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Research Project - FISH 441

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**Heat Shock Priming of a Temperate Intertidal Anemone, *Anthopleura elegantissima***

**Abstract:**

**Introduction:**

Unlike intertidal fishes and mobile invertebrates, sessile intertidal invertebrates cannot retreat to the relatively stable environment of the sea with the falling tide. Nor can they readily move to take refuge in tide pools. For many sessile intertidal invertebrates, tolerance rather than avoidance is the approach to surviving emersion at low tide. Emersion involves a complex of stressors including hypoxia, high irradiance and thermal stress. And these stressors have a strong influence on the structure of intertidal communities. Tiexiera, T. (2013)

One method of tolerating heat stress employed by organisms across many animal taxa is the expression of suites of chaperone proteins commonly referred to as heat shock proteins. These heat shock proteins act to stabilize and thus maintain the function of enzymes and other proteins which would otherwise be denatured by the heat stress. Once manufactured by the organism, these heat shock proteins do not degrade immediately and persist for a period of time after the heat stress has abated. Thus organisms exposed to non-lethal heat stress may be better prepared for another heat stress event by having residual heat shock proteins already in their cells at the onset of the event rather than having to wait until the proteins can be made to responds. This is referred to as heat shock priming.

This work seeks to assess heat shock priming in *Anthopleura elegantissima*, an intertidal anemone of the Pacific Northwest. Anemones collected from a beach on Puget Sound in Seattle Washington will be subjected to emersion and heat stress daily for one week and before and after hsp90 expression levels will then be compared to control anemones via hsp90 mRNA quantification.

**Methods:**

**Collection**Specimens of Anthopleura elegantissima were collected from South Alki Beach on Elliot Bay in Seattle, Washington during a low tide on November 1st, 2013 between 10 AM and 12 PM. Ambient air temperatures during the collection period were between 7C and 13C and water temperature in Puget Sound was 10C + 2C. Anemones were collected attached to small, buried stones. Most were collected while fully exposed to the air and some were collected submerged in 5-10 cm of tide pool water. The anemones were held in 5 gallon buckets with native seawater at ambient temperatures and were transported to the holding tank within approximately 3 hours of collection.

The holding tank was a large, well-established temperate marine system containing many local marine invertebrate species. Salinity in the holding tank approximated local seawater. Temperature of seawater in the holding tank was a steady 13C and remaining so throughout the treatment period. Anemones were held attached to their rocks in individual plastic containers with lids to prevent roaming. The containers were drilled for water circulation. Lighting above the holding tank was minimal and consisted of a single small fluorescent bulb on 24 hours per day. The lighting did not approximate natural lighting and was not intending to feed the symbiotic zooxanthellae in the anemone tissue. The anemones were not fed for the duration of the treatment.

**Treatment**

The sample set consisted of 2 treatment anemones and 2 control anemones. To simulate the heat stress of emersion during a low tide in summer, treatment animals were subjected to ~90% emersion and a temperature of 23C via heat lamp. Animals subjected to the treatment remained in their individual containers which were removed from the holding tank. Approximately 0.5 cm of holding tank water was allowed to remain in each container. No attempt was made to orient the anemones such that they were partially submerged. Treatment animals were subjected to emersion and heat daily for 2 hours for a period of 7 days. Control animals remained in their individual containers fully submerged in the holding tank water for the duration of the 7 day treatment.

**Sampling**

Tissue samples were taken twice, once just prior to the first treatment and once on the day following the final treatment. The samples were taken from the column and tentacle crown of the anemones and were approximately 8 mm3 in size. Samples were immediately placed in flip-top tubes and put on dry ice. Samples were placed into storage at -80C with 2 hours of being taken.

**RNA Extraction/Isolation**

RNA Extraction: 500uL of TriReagent were added to the 1.5mL snap cap tube containing the thawed tissue. The tissue was carefully homogenized using a disposable pestle. After the sample was completely homogenized, an additional 500uL of TriReagent was added to the tube and the tube closed tightly. The sample was then Vortexed vigorously for 15s. Samples were stored on ice throughout the process. The homogenized tissue was then stored at -80 C.

RNA Isolation: The tissue samples were incubated for 5 minutes at room temperature. Under a fume hood, 200 uL of chloroform was added to each sample and the tubes vortexed vigorously for 30 seconds. The tubes containing the emulsions were placed into a refrigerated microfuge for 15 minutes at maximum speed. The aqueous layer containing the RNA from each sample was transferred to a fresh microfuge tube. To the isolated aqueous layer was added 500 uL isopropanol which was mixed by inverting the tube until the contents appeared uniform. The samples were then incubated at room temperature for 10 min. Next, the samples were centrifuged at maximum speed for 8 minutes. A small greyish pellet of presumed RNA could then be seen affixed to the bottom of each spin tube. All supernatant was removed from the sample. Next we resuspended each pellet of RNA in 100 uL of 0.1% DEPC-H20 by pipetting up and down until the pellet dissolved. Then the samples were incubated at 55C for 5 minutes to help solubilize the RNA. The samples were then removed from the heat, flicked a few times to mix and then placed in storage at -80C.

**Reverse Transcription**

5uL of RNA sample, A first master mix was created using 1 uL Oligu dT and 4 uL of nuclease-free H20 for each trial. 5 uL of this first master mix plus 5 uL of RNA sample were added to 0.5 mL tubes for each of the eight RNA samples and incubated for 5 minutes at 70C. 14 uL A second master mix was created using 5 uL of M-MLV 5X reaction buffer, 1.25 uL of dNTPs, 0.5 uL of M-MLV RT and 0.5 uL of nuclease-free water per trial. 7.25 uL of this second master mix were added to each tube. The tubes were then vortexed and briefly centrifuged. The tubes were then incubated for 60 minutes at 42C and then heat inactivated at 70C for 3 minutes. The tubes were then centrifuged again and the products were put in storage at -80C.

**Quantitative PCR**

In order to test the primers with minimal potential waste of the other components, a qPCR was prepared for 2 cDNA sampes plus a water control. A master mix was created with enough material for 4 trials. The mix for a single trial contained 10 uL of 1X SsoFast EvaGreen Supermix, 8 uL of ultra-pure water, 0.5 uL of 2.5 uM upstream primer and 0.5 uL of 2.5 uM downstream primer. The hsp90 primers for Nematostella vectensis were as follows. Species specific primer sequences for heat shock proteins in *A. elegantissima* were not available.

Forward primer: 5’ -> 3’ ACCTCGGTACCATTGCCAAG

Reverse primer: 5’ -> 3’ ACACTAAAAGCACATACAACTTGA

20 uL of this master mix were added to each of 3 PCR wells. To well 1 was added 1 uL of cDNA from anemone 1 before treatment. To well 2 was added 1 uL of cDNA from anemone 1 after treatment. To well 3 was added ultra-pure water. qPCR was performed with the following conditions. 95°C for 10 minutes, 95°C for 15 seconds, 55 °C for 15 seconds, 72°C for 15 seconds, repeat steps 2 through 4 39 more times, then 95°C for 10 seconds.

**Results:**

Physical Responses of Anemones – Visual Observations – General Stressors ( collection, low light, nutrient levels in water, suboptimal substrate.) Response to tissue sampling. Response to treatment. Response to collection/holding.

Quantification of RNA and cDNA samples

Results of qPCR – 2 runs

**Discussion:**

**References:**

Teixeira, T (Teixeira, Tatiana)[ 1 ] ; Diniz, M (Diniz, Mario)[ 2 ] ; Calado, R (Calado, Ricardo) Coral physiological adaptations to air exposure: Heat shock and oxidative stress responses in Veretillum cynomorium [ 3,4 ] ; Rosa, R (Rosa, Rui)[ 1 ] Source: JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY Volume: 439 Pages: 35-41 DOI: 10.1016/j.jembe.2012.10.010 Published: JAN 2013