Gene expression of settled and metamorphosed *Orbicella faveolata* during establishment of symbiosis

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Abstract

Corals rely on a symbiosis with dinoflagellate algae (*Symbiodinium* spp.) to thrive in nutrient poor tropical oceans. However, the coral-algal symbiosis can break down during bleaching events, potentially leading to coral death. While genome-wide expression studies have shown the genes associated with the breakdown of this partnership, the full conglomerate of genes responsible for the establishment and maintenance of a healthy symbiosis remains unknown. Results from previous studies suggested little transcriptomic change associated with the establishment of symbiosis. We examined the transcriptomic response of the coral *Orbicella faveolata* in the presence (symbiotic) and absence (aposymbiotic) of *Symbiodinium minutum*, one of its associated symbionts. 9 days post-metamorphic aposymbiotic coral polyps of *O. faveolata* were compared to symbiotic coral polyps and the subsequent differential gene expression between control and treatment was quantified using cDNA microarray technology. Coral polyps exhibited differential expression of genes associated with nutrient metabolism and development, providing insight into control of pathways as a result of symbiosis driving early polyp growth. Furthermore, genes associated with lysosomal fusion were also up-regulated, suggesting host regulation of symbiont densities soon after infection.

Introduction

Coral reefs are found in nutrient poor areas (Hoegh-Guldberg 1999; Muscatine & Porter 1977) and they are metabolically maintained by a symbiosis with the photosynthetic dinoflagellates of the genus *Symbiodinium*. Under this stable condition, the symbiont provides photosynthetic products in the form of glucose, succinate/fumarate, and glycerol (Burriesci et al. 2012; Muscatine 1990). In return, the coral provides shelter, nitrogen and inorganic carbon to the symbiont (Muscatine & Cernichiari 1969). As a result of this nutrient exchange, coral calcification increases during *Symbiodinium* photosynthesis (Colombo-Pallotta et al. 2010; Holcomb et al. 2014). While much of the research on coral-dinoflagellate symbiosis focus on its breakdown during bleaching (DeSalvo et al. 2008; Weis et al. 2008), we lack a mechanistic understanding on how the symbiosis is established and maintained.

Previous studies have suggested surface binding proteins to be involved in early recognition of the symbiont (Davy et al. 2012). Pattern recognition receptors (PRR) (e.g. lectins) bind and detect surface molecules (e.g. glycans) to establish contact and induce a subsequent signaling cascade (Fransolet et al. 2012; Kvennefors et al. 2010; Wood-Charlson et al. 2006), Upon phagocytosis, the symbiont is taken up by an endosome and is either digested by lysosomal degradation (Hohman et al. 1982) or maintained in the early endosome. This stage represents a modified vacuole known as the symbiosome, where the symbiont resides without further progression into a phagosome (Fitt & Trench 1983; Wakefield & Kempf 2001). This phagosomal maturation is arrested by members of the Rab family through prevention of lysosomal fusion (Chen et al. 2004; Chen et al. 2005).

Studies have utilized both genomic and transcriptomic approaches to uncover the genes involved in the establishment of symbiosis. Two such studies have suggested that there are

76 little to no changes in the transcriptomic profile during the early onset of symbiosis, with the
77 number of DEGs and their respective fold changes being generally small (Schnitzler & Weis
78 2010; Voolstra et al. 2009). This led authors to suggest that the symbiont is evading host
79 detection-or alternatively, that a modulation of existing pathways could be the hallmark of
80 establishing and maintaining a successful symbiotic relationship. The process of escaping the
81 host immune system is a common strategy employed by both symbiotic and pathogenic
82 microorganisms. For example, the pathogen *Mycobacterium turberculosis* prevents
83 phagosome-lysosome fusion in order to escape degradation by manipulating localization of
84 Rab proteins to the phagosome (Vergne et al. 2005). One study has found the involvement of
85 the TGF-β cytokine pathways in the symbiont tolerance of *Exaiptasia pallida* (Detournay et
86 al. 2012) where infection by the symbiont was reduced when the pathway was blocked.
87 Hence, current research suggests manipulation of the host immune system to be involved in
88 symbiont entry and maintenance. Modulation of additional existing pathways has been shown
89 in other microarray studies, which investigated the differences between the symbiotic and
90 aposymbiotic state and reported expressional changes for genes involved in cell adhesion,
91 cytoskeletal activity, cell cycle, protein biosynthesis, response to stress, metabolism,
92 transcriptional regulation, immune response, and RNA modification (DeSalvo et al. 2008;
93 Rodriguez-Lanetty et al. 2006; Schnitzler & Weis 2010; Voolstra et al. 2009).
94
95 Previous experiments performed in symbiotic corals have been conducted with competent
96 larva (DeSalvo et al. 2008; Rodriguez-Lanetty et al. 2006; Schnitzler & Weis 2010) sampled
97 at different time points (Meyer et al. 2011). In this study, we compared the transcriptomic
98 response of the coral *Orbicella faveolata* during the onset of infection with *Symbiodinium*
99 *minutum* to aposymbiotic polyps. The analysis we present here sampled post-metamorphic
100 polyps stage to eliminate confounding factors as a result of larval development. A 9-day post-

infection time point (sampling 16 day old polyps) was chosen to evaluate the genes within the host responsible for the maintenance of symbiosis. We hypothesize that this later stage presents the best proxy for the adult polyp in which we still can compare the aposymbiotic to the symbiotic transcriptomic response.

Materials and Methods

1. Larval Collection, Rearing, and Experimental Setup

For infection of juvenile coral polyps, we used *Symbiodinium minutum* (type Mf1.05b), a Clade B1 symbiont. This *Symbiodinium* type has been shown to successfully re-infect and establish a stable endosymbiosis with *O. faveolata* (Voolstra et al. 2009). Cultures of *S. minutum* were maintained in Puerto Morelos at 24°C under a 12hr:12hr light (fluorescent light with 50 $\mu molm^{-2}s^{-1}$) dark cycle in ASP-8A medium. Egg-sperm bundles were collected from adult colonies of *O. faveolata* on September 10th, 2009 in Puerto Morelos, Quintana Roo Mexico from the La Bocana site (20° 52'28.77"N and 86°51'4.53"W) at four meters depth. The collection permit was provided by SAGARPA (No. DGOPA 12035.121108.2312) Fine mesh nets (1.75 m wide, 2 m high) were placed over six colonies before spawning and secured to surrounding rocks by small weights. Buoyant gamete bundles were collected in plastic jars fixed to the top of each cone-shaped net. Bundles of different colonies were mixed in a cooler with 1 µm filtered seawater (FSW) that was sterilized using ultraviolet light. The egg-sperm solution was mixed gently to break the bundles and increase fertilization rates. After a one-hour incubation, excess sperm was removed by repeatedly washing with FSW until the water was clear. The embryos were initially raised in large plastic coolers (150 liters), containing UV treated FSW and kept at a constant 29ºC. Healthy embryos were then evenly distributed to smaller polypropylene containers (6 liters) at a density of >5 embryos per ml. Water was changed every other day and kept at a constant 29ºC. Once the embryos

126 developed into the planula stage, they were randomly assigned to the infection treatment (*S.*
127 *minutum*) and control (n = 3 replicates per treatment, approximately 1,000 coral larvae per
128 replicate). Cultures of *Symbiodinium minutum* were grown in ASP-8A media at 12:12 light
129 dark cycle at 150 μ mol quanta $m^{-2}s^{-1}$. Planula larvae settled and metamorphosed into sessile
130 polyps seven days after fertilization. For infection (i.e. symbiotic treatment), *S. minutum* was
131 added to three replicates at an initial concentration of 3 x 10^5 cells/ml and stable
132 concentrations of *S. minutum* were ensured by regular reinfection after daily water changes
133 for nine days. The state of infection by *S. minutum* was confirmed every two days using
134 microscopy by sampling ten polyps and flattening them under a microscope slide. For the
135 control treatment, the growth media (ASP-8A) without *S. minutum* was added to another set
136 of three replicates. 9 days after infection (16 days post-fertilization), settled polyps were
137 cotton-swabbed from sides of the polypropylene containers, preserved in RNAlater (Ambion),
138 and stored at -80°C for further processing.
139
140 2. RNA isolation and amplification
141 To isolate total RNA from the cotton swabs, microcentrifuge tubes with RNAlater and cotton
142 swab heads were centrifuged for 10 min at 12,000 x g. Swabs were wiped across the interior
143 surfaces of the tubes using tweezers to collect any pelleted coral tissue from the tubes. Swabs
144 were then placed in a mortar containing liquid nitrogen and ground into a powder. The
145 powder was removed with a spatula and placed in a 2 ml screw cap tube. To each tube, 1.5
146 ml of Qiazol (Qiagen) was added. Samples were then homogenized for 2 min using a Mini
147 Bead-Beater (Biospec) with both 0.1 mm and 0.55 mm silica beads. To each tube, 450 µl of
148 chloroform was added. Tubes were then vortexed for 30 seconds and incubated at RT for 3
149 min. Each sample was centrifuged at 12,000 g for 15 min at 4°C. From the aqueous layer,
150 500 µl were transferred to a new tube and RNA was precipitated by adding 500 µl of 100%

isopropanol and 5 µl of glycerol (20 ng/µl). To pellet the RNA, tubes were vortexed for 30 s, then incubated at RT for 10 min, then centrifuged for 15 min under the same conditions as above. The isopropanol was removed and RNA pellets were washed twice with 70% EtOH and centrifuged at maximum speed for 5 min at 4°C. The wash and centrifugation step was repeated a second time. RNA pellets were then air-dried for 10 min and resuspended in 50 ul RNase-free water. The RNA was further purified using the RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. RNA was assessed using a NanoDrop ND-1000 spectrophotometer. For each experimental replicate, 1 ug of total RNA was amplified using the MessageAmp II aRNA kit (Ambion) according to manufacturer's instructions.

3. Microarray hybridization

Microarray hybridization was completed following the protocol described by DeSalvo et al. (2008) with some modifications. Prior to hybridization, microarrays were post-processed by1) ultraviolet crosslinking at 60mJ 30 seconds, 2) a 'shampoo' treatment (3x SSC, 0.2% SDS at 65°C 2 minutes), 3) a blocking step by incubating microarrays in 5.5 g of succinic anhydride dissolved in 335 ml 1-methyl-2-pyrrolidinone and 15 ml of sodium borate, and 4) drying by centrifugation. The microarray consisted of 10,930 PCR-amplified cDNAs spotted in duplicate on poly-lysine-coated slides yielding a microarray with 21,860 total features and is referenced as Mfaveolata_11k_v1. Spotted cDNAs were chosen from EST libraries partially described by Aranda et al. (2011). For annotation, ESTs from the Mfav_v1 microarray (GEO accession No: GPL13114) were downloaded from the EST database at http://sequoia.ucmerced.edu/SymBioSys and successively queried against the UniProt, SwissProt, and TrEMBL databases (2015) using BLASTX (Evalue cutoff ≤1e-5). Gene Ontology (GO) terms were subsequently assigned using the GOA database (Dimmer et al. 2012). In addition, the ESTs were annotated against the Kyoto Encyclopedia of Genes and

Genomes (KEGG) database using the KEGG Automatic Annotation Server (KAAS) (http://www.genome.jp/tools/kaas/) and the bi-directional best hit (BBH) method. For each experimental treatment (i.e., 3 control polyps and 3 polyps infected with *S. minutum*), 3 µg of aRNA were primed with 10µM random pentadecamers for 10 min at 70°C for subsequent cDNA generation (see below). A pooled reference was created by combining 3 µg of aRNA from both experimental treatments and processed accordingly. Reverse transcription was carried out for 2 hours at 50°C using SuperScript III Reverse Transcriptase (Invitrogen) containing a 4:1 ratio of aminoallyl-dUTP to TTP (Ambion). After reverse transcription, RNA was hydrolyzed by adding EDTA and NaOH for a final concentration of 0.1M and 0.2M, respectively, for 15 minutes at 65°C. Following hydrolysis, HEPES was added at a final concentration of 0.5M. Reactions were cleaned using the MinElute Cleanup kit (Qiagen) according to manufacturer's instructions. cDNAs were labeled with Cy3 and Cy5 fluorescent dyes for sample and reference respectively. Briefly, cDNA was added to 4.5 nmol of dye dissolved in 1 M DMSO, and incubated in the dark for two hours. Dye-coupled cDNAs were cleaned using the MinElute Cleanup kit (Qiagen) according to manufacturer's instructions. Each Cy3-labeled treatment was hybridized to the array together with a Cy5-labeled pooled reference sample. Briefly, 12µl of treatment and 12µl of reference were combined with 6µl of hybridization buffer containing 0.25% SDS, 25 mM HEPES, and 3X SSC. Samples were heated to 99°C for 2 min and pipetted into the space between a microarray glass slide and an mSeries Lifterslip (Erie Scientific). Microarrays were hybridized for 14 hours at 63°C and subsequently washed twice in 0.6X SSC and 0.01% SDS followed by a rinse in 0.06X SSC and dried via centrifugation. Slides were immediately scanned using an Axon 4000B scanner.

4. Data analysis

After scanning the microarrays, annotation grid files were overlaid and fit for feature extraction of scanned images. GenePix Pro (Molecular Devices) software was used to extract background-subtracted spot intensities that gave rise to 6 GPR files. GPR files were subsequently converted to MEV files using TIGR Express Converter 4.0 (Saeed et al. 2003). Microarray data were normalized using TIGR MIDAS 2.21 with printtip-specific LOWESS followed by in-slide replicate analysis. Genes were included in subsequent statistical analyses only if present in two out of three replicates. DEGs between uninfected (control) and *S. minutum*-infected (treatment) polyps were determined via 2-class unpaired SAM analyses and a FDR ≤ 0.05 in MeV software. A GO enrichment analysis was performed using the R package topGO (Dimmer et al. 2012) in order to identify biological processes that are overrepresented among the DEGs. Enriched GO terms (FDR ≤ 0.05) were imported to REViGO (Supek et al. 2011) for redundancy removal and data visualization. Nodes in the resulting clusters were mapped back to common differentially expressed ESTs. Genes shared within the clusters were referenced to the KEGG database for pathway information. The expression data was deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number (GSE 92695).

Results

The two-class unpaired SAM analysis identified 866 significantly DEGs (FDR ≤ 0.05, Table S1), representing about 7.94% of all genes assayed on the microarray. Of those, 862 genes were up-regulated and only 4 genes were down regulated in the symbiotic state. The $\log_2$ fold-change for up-regulated genes ranged between 0.74 and 3.19 (mean = 1.35), while down-regulated genes ranged from -1.79 to -2.54 (mean = -2.18).

Of the 862 up-regulated genes, 331 had identifiable homologs within the UniProt database (Table S1). 38 biological processes were significantly enriched among the DEGs according to the topGO analysis (FDR ≤ 0.05, Table 1).

	GO ID	Term	Expected	result1
1	GO:0046473	phosphatidic acid metabolic process	0.29	0.0017
2	GO:1901698	response to nitrogen compound	9.04	0.0032
3	GO:0006662	glycerol ether metabolic process	0.35	0.0033
4	GO:0010976	positive regulation of neuron projection development	1.73	0.0035
5	GO:0071398	cellular response to fatty acid	0.4	0.0055
6	GO:0060324	face development	0.52	0.0095
7	GO:0009154	purine ribonucleotide catabolic process	0.52	0.0122
8	GO:0030878	thyroid gland development	0.52	0.0122
9	GO:0010498	proteasomal protein catabolic process	5.35	0.018
10	GO:0016079	synaptic vesicle exocytosis	0.63	0.0182
11	GO:0007492	endoderm development	0.81	0.0183
12	GO:0061077	chaperone-mediated protein folding	1.09	0.0209
13	GO:0016042	lipid catabolic process	5.24	0.0217
14	GO:0006508	proteolysis	18.88	0.0218
15	GO:0035315	hair cell differentiation	1.21	0.0219
16	GO:0006376	mRNA splice site selection	0.63	0.022
17	GO:0010888	negative regulation of lipid storage	0.29	0.0294
18	GO:0010889	regulation of sequestering of triglyceride	0.29	0.0294
19	GO:0043486	histone exchange	0.29	0.0294
20	GO:0045616	regulation of keratinocyte differentiation	0.29	0.0294
21	GO:0045606	positive regulation of epidermal cell differentiation	0.29	0.0294
22	GO:0043252	sodium-independent organic anion transport	0.29	0.0294
23	GO:0090208	positive regulation of triglyceride metabolic process	0.29	0.0294
24	GO:0042853	L-alanine catabolic process	0.29	0.0294
25	GO:0060253	negative regulation of glial cell proliferation	0.29	0.0294
26	GO:0045599	negative regulation of fat cell differentiation	0.29	0.0294
27	GO:0060872	semicircular canal development	0.29	0.0294
28	GO:0035239	tube morphogenesis	3.97	0.0298
29	GO:0031338	regulation of vesicle fusion	0.35	0.0424
30	GO:1901983	regulation of protein acetylation	0.35	0.0424
31	GO:1902106	negative regulation of leukocyte differentiation	0.35	0.0424
32	GO:0021772	olfactory bulb development	0.35	0.0424
33	GO:0060416	response to growth hormone	0.35	0.0424
34	GO:0010165	response to X-ray	0.35	0.0424
35	GO:0097150	neuronal stem cell population maintenance	0.35	0.0424
36	GO:0043647	inositol phosphate metabolic process	0.35	0.0424
37	GO:0061462	protein localization to lysosome	0.35	0.0424
38	GO:0046717	acid secretion	0.81	0.0427

Table 1: enriched biological process using topGO. 38 biological processes were significantly enriched among DEGs according to topGO analysis.

Terms related to development, cellular homeostasis, gene expression, and metabolic/catabolic processes comprised a major portion of the overrepresented terms. To remove redundant GO terms, overrepresented biological processes were further analyzed using REViGO (Figure 1).

Terms grouped into three main clusters associated with protein and nutrient recycling, development, and lysosomal activity. One large cluster consisted of 8 nodes, which loosely groups to protein and lipid metabolism: purine ribonucleotide catabolism (GO:0009154), glycerol ether metabolism (GO:0006662), proteolysis (GO:0006508), chaperone-mediated protein folding (GO:0061077), proteasomal protein catabolism (GO:0010498), L-alanine catabolism (GO:0042853), regulation of protein acetylation (GO:1901983), and inositol phosphate metabolism (GO:0043647). The second cluster consisted of terms associated with development, including mRNA splice site selection (GO:0006376), endoderm development (GO:0007492), histone exchange (GO:0043486), positive regulation of neuron projection development (GO:0010976), face development (GO:0060324), phosphatidic acid metabolic process (GO:0046473), positive regulation of triglyceride metabolic process (GO:0090208).

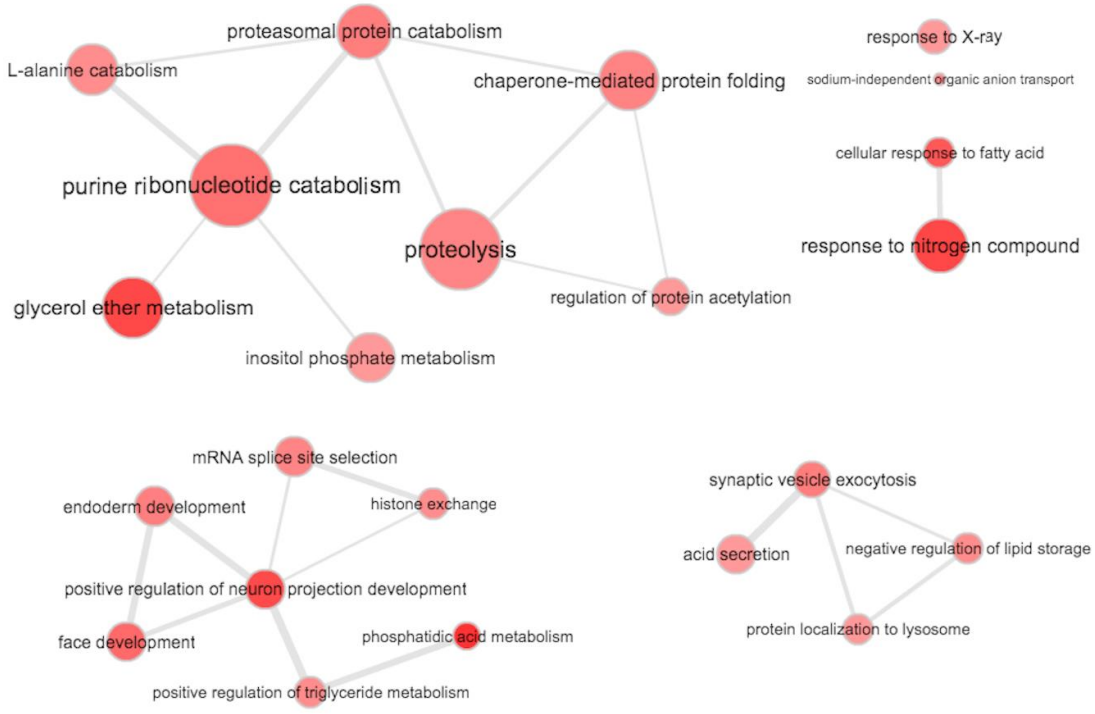

Figure 1: GoTerms grouped into three main clusters when analyzed in Revigo. These terms are associated with protein and nutrient recycling, development, and lysosomal activity.

Contrary to the greater number of up-regulated genes at 9 days, only 4 genes were found to be down-regulated. Three genes had identifiable homologs in the UniProt database. The three genes were identified as 40S ribosomal protein S4 (AOSF1416), 60S ribosomal protein L7a (CAON914), and Guanine nucleotide-binding protein (CCHW9377).

Discussion

One particular challenge we face with understanding symbiosis in scleractinian corals is that coral hosts do not exist in an aposymbiotic state, i.e. adult corals always host symbionts and hence a non-symbiotic control is unavailable (even during coral bleaching, a percentage of *Symbiodinium* cells remain in the coral tissue). Examining *Symbiodinium* infection in larvae of free spawning corals provides an opportunity to shed light on the onset of coral-algal symbiosis. Fertilized *Orbicella faveolata* eggs develop from embryos into sessile polyps in approximately 7 days (Szmant 1991) at which point embryonic development is assumed to be complete, providing us with an adult-like aposymbiotic system. Here, we analyzed transcriptional changes of 16-day old, settled polyps that were exposed to competent symbionts five days prior to sampling to achieve two objectives: 1) minimize the confounding factor of development and associated gene expression changes, and 2) compare gene expression during establishment and maintenance of symbiosis to an aposymbiotic state.

In line with other studies that target corals (Grasso et al. 2008; Schwarz et al. 2008), a large number of DEGs are often of unknown function. Of the 828 DEGs, 331 were annotated. DEG analysis of overrepresented biological processes revealed many of the themes that have been identified in other studies targeting symbiosis (Davy et al. 2012; Fransolet et al. 2012; Reyes-Bermudez et al. 2009; Richier et al. 2008) but also some new genes and processes that may help to further decipher the genetics of symbiosis in corals. Previous microarray

273 experiments reported few changes in post-infection expression in *Acropora palmata* and *O.*
274 *faveolata*, exhibiting 42 and 17 DEGs at 6 hours post-infection, respectively (Voolstra et al.
275 2009). Gene expression in *Fungia scutaria* 48 hours post infection also exhibited few
276 changes, with only 17 genes found to be differentially expressed (Schnitzler & Weis 2010).
277 In congruence with the microarray studies, an RNAseq experiment measuring gene
278 expression at 4, 12, and 48 hours post-infection in *Acropora digitifera* revealed no
279 measurable changes in gene expression at 12 and 48 hours (Mohamed et al. 2016). However,
280 in stark contrast, at 4 hours post-infection 1073 (2.91%) of genes showed differential
281 expression, indicating differential gene expression with onset of symbiosis to occur within
282 minutes to hours after infection.
283
284 In our study, of the 10,930 genes assayed, 7.92% were differentially expressed. This is
285 potentially an underestimate of the total number of DEGs, with the *Orbicella* genome
286 containing approximately 47,000 genes (Kamel et al. *In Review*). Thus, the data presented
287 here appears to indicate the symbiotic state of *O. faveolata* polyps exhibiting maintenance
288 and symbiosis supported growth. Results of the differential expression analysis revealed
289 genes previously identified to prevent lysosomal fusion, and thus maintenance of the
290 symbiosis. A number of Rab homologs have been associated with the establishment of
291 symbiosis (Chen et al. 2003; Chen et al. 2004; Chen et al. 2005). Rab proteins are members
292 of the wider Ras superfamily of GTPases (Wennerberg et al. 2005) and regulate membrane
293 and vesicle trafficking. In our analysis we identified a homolog of *Rab-3* to be up-regulated
294 in the symbiotic state of *O. faveolata*. Rab-3 has been shown to localize to the symbiosome
295 of the symbiotic non-calcifying cnidarian, *Exaiptasia pallida* (Hong et al. 2009), potentially
296 implicating its role in symbiosome biogenesis and phagosome maturation. Along with *Rab-3*,
297 *Rab-21* was also found to be differentially expressed. Rab-21 has been associated with

298 vesicle transport in addition to having a potential role in membrane recycling (Opdam 2000).
299 In addition to the *Rab-3* and *Rab-21*, we identified other up-regulated genes belonging to the
300 Ras superfamily, including Ras-related protein Rab-10, Ras-related and estrogen-regulated
301 growth inhibitor (*RERG*), and Ras-related protein SEC4. Ras-related protein SEC4 has been
302 shown to be responsible for regulation of vesicular transport in yeast (Haubruck et al. 1990),
303 while RERG has been shown to be involved in transcription regulation and cell proliferation,
304 Expression of the Ras-related protein Rab-21 (CCHW3870) and Ras-related and estrogen-
305 regulated growth inhibitor (CCHW1401) on the symbiosome may help to signal exocytosis
306 of the symbiosome contents. This is implied from their KEGG classification as genes
307 affiliated with the exosomal proteins of haemopoietic cells (B-cell, T-cell, DC-cell,
308 reticulocyte, and mast cells). Terms associated with vesicle fusion (GO:0031338), regulation
309 of protein acetylation (GO:1901983), protein localization to lysosome (GO:0061462), and
310 acid secretion (GO:0046717) suggests dynamic processing and turnover of infecting
311 symbionts.
312
313 In addition to digestion of *Symbiodinium* cells, some of these genes may also be playing a
314 role in autophagy associated with the normal developmental growth of the polyp (Levine &
315 Klionsky 2004). As discussed, only the polyps that had undergone settlement and
316 metamorphosis were assayed in this study to ensure that transcripts that were differentially
317 expressed as a result of metamorphosis were eliminated. Regardless, we identified several
318 genes that are classically regarded as important for development including three up-regulated
319 genes: Forkhead box protein O1 (*FOXO1*; CCHW1209), Mothers against decapentaplegic
320 homolog 3 (*Smad3*; CCHW6627), and Protein bicaudal C homolog 1 (*BICC1*; CCHW1182)
321 (Table 1). GO enrichment analysis identified several terms associated with development
322 (Table 1, Figure 1), including face development (GO:0060324), endoderm development

(GO:0007492), semicircular canal development (GO:0060872). Given our selected time point focusing on post-metamorphic polyps, the up-regulation of developmental genes may suggest their involvement in maintenance of symbiosis. *Smad3* stands out as a gene relevant to symbiosis because of its involvement in the TGF-β pathway (Moustakas et al. 2001). The overexpression of the *TGF-β* has been shown to allow for symbiont infection by suppressing the immune response in *E. pallida* (Detournay et al. 2012). This observation points to *Smad3* as a probable symbiosis hub gene involved in biological processes important for maintenance of symbiosis. As further qualification, mice that are deficient in Smad3 are shown to develop colon cancer after infection with *Helicobacter pylori* (Maggio-Price et al. 2009). This is the result of Smad3-deficient mice having reduced IgA responses where IgA is commonly associated with protection against pathogens and is also believed to reinforce mutualism between the host and its commensal gut microbiota (Feng et al. 2011). A polyp working within the constraints of innate immunity may up-regulate *Smad3* in order to maintain its endosymbiont as well as overall equilibrium after infection. This is similar to what has been observed in *E. pallida* where phosphorylated Smad2/3 is more highly expressed in the symbiotic state. These Smads then act as transcription factors assumed to support a tolerogenic immune response (Detournay et al. 2012). Alternatively, up-regulation of genes involved in development may indicate normal growth of the polyps supported by the presence of *Symbiodinium*. Watanabe et al. (Watanabe et al. 2007) showed growth rates of symbiotic *Acropora tenuis* polyps to be greater than aposymbiotic polyps and in *E. pallida*, genes related to protein synthesis were up-regulated in the symbiotic state (Kuo et al. 2004). Taken together, symbiotic polyps compared to aposymbiotic polyps appear to undergo metabolic processes related to normal cell growth. In support of this, genes related to protein and amino acid recycling as well as lipid processing were also up-regulated in *O. faveolata.*

However, 2 out of the 4 genes found to be down-regulated were ribosomal subunits 40S and 60S, potentially suggesting reduced protein synthesis.

Conclusion

In our analysis of the transcriptomic changes that take place in settled symbiotic coral polyps sampled nine days after infection, we see changes to expression potentially associated with turnover of the symbiosome and associated *Symbiodinium*. Host cellular processes tightly manage exocytosis and digestion of symbiont cells during the populating of host endodermal tissue. This can be observed through the up-regulation of genes related to vesicular transport and phagosome maturation. Genes associated with development were also up-regulated, along with protein recycling, suggesting resource management by the host to permit normal polyp growth in the presence of the symbiont. While some of these genes may be directly responsible for the establishment and maintenance of the symbiosis, further experiments directly manipulating gene expression of host and symbiont will be required to support these hypotheses.

Acknowledgement

We thank members of the Medina Laboratory for aid in execution and analysis of the experiment as well as assistance in editing of the manuscript. We thank Roberto Iglesias-Prieto and his team for facilitating research at the UNAM field station in Puerto Morelos, Mexico. This work was done in partial fulfillment of Aubrie O'Rourke's masters thesis in Quantitative Systems Biology at the University of California Merced.

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