INCREASED LIGHT INTENSITY INDUCES HEAT SHOCK PROTEIN HSP60 IN CORAL SPECIES

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Introduction

- The effect of increased light intensity and heat stress on heat shock protein Hsp60 was examined in two coral species using a branched coral and a laminar coral, selected for their different resistance to environmental perturbation.
- Coral reefs are one of the most diverse and valuable marine ecosystems on earth.
- Coral bleaching, which results in massive death of coral beds, can be triggered by environmental stresses.
- Heat shock proteins (Hsps) play important roles in cellular repair and enable cells to acquire cytoprotection against stressful conditions.
- Investigations of Hsps in most organisms have not examined increased light intensity as a stressful stimulus.
- The observation that light intensity affects the health of coral beds and a recent report demonstrating the effect of light on heat shock protein Hsp60 homologs in cyanobacteria influenced the design of our present coral investigation that focuses on Hsp60 and includes, as stressful conditions, both light stress and elevation of water temperature.

Materials and Methods

Collection and maintenance of corals. *S. pistillata* (Sty, an environmentally sensitive branched coral) and *T. reniformis* (Tur, a more resistant laminar coral), collected from the Red Sea, were maintained under laboratory culture conditions. Nubbins (coral tips) were cultured in aquarium tanks with sea water of constant salinity and pH at normal temperature of 27°C.

Heat and light stress of corals. Nubbins were subjected to the following conditions: (a) control (27°C, low light [LL; 200 µmol photon m- 2 s- 1]), (b) high light (HL; 27°C, high light [400 µmol photon m- 2 s- 1]), (c) heat shock (HS) at 32°C under LL, and (d) HS under HL. Three nubbins of each species were then collected after 24 and 48 h, frozen in liquid nitrogen, and stored at - 80°C.

Photosynthetic efficiency measurement. Maximal quantum yield (dark adapted Fv/Fm), a parameter for photosynthetic efficiency, was measured at each condition on six nubbins using a DIVING PAM (Walz, Germany) after either 24 or 48 h of stress treatment.

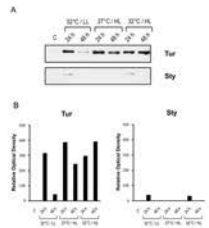
Coral sample preparation and Western blotting. Nubbins were reduced to a powder by grinding under liquid nitrogen, weighed, and proteins extracted in 20 µl/g of cold buffer containing 500mM potassium phosphate (pH 7.8), 10% L-ascorbic acid, 20 mM phenyl methyl sulfonyl fluoride, and 10 mg/ml protease inhibitor cocktail. Skeleton fragments were removed by centrifugation on a nylon mesh (1,000×g, 10 min, 4°C) and extracted proteins were freeze-dried. Samples were solubilized in Laemmli buffer and boiled for 20 min, before centrifugation for 15 s at 13,000 rpm to remove insoluble particles. Equal loadings of 50 µg of protein per lane were separated by 12% SDS-PAGE and subjected to Western blotting analysis with specific antibody for Hsp60 (SPA-807, StressGen). Results were substantiated using a second Hsp60 antibody (SPA-805).

Sequence and structural analysis of HSP60/GroEL sequences. Protein structure and sequence of GroEL were obtained from Protein Data Bank (PDB id: 1GRU). Viewed by PyMol (http://www.pymol.org), the apical, hinge, and equatorial domains were color annotated on the protein sequence. Hsp60 amino acid sequences for human, sea anemone, and plant (*Arabidopsis thaliana*) were obtained from the National Center for Biotechnology Information database, with sequence ids P10809, AAR88509, and NP_189041, respectively. They were aligned against GroEL sequence, using ClustalW multiple sequence alignment (http://align.genome.jp/).

Statistical analysis. Comparison of Fv/Fm between treatments was tested using two-way analysis of variance (ANOVA).

Results

Figure 1. Effect of light and thermal stresses on induction of heat shock protein Hsp60 in the environmentally sensitive branched coral *S. pistillata* (Sty) and the more resistant laminar coral *T. reniformis* (Tur)

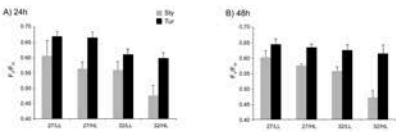


A) Immunoblotting demonstrated a robust transient induction of Hsp60 in Tur at 24 h in response to heat stress (32°C) or high light conditions (HL; 400 µE).

- A more sustained Hsp60 induction at 48 h was observed after heat stress in the presence of high light.
- Sty exhibited a slight transient induction of Hsp60 at 24 h in response to heat stress but not after increased light intensity.
- Hsp60 signal was not detected at control conditions (C; 27°C and low light [LL; 200 µE]).

B) Relative optical density of the Western blot.

Figure 2. Effect of light and heat stresses on coral photosynthetic activity



Photosynthetic efficiency of the branched coral (Sty) and the laminar coral (Tur) were measured after 24 (a) and 48 h (b) at 27°C or 32°C under low (LL) or high (HL) light intensity.

- Light stress and heat stress decreased photosynthetic efficiency at 24 h and 48 h in Sty. No recovery was observed.
- Heat stress, but not light stress, decreased photosynthetic efficiency at 24 h in Tur. By 48 h, Tur had recovered and exhibited control values of photosynthetic activity.
- The laminar coral (Tur) has been reported to better tolerate adverse conditions in the natural environment compared to the branched coral (Sty).
- Tur demonstrated an enhanced ability to induce Hsp60 in response to light and heat stress, and its photosynthetic apparatus demonstrated the ability to withstand these stressful conditions more effectively.

Table 1. Two-way ANOVA testing the effect of light and heat stresses on maximal photosynthetic efficiency of the branched coral (Sty) and the laminar coral (Tur) after 24 and 48 h of incubation

	After 24 h		After 48 h	
	F	*p*	*F*	*p*
Sty				
Heat	13.95	**0.002**	75.50	**<0.001**
Light	13.67	**0.003**	43.19	**<0.001**
Heat × light	1.32	0.279	12.34	**0.004**
Tur				
Heat	13.81	**0.002**	3.93	0.071
Light	0.34	0.568	1.13	0.312
Heat × light	0.07	0.795	1.34	0.365

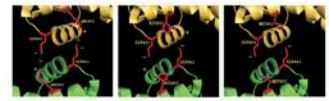
Significant values are in bold

Figure 3. Multiple sequence alignment of Hsp60/GroEL

Comparative amino acid sequence analysis was performed to gain insights into features of Hsp60.

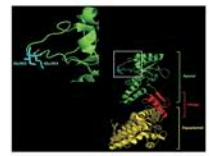
- GroEL, the Hsp60 bacterial homolog, has been the focus of more extensive structural and functional studies.
- Amino acid sequence information for sea anemone, a closely related Cnidarian sessile marine animal, was employed since coral Hsp60 sequences are lacking in the literature.
- The three major domains of GroEL are the equatorial domain (yellow boxes with solid triangle, alignment positions 1–189 and 421–590), the hinge domain (pink boxes with solid squares, alignment positions 190–224 and 410–420), and the apical domain (dark green box with solid circle, alignment positions 226–408).
- The salt-bridge: The two red boxes correspond to GroEL amino acids R452 (asterisk, alignment position 487) and E461 (number sign, alignment position 496) that take part in salt-bridge formation, which stabilizes the double-ring structure in GroEL.
- The eight amino acids involved in polypeptide binding: The six blue boxes (open circle, within alignment position 233 to 298) represent the eight hydrophobic amino acids that are required for the binding of the unfolded polypeptide to the interior wall of the cavity created by the GroEL–GroES complex.
- Ala/Gly insertion at the apical domain of Hsp60: The insertion of a small amino acid at alignment position 339 is shown using a cyan box (open triangle, alignment positions 338–340; Gly insertion in human, Ala insertion in sea anemone).
- The conserved Gly-Gly-Met motif: The Gly-Gly-Met repeats at the C-terminal of Hsp60 are shown in an orange box (open square, alignment positions 572–590).

Figure 4A. The salt-bridge



- R452-E461 salt-bridge formation (GroEL) allows GroEL to exist as a double heptameric ring.
- In human Hsp60, substitution of Arg by Met disrupts salt-bridge formation, resulting in a single ring structure.
- Substitution of His in sea anemone could enable adoption of either a single- or double-ring structure depending on environmental conditions.
- At low pH, His acquires a net positive charge allowing sea anemone Hsp60 to mimic the GroEL double-ring structure; at high pH, basic groups of His remain uncharged and sea anemone Hsp60 could behave as its single-ringed human homolog.

Figure 4B. The Ala/Gly insertion at the apical domain of Hsp60



- Compared to plant (Arabidopsis) Hsp60 and GroEL, insertion of an extra amino acid in the apical domain was observed for sea anemone (Ala insertion) and human (Gly insertion).
- As indicated by a white arrow, the insertion corresponds to the apical domain of GroEL, between Glu (GroEL amino acid E304) and Ile (GroEL amino acid I305).
- As the apical domain is involved in polypeptidebinding, it is possible that Cnidarian and human Hsp60 may have different protein substrate specificity when compared to other Hsp60 homologs.

Discussion

- The laminar coral (Tur) demonstrated a robust transient induction of Hsp60 in response to both light and heat stress in contrast to the branched coral (Sty).
- This observation is consistent with the relative susceptibility of branched coral (Sty) to bleaching triggered by environmental conditions resulting in the massive death of coral beds of this species.
- Our observations also demonstrate that increased light intensity and heat stress exhibited a greater negative impact on the photosynthetic capacity of environmentally sensitive branched coral (Sty) than the more resistant laminar coral (Tur).
- These results support a correlation between stress induction of Hsp60 and (a) ability to counter perturbation of photosynthetic capacity by light and heat stress and (b) resistance to environmentally induced coral bleaching.
- Sublethal light stress encountered at midday periods of peak light intensity could trigger Hsp60 synthesis in the laminar coral and protect it from adverse environmental conditions.
- Hsp60 could protect the photosynthetic machinery using a different mechanism other than thermotolerance.
- In Red Sea reefs, where our samples were originally acquired, the branched coral (Sty) is one of the most susceptible coral species to tissue lost and damage.
- We suggest that Hsp60 induction levels could be a potential indicator of resistance to stress-induced coral bleaching in diverse coral species.
- Comparative amino acid sequence analysis was performed to gain insights into features of Hsp60. Although the physiological significance and potential benefits of these sequence variations in Cnidarian Hsp60 are not fully understood, changes in critical domains could potentially affect the regulation and hence kinetics of Hsp60-mediated protein folding in corals.
- Hsp60 in sessile Cnidarian marine animals may exhibit adaptability to changing environmental conditions.
- Single-/double-ring interchangeability in sea anemone, a Cnidarian closely related to corals, could enable Hsp60 to cater for specific sets of binding substrates that may require protein conformational adjustments following stressful environmental conditions.

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