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Associations between transcriptional changes and protein phenotypes provide insights into immune regulation in corals

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ABSTRACT

Disease outbreaks in marine ecosystems have driven worldwide declines of numerous taxa, including corals. Some corals, such as *Orbicella faveolata*, are particularly susceptible to disease. To explore the mechanisms contributing to susceptibility, colonies of *O. faveolata* were exposed to immune challenge with lipopolysaccharides. RNA sequencing and protein activity assays were used to characterize the response of corals to immune challenge. Differential expression analyses identified 17 immune-related transcripts that varied in expression post-immune challenge. Network analyses revealed several groups of transcripts correlated to immune protein activity. Several transcripts, which were annotated as positive regulators of immunity were included in these groups, and some were downregulated following immune challenge. Correlations between expression of these transcripts and protein activity results further supported the role of these transcripts in positive regulation of immunity. The observed pattern of gene expression and protein activity may elucidate the processes contributing to the disease susceptibility of species like *O. faveolata*.

1. Introduction

Marine disease outbreaks have become one of the most serious threats to marine ecosystems (Burge et al., 2014; Harvell et al., 1999, 2004; Ward and Lafferty, 2004). Increases in marine disease have affected a diversity of taxa including: turtles, corals, marine mammals, mollusks, and urchins (Ward and Lafferty, 2004). Scleractinian corals form the basis of coral reefs (Bozec et al., 2013; Graham and Nash, 2013), which are some of the most diverse ecosystems on the planet (Bellwood and Hughes, 2001; Bellwood et al., 2006; Odum and Odum, 1955; Renema et al., 2008; Roberts, 1995; Sebens, 1994). However, coral reefs are currently in decline due to losses in coral coverage as a result of increasing disease prevalence (Daszak et al., 2001; Harvell et al., 1999; Sutherland et al., 2004).

Some corals, such as the major reef building Caribbean coral *Orbicella faveolata*, are particularly susceptible to disease (Sutherland et al., 2004; Weil, 2004; Weil and Rogers, 2011). *O. faveolata* is affected by as many as eight different diseases, including single pathogen bacterial diseases such as white plague type II, as well as microbial consortium diseases such as yellow band, black band, red band and darks spot syndrome, and diseases of unknown cause such as tumor growth syndrome (Weil, 2004; Weil and Rogers, 2011). Outbreaks of these diseases, coupled with other stressors, have driven massive declines in populations of this species across the Caribbean (Bruckner and Hill, 2009; Nugues, 2002; Weil et al., 2009). Our understanding of *O. faveolata* susceptibility is limited due to a lack of knowledge in coral immunity.

Components of each of the three main processes of invertebrate immunity (i.e. pathogen recognition, signaling pathways, and effector responses) have been documented in corals and are essential to the host's defense against disease (Palmer and Traylor-Knowles, 2012). Pattern recognition receptors (PRRs) are essential for pathogen recognition in invertebrate systems. These molecules recognize and bind to potential microbial pathogens, triggering molecular pathways and inducing immune responses through signaling pathways (Akira and Takeda, 2004; Akira et al., 2006; Janeway and Medzhitov, 2002; Kawai and Akira, 2011; Kumar et al., 2011; Takeuchi and Akira, 2010). Signaling pathways consist of a number of intermediate molecules which promote the necessary changes in gene expression and protein activity to generate an

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effective defense response against potential pathogen(s) (Aderem and Ulevitch, 2000; Akira and Takeda, 2004; Akira et al., 2006; Arthur and Ley, 2013; Newton and Dixit, 2012; O'Shea and Plenge, 2012). This chain of immune events is recognized as the "effector responses". Effector responses include, but are not limited to, the production of antimicrobial compounds and the activation of phagocytic cells (Aderem and Ulevitch, 2000; Medzhitov, 2007; Underhill and Ozinsky, 2002).

Representative segments of all three immune processes have been described in corals and other cnidarians. A number of different immune receptors have been identified, including Toll-like receptors (TLRs) (Burge et al., 2014; Franzenburg et al., 2012; Miller et al., 2007; Poole and Weis, 2014; Shinzato et al., 2011; Wolenski et al., 2011), and various types of lectins (Hayes et al., 2010; Kvennefors et al., 2010; Schwarz et al., 2007; Wood-Charlson and Weis, 2009). Unfortunately, there is a paucity of data supporting their functional role in the recognition of pathogens in coral systems (Burge et al., 2013; Libro et al., 2013). In addition to signaling pathways, such as the melanin synthesis (Mydlarz et al., 2008; Palmer et al., 2008) and complement pathways (Brown et al., 2013; Miller et al., 2007), corals and other cnidarians, have been found to produce a number of immune effector responses such as the antioxidants catalase (Dash and Phillips, 2012; Merle et al., 2007; Palmer et al., 2011), peroxidase (Mydlarz et al., 2009; Mydlarz and Harvell, 2007; Palmer et al., 2011), and superoxide dismutase (Couch et al., 2008; Dash et al., 2007; Richier et al., 2003), as well as antimicrobial compounds (Jensen et al., 1996; Vidal-Dupiol et al., 2011a, 2011b).

Advancements of next generation technology have led to rapid increases in our understanding of coral genomics and transcriptomics (Barshis et al., 2013, 2014; Miller et al., 2011; Palumbi et al., 2014; Pinzon et al., 2015; Shinzato et al., 2011). Many studies have examined modulation of coral immunity in response to a variety of stressors, including immune challenge (Anderson et al., 2016; Burge et al., 2013; Libro et al., 2013; Libro and Vollmer, 2016; Pinzon et al., 2015; Weiss et al., 2013; Wright et al., 2015). Additionally, many studies have described and documented changes in immune proteins associated with the coral response to disease (Couch et al., 2008; Mann et al., 2014; Mydlarz and Harvell, 2007; Mydlarz and Palmer, 2011; Vidal-Dupiol et al., 2011a, 2011b). However, few studies have used both a genomic and protein-based approach to study coral immune response, resulting in limited understanding of the connections between changes in gene expression and phenotypic response.

In order to better understand the genomic mechanisms underlying the coral immune response, and observed phenotypic differences in this non-model system, this study experimentally exposed colonies of *Orbicella faveolata* to bacterial pathogen-associated molecular patterns (PAMPs) and examined associated changes in both protein activity and gene expression. By leveraging existing knowledge and well-developed protein activity assays, gene expression and protein activity data were correlated to one another using novel analytical techniques in order to better understand the connections between gene expression and proteins. Using these techniques we show that complementing gene expression with protein activity data can provide new insight regarding the response of corals to immune challenge and provide a more holistic image of coral response to immune challenge.

2. Methods

2.1. Sample collection

Coral fragments were collected in July of 2012 from five randomly selected *Orbicella faveolata* colonies from on Media Luna reef (17° 56.096 N; 67° 02.911 W) near La Parguera, Puerto Rico. Six small fragments (5 × 5 cm) were chipped off randomly from each colony with a hammer and chisel for a total of 30 fragments. Upon collection, the fragments were placed in labeled zip-lock bags and transported in ambient seawater to an indoor running saltwater facility at the Dept. of Marine Sciences (University of Puerto Rico – Mayagüez in Isla Magueyes). At the facility, three fragments from each colony were randomly assigned to one of the two treatment groups (control and PAMP exposure).

Five fragments from the same treatment were placed in one of six large plastic containers. Each container was aerated using an electric air pump and supplied with continuous flow of seawater. To control for temperature, the water was initially contained in a 500 gallon barrel where the temperature was maintained at 26 °C using electric heaters and chillers when needed. Overhead lamps were used to maintain a 12 h light/dark cycle. Fragments were maintained in these conditions for seven days prior to experimentation to allow for acclimatization and healing from fragmentation.

2.2. Experimental design

Following the acclimatization period, continuous water flow and aeration were ceased and water levels in each of the large containers reduced to 3L. A piece of PVC pipe (6.35 cm high and 5.08 cm wide) was placed around each coral fragment, making a temporary microenvironment. Using a micropipette, 1 mL of 7.57 mg/mL lipopolysaccharides (LPS), a pathogen-associated molecular pattern (PAMP), from *Escherichia coli* 0127:B8 (Sigma-Aldrich L3129-100MG) was added just above the surface of each treatment fragment. Final concentration of LPS in the container was 10 μg/mL spread over the five fragments on each PAMP exposure container. Control fragments received 1 mL of sterile seawater used in preparation of the LPS solution.

Exposure conditions were maintained for 30 min to ensure the LPS was taken into the coral, after which the aeration was resumed. Then the fragments were allowed in continuous flow for an additional 4 h before being removed and frozen in liquid nitrogen. All samples were shipped on dry ice to the University of Texas at Arlington, where they were divided in two, leaving a small (~1 cm^2) piece for RNA extraction and the rest for protein extraction. Samples were then stored at −80 °C until tissues were collected.

2.3. Protein extraction

Proteins were extracted over ice using a Paansche airbrush (Chicago, IL, USA) with coral extraction buffer (50 mmol tris buffer, pH 7.8, with 0.05 mmol dithiothreitol). Tissues were then homogenized using a Power Gen 125 tissue homogenizer with a medium saw tooth generator (Fisher Scientific, Pittsburgh, PA, USA) for 60 s on ice. Samples were then left on ice for 10 min. From the resulting extract, 1 mL was reserved for melanin analysis. The remaining volume was centrifuged for 5 min at 4 °C and 3500 RPM in an Eppendorf centrifuge 5810R. The resulting supernatant, or coral extract, was split into two ~2 mL aliquots mL which were frozen in liquid nitrogen and stored at −80 °C (Mydlarz and Palmer, 2011).

Total protein in each sample was determined using the Red660 protein assay (G Biosciences, St. Louis, MO) standardized to BSA. These concentrations were used to standardize all biochemical assays conducted on the samples. All colorimetric assays were run in duplicate on 96 well plates using a Synergy two multi-detection microplate reader and Gen5 software (Biotek Instruments, Winooski, VT, USA).