Enhanced photoprotection pathways in symbiotic dinoflagellates of shallow-water corals and other cnidarians

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Edited by Elisabeth Gantt, University of Maryland, College Park, MD, and approved July 22, 2008 (received for review May 28, 2008)

Photoinhibition, exacerbated by elevated temperatures, underlies coral bleaching, but sensitivity to photosynthetic loss differs among various phylotypes of *Symbiodinium*, their dinoflagellate symbionts. *Symbiodinium* is a common symbiont in many cnidarian species including corals, jellyfish, anemones, and giant clams. Here, we provide evidence that most members of clade A *Symbiodinium*, but not clades B–D or F, exhibit enhanced capabilities for alternative photosynthetic electron-transport pathways including cyclic electron transport (CET). Unlike other clades, clade A *Symbiodinium* also undergo pronounced light-induced dissociation of antenna complexes from photosystem II (PSII) reaction centers. We propose these attributes promote survival of most cnidarians with clade A symbionts at high light intensities and confer resistance to bleaching conditions that conspicuously impact deeper dwelling corals that harbor non-clade A *Symbiodinium*.

coral bleaching | light-harvesting complexes | photoinhibition | *Symbiodinium* | cyclic electron transport

Corals and other cnidarians thrive in tropical, near-shore oceans due to intracellular symbioses with dinoflagellates of the genus *Symbiodinium*, which provide their hosts essential photosynthates (1, 2). Coral species distribution largely correlates with prevailing light and temperature gradients and likely is influenced by the differing photosynthetic and light tolerance properties of their symbionts coupled with host-symbiont specificity and biogeographical distribution (3–6). Hence, *Symbiodinium* phylotypes, predominantly clades A, B, and C and sometimes clade D and their respective hosts are found at characteristic depth ranges (4, 7). Clade A *Symbiodinium* are especially prevalent in shallow-water cnidarians including corals, jellyfish, and anemones in the Caribbean (4). Clade A isolates abundantly produce UV-protective mycosporine-like amino acids (MAAs) in culture whereas all clades, with a depth-dependent correlation, synthesize MAAs *in hospice*, especially mycosporine-glycine, which effectively absorbs harmful UV-B wavelengths (8). Little else is known about distinct physiological properties of *Symbiodinium* species and their susceptibilities to temperature and high light stress, although corals harboring clade D have been observed to be high-temperature tolerant (9).

Symbiodinium, like other photosynthetic organisms, are susceptible to photodamage of PSII (10). The resulting photoinhibition can lead to breakdown of symbioses and is an initial event in the onset of coral bleaching during episodes of elevated ocean temperatures (11). Differences in temperature sensitivity to photodamage (12) and rates of replenishment of the PSII D1 reaction center protein are documented in a few representatives of *Symbiodinium* in culture and *in hospice* (10, 12). Low degrees of fatty acid desaturation in thylakoid lipid membranes of cultured dinoflagellates have been correlated with elevated temperature tolerance and reduced production of reactive oxygen species under high irradiance, but there is not a strict correlation with heat sensitivity along *Symbiodinium* cladal lines (12).

Some corals bleach seasonally and others rarely (13), suggesting differential survival capabilities of the symbionts and their hosts when subject to environmental extremes. In the Caribbean, deeper water corals that commonly harbor clades B and C *Symbiodinium* are generally more susceptible to bleaching at elevated temperatures than are shallow-water corals in symbiosis with clade A (10), indicating that thermal and high irradiance tolerance can be interrelated. Photoprotection mechanisms identified in *Symbiodinium* include engagement of xanthophyll deepoxidation associated with nonphotochemical quenching (NPQ) within intrinsic light-harvesting complexes (LHCs) (14, 15) and longer-term photoacclimation processes that modulate the abundance and size of photosynthetic units and peripheral peridinin–chlorophyll-*a*–protein (PCP) antenna complexes (16, 17).

We show here that members of clade A *Symbiodinium* in culture and those inhabiting shallow-water cnidarians are conspicuously capable of PSII-independent CET. Evidence is also presented for the occurrence of chlororespiration, wherein oxygen serves as an alternative photosynthetic electron transport acceptor and is supplemental to CET. Both chlororespiration and CET can sustain ATP synthesis when PSII is inactivated (18). These studies employ a modified pulse amplitude modulated (PAM) fluorometry method that is easily applied in field studies. Clade A *Symbiodinium* are also found to readily undergo high light-induced dissociation of antenna complexes from PSII, particularly the "soluble" PCPs found only in dinoflagellates.

Conventional PAM fluorometry uses dark-adapted photosynthetic samples to assess PSII photosynthetic efficiency from minimal and maximal chlorophyll fluorescence levels. Emission from a weak modulated measuring light (ML) (F_0) and maximal fluorescence (F_m) induced by a saturating light pulse estimates maximum quantum yield as $F_v/F_m = F_m - F_0$. Typically, continuous actinic irradiation is subsequently imposed along with intermittent saturating flashes to monitor decreases of maximal fluorescence (F_m') due to NPQ $[(F_m - F_m')/F_m')]$. We show a simple method to detect nonlinear photosynthetic pathways by measuring chlorophyll-*a* (chl-*a*) fluorescence in response to serial irradiation pulses (SIP) of 1-s duration without concurrent actinic irradiation.

Results and Discussion

Evidence for CET in clade A *Symbiodinium* by Using SIP Protocol. When using the SIP method with dark-adapted symbiotic clade A *Symbiodinium* cells in culture and *in hospice*, incremental

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Author contributions: J.M.R. and G.W.S. designed research; J.M.R., B.U.B., W.K.F., and G.W.S. performed research; B.U.B. and G.W.S. contributed new reagents/analytic tools; J.M.R., W.K.F., and G.W.S. analyzed data; and J.M.R., B.U.B., W.K.F., and G.W.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0805187105/DCSupplemental.

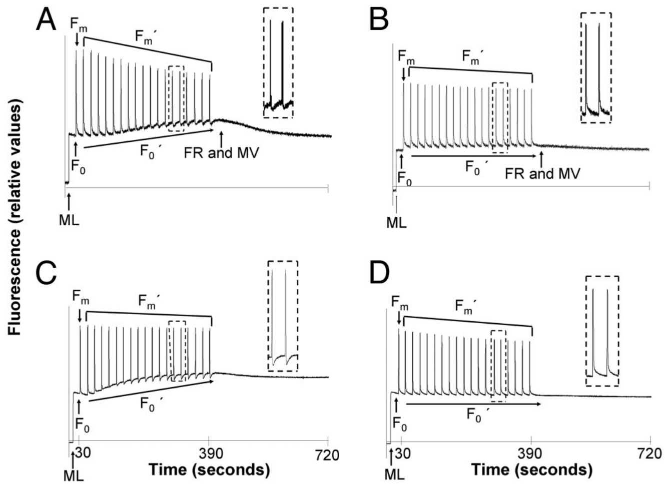

Fig. 1. SIP detection of postillumination PQ reduction in clade A but not B *Symbiodinium* in culture and *in hospice*. Dark-adapted cultured cells and corals were subjected to saturating light pulses every 20 s after applying low-intensity ML. A progressive increase in F_0' levels reflect substantial reduction of the PQ pool in clade A3 cultured cells (*A*) but not in clade B1 cultured cells (*B*). (*Insets*) Light-induced PQ oxidation and dark rereduction between the 13th and 15th saturating pulses in clade A but not clade B phylotypes. (*A* and *B*) After clade A3 SIP treatment, 50 μM MV was used in combination with FR. (*C* and *D*) A Diving-PAM was used for SIP with dark-adapted corals. (*C*) *Symbiodinium* phylotype A4a in shallow-dwelling *P. astreoides* exhibits progressive PQ reduction and postillumination PQ reduction. (*D*) Fluorescence responses of *M. faveolata* harboring B1 *Symbiodinium* show no change with SIP.

increases in the levels of F_0 chl-*a* fluorescence are initially detected, reflecting progressive reduction of the electron acceptor pool for PSII, plastoquinone (PQ) (Fig. 1 *A* and *C*). SIP treatment clearly does not lead to reduction of PQ in clade B *Symbiodinium* (Fig. 1 *B* and *D*) indicating sustained linear electron transport activities between PSII and PSI in contrast to clade A cells. The lengthy duration of flashes and limited sensitivity of the instruments and detectors used enable only an approximation of F_m and F_m' levels (19). However, the multiturnover excitation of PSII to drive water oxidation is beneficial to initiating and poising PQ in a reduced state as is apparent from clade A F_0' increases. As shown below, dissociation of PSII LHCs, particularly PCPs, lead to preferential PSI excitation in later phases of the clade A SIP response.

When the PQ pool becomes significantly reduced in clade A samples at approximately the fifth flash and thereafter, abrupt F_0' decreases are then induced by each subsequent flash (Fig. 1*A*). These signals are attributed to transient PSI oxidation of the electron transport interchain, including PQ. During following dark phases, PQ reduction (F_0') then returns to levels above that of the prepulse signal with approximate half-times of ≈10 s in culture (Fig. 1*A Inset*) and 5 s *in hospice* (Fig. 1*C Inset*). A similar phenomenon has been described in a few other algae, but under conditions of limited electron acceptors and/or anaerobiosis (20). Such postillumination PQ reduction signals have also been observed after prolonged illumination of isolated spinach chloroplasts (21) and in leaves of C4 plants (22). The SIP response is significantly enhanced when clade A *Symbiodinium* are subjected to anaerobic conditions [supporting information (SI) Fig. S1]. Overall, the clade A fluorescence responses are ascribed to PSI-dependent plastoquinol oxidation followed by PQ rereduction by CET and/or chlororespiration (23, 24). The postillumination PQ reduction signal is likely to reflect only a small portion of CET activity that occurs during constant illumination.

Further evidence that PQ reduction/oxidation is PSI and CET-dependent in SIP-subjected clade A cells is that irradiation with far-red light (FR) and then administration of methyl viologen (MV), an artificial PSI acceptor, leads to decline of F_0' to the initial dark-adapted F_0 level (Fig. 1*A*). Because MV interacts directly with PSI (17), it intercepts PSI electron transfer and downstream ferredoxin and NADPH-mediated processes, including ferredoxin-mediated CET through cytochrome b_6/f and/or NAD(P)H-driven chlororespiration. The MV effect also indicates that PSII photodamage is not responsible for the SIP-induced F_0' increase.

Negative results of SIP treatments with clade B *Symbiodinium* certify the fluorescence phenomena in clade A are not an experimental artifact (Fig. 1 *B* and *D*). Also, measurements with clade A yield identical SIP responses when PAM fluorometry is used with settled cells in culture flasks (data not shown). Hence, the postillumination decrease and then increase of F_0' is not due to sample stirring and detector array configuration (25).

SIP Induced CET *in Hospice*.

The cultured clade A representative in Fig. 1*A* is of western Pacific origin (from the giant clam *Tridacna gigas*). Fig. 1*C* shows that clade A *Symbiodinium* from a Caribbean coral also exhibits diagnostic clade A SIP responses *in hospice*. *Porites astreoides* specimen were collected from shallow water (2 m), and their symbionts show an identical response to cultured clade A cells (Fig. 1*A*) when SIP-assayed with the submergible Diving-PAM (Fig. 1*C*). Subsequent genotyping by using PCR-denaturing gradient gel electrophoresis (DGGE) analysis of the rDNA ITS2 region (5) confirmed the predominance of subclade A4a symbionts. Symbionts of deeper dwelling *P. astreoides* consistently show no SIP-induced F_0 fluctuations and harbor C1 *Symbiodinium* (4). Similarly, corals like *Montastraea faveolata* hosting subclade B1 *Symbiodinium* exhibit flat line F_0' and F_m' when SIP-probed (Fig. 1*D*). We additionally surveyed many other species of cnidarians known to host clade A *Symbiodinium* in the Caribbean including corals *Acropora cervicornis* and *Acropora palmata*, anemones *Aiptasia pallida* and *Bartholomea annulata*, and the jellyfish *Cassiopeia xamachana*, as well as others known to host clades B and C. Employing SIP, only cnidarians harboring clade A *Symbiodinium* consistently display CET signals and flash-induced NPQ *in hospice*. Among cultured clade A, only subclade A2 isolates from a sea fan and a clam do not yield a CET SIP response (Table S1).

Porites furcata harbors multiple phylotypes of *Symbiodinium* in the Caribbean (4). A blind test was conducted by using SIP with *P. furcata* collected from a 1-m depth off Key Largo, Florida, revealing fluorescence patterns indicative of clade A, but not in this species when found at a 1.5-m depth (Fig. 2 *A* and *B*). Presence of the dominant phylotype A4a (in combination with B1) in the shallowest samples was confirmed by PCR-DGGE analysis of the rDNA ITS2 region. Thus, the SIP technique provides a noninvasive means for detecting the probable presence of clade A *Symbiodinium* and is potentially applicable to tracking symbiont genotypic and/or phenotypic fluctuations in the course of seasonal and long-term environmental change.

SIP induces a marked decrease in F_m' levels in clade A but not clade B *Symbiodinium* (Fig. 1 *A* and *B*) reflecting strong induction of NPQ and decreased light energy transfer to PSII. Analogous to model systems subjected to continuous illumination, this F_m' decrease can follow from reduced cytochrome b_6/f-dependent activation of chloroplast protein kinases to phosphorylate/dissociate PSII LHCs, conformational changes of LHCs by means of a large decrease of thylakoid lumen pH, activation of xanthophyll deepoxidase on lumen pH decline, or a combination of such excitation energy dissipation mechanisms (18). Dinoflagellates uniquely possess peripheral antennae PCP complexes, presumed to reside in the thylakoid lumen, that function to maximize green light energy transfer to PSII (26, 27). Shown below, dissociation of PCPs from PSII and possibly

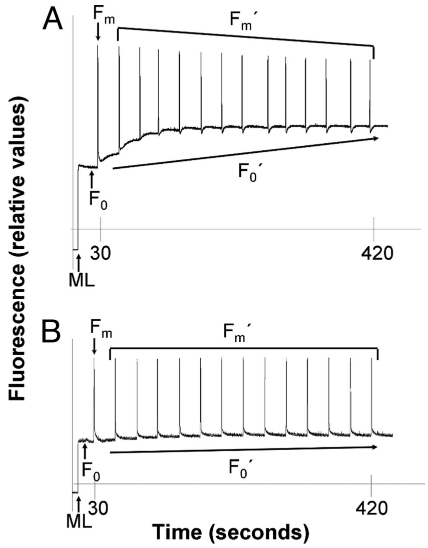

Fig. 2. Detection of clade A *Symbiodinium* in coral hosts. SIP of dark-adapted *P. furcata*, from a 1-m depth, reveals a fluorescence pattern typical of clade A. (*A*) Genetic analysis by using DGGE revealed the presence of a combination of A4a and some B1 phylotypes of *Symbiodinium*. (*B*) Dark-adapted *P. furcata* from a 1.5-m depth, and found to harbor only B1 *Symbiodinium*, does not display a clade A specific fluorescence pattern SIP response.

integral antennae complexes are conspicuous features of NPQ in clade A *Symbiodinium.*

Anaerobic Enhancement of Dark PQ Reduction and Engagement of NPQ in clade A *Symbiodinium*. Clade A cells do not exhibit a transient PQ reduction/F_0' increase after termination of prolonged irradiation with moderate actinic light (250 μmol quanta $m^{-2}{\cdot}s^{-1}$) and after NPQ approaches steady-state (Fig. 3*A*), likely due to activation of processes that divert PSI electron acceptors from CET such as carbon fixation, nitrate/sulfate reduction, the Mehler/ascorbate peroxidase pathways, or photorespiration. PSII-dependent oxygen evolution would promote the two latter pathways. We found that anaerobic conditions double the rate of SIP-induced PQ reduction (Fig. S1). Imposing anaerobic conditions (Fig. 3C), light-adapted clade A cells exhibit a postillumination F_0' increase with an overall half-time of 30 s. A similar signal, although weaker, is observed in clade B *Symbiodinium* but under both aerobic and anaerobic conditions (Fig. 3 *B* and *D*). Clearly, clade A *Symbiodinium* is capable of more complete engagement of NPQ as indicated by the fluorescence decrease in response to continuous illumination as compared with clade B cells. Additionally, substantial but gradual dark PQ reduction, likely due to partial engagement of a chlororespiration pathway, is discernable when the oxygen electron acceptor for photorespiration, chlororespiration, and/or pathways related to the Mehler reaction is diminished. Collapse of a transthylakoid pH gradient at the onset of darkness in clade B *Symbiodinium* and, partially in clade A cells, could also contribute to the F_0' postillumination increase.

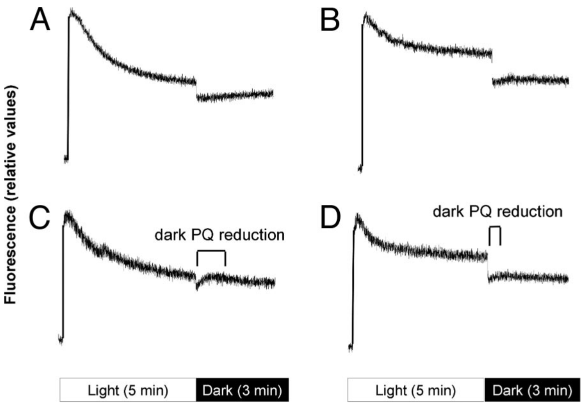

Fig. 3. Enhanced dark PQ reduction in anaerobic clade A *Symbiodinium*. After dark incubation for 10 min and supplementation with 10 mM bicarbonate to maximize carbon fixation, cultures of subclades A3 (*A* and *C*) and B2 (*B* and *D*) were bubbled with air (*A* and *B*) or argon (*C* and *D*) to impose aerobic and anaerobic conditions, respectively. Fluorescence decreases after onset of continuous actinic illumination reflect NPQ processes, which are almost complete after 5-min exposure to 250 μmol quanta m^{-2} s^{-1}. During subsequent darkness, anaerobic clade A (*C*) and, to a lesser extent, both aerobic and anaerobic clade B exhibit PQ reduction (*B* and *D*).

Light-Induced Dissociation of PSII Antennae in clade A *Symbiodinium*. High light induction of NPQ is fairly well documented in *Symbiodinium* in culture and *in hospice* (11, 14, 15, 28). Only the initiation of xanthophyll deepoxidation to facilitate a NPQ thermal decay of excitation energy within integral LHC chl-a/c_2 complexes has been documented (29). Other photoprotective processes akin to state transitions, in which LHCs dissociate from PSII, also have been theoretically deduced (30, 31). However, no definitive evidence for state transition-like mechanisms in *Symbiodinium* or other dinoflagellates have been documented. Fluorescence spectra at 77 K of dark-acclimated clade A *Symbiodinium* show that both intrinsic LHCs and extrinsic PCPs are fully connected to PSII as is apparent in the major fluorescence emission peak at 686 nm, presumably from the CP43 core antennae (Fig. 4*A*) (32). Excitation spectra of 686 nm show a strong contribution of PCP with novel peridinin absorption in the 530-nm region, along with the ≈400-nm Soret bands of chl (Fig. 4*B*). That PCPs are fully PSII-associated in dark-adapted cells is evidenced by minimal emission at 673 nm where a fluorescence shoulder from unassociated PCP chl-*a* would be selectively detected (Fig. 4*B*) (26, 33).

After exposure to actinic white light (250 μmol $m^{-2}{\cdot}s^{-1}$) for 100 min, fluorescence emission spectra of clade A cells exhibit dual peaks at 675 nm and 686 nm on preferential excitation of either chl (440 nm) or peridinin (530 nm) (Fig. 4*C*). The 675-nm peak is close to the PCP chl-*a* emission band of purified complexes (26, 27, 33). Peridinin, as a major contributor to the 675-nm emission peak, is further evidenced by an elevated 530 nm-induced excitation signal as compared with that obtained by the 440-nm excitation spectrum (Fig. 4*D*). We estimate from light/dark fluorescence spectral differences that almost half of the PCP population, perhaps together with integral LHCs dissociates from PSII as a major component of NPQ on prolonged light exposure in clade A *Symbiodinium*. No modifications of fluorescence emission spectra are detected in clade B and C *Symbiodinium* in response to these illumination conditions (Fig. S2).

Enhancement of fluorescence peaks at wavelengths longer than 700 nm would reflect intrinsic antenna translocation from PSII to PSI, the classical state transition earmark in green algal systems (34). In *Symbiodinium*, such a State 2 antenna association with PSI is not substantial (Fig. S2). When exposed to prolonged FR, the classical algal state transition response is antenna reversion from PSI to PSII connectivity (State 1) (35). Surprisingly, clade A cells respond to far-red irradiation differently, displaying dual fluorescence peaks at 675 nm and 683 nm, whereas 686-nm emission from PSII is minimized (Fig. 4*C*). The emergent 683-nm peak is best ascribed to disconnected intrinsic chl-*a*/*c* LHCs (33) antenna as evidenced by a chl-*c* contribution near 458 nm (27). The loss of 686-nm fluorescence could be due to diminished PSII integrity resulting from dissociation of core antenna CP43 in combination with the PSII disconnection of LHCs (Fig. 4 *A* and *C*). We attribute pronounced CET and

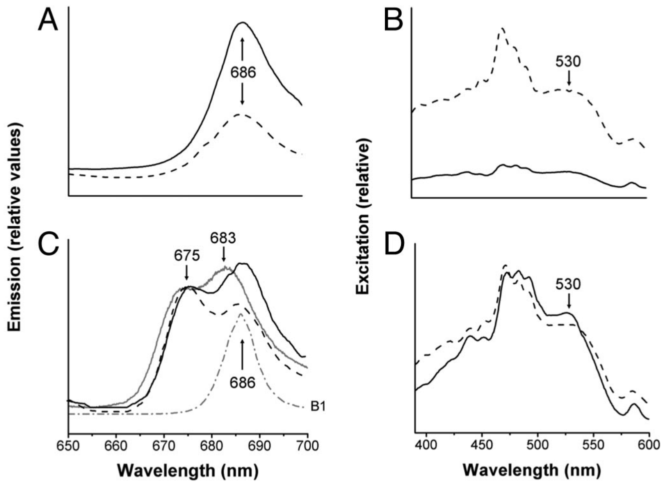

Fig. 4. Light-Induced PCP and Intrinsic LHC Dissociation from PSII in clade A3 *Symbiodinium*. (*A*) The 77 K fluorescence emission spectra at excitation wavelengths 440 nm (solid line) and 530 nm (dashed line). (*B*) Excitation spectra of the same clade A3 cells detected at 673 nm (solid line) and 683 nm (dashed line) after 60-min dark adaptation. The dark-acclimated excitation spectrum, specifically the peridinin excitation shoulder at 530 nm, is higher at 683 nm than 673 nm as a result of the greater PSII connectivity of the PCP antenna complexes and resulting fluorescence. (*C*) Fluorescence emission spectra at excitation wavelengths of 440 nm (solid line) and 530 nm (dashed line) after exposure to 100 min of white light. The two emission peaks (675 and 686 nm) in both excitation spectra from white light-adapted clade A3 cells reflect partial dissociation of LHCs from PSII. Prolonged FR illumination (>690 nm) results in further loss of PSII 686 nm fluorescence, whereas 683-nm emission from PCPs and intrinsic LHCs is enhanced (solid line). No far-red-induced fluorescence shifts occur in clade B (dotted-dashed line). (*D*) Fluorescence excitation spectra of the same clade A3 cells as in *C* at detection wavelengths of 673 nm (solid line) and 683 nm (dashed line) after 100 min of actinic light exposure, showing a pronounced peridinin contribution to 675-nm emission by 530-nm excitation in comparison with that of dark-adapted cells in *B*.

cytochrome b_6/f reduction as an underlying basis for the distinctive response of clade A *Symbiodinium* to far-red and high light illumination. Clades B and C exhibit no fluorescence changes as a consequence of far-red (Fig. 4*C*) or prolonged high light acclimation (Fig. S3).

Conclusions

Clade A is the most ancestral *Symbiodinium* phylotype (36). Most closely related subphylotypes found in shallow water appear to have evolutionarily retained constitutive CET/chlororespiration and PCP/LHC dissociation mechanisms. Moreover, when thermal perturbation of PSII photodamage and repair occurs, CET conceivably would sustain symbiont/host survival together with likely synergisms of xanthophyll deepoxidation contribution to NPQ and production of UV-protective MAAs.

Clades B, C, and D symbionts are analogous to shade plants because they are usually found in symbioses in deeper waters than clade A *Symbiodinium* and would benefit from enhanced light-harvesting capability. Some subclade B *Symbiodinium* are found in symbiosis with corals in shallow water but they likely employ photoprotection mechanisms other than antenna translocation under such circumstances. *Symbiodinium* clades B and C of deeper-dwelling corals, normally more susceptible to bleaching, can engage NPQ and varying degrees of chlororespiration (data not shown). However, kinetics and magnitude of their SIP-induced fluorescence patterns indicate they primarily sustain photosynthesis through linear photosynthetic electron transport and not CET.

Understanding *Symbiodinium* photoprotection and thermal tolerance mechanisms is crucial to predicting how coral-dinoflagellate symbioses will respond to increased ocean temperatures, irradiance conditions, and perhaps disease pressures. From the studies presented here, corals with clade A *Symbiodinium* are likely more resistant to combinations of high light and high temperature, conditions common at the end of summer (13), because of their enhanced and constitutive alternative photosynthetic electron pathways and photoprotection processes. CET and chlororespiration (37) may also serve to minimize oxygen evolution thereby decreasing the probability of the formation of and damage by reactive oxygen species. We propose that CET can sustain ATP synthesis and underlies clade A symbiont survival during warm conditions that can exacerbate PSII loss. Analogous to the case of PSII-less tobacco mutants (38), CET and/or chlororespiration could become dominant means for photosynthetic energy transduction during bleaching episodes. However, whether some non-clade A symbionts might conditionally develop CET and significant antenna dissociation capacity in response to elevated temperature deserves further study.

Materials and Methods

***Symbiodinium* Cultures and *in Hospice*.** Cultures of *Symbiodinium* were grown in ASP-8A medium (39) and maintained at 50 μmol quanta $m^{-2} \cdot s^{-1}$ under cool white fluorescent lamps and at a constant temperature of 26°C under a 14:10-h light/dark photoperiod (16). Cells were harvested during exponential growth.

Coral samples gathered from Admiral Reef, Alligator Reef, or Little Grecian Reef in the northern Florida Keys included *P. astreoides*, *M. faveolata*, and *P. furcata*. Other samples were collected from Florida Bay directly offshore such as coral *Siderastrea radians*, anemones *A. pallida* and *B. annulata* and jellyfish *C. xamachana* (see Table 1 for a list of cnidarians analyzed). Specimen were immediately placed in fresh seawater and dark adapted for at least 60 min before SIP assays. *Symbiodinium* were genotyped by using PCR-DGGE analysis of the rDNA ITS2 region as described by LaJeunesse and Trench (40) and LaJeunesse (5).

SIP Fluorescence Analyses. Chlorophyll fluorescence of cultured *Symbiodinium* was measured by using a 101/103 PAM fluorometer (Walz) with the ED-101US/MD optical unit and HPL-L470 blue light-emitting diode (LED) source for saturating flashes. A Hansatech LS2 halogen light source was used for actinic white light (250 μM quanta $m^{-2} \cdot s^{-1}$) and when filtered, far-red irradiation (>690 nm). Culture samples were concentrated 10-fold by centrifugation, resuspended in fresh media, and dark-adapted for at least 20 min. Except for the comparison of anaerobic and aerobic experiments described below, cells were constantly stirred by using a cuvette mixer.

Cnidarian samples and cultured cells settled at the bottoms of culture flasks were assayed after dark-adaptation for at least 1-h (cnidarians) and 20 minutes (culture cells) with the submergible Diving-PAM (Walz), configured with a blue LED ML source. FR was not used for these samples but the fluorescence responses among clades were similar in cultures and *in hospice* specimens.

Baseline fluorescence (F_0 and F_0') was monitored by a modulated ML (<1 μmol quanta $m^{-2} \cdot s^{-1}$) to assess minimal fluorescence from PSII in dark periods. For SIP, maximal fluorescence (F_m and F_m') was measured by administering a 1-s saturating flash of 470 nm of light (≈5000 μmol quanta $m^{-2} \cdot s^{-1}$) every 20 s beginning at 30 s into the sampling period. When used, MV (1,1′-dimethyl-4,4′-bipyridium-dichloride) was added at a final concentration of 50 μM.

Anaerobic Measurements. Cultures were incubated in the dark for 10 min in the presence of 10 mM bicarbonate to maximize carbon fixation rates during subsequent light treatments. The samples also were bubbled with air or argon without stirring to impose aerobic and anaerobic conditions, respectively, during the dark adaptation and fluorescence measurement periods.

Fluorescence Spectra. Whole cell low-temperature (77 K) emission and excitation spectra were measured by using a SLM-Aminco SPF-500 spectrofluorometer. Cells were harvested and maintained for 100 min in darkness or 250 μM quanta $m^{-2} \cdot s^{-1}$ actinic light. Immediately after treatment, samples were quickly suspended in 40% glycerol and frozen in liquid nitrogen in a custom glass Dewar. Emission spectra were measured with 440-nm or 530-nm excitation. Excitation spectra were measured at 673 nm or 683 nm and corrected for instrument response.

ACKNOWLEDGMENTS. We thank D. W. Kemp, D. J. Thornhill, and T. C. LaJeunesse for field and laboratory support and A. A. Reynolds for reviewing the manuscript. Research Funding was provided by National Science Foundation Grant OCE-0137007. J.M.R. is supported by a Dr. Nancy Foster Scholarship through the National Oceanic and Atmospheric Association.

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