The Molecular Stress Response in the Indo-Pacific Model Scleractinian Coral, *Acropora millepora*

Thesis submitted by

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STATEMENT ON THE CONTRIBUTION OF OTHERS

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Publications resulting from the research in this thesis

Seneca F, Forêt S, Ball E, Smith-Keune C, Miller D J and van Oppen M. (2009) Patterns of gene expression in a scleractinian coral undergoing natural bleaching. *Marine Biotechnology*: 10.1007/s10126-009-9247-5.

Forêt S[#], **Seneca F[#]**, de Jong D, Bieller A, Hemmrich G, Augustin R, Hayward DC, Ball EE, Bosch TCG, Agata K, Hassel M and Miller DJ (Accepted) Phylogenomics reveals an anomalous distribution of USP genes in metazoans. *Molecular Biology and Evolution*. [#]These authors contributed equally.

ABSTRACT

A better understanding of the molecular coral bleaching response to environmental stressors is essential to determine how global climate change and water quality degradation affect corals. A central part of an individual's response to biotic or abiotic stressor exposure is the regulation of genes within the cells. The expression pattern of particular genes, which play key roles in the fate of cells under stress, can indicate the level and source of intra-cellular disturbances. The study of gene expression, or transcriptomics, can therefore be used to diagnose the health of an organism or population. The research reported here has for goal to improve our current knowledge of the coral transcriptomic stress response by: (1) providing optimized methods for the measurement of gene expression during coral bleaching in the field (Chapter 2); (2) exploring the changes in gene expression during bleaching and subsequent recovery period (Chapter 3); and, (3) describing a novel coral gene family potentially involved in the stress response (Chapter 4).

Coral bleaching is a major threat to coral reefs worldwide and is predicted to intensify with increasing global temperature. The present study represents the first investigation of gene expression in an Indo-Pacific coral species undergoing natural bleaching which involved the loss of algal symbionts. Quantitative real-time PCR (qRTPCR) experiments were conducted to select and evaluate coral internal control genes (ICGs), and to investigate selected coral genes of interest (GOIs) for changes in gene expression in nine colonies of the scleractinian coral *Acropora millepora* undergoing bleaching at Magnetic Island, Great Barrier Reef (GBR), Australia. Among the six ICGs tested, glyceraldehyde 3-phosphate dehydrogenase and the ribosomal protein genes S7 and L9 exhibited the most constant expression levels between samples

from healthy looking colonies and samples from the same colonies when severely bleached a year later. These ICGs were therefore utilized for normalization of expression data for seven selected GOIs. Of the seven GOIs, homologs of catalase, C-type lectin and chromoprotein genes were significantly up-regulated as a result of bleaching by factors of 1.81, 1.46 and 1.61 (linear mixed models analysis of variance, $p < 0.05$), respectively. I present these genes as potential key coral bleaching response genes. In contrast, three genes, including one putative ICG, showed highly variable levels of expression between coral colonies. Potential variation in microhabitat, gene function unrelated to the stress response, and individualized stress responses may influence such differences between colonies, and need to be better understood when designing and interpreting future studies of gene expression in natural coral populations.

In the next experiment, I used an extensive coral cDNA microarray assay to conduct a novel investigation of transcriptomic changes in colonies undergoing a natural summer bleaching event in the field environment. Four colonies of the Indo-Pacific model reef-building coral, *Acropora millepora*, were sampled *in situ* during a bleaching event and the subsequent recovery period on the GBR, in summer 2000-01. Significant change in the expression of hundreds of genes representing approximately 14 different processes/mechanisms shows that the natural bleaching stress response is fundamentally a general rearrangement involving almost all aspects of the cellular machinery. I identified a large number of genes, additional to those published in prior laboratory experiments, that are involved in previously identified cellular processes as well as new processes and gene groups unique to this study, such as exo- and endocytic pathway, defense and inflammation response, protein/cell degradation and death, cell cycle and division, and DNA/protein repair. Surprisingly, the relationship between the level of stress and changes in expression of hallmarks genes of the stress response, such

as HSPs and antioxidants, remains equivocal. However, the detection of key transcription factors, calcium binding, cytoskeleton and extracellular matrix protein genes provide further information about the characteristics of natural coral bleaching. Moreover, my results from a natural bleaching event enable comparisons to laboratory studies, which both enhance our understanding of the genes and processes implicated in the stress response as well as suggest the limitations and benefits of both approaches.

In the last chapter, I describe a novel coral stress related gene family, the Universal Stress Proteins (USP). Members of the USP family were identified in bacteria in the context of the stress response, and the USP-like domain occurs in a phylogenetically diverse range of prokaryotes, fungi, protists and plants. Here, I report that members of the USP family also occur in the animal kingdom, but that their distribution follows an unusual pattern. USP genes are present in urochordates as well as all Cnidaria and Lophotrochozoa examined, but are not present in any ecdysozoans or non-urochordate deuterostomes. The vast majority of the metazoan USPs are short, single domain proteins, and phylogenetically distinct from the prokaryotic, plant, protist and fungal members of the protein family. Phylogenetic analyses imply that one or a few USP loci were present in the common metazoan ancestor, and have undergone independent expansions in some lineages but have been lost from others. Most of the metazoan USP genes contain introns, the position of one of which is conserved across the Metazoa and possibly also with some of the plant sequences. By contrast, most (22 of 24) of the *Hydra* genes encoding USPs are atypical in that they are intronless and these clustered together in phylogenetic analyses, whereas the remaining *Hydra* sequences contain introns and seem to have counterparts in other cnidarians, urochordates and lophotrochozoans. Expression patterns were determined for several cnidarian USPs, including two genes belonging to the intronless clade, and these imply

a diversity of functions. The apparent paradox of implied diversity of roles despite high overall levels of similarity parallels the situation in bacteria. I hypothesize that the absence of USP genes in ecdysozoans and most deuterostomes may be a consequence of functional redundancy or specialization in taxon-specific roles, for example USP genes have been recently detected in bleaching experiments and may play an important role in the coral stress response. This calls attention to an exciting novel coral gene family for future research.

This thesis provides future research aiming to explain the molecular stress response of reef-building corals with relevant information about the genes involved in the natural coral bleaching response. With greater understanding of the molecular effect of particular stressors and/or stress events on corals, we can better comprehend the expected changes to corals under global climate change, improve our forecasting of coral reef deterioration, and prioritize management of the most serious threats.

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