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Temporal analysis of gene expression in a field population of the Scleractinian coral *Montastraea faveolata*

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Abstract

Organisms maintain homeostasis and abate cellular damage by altering gene expression. Coral colonies have been shown to produce unique gene expression patterns in response to different environmental stimuli. In order to understand these induced changes, the natural variation in expression of genetic biomarkers needs to be determined. In this study, an array of genes isolated from Scleractinian coral was used to track changes in gene expression within a population of *Montastraea faveolata* from April to October 2001 in the Florida Keys. The profiles of genes observed in this study can be divided into two groups based on expression over this time period. In spring and early summer, May through July, most of the genes show little deviation from their average level of expression. In August and September, several genes show large deviations from their average level of expression. The physiological and environmental triggers for the observed changes in gene expression have not yet been identified, but the results show that our coral stress gene array can be used to track temporal changes in gene expression in a natural coral population.
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Keywords: Biomarker; cDNA array; Coral population; *Montastraea faveolata*; Natural variation; Temporal gene expression

1. Introduction

It has been well documented that coral populations around the world are in serious decline (Done, 1992; Wilkinson, 2000; Lesser, 2004). Decreased recovery from bleaching events, increased susceptibility to disease, impacts on reproduction, lowered diversity and death are some of the physiological responses to factors impacting coral populations. Molecular ecology is a rapidly expanding field of biology that is concerned with applying molecular techniques to address traditional ecological questions. The use of genomic technology, such as DNA arrays, can identify coral responses to environmental change before physiological decline is evident (Snell et al., 2003). The unique expression of a specific suite of genes can provide insight into the molecular mechanisms involved in an organism's response to its environment. Edge et al. (2005) review the use of molecular genetic technology as a method to diagnose coral health.

Organisms alter the expression of specific genes in order to maintain homeostasis and abate cellular damage. For example, coral colonies produce unique gene expression patterns in response to different environmental conditions (Edge et al., 2005). In order to understand these induced changes, the natural variation in expression of genetic biomarkers needs to be investigated. While most of the gene expression studies of non-model organisms are conducted in controlled laboratory conditions, field studies are becoming more common (Wiens et al., 2000; Bais et al., 2003; Morgan et al., 2005). However, few of these studies have investigated the natural variation of gene expression within a population (Lejeusne et al., 2006). In order for gene array technology to be a useful tool for detecting population responses in coral, it needs to be determined whether changes in gene expression can be detected

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above the natural variation in expression within a population over time (Klaper and Thomas, 2004).

The analysis and interpretation of changes in gene expression by Scleractinian coral may pose challenges not encountered in the study of model organisms due to their colonial morphology, ability to reproduce sexually and asexually, and the blurred distinction between species (Knowlton et al., 1997). In addition, gene expression within a single cell varies in complexity and activation (Levsky and Singer, 2003; Oleksiak et al., 2004; Raser and O'Shea, 2005). Some genes are static, exhibiting little variability in expression over time and under different environmental conditions. Other genes exhibit stochastic expression, fluctuating unpredictably over time in response to a variety of conditions. However, inducible genes fluctuate in a predictive manner in response to specific cues from the extracellular or intracellular environment. The expression patterns produced by a suite of these inducible genes incorporated onto an array can provide information on how a population responds under different conditions.

In this study, an array of genes isolated from coral and tracked changes in gene expression in a population of coral through time. Results show that the targeted DNA array can be used to detect changes in gene expression and that subsets of genes show similar patterns of expression over time. The expression profiles of some of the individual genes revealed significantly different levels of expression which were detectable above natural variation within the population of corals investigated. In addition, other genes whose expression was not detected also provided useful information about the presence or absence of some anthropogenic stressors. For example, genes responsive to organopesticides and other xenobiotics were not detected/induced at the time of sampling.

2. Materials and methods

2.1. Coral collections

Fragments of *Montastraea faveolata*, approximately 2 cm^2, were collected from five colonies at a depth of 4 m from East Turtle Shoal (24°43'15"N, 80°55'50"W) in the middle Florida Keys, USA, in 2001. Samples were collected twice a month from this inshore patch reef during a seven month period (April to October) with the exception of a single collection in late April and early October. Coral fragments were transported to the Florida Keys Marine Laboratory (FKLM) on Long Key in closed containers of natural, recirculating seawater. Samples were then processed for subsequent molecular analysis. Excess skeleton was removed with a hammer and chisel, and the samples were ground in 25–30 ml of a phenol based solution (TRIzol®, Invitrogen™) with a mortar and pestle. Homogenization in TRIzol stops cellular activity for long-term storage and preservation of samples used in molecular analyses.

2.2. Environmental data

Environmental parameters including ocean temperature (degrees Celsius), salinity (ppt), photosynthetically active radiation (PAR, umol/m^2/s), and transmissometry (Formazine Turbidity Units, FTU) for April through October, 2001 were downloaded from NOAA's SeaKeys/C-MAN database recorded by the station at Long Key (LONF1, 24° 50' 24" N, 80° 51' 36" W). If environmental data was not available for a collection date in this study, then a calculated mean consisting of two dates before and two dates after the particular date was used (Fig. 1A and B).

2.3. Target development

Total RNA was isolated from a 2 ml aliquot of each homogenized coral fragment following the manufacturer's protocol for TRIzol® (based on Chomezynski and Sacchi, 1987). RNA concentrations were estimated by ultraviolet absorbance at 260 nm and integrity of the ribosomal subunits was confirmed by electrophoresis on a 1% formaldehyde agarose gel. Replicate aliquots of up to 2 μg of total RNA from each sample collection were reverse transcribed using SuperScript™ II reverse transcriptase (Invitrogen) and an oligo (dT) primer (Operon Biotechnologies, Inc.). During reverse transcription, DIG labeled dUTPs (digoxigenin-11-2'-deoxy-uridine-5'triphosophate, alkali-labile; Roche Diagnostics) were incorporated into the transcribed cDNA for subsequent detection using chemiluminescence. Specific conditions of the reverse transcription reaction are described in Edge et al. (2005). For each sample collection, an aliquot of cDNA was added to a high sodium-dodecyl-sulfate (SDS) buffer (Roche Diagnostics) resulting in cDNA concentrations ranging from 30–50 ng ml^{-1}. These DIG-labeled cDNA solutions represent the targets used with a coral array to assess differences in gene expression across collection date.

2.4. Expression profiling

An experimentally designed coral gene array was used to evaluate differential gene expression in the field samples. ESTs on the array correspond to 32 different genes isolated from *Acropora cervicornis* and *Montastraea faveolata* (Morgan et al., 2001; Morgan and Snell, 2002; Edge et al., 2005). These gene fragments cover a range of functions including response to xenobiotic exposure and oxidative stress, maintenance of cellular integrity and respiration, post-translational processing and apoptosis. The cDNAs representing each gene were spotted in triplicate onto each nylon array and samples were analyzed using three replicate arrays to estimate technical error. Edge et al. (2005) describe the development of the array and the preparation of probes on the array.

2.5. Hybridization

DIG-labeled targets were hybridized to the array in order to visualize probes expressed in the total RNA from colonies collected at each date. The hybridization protocol is described in Morgan et al. (2001) and Morgan and Snell (2002). Hybridizations of samples from each date were performed three times using labeled cDNA from different colonies. Nylon membranes were used only once to ensure a consistent correlation between spot intensity and transcript concentration. Membranes exposed

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