deduced from the absorption of the denatured protein in 0.1 N NaOH (Gross et al. 2000, Oswald et al. 2007); CFP: cyan fluorescent protein; CP: chromoprotein; OFP: orange fluorescent protein; RFP: red fluorescent protein

	Pigment class	$\lambda_{Abs/Ex}/\lambda_{Em}$ (nm)	Protein name	Chromophore structure	GenBank accession no.
Hydnophora grandis	CFP	443/492[a]	hgraFP492	GFP	–
Seriatopora hystrix	CP	562[a]	shysCP562	dsRed	–
Montipora digitata	CFP	470/486[a]	mdigFP486	GFP	–
	GFP	508/514[a]	mdigFP514	GFP	–
	OFP	556/572[a]	mdigFP572	dsRed	–
Acropora pulchra	CFP	420/483[b]	apulFP483	GFP	apulFP483 EU709806
	CP	584[b]	apulCP584	dsRed	apulCP584 EU709807
Acropora millepora	CFP	420/484[b]	amilFP484	GFP	amilFP484 EU709808
	GFP	477/497[b]	amilFP497	GFP	amilFP497 EU709809
	GFP	500/512[b]	amilFP512	GFP	amilFP512 EU709810
	RFP	558/597[b]	amilFP597	dsRed	amilFP597 EU709811

Peak positions were determined from spectra [a]of GFP-like proteins purified from tissue extracts by size exclusion chromatography, or [b]of purified proteins recombinantly expressed in *Escherichia coli*

from *A. millepora* (Cox et al. 2007) (GenBank accession numbers DQ206400, AY646073 and AY646070). The identified pigments represent major color classes of GFP-like proteins, namely cyan, green, orange and red fluorescent proteins and also non-fluorescent pink or purple-blue chromoproteins (Table 1).

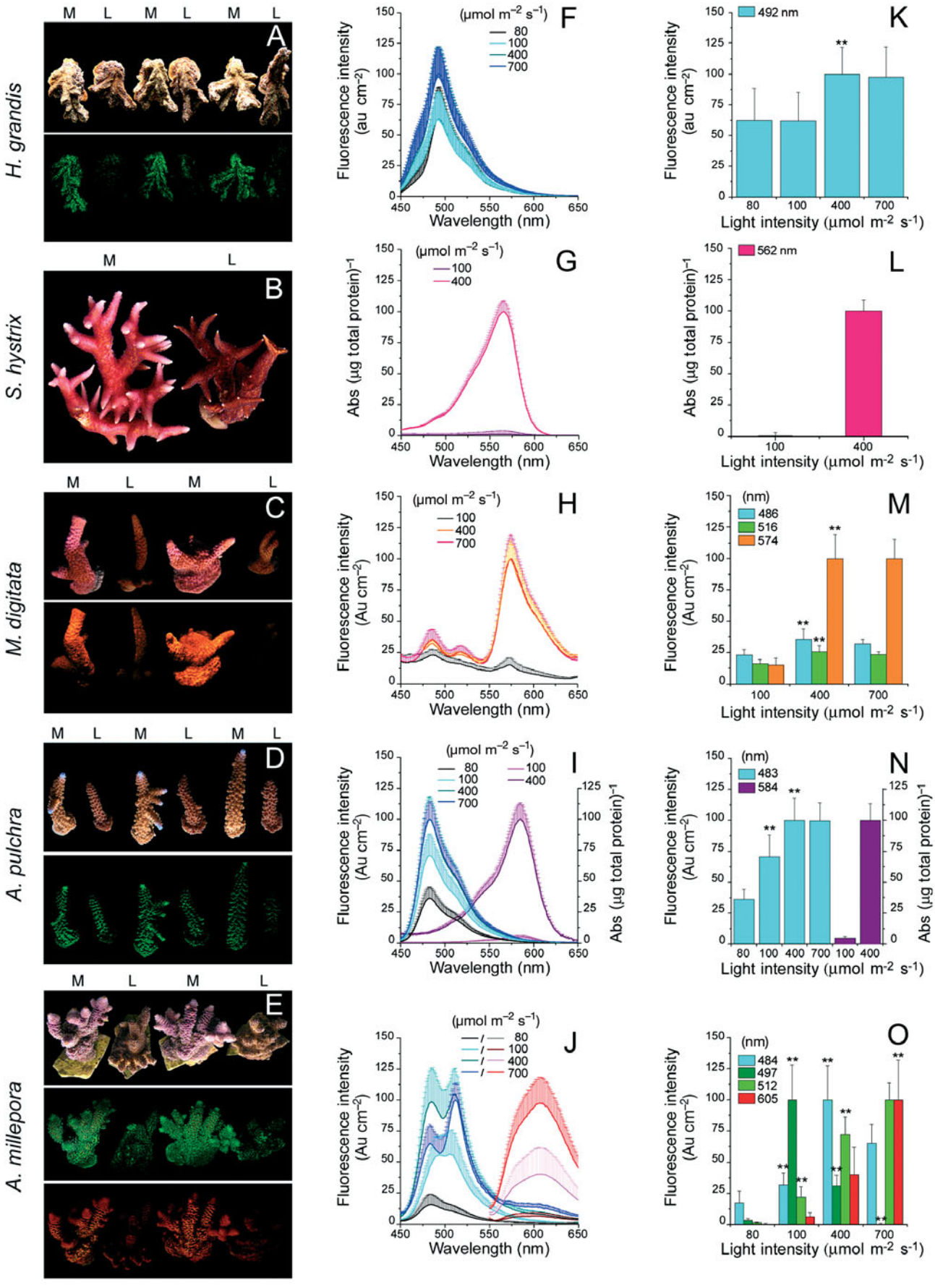
Effect of light intensity on GFP-like protein expression

Coral pigmentation was analyzed after subjecting colonies of each species for 6 wk to 4 different light intensities (photon flux densities), which we classify as very low (80 µmol m^{-2} s^{-1}), low (100 µmol m^{-2} s^{-1}), moderate (400 µmol m^{-2} s^{-1}) and high (700 µmol m^{-2} s^{-1}). As indicated by the gain of wet weight of coral colonies, growth rates under low light reached 20 to 40% of the rates determined for specimens under moderate light (data not shown). Consequently, colonies from low light treatments were smaller than their counterparts grown under moderate light (Fig. 1A–E).

Colonies illuminated with low-intensity light appeared brownish, whereas those growing under moderate light displayed distinctive hues ranging from blue over green to reddish. The increase in pigmentation of moderate-light-treated specimens was striking when the tissue fluorescence was excited with blue or green light (Fig. 1A–E).

The amount of pigments was quantified by measuring the emission of FPs directly on the corals or by collecting absorption spectra of size-fractionated tissue extracts. In 4 cases, varying light treatments were observed to modulate the spectral amplitude without changing the overall shape (Fig. 1F–I). An exception was the change in the ratio of the 2 emission bands at ~490 and ~510 nm of *Acropora millepora* upon light intensity variation (Fig. 1J). These overall emission spectra taken from tissues can be decomposed using the spectra of the 3 recombinant proteins amilFP484, amilFP497 and amilFP512 (Table1) cloned from one of the individuals used in the experiments.

Fig. 1. *Hydnophora grandis, Seriatopora hystrix, Montipora digitata, Acropora pulchra,* and *A. millepora.* Effect of light intensity on the content of green fluorescent protein (GFP)-like proteins: (A–E) Images of corals kept for 6 wk in photon flux densities of 100 µmol m^{-2} s^{-1} (low light, L) or 400 µmol m^{-2} s^{-1} (moderate light, M). All species were photographed under white light illumination (upper panels). Lower panels in (A,C,D) and middle panel in (E) depict tissue fluorescence imaged under blue light excitation. Lower panel in (E) shows red tissue fluorescence excited by green light. (F–J) Fluorescence and/or absorption (Abs) spectra after light exposure (mean + SD from 12 to 33 measurements). Maximum peak in every graph was set to 100 while the others were normalized accordingly. Fluorescence was measured directly on the replicate colonies, whereas the chromoprotein absorption spectra of *S. hystrix* (G) and *A. pulchra* (I) were determined from the peak fraction of size-fractionated tissue extracts and normalized to the total protein content of the samples. The photon fluxes of the treatments (µmol m^{-2} s^{-1}) are given in the keys. (K–O) Tissue fluorescence and/or absorption at the peak wavelength (nm) specified in the key of each graph. Cyan and green fluorescence intensities in (O) represent results from the spectral decomposition. **Highly significant difference ($p < 0.01$, independent *t*-test) compared to the closest lower photon flux


H. grandis
S. hystrix
M. digitata
A. pulchra
A. millepora
M L M L M L
M L
M L M L
M L M L M L
M L M L
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
(μmol m−2 s−1)
80
100
400
700
Fluorescence intensity (au cm−2)
Fluorescence intensity (Au cm−2)
Abs (μg total protein)−1
Wavelength (nm)
492 nm
562 nm
(nm)
486
516
574
483
584
484
497
512
605
Light intensity (μmol m−2 s−1)

Based on the response to light intensity, 2 different groups of proteins could be distinguished: a low-threshold group that includes all cyan fluorescent proteins (CFPs) in the present study, mdigFP514, mdigFP572 and amilFP497, and a high-threshold group consisting of amilFP512, amilFP597, and all CPs from *Acropora pulchra* and *Seriatopora hystrix*. Proteins of the high-threshold group were essentially absent when the light intensity fell below 100 µmol photons m^{-2} s^{-1}, and they accumulated nearly in proportion to light intensity up to 700 µmol photons m^{-2} s^{-1}. In contrast, representatives of the low-threshold group were expressed in considerable amounts at low light intensity, reaching their highest expression levels at a photon flux of ≤400 µmol photons m^{-2} s^{-1} and either saturating or even decreasing at higher light intensity. These results demonstrate unambiguously that the expression of GFP-like proteins in these corals is tightly controlled by light. Consequently, a variety of genes encoding GFP-like proteins regulated by environmental conditions can be clearly distinguished from another set constitutively expressed in the coral tissue (Kelmanson & Matz 2003, Leutenegger et al. 2007b, Oswald et al. 2007).

Effect of light color on GFP-like protein expression

Exposure of replicate colonies of each species to red, green or blue light at a photon flux of 200 µmol m^{-2} s^{-1} showed that corals incubated with blue light had the highest amounts of GFP-like proteins. Pigments of the low-threshold group were consistently found in corals growing in red light and reached ≥50% of the expression levels achieved with green light irradiation (Appendix 5). In contrast, representatives of the high-threshold group were essentially undetectable in corals grown under red light, and green-light-exposed specimens did not reach 50% of the levels observed under blue light.

Western blot analyses to further characterize the differential regulation of GFP-like protein content showed that, in all examined coral species, the antibody revealed the ~26 kDa-band typical of GFP-like proteins (Fig. 2A–F). The observed band intensities further confirmed that the concentrations of FPs or CPs were always highest for blue-light-treated specimens.

The differences in pigment content between corals incubated under low and moderate light are similar to those between green and blue-light-exposed specimens (Appendix 5). The metal halide lamps used to illuminate the corals emit ~50% of the photons in the blue spectral region. Therefore, corals subjected to low or moderate light treatment experienced a photon flux of ~50 or ~200 µmol m^{-2} s^{-1} of blue light, respectively. The green filter utilized in our experiments still transmits ~25% of the photons, corresponding to a photon flux of ~50 µmol m^{-2} s^{-1}, in the blue region of the spectrum (Appendix 1). Therefore, the light-driven accumulation of GFP-like proteins observed upon green light exposure is likely due to residual blue light passing the green filter. The expression of GFP-like proteins in members of the low-threshold group under red light suggests that a constitutive basal expression exists.

Regulation of GFP-like protein expression in corals

Semiquantitative RT-PCR was performed to determine whether the pigment content was regulated at the transcriptional level. As expected from the similarity between cyan and green FPs from *Acropora millepora* that were sequenced earlier (Cox et al. 2007), PCR with amilFP484 primers also led to amplification of cDNAs encoding amilFP497 and amilFP512 (Fig. 3A,B). Low transcription levels of the amilFP484/497/512 group and amilFP597 were detected in *A. millepora* exposed to red light. A major increase in the amount of FP transcripts occurred with green light, and a further increment with blue light exposure, indicating a regulation of the tissue pigment content at the transcriptional level. The transcript of the low-threshold protein apulFP483 was already present in red-light-illuminated *A. pulchra*; its amount increased to a maximum level for green-light-exposed specimens. This result agrees well with the small changes in tissue fluorescence due to proteins of the low-threshold group for illumination with blue and green light. In contrast, the transcript level of the high-threshold protein apulCP584 was significantly higher upon exposure to blue light.

RT-PCR analysis was performed to determine the time the corals require to increase transcript levels in response to a changed light stimulus. FP transcripts accumulated at very low levels at time point 0 (i.e. immediately before exposure to blue light, Fig. 3C,D) and in specimens treated for another 8 h with red light. In contrast, transcript levels were significantly increased after 8 h of exposure to blue light. However, after 8 h of stimulation, the maximal transcript level was not yet reached, as shown by comparison with the levels in the positive control group after 4 wk of blue-light exposure. This rather slow increase suggests that the accumulation of pigments is a long-term adaptive process.

Fluorescence in primary polyps of *Acropora millepora*

Further investigations were conducted to test whether the presence of endosymbiotic algae is mandatory for the regulation of GFP-like proteins. Examination of azooxanthellate primary polyps (exposed to red or blue light

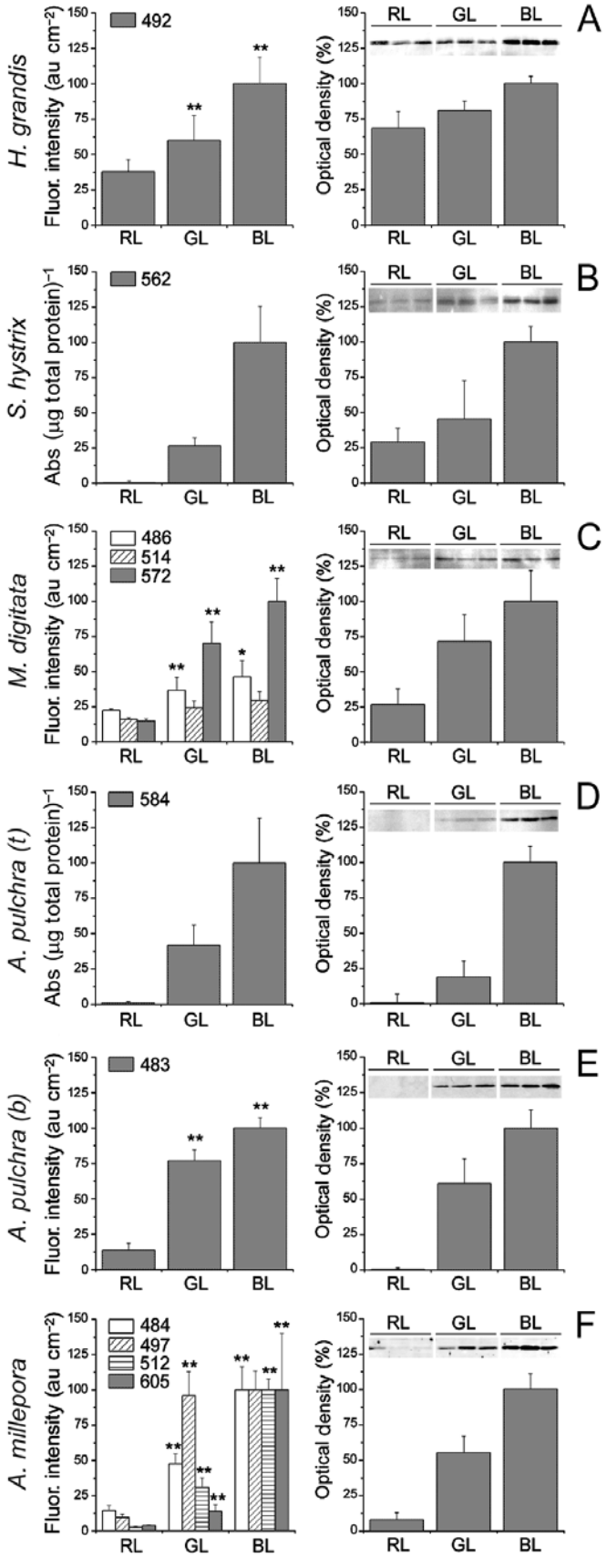


Fig. 2. *Hydnophora grandis, Seriatopora hystrix, Montipora digitata, Acropora pulchra,* and *A. millepora.* Effect of light energy (color) on the accumulation of green fluorescent protein (GFP)-like proteins. Replicate colonies were exposed to red (RL), green (GL) or blue light (BL) (photon flux of 200 µmol m^{-2} s^{-1}). Left graph of each panel gives the fluorescence emission or absorption (Abs) of fluorescent proteins (FPs) and chromatoproteins (CPs), respectively (corresponding wavelengths in nm indicated in the keys). Bars represent the mean (+SD) of 12 to 33 measurements. Significant (*$p < 0.05$) or highly significant (**$p < 0.01$) differences, as calculated by independent *t*-test between GL and RL exposed specimens (asterisk on the GL bars), or between BL and GL exposed specimens (asterisks on the BL bars). Right graph of each panel displays the results of western blot analyses of tissue extracts prepared at the end of the experiment. Three independent measurements of the optical density of the bands specific for GFP-like proteins (insets) were made. Data are means (+SD), with values from BL exposure set to 100 and all other data normalized accordingly

immediately after metamorphosis) by fluorescence microscopy after 5 d of illumination revealed a significant increase of tentacle fluorescence in the green spectral region (Fig. 4). This suggests that photoreceptors of the host are involved in the regulation of pigment levels. Perception of blue-green light by the ectodermal, zooxanthellae-free cells of *Montastrea cavernosa* and *Eusmilia fastigiata* was demonstrated earlier (Gorbunov & Falkowski 2002), and only very recently, cryptochromes were identified in *Acropora millepora*, providing putative blue-light photoreceptors (Levy et al. 2007).

Implications for biological function

Among the known FPs and CPs, only the absorption properties of CFPs spectrally match the major absorption band of chlorophyll *a* and *c* at ~430 nm, making them suitable for effective shielding of the photosynthetic system of the zooxanthellae. Our results have shown that the amounts of these proteins in coral tissue increased at light intensities >80 µmol m^{-2} s^{-1} and saturated at ~400 µmol m^{-2} s^{-1}. This response is in striking agreement with photosynthesis-irradiance curves of zooxanthellae from different coral species that show a similar increase and saturation at 400 µmol m^{-2} s^{-1} (Falkowski et al. 1990, Smith et al. 2005). However, the detrimental effects of excessive light levels become apparent once photosynthesis is saturated and the generation of reactive oxygen species (ROS) increases (Smith et al. 2005, Lesser 2006). If CFPs and other members of the low-threshold group were exclusively involved in a photoprotective function, an upregulation of these proteins should be expected, especially at photon fluxes higher than 400 µmol m^{-2} s^{-1}. Our data do not favor such a scenario, so these pigments might also play other roles in the interaction of the coral host and its algal symbionts. Possible functions might include sensing of

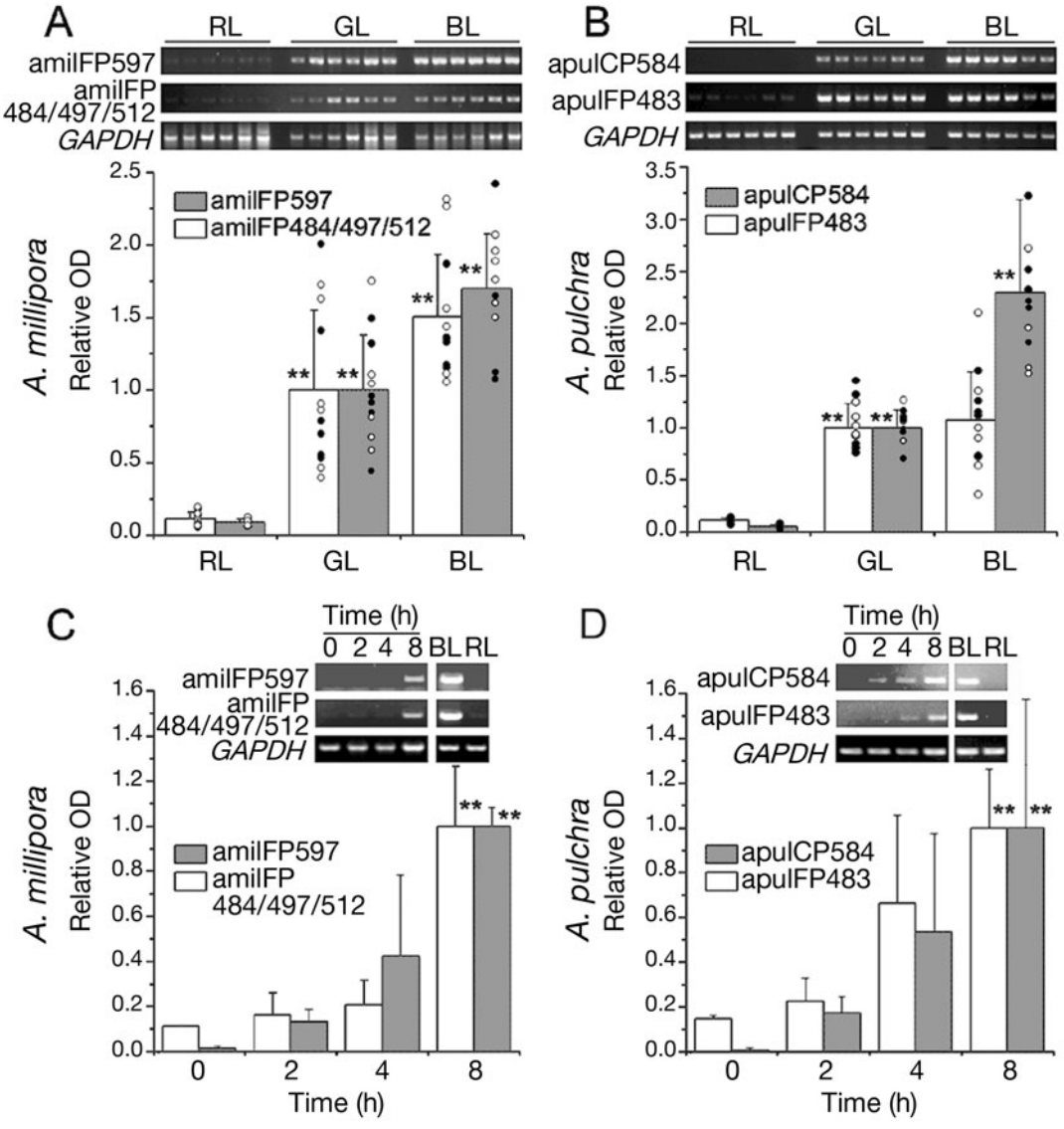


Fig. 3. *Acropora pulchra* and *Acropora millepora*. Semi-quantitative RT-PCR analysis of green fluorescent protein (GFP)-like protein transcripts: (A,B) Semi-quantitative analysis of transcript levels after treatment with red (RL), green (GL) or blue light (BL) (photon flux of 200 µmol m^{-2} s^{-1}). Representative ethidium bromide-stained gels in the upper part of the panels show the bands corresponding to amplified transcripts of GFP-like proteins and the housekeeping gene *GAPDH*. As gel analysis of transcript levels of the housekeeping gene *AmES* yielded results comparable to *GAPDH*, the respective panels were omitted for space reasons. Data are means (+SD) of the intensities of replicate bands from 6 independent measurements, normalized to the corresponding bands of the housekeeping genes *GAPDH* and *AmES*. Open and filled circles depict values obtained by normalization to *GAPDH* and to *AmES*, respectively. (C,D) *A. millepora* and *A. pulchra* specimens transferred from RL to BL (time point 0 h). Samples were taken after 2, 4 and 8 h of BL exposure and used for RT-PCR analysis. Control specimens incubated for 8 h under RL and 4 wk under BL were processed in parallel. OD: optical density, **highly significant differences ($p < 0.01$, independent *t*-test)

photosynthetic activity of zooxanthellae, support of algal productivity by the addition or removal of rate-limiting substances, or accumulation of the pigments for amino acid or nitrogen storage purposes. A close spatial association with zooxanthellae required for such functions was previously observed for certain coral FPs (Mazel et al. 2003, Oswald et al. 2007).

The light-induced upregulation of GFP-like proteins from the high-threshold group resembles the increasing accumulation of well known photoprotectants such as MAAs, β-carotene, xanthophylls or melanins (Shick et al. 1995, Bandaranayake 2006). However, none of the members of the high-threshold group absorbs light at wavelengths that would provide an efficient shielding of the photosynthetic apparatus of the zooxanthellae. The screening effect could be enhanced if these proteins were to act as acceptors in fluorescence resonance energy transfer (FRET) (Gilmore et al. 2003, Cox

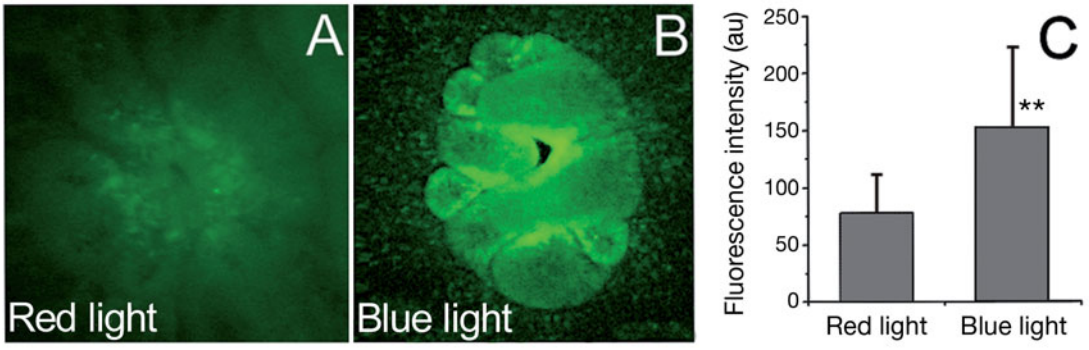


Fig. 4. *Acropora millepora.* Upregulation of green fluorescent protein (GFP)-like protein expression in primary polyps. Fluorescence photomicrographs of tentacles treated with (A) red and (B) blue light. (C) Green fluorescence (mean + SD) of tentacles from red and blue light-treated polyps from 28 independent measurements. **Highly significant differences ($p < 0.01$, independent *t*-test)

et al. 2007). Alternatively, protection from harmful light effects could also involve an as yet undiscovered function, for instance ROS scavenging (Bou-Abdallah et al. 2006) or antimicrobial defense of corals becoming vulnerable under light stress. Interestingly, in the case of *Acropora pulchra*, the strong accumulation of apulCP584 was limited to the tips of the branches. The apical polyps are essentially free of zooxanthellae, but are the areas with the highest growth rates (Fang et al. 1989). The constrained localization of some FPs in zooxanthellae-free parts of certain corals (e.g. skeletal ridges) has been noticed earlier (Mazel et al. 2003). Therefore, GFP-like proteins, particularly representatives of the high-threshold group, could play a role in metabolic pathways related to coral growth.

Coral color as an indicator of environmental conditions

In this work, we have established the tight regulation of GFP-like proteins in corals from different taxonomic groups in response to the intensity and spectral composition of the irradiating light. Consequently, the pigment complement of coral species that express representatives of both high- and low-threshold groups, might serve as a predictable 'ratiometric' indicator of light conditions in the habitat. In conclusion, coral pigmentation appears promising as a sensitive indicator of environmental conditions. Future work will further refine our knowledge about the influence of diverse environmental factors on the expression levels of GFP-like proteins in reef-building corals.

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