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Shotgun Proteomic Analysis of Thermally Challenged Reef Corals

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Although coral reef ecosystems across the globe are in decline due to climate change and other anthropogenic stressors, certain inshore reefs of the Upper Florida Keys reef tract have persisted, with some even thriving, under marginalized conditions. To better understand the molecular basis of the thermotolerance displayed by these corals, a laboratory-based temperature challenge experiment that also featured conspecifics from a more stress-susceptible offshore reef was conducted with the common Caribbean reef-builder *Orbicella faveolata*, and the proteomes of both the coral hosts and their endosymbiotic dinoflagellate communities were profiled in (1) controls, (2) corals that succumbed to high-temperature stress and bleached, and (3) those that instead acclimated to high temperatures *ex situ*. Proteomic signatures varied most significantly across temperatures, host genotypes, and Symbiodiniaceae assemblages, and the two eukaryotic compartments of this mutualism exhibited distinct proteomic responses to high temperatures. Both partners maintained high levels of molecular chaperones and other canonical (eukaryotic) stress response (CSR) proteins in all treatments (including controls). Instead, proteins involved in lipid trafficking, metabolism, and photosynthesis played greater roles in the holobionts' high-temperature responses, and these energy mobilization processes may have sustained the elevated protein turnover rates associated with the constitutively active CSR.

Keywords: coral reefs, dinoflagellates, global climate change, proteomics, symbiosis

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INTRODUCTION

Corals reefs the world over are threatened by global climate change (GCC; Hoegh-Guldberg et al., 2017), and especially the rapidly increasing seawater temperatures associated with this phenomenon (Brown, 1997). South Florida's coral reefs, in particular, have deteriorated dramatically over the past decades (Manzello, 2015) as a result of land-based pollution, GCC, and disease, yet certain inshore reefs continue to persist (even thriving in certain cases; Gintert et al., 2018). These seemingly marginalized reefs are characterized by high summer seawater temperatures, high sediment loads, extreme high or low light levels (depending on season), high nutrient levels, and otherwise poor seawater quality conditions for coral survival (Manzello et al., 2015). Nevertheless, they feature a large number of massive coral colonies that do not regularly succumb to bleaching or disease [including the now-prevalent stony coral tissue loss disease (SCTLD)]. Only 1–2 km away, the offshore reefs have suffered to a greater extent (<5% live coral cover), and their resident corals appear to have lower resilience with respect to elevated

temperatures and disease (Manzello et al., 2019); this is despite the presumably more favorable seawater quality associated with these habitats.

To gain insight into the thermotolerance of massive corals of the Upper Florida Keys' inshore reef tract, we previously exposed *Orbicella faveolata* genotypes from sites of differing environmental tolerances to each of two high-temperature conditions: a 5-day (d) extreme high-temperature hold at 33°C and a 31-day exposure to 32°C (a temperature these corals encounter *in situ*, albeit not typically over multi-week durations; Manzello et al., 2019). As hypothesized, offshore corals were less thermotolerant than inshore genotypes, which failed to bleach at 33°C. Furthermore, some corals shuffled their endosymbiotic dinoflagellate (family Symbiodiniaceae) communities over the course of the study, though switching from *Breviolum*-dominated assemblages to those with higher proportions of the hypothetically more thermotolerant genus *Durusdinium* did not demonstrate improved survival at 32°C. Although the inshore-offshore gradient in thermotolerance was confirmed, even the most resilient inshore genotypes had at least begun to bleach slightly after 31 days at 32°C.

We sought to exploit this sample set to better understand the molecular basis of thermotolerance in this Caribbean reef-builder by specifically characterizing the cellular processes underlying (1) acclimation in corals found to be relatively more high-temperature resilient and (2) the stress response in those that were beginning to bleach. A mass spectrometry (MS)-based proteomics approach was taken because, although many works have attempted to characterize the cellular biology of thermotolerant corals based on mRNA profiling alone (e.g., Bellantuono et al., 2012; Pinzón et al., 2015), the lack of correlation between gene expression and concentration of the respective protein in both scleractinian corals and Symbiodiniaceae dinoflagellates (Mayfield et al., 2016, 2018a,b) signifies that those seeking to make inferences about the cellular biology of corals and their endosymbionts must instead profile proteins; unlike mRNAs, these macromolecules directly effect behavioral changes in cells.

MATERIALS AND METHODS

The Experiment

Key features of the experiment have been detailed in the **Supplementary Material**. Briefly, 20 *O. faveolata* colonies from each of three sites (**Table 1**; see Manzello et al., 2019 for maps and detailed site information.)-"Little Conch" (UKO2; offshore), "The Rocks" (UKI2; inshore), and "Cheeca Rocks" (UKI1; inshore)-were tagged, genotyped, and cored. After *in situ* recovery of the cores, they were moved to the laboratory, acclimated to aquarium conditions for several days, adhered to tile "pucks" (∼4 × 4 cm), again allowed to recover for several days, and then exposed to the treatments described above (33°C for 5 d or 32°C for 31 d, with a 7-d ramping period between the acclimation temperature of 30°C and the target temperature). The trailing two-week mean at the time of sampling (July 2017), 30°C, was used for the control treatment. Historical temperature data from Cheeca Rocks can be found at https://www.pmel.noaa.gov/co2/pco2data/cheeca/alldata/.

Extractions and MS

A subset of 16 samples was chosen for proteomic analyses (**Table 1**), and an "identical twin" design was employed in which the responses of fragments made from the same source genotype were tracked across all treatments. Genetic analyses (Manzello et al., 2019) revealed that all 20 offshore colonies from Little Conch are unique genotypes; one such clone of the black(a) genotype (2b-RAD nomenclature), colony C5, was randomly chosen for proteomics. In contrast, the inshore reef The Rocks lacks genetic diversity and is dominated by a single *O. faveolata* clone: skyblue (Manzello et al., 2019). One skyblue colony, A4, was chosen for proteomics. Randomly selected colonies from two genotypes of Cheeca Rocks, light-yellow (B5) and grey60 (D5), constituted the final two colonies whose fragments were analyzed in each of the four treatments. Four and two host coral and Symbiodiniaceae samples yielded few proteins upon MS (discussed below) and were omitted. This resulted in a total sample size of 12 and 14 for the host and endosymbionts, respectively (**Table 1**), with 3–4, 3, and 6–7 samples from Little Conch, The Rocks, and Cheeca Rocks, respectively (3–4 offshore and 9–10 inshore samples).

Coral pucks were flash-frozen in liquid nitrogen (LN_2) immediately upon sampling and quickly pulverized in additional LN_2 with a Baileigh Industrial hydraulic press (United States) into a wet, sand-like consistency prior to freezing (−80°C). Later, RNAs, DNAs, and proteins were extracted from ∼100 mg of partially ground tissue (including powdered skeleton) as described in the **Supplementary Material**. Briefly, coral and endosymbiont proteins were extracted with a modified TRIzol™ (Invitrogen, United States) protocol, and 15 μg were dissolved in ammonium bicarbonate (AB) supplemented with sodium dodecyl sulfate, quantified, electrophoresed [for protein quality control (QC)], repurified, resuspended in AB, denatured with urea, alkylated with iodoacetamide, digested overnight with trypsin, repurified, and resuspended in 20 μl of 2% acetonitrile with 0.1% formic acid (to aid in ionization) prior to nano-liquid chromatography (LC) on an Easy Nano LC™ 1000 [Thermo Fisher Scientific (TFS), United States] with a Nanospray Flex ion source (TFS) as described in Musada et al. (2020). Peptide eluates from a 2–98% acetonitrile gradient (84 min) were run on a Q Exactive Orbitrap MS (TFS) as in Musada et al. (2020). Details for all aforementioned steps can be found in the **Supplementary Material**.

MS Data Analysis

RAW data files from the MS were uploaded into Proteome Discoverer (ver. 2.2, TFS), and each of three fasta mRNA sequence libraries (conceptually translated to proteins) was queried: an *O. faveolata* transcriptome, a Symbiodiniaceae (*in hospite* with *O. faveolata*) transcriptome, and a *Pocillopora acuta*-Symbiodiniaceae transcriptome (Mayfield et al., 2014). All three sequence databases are described in detail in the **Supplementary Material**. The peak integration tolerance was 20 ppm, and the peak integration method was based on

TABLE 1 | *Orbicella faveolata* samples analyzed by shotgun proteomics.

Sample code	Temp. (°C) (level)	Time (days)	Temp. × time	Genotype (2b-RAD)	Shelf	Health status
A4-1	30 (control)	5	Control-short	Skyblue	Inshore	Healthy control
A4-5	32 (high)	31	High-long	Skyblue	Inshore	Stressed @ high temp.
A4-7	33 (very high)	5	High-short	Skyblue	Inshore	Healthy @ high temp.
A4-8*	30 (control)	31	Control-long	Skyblue	Inshore	Healthy control
B5-1	30 (control)	31	Control-long	Lightyellow	Inshore	Healthy control
B5-2*	32 (high)	31	High-long	Lightyellow	Inshore	Stressed @ high temp.
B5-4 bu	33 (very high)	5	High-short	Lightyellow	Inshore	Healthy @ high temp.
B5-7 bu	30 (control)	5	Control-short	Lightyellow	Inshore	Healthy control
C5-1	30 (control)	5	Control-short	Black(a)	Offshore	Healthy control
C5-2	32 (high)	31	High-long	Black(a)	Offshore	Stressed @ high temp.
C5-7	30 (control)	31	Control-long	black(a)	Offshore	Healthy control
C5-8[a,b]	33 (very high)	5	High-short	Black(a)	Offshore	Stressed @ high temp.
D5-2[a]	30 (control)	5	Control-short	Grey60	Inshore	Healthy control
D5-3	32 (high)	31	High-long	Grey60	Inshore	Stressed @ high temp.
D5-5	33 (very high)	5	High-short	Grey60	Inshore	Healthy @ high temp.
D5-8 bu[b]	30 (control)	31	Control-long	Grey60	Inshore	Healthy control

Underlined samples possessed Durusdinium *spp.-dominated Symbiodiniaceae (Sym) communities; all others were instead* Breviolum *spp.-dominated unless noted otherwise. In the "health status" column, designations were made from Coral Watch scores (Siebeck et al., 2006); those samples colored < E3 were considered to be potentially stressed. Samples denoted by asterisks (*) were omitted from analysis due to low host coral protein yield (<30 μg). "bu" = backup (i.e., original extraction attempt failed). temp. = temperature.*

[a]*Assessed Sym proteins only.* [b]*Mix of* Breviolum *spp. and* Durusdinium *spp. dinoflagellates.*

the most confident centroid. Precursor and fragment mass tolerances were 10 ppm and 0.02 Da, respectively, and up to two missed cleavages were permitted. Each of the 16 RAW files was queried separately against the three fasta libraries, and a false discovery rate (FDR)-adjusted *q*-value of 0.01 was set *a priori*. It should be noted that these stringent search parameters reduced the overall number of peptide "hits" (see "Results.") yet ensured confidence in assignment of the peptide to the correct compartment of origin (host coral vs. dinoflagellate endosymbiont). All proteomic data (RAW MS, MZML, and MZID files), as well as the fasta libraries against which the MZML files were queried, were submitted to "MassIVE" (University of California, San Diego; accession: MSV000086098), ProteomeXchange (Vizcaíno et al., 2014; accession PXD021349), and NOAA's Coral Reef Information System (CoRIS; accession 0227133). Finally, a tab-delineated **Online Supplementary Data File (OSDF)** accompanies this article and includes all data featured in analyses, figures, and tables.

Statistical Analysis-Overview

Sequenced proteins were manually checked by BLASTp (against NCBI nr) to determine if any represented duplicate hits found in both the (1) *O. faveolata* and *P. acuta* holobiont transcriptomes and (2) Symbiodiniaceae and *P. acuta* holobiont transcriptomes; duplicate data were concatenated and scored as presence (1) vs. absence (0) for each protein. Those host and endosymbiont proteins synthesized by only 1–2 samples were excluded from differentially concentrated protein (DCP) analyses, as were those sequenced from ≧11 and ≧12 samples, respectively ("housekeeping proteins"). This difference in criteria between compartments arises from the fact that two samples that were eliminated from the host analysis due to low number of peptide hits, C5-2 and D5-2, were *included* in the Symbiodiniaceae analysis (**Table 1**). When the host and endosymbiont proteins were combined for "total holobiont" analysis, these two samples were excluded ($n = 12$). DCP identification was therefore carried out with only the host and endosymbiont proteins found in 3–10 and 3–11 of the 12 and 14 samples, respectively (hereafter "variable proteins"). In contrast, multivariate statistical analyses (MSA) were conducted with all host coral, endosymbiont, and total holobiont proteins.

MSA

Multi-dimensional scaling (MDS) was undertaken to depict similarity among the 5-day, 31-day, and all 12–14 samples. To assess multivariate differences across eight experimental parameters (EP)- temperature (analyzed as 1: control vs. high and 2: 30, 32, or 33°C), (3) temperature × time, (4) genotype ($n = 4$), (5) site ($n = 3$), (6) shelf (inshore vs. offshore), (7) endosymbiont assemblage (*Breviolum*-dominated, *Durusdinium*-dominated, or mixed-lineage; **Table 1**), and (8) pigmentation (i.e., "health status;" **Table 1**)- PERMANOVA was carried out with PRIMER ver. 6 (United Kingdom) after ensuring that multivariate dispersion was similar across bins with PERMDISP (alpha = 0.05 for all MSA). PRIMER's "relate" algorithm was used to determine whether patterns in the proteomic dataset correlated with those in the experimental one after converting categorical EP data to integers (e.g., inshore = 1, offshore = 2). A Bray-Curtis (BC) similarity matrix was constructed from these recoded EP data and compared to the BC similarity matrix of the presence-absence proteomic data. These same two matrices were compared against one another with PRIMER's "BEST" algorithm to identify the EP that accounted for the greatest degree of variation in the proteomic dataset's similarity structure. As a

second, arguably more robust approach for selecting influential EP, PRIMER's distance-based linear modeling (DistLM) was used to generate backward selection models for which the Bayesian information criterion (BIC) was minimized.

DCPs

We sought to identify proteins sequenced in *all* samples of one treatment (or exclusively within one bin, e.g., genotype) and in *none* of the remaining. Since few fulfilled this criterion, five additional DCP identification methods were executed in JMP Pro® (United States; ver. 15): response screening (RSA; FDR-adjusted multiple comparisons), predictor screening (PSA; proteins accounting for the largest proportion of variation associated with each EP), stepwise discriminant analysis (SDA), stepwise regression (SRA), and generalized multivariate regression (GMR). Each approach is described in detail in the **Supplementary Material**.

RESULTS

Overview of Proteomic Data

Across all samples, 20,185 host and 3,470 Symbiodiniaceae peptides were sequenced (1,673,323 and 495,276 amino acids, respectively); these mapped to 3,824 and 1,116 proteins, respectively, in the coral and Symbiodiniaceae sequence databases queried (FDR-adjusted $q < 0.01$). It should be reiterated that this represents a subset of the total number of proteins sequenced given the high degree of stringency employed; had we been less concerned with correctly elucidating the compartment of origin, host or endosymbiont, a larger number would have been mapped and included in downstream analyses. Details on protein yield per sample, host/endosymbiont protein ratios, and protein coverage can be found in the **Supplementary Material** (notably **Supplementary Figure 1**). Of the 769 presumably unique anthozoan proteins, 300 were later found to be repeated in both the *O. faveolata* and *P. acuta* databases; MSA were carried out with the remaining 469 [of which all but 44 (9.4%) could be identified using alignment-based bioinformatics approaches]. Of the 1,116 endosymbiont proteins, 315 were unique and featured in such analyses [of which all but 74 (23%) could be identified]. Of these 469 host and 315 endosymbiont proteins, 96 and 15, respectively, were housekeeping proteins (**OSDF** and **Supplementary Figure 2**), and 160 and 212, respectively, were only identified in only 1–2 samples. Univariate DCP identification was carried out with the remaining 213 and 88 "variable" host and endosymbiont proteins, respectively.

Multivariate Effects

A number of multivariate effects (**Table 2**) were uncovered for the holobiont (**Figures 1A–C**), host coral (**Figures 1D–F** and **Supplementary Figure 2A**), and Symbiodiniaceae (**Figures 1G–I** and **Supplementary Figure 2B**) proteomes. Predominant findings of interest have been included below, with details found in the **Supplementary Material**. The holobiont proteome was affected by temperature (BEST and DistLM), site (BEST, DistLM, and PERMANOVA), and pigmentation (DistLM), though in the MDS plot (**Figure 1C**) some influence of Symbiodiniaceae assemblage is also evident. For the host proteome alone (**Figures 1D–F** and **Table 2**), site (BEST and PERMANOVA) and genotype (DistLM and PERMANOVA) best distinguished samples. Unlike the host, the PERMANOVA effect of temperature was marginally significant

TABLE 2 | Statistically significant multivariate effects on the *Orbicella faveolata*-Symbiodiniaceae (Sym) proteome.

Experimental parameter	df	Pseudo *F*	*p*	#Permutations	BEST model?	DistLM model?	PERMANOVA multiple comparisons
Holobiont (*n* = 784 proteins)*							
Correlation of BEST model = 0.401. DistLM BIC = 82.9 (R^2 = 0.334)							
Temp. (control vs. high)	1	1.079	0.306	406	Yes	Yes	
Site (*n* = 3)	2	1.543	**0.048**[a]	958	Yes	Yes	The Rocks≠Cheeca Rocks
Health status (*n* = 2)	1	1.216	0.245	216		Yes	
Host coral (*n* = 469 proteins)							
Correlation of BEST model = 0.261. DistLM BIC = 75.7 (R^2 = 0.158)							
Site	2	1.738	**0.030**	942	Yes		The Rocks≠Cheeca Rocks
Genotype	3	1.591	**0.045**[b]	973		Yes	No *post-hoc* differences
Endosymbiont (*n* = 315 proteins)*							
Correlation of BEST model = 0.366. DistLM BIC = 107 (R^2 = 0.130)							
Sym assemblage	2	1.53	**0.014**[c]	988		Yes	*Breviolum*-dominated≠*Durusdinium*-dominated
Health status	1	1.55	0.053	630	Yes		

*Experimental factors that either significantly affected the holobiont, host coral, or Symbiodiniaceae proteomes (PERMANOVA $p < 0.05$; bold font) or were included in PRIMER's BEST and/or DistLM models have been included in the table. For non-statistically significant model terms, please see **Supplementary Table 1**. Note that, although "health status" features three categories in **Table 1**, only two (healthy vs. bleached) were considered in the PERMANOVA. BIC = Bayesian information criterion. Temp. = temperature. *significant rho (Spearman rank-based) between experimental parameter data matrix and proteomic data matrix.*

[a] *See **Figures 1A,B**.* [b] *See **Figures 1D–F**.* [c] *See **Figures 1C,G–I**.*

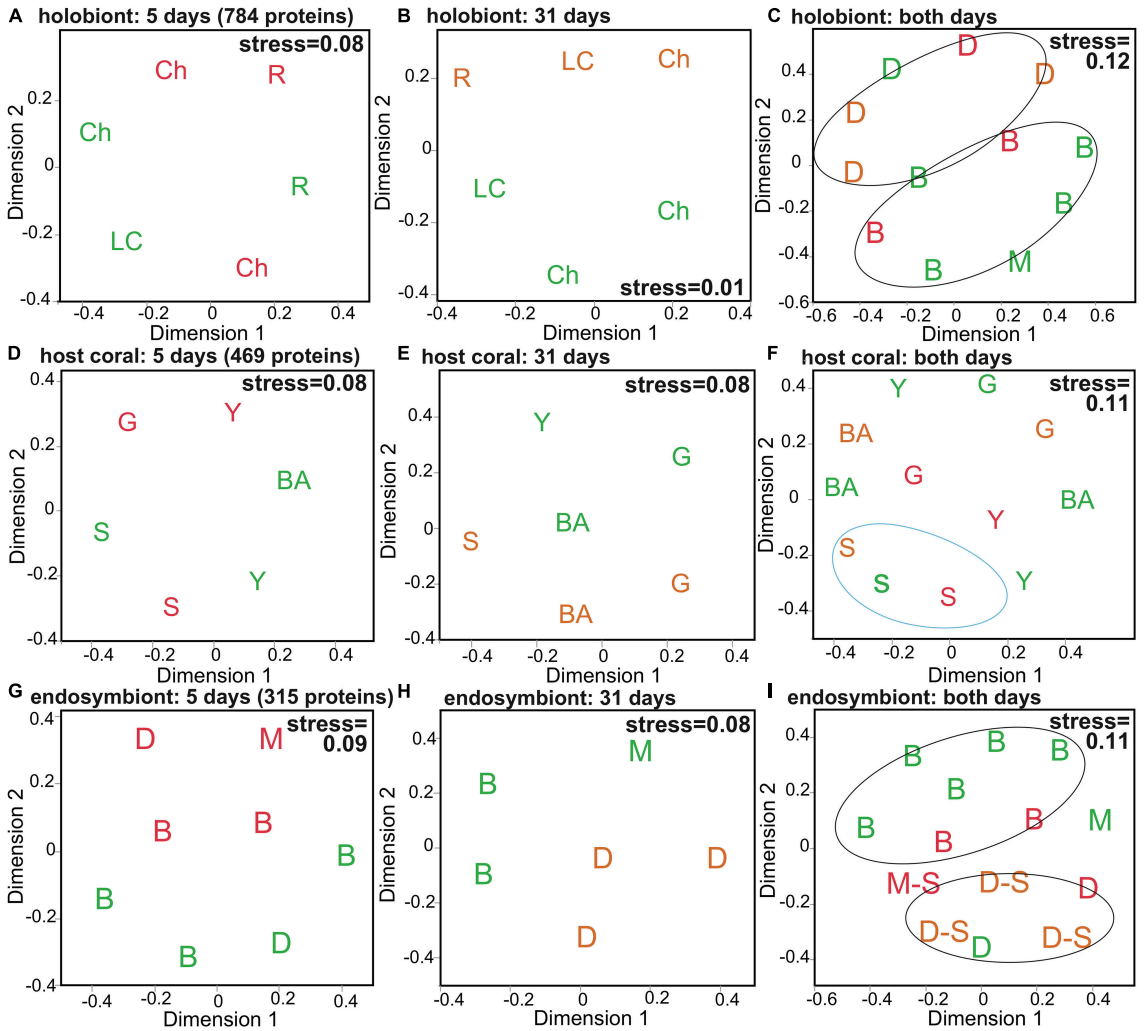

FIGURE 1 | Multivariate analysis of the *Orbicella faveolata*-Symbiodiniaceae (Sym) proteome. Multi-dimensional scaling (MDS) depicting similarity among samples at the 5-day **(A,D,G)**, 31-day **(B,E,H)**, and 5+31-day **(C,F,I)** sampling times for all 784 holobiont proteins **(A–C)**, the 469 host coral proteins **(D–F)**, and the 315 Sym proteins **(G–I)**. Icons have been coded to denote dominant PERMANOVA effects (**Table 2**). Site names **(A,B)** are abbreviated as "Ch" (Cheeca Rocks), "R" (The Rocks), and "LC" (Little Conch); Sym assemblages **(C,G–I)** as "B" (*Breviolum*-dominated), "D" (*Durusdinium*-dominated), and "M" (mixed-lineage; *Breviolum* and *Durusdinium*); and host genotypes **(D–F)** as "S" (skyblue), "G" (grey60), "Y" (lightyellow), and "BA" [black(a)]. In all panels, icons colored green, orange, and red correspond to control (30°C), high temperature (32°C), and very high temperature (33°C) samples, respectively, and samples that were Stressed at the time of sampling have been denoted by "–S" in **I**.

for the Symbiodiniaceae proteome (**Figures 1G–I** and **Table 2**), though Symbiodiniaceae assemblage most strongly affected the endosymbionts' proteomes (DistLM and PERMANOVA; i.e., the *Breviolum* and *Durusdinium* proteomes differed significantly from one another.).

DCP Overview

The five DCP identification approaches tended to yield the same suites of proteins as being responsive to each EP of interest (**Supplementary Figure 3** and **Supplementary Tables 2, 3**), and host genotype and temperature were the EP across which the largest number of proteins differed in abundance: 18/47 DCPs (38.3%) for each (**Supplementary Figure 4**). Host genotype also most strongly affected the host proteome at the multivariate scale (see above.), and temperature best accounted for the structure of the holobiont proteomic dataset (**Table 2**). In contrast, although site significantly affected both holobiont and host proteomes (**Table 2**), only three site-responsive DCPs were uncovered (**Supplementary Figure 4C**); this is because, with the exception of Cheeca Rocks (for which two genotypes were analyzed), only one genotype was analyzed at each of the remaining sites. For this reason, we generally discuss genotype and site effects in tandem hereafter. The functional breakdowns of the 27 coral (**Table 3**), 20 endosymbiont (**Table 4**), and 47 holobiont DCPs can be seen in **Figures 2A,B,** and **C**, respectively, and the most dominant cellular processes (gene ontology) of the coral hosts and endosymbiotic dinoflagellates were metabolism (**Figure 2A**) and photosynthesis (**Figure 2B**), respectively. When analyzing all

TABLE 3 | Host coral differentially concentrated proteins (DCPs).

Contig	Identity	Function	Trend(s)
Temperature-responsive proteins (*n* = 8; Figure 3)			
comp111800_c2_seq1	Ras-related protein rab11	Vesicle fusion and trafficking	High (32°C) > all others, stressed > healthy
contig8136	Large subunit ribosomal protein L18e	Translation	Control-temp. only, healthy > stressed
OFAVBQ_DN204682_c1_g1_i2	40S ribosomal protein S3	Translation	Control temp. only
OFAVBQ_DN211578_c1_g2_i4	Citrate synthase	Metabolism	Not expressed by stressed corals
OFAVBQ_DN212753_c2_g1_i4	Erlin-1	Cholesterol/lipid homeostasis	Control-5-day > all others
OFAVBQ_DN214053_c3_g2_i3	Calumenin	Protein folding and sorting	High > control temp., stressed > healthy
OFAVBQ_DN215575_c0_g1_i3	Glutamine synthetase-like	Metabolism	Control > high temp.
OFAVBQ_DN222739_c0_g1_i5	Unknown[a]	Unknown	High (32°C) > all others, stressed > healthy
Genotype-responsive proteins (including site effects; *n* = 14; Figure 4)			
OFAVBQ_DN221283_c3_g2	Collagen alpha-1(I) chain-like	Structural	Offshore only
OFAVBQ_DN218618_c3_g1_i10	Galaxin-like	Calcification	Not found in lightyellow
OFAVBQ_DN208448_c1_g1_i1	Protein DD3-3-like	Cell-cell interactions	Not found in lightyellow
OFAVBQ_DN224869_c0_g1_i2	Laccase-25-like isoform X1	Metabolism	Grey60 > other genotypes
OFAVBQ_DN218284_c7_g2_i4	Dermatopontin	Cell-cell interactions	Grey60 only
OFAVBQ_DN205148_c1_g2_i1	Rhamnose-binding lectin	Cell-cell interactions	Grey60 only
OFAVBQ_DN103730_c0_g1_i1	14-3-3 protein epsilon	Signaling	Grey60 > all other genotypes
OFAVBQ_DN210981_c4_g1_i3	Legumain-like	Protease	Not expressed by skyblue
OFAVBQ_DN213163_c3_g1_i11	Transaldolase	Metabolism	Cheeca genotypes > others
OFAVBQ_DN218286_c0_g1_i1[c]	Glycerol kinase	Metabolism	Cheeca genotypes > others
OFAVBQ_DN199317_c0_g3_i1	Collagen triple helix repeat-containing protein 1	Structural	Lightyellow only
OFAVBQ_DN197100_c0_g1_i1	Protein PRY1-like	Lipid secretion	Not found in offshore samples
OFAVBQ_DN203970_c3_g5_i1	Elongation factor 1-beta-like	Translation	Lightyellow > other genotypes
OFAVBQ_DN197154_c0_g3_i1	Galaxin-like	Calcification	Lightyellow only
Shelf-responsive proteins (*n* = 3; Supplementary Figure 5)			
OFAVBQ_DN217467_c0_g1_i3	40S ribosomal protein S4-like	Translation	Offshore > inshore
OFAVBQ_DN204853_c0_g1_i1	Nitrilase with biotinidase and vanin domains	Metabolism	Offshore > inshore
OFAVBQ_DN192542_c0_g1_i1	Nucleoside diphosphate kinase	Metabolism	Not found in offshore samples
Other effect types (*n* = 2; Supplementary Figure 5)			
OFAVBQ_DN198838_c3_g2_i3	40S ribosomal protein S20	Translation	*Breviolum* assemblages only
OFAVBQ_DN218234_c4_g1_i2[b]	MAC/perforin domain	Immunity	Stressed > healthy

For details on the analytical approach(es) used to identify the 27 DCPs, please see ***Supplementary Table 2****, and for a comparison with other studies, please see* ***Supplementary Table 4****. Sym = Symbiodiniaceae. temp. = temperature.*
[a]*also known as sushi, von Willebrand factor type A, EGF, and pentraxin domain-containing protein 1.* [b]Durusdinium-*dominated assemblages only.*

47 DCPs together (**Figure 2C**), these two processes accounted for nearly 40% of all DCPs, with translation being the third most affected process.

Host Coral DCPs

Of the 27 host DCPs (**Table 3** and **Supplementary Tables 2, 4**), 8 were significantly affected by temperature (**Figure 3**), and a PCA with these separated samples of the control, 32, and 33°C treatments (**Figure 3A**). Only one DCP, ras-related protein rab11 (**Figure 3B**), fulfilled our optimal criterion of being synthesized only by corals of a high-temperature treatment (32°C). Three additional DCPs (**Figure 3B**)- large subunit ribosomal protein L18e, 40S ribosomal protein S3, and glutamine synthetase- were instead *only* translated by controls. Metabolism and translation were the two cellular processes featuring the largest number of temperature-sensitive host coral proteins (**Figure 3C**). There was also a strong effect of host genotype on 14 of the 27 DCPs (**Figure 4**, **Table 3**, and **Supplementary Tables 2, 4**), and 5 of these were only found in one of the four genotypes (**Figure 4B**). For a detailed treatise of these 14 DCPs, please consult the **Supplementary Material**.

Symbiodiniaceae DCPs

Temperature and Symbiodiniaceae assemblage were the predictors that generated the greatest number of Symbiodiniaceae DCPs (**Supplementary Figure 4B**, **Table 4**, and **Supplementary Tables 3, 5**): 9 and 7, respectively (with 2 of these affected by both temperature and assemblage). These EP were also associated with the lowest PERMANOVA *p*-values (**Table 2**). The nine temperature-responsive DCPs led to separation among samples in a PCA (**Figure 5A**). Although none were maintained only in high-temperatures and not in controls, four were absent from high-temperature samples (**Figure 5B**): photosystem II chlorophyll-binding protein CP43, an unknown protein, a phosphoglycerate

TABLE 4 | Symbiodiniaceae (Sym) differentially concentrated proteins.

Contig	Identity	Function	Trend(s)
Temperature-responsive proteins (*n* = 9; Figure 5)			
SYMBOF_DN176524_c0_g1_i1.p1	Rab-related GTPase family rab5	Molecular trafficking	High-long > control long
SYMBOF_DN190166_c0_g1_i2.p1	ADP-ribosylation factor	Molecular trafficking	H33 < others
SYMBOF_DN201098_c1_g4_i1.p1	Photosystem II chlorophyll-binding protein CP43	Photosynthesis	Control temp. only
SYMBOF_DN192802_c0_g1_i2.p1	Unknown	Unknown	Control-short > all others
SYMBOF_DN196424_c4_g3_i3.p1	Phosphoglycerate kinase	Metabolism	Control > high temp.
SYMBOF_DN201284_c0_g2_i5.p1	Chaperone protein DNAJ (HSP40)	Stress response	Control > high temp., Healthy > stressed
SYMBOF_DN201349_c3_g4_i2.p1	Fucoxanthin-chlorophyll a-c binding protein B	Photosynthesis	Control > high temp., Healthy > stressed
SYMBOF_DN163023_c0_g2_i2.p1	60S ribosomal protein L3 (see also **Figure 6C**.)	Translation	Control > high temp., Mostly D < others
SYMBOF_DN218526_c0_g1_i5.p1	Chloroplast soluble PCP precursor-1 (see also **Figure 6C**.)	Photosynthesis	H32 < all others, Healthy > stressed, Mostly D < others
Sym assemblage-responsive proteins [*n* = 5 (excluding two that were also affected by temp. and instead found above); Figure 6]			
SYMBOF_DN191788_c0_g1_i1.p1	Peridinin chlorophyll a binding protein	Photosynthesis	Mostly B > all others, Lightyellow > others
SYMBOF_DN208331_c3_g1_i2.p1	Fucoxanthin-chlorophyll a-c binding protein F-1	Photosynthesis	Healthy > stressed, Mostly D < others
SYMBOF_DN211305_c3_g1_i1.p1	Chloroplast soluble PCP precursor-2	Photosynthesis	Mostly D only
SYMBOF_DN215694_c5_g7_i1.p1	Fucoxanthin-chlorophyll a-c binding protein F-2	Photosynthesis	Mostly B only
SYMBOF_DN217254_c0_g1_i1.p1	Heat shock protein 90 (HSP90)	Stress response	Not in mix of B and D
Other effects (*n* = 6; Supplementary Figure 5)			
SYMBOF_DN192258_c0_g6_i3.p1	Unknown	Unknown	Lightyellow > all others
SYMBOF_DN223629_c2_g4_i1.p2	Peridinin-chlorophyll A binding protein	Photosynthesis	Lightyellow < all others
SYMBOF_DN184263_c0_g2_i1.p1	Acyl-CoA-binding protein	Metabolism	Skyblue only
SYMBOF_DN181028_c0_g1_i1.p1	Luminal-binding protein 5 (BIP5)	Stress response	Grey60 only
SYMBOF_DN214774_c2_g1_i1.p1	Fucoxanthin-chlorophyll a-c binding protein F	Photosynthesis	Inshore > offshore, Healthy > stressed
SYMBOF_DN214550_c0_g1_i1.p1	Ubiquitin	Stress response	Cheeca Rocks < others

For details on the analytical approach(es) used to identify the 20 DCPs and a comparison with other published works, please see ***Supplementary Tables 3*** *and* ***5****, respectively. H32 and H33 = high-temperature (temp; 32°C) treatment and very high temp. (33°C) treatments, respectively. "mostly B," "mostly D," and "mix of B and D" correspond to* Breviolum*-dominated,* Durusdinium*-dominated, and mixed* Breviolum+Durusdinium *Sym assemblages, respectively. "Short" and "long" in the "Trend(s)" column correspond to the 5- and 31-day sampling times, respectively. PCP = peridinin chlorophyll-a binding protein. QC = quality control (protein trafficking and folding).*

kinase, and a DNAj chaperone protein (i.e., HSP40). Two additional proteins were not synthesized by any 32°C sample (**Figure 5B**): 60S ribosomal protein L3 and a chloroplast-soluble peridinin chlorophyll a-binding protein (PCP). One-third of the Symbiodiniaceae temperature-sensitive DCPs were involved in photosynthesis (**Figure 5C**). To gain greater insight into Symbiodiniaceae assemblage effects (**Figure 6**), the mRNA read breakdowns for the 14 samples were plotted (**Figure 6A**). For concentration trends and functional breakdowns of the seven Symbiodiniaceae assemblage-responsive DCPs (**Figures 6B,C**), please consult the **Supplementary Material** (particularly **Supplementary Figure 5**).

DISCUSSION

Herein we sought to uncover high-temperature effects on cellular biology of massive coral genotypes from distinct thermal habitats that varied in their capacity to acclimate to experimentally elevated temperatures. There were clear proteomic differences among the four host genotypes and three Symbiodiniaceae assemblages, and the Symbiodiniaceae proteomes were relatively more affected by high-temperature exposure at the multivariate scale than their hosts. Symbiodiniaceae dinoflagellates also demonstrate more pronounced mRNA-level responses to high temperatures than their cnidarian hosts (Mayfield et al., 2014), and their proteomic strategy for confronting environmental change is also different from that of other corals studied to date (Mayfield et al., 2016); the latter finding was corroborated herein. Proteins involved in metabolism and translation were most likely to be affected by high-temperature exposure in the cnidarian hosts, whereas the temperature-sensitive endosymbiont DCP pool was dominated by photosynthesis proteins; this substantiates findings dating back over 20 years (e.g., Jones et al., 1998). That being said, molecular trafficking proteins (e.g., rab) were affected by thermal stress in both compartments and are discussed in greater detail below.

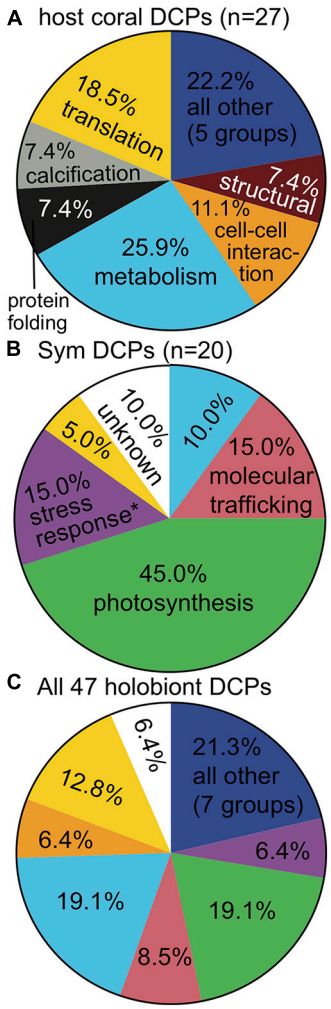

FIGURE 2 | Functional breakdown of differentially concentrated proteins (DCPs). The cellular process color codes are shown only the first time they are used (the exception being the "all other" category). When proteins involved in a cellular process (e.g., stress response) were documented at a significantly higher proportion in one compartment vs. the other (Fisher's exact test, $p < 0.01$), the process name in the compartment with the higher value has been denoted by an asterisk (*). Processes specific to anthozoan hosts **(A)** (i.e., calcification) and dinoflagellates **(B)** (i.e., photosynthesis) were excluded from this analysis.

Of the 784 proteins profiled, only 17 (2.2%) were significantly affected by temperature. This could be due to one of at least two reasons. First, there was high proteomic fidelity to host genotype. Even after several weeks of high-temperature exposure, the host proteomes clustered as much by genotype as by their temperature treatment. Such distinct differences in protein signatures (and the associated high inter-genotype variability) may have thwarted our ability to uncover temperature-sensitive host DCPs. Instead, those eight identified represent "core" proteins exploited by all four genotypes upon high-temperature exposure. Since the low number of universal high-temperature-sensitive proteins uncovered (including nine of endosymbiont origin) could have also been due to having pooled the data from samples that were bleaching with those that had instead potentially acclimated, a separate, genotype-to-genotype analysis has been provided in the **Supplementary Material**. Briefly, the four host genotypes manifested distinct stress responses, as well as dissimilar strategies for acclimating to high temperatures. For instance, of the 80 and 63 proteins found to be involved in high-temperature acclimation ($n = 3$ controls vs. $n = 3$ actively acclimating corals) and bleaching ($n = 3$ controls vs. $n = 3$ bleaching corals), respectively, only 4 (5%; **Supplementary Figure 6**) and 5 (8%; **Supplementary Figure 7**) were utilized in the acclimation and bleaching responses, respectively, of multiple genotypes. The fact that coral fragments sampled from colonies that were within even several meters of each other *in situ* utilized entirely different means of acclimating (or succumbing) to high temperatures is puzzling, unexpected, and is under active investigation by ourselves and others working in South Florida. Perhaps, the location on the colonies from which cores were made could accommodate some of the variation (despite efforts made to be consistent with respect to the relative positioning of the drill-bit); biopsies from more shaded areas might display differing means of thermo-acclimation on account of entrained light effects, even after acclimation in a common garden prior to experimentation. Given the low overlap in the acclimation and stress responses across genotypes/sites, as well as the current inability to rigorously account for such variation, we have instead focused the remainder of our discussion on the common elements uncovered.

Of the 17 temperature-sensitive DCPs, three differed in abundance between samples that were bleaching (C5-2) or beginning to bleach (A4-5 and D5-3) at the time of sampling vs. high-temperature samples that resisted bleaching: a host ras-related protein rab11 involved in vesicle trafficking and fusion [only synthesized by visibly stressed samples (color score < E3)], an unknown host coral protein with sushi, von Willebrand factor type A, EGF, and pentraxin domains (OFAVBQ_DN222739_c0_g1_i5; only synthesized by visibly stressed samples), and an endosymbiont fucoxanthin-chlorophyll a-c binding protein F (FCPE; chloroplastic; SYMBOF_DN208331_c3_g1_i2.p1-1) involved in photosynthesis (not translated by visibly stressed samples). Given the large number of FCPE proteins even within the endosymbiont DCP pool, it is unclear whether the absence of one isoform would have significant implications for photosynthetic function. In contrast, the two host proteins were distinct, and numerous rab proteins involved in vesicle and lipid trafficking were up-regulated at high temperatures in bleaching offshore samples in the genotype-by-genotype analysis (**Supplementary Figures 6, 7**). Furthermore, an endosymbiont rab5 protein was up-regulated in the three bleaching samples.

Prior works have also uncovered proteins involved in vesicle, lipid, and lipid body trafficking to be affected by high-temperature exposure in host corals and their endosymbionts (Mayfield et al., 2018a,b), and the role of lipid trafficking within the anthozoan-dinoflagellate endosymbiosis has been well-studied (Chen et al., 2012, 2015, 2017). Prior work on

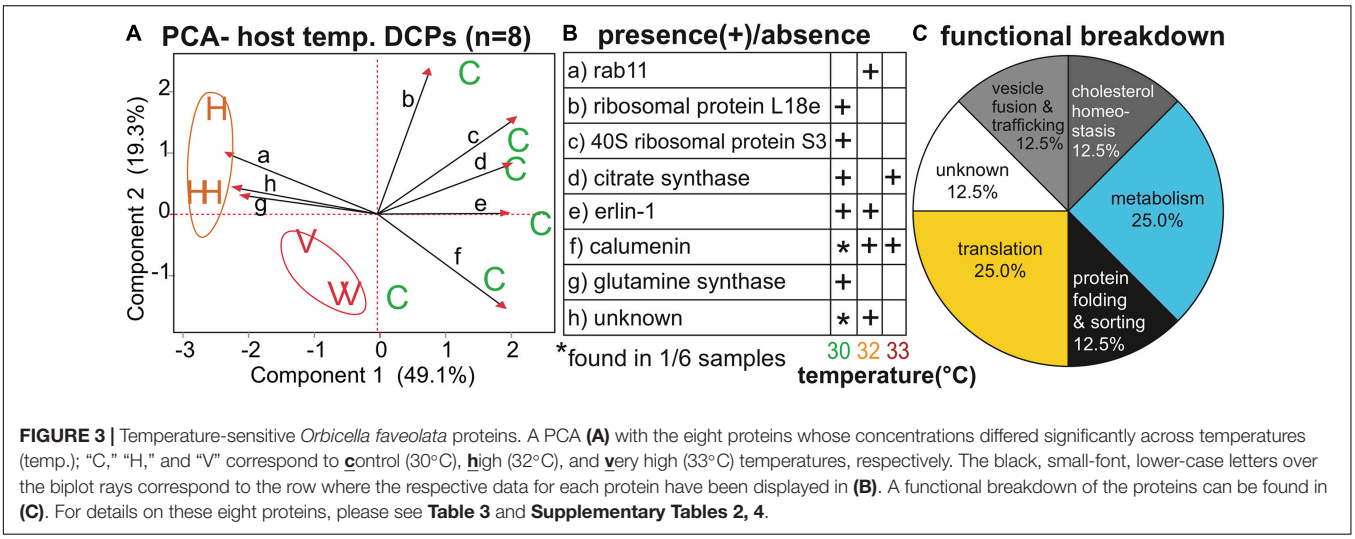

FIGURE 3 | Temperature-sensitive *Orbicella faveolata* proteins. A PCA **(A)** with the eight proteins whose concentrations differed significantly across temperatures (temp.); "C," "H," and "V" correspond to **c**ontrol (30°C), **h**igh (32°C), and **v**ery high (33°C) temperatures, respectively. The black, small-font, lower-case letters over the biplot rays correspond to the row where the respective data for each protein have been displayed in **(B)**. A functional breakdown of the proteins can be found in **(C)**. For details on these eight proteins, please see **Table 3** and **Supplementary Tables 2, 4**.

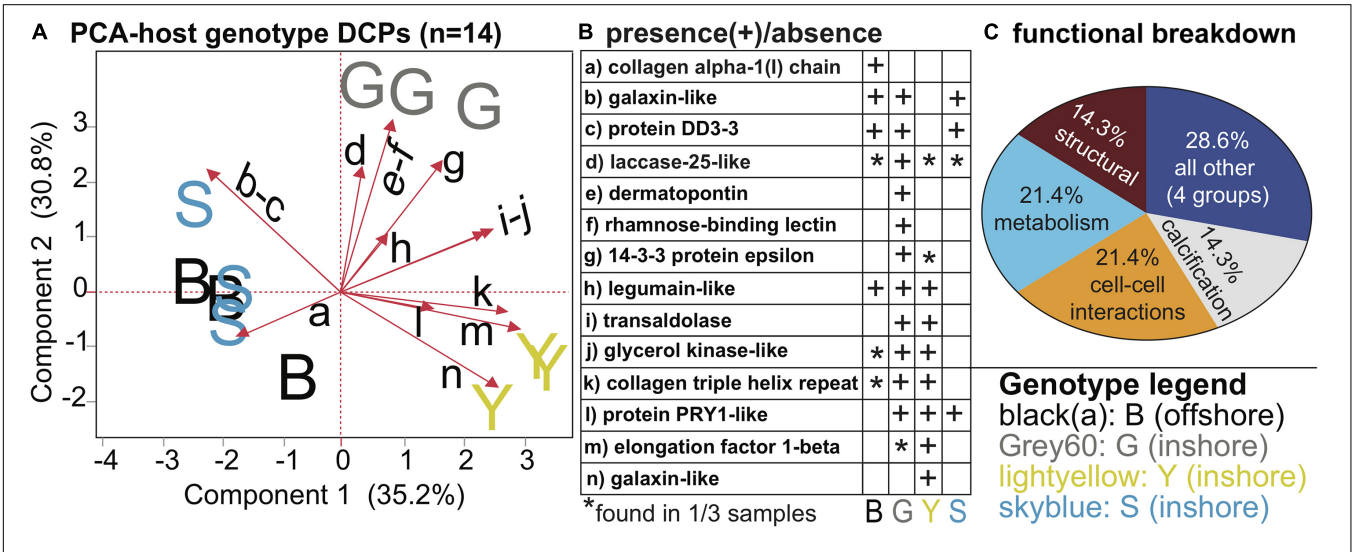

FIGURE 4 | Genotypic differences in *Orbicella faveolata* proteins. A PCA with the 14 proteins whose concentrations differed significantly across host genotypes **(A)**. Please note that the black, small-font, lower-case letters adjacent to the biplot rays correspond to the row where the respective data for each protein have been displayed in **(B)**, and the legend for the sample icons can be found in the bottom right corner. A functional breakdown of these proteins is depicted in **(C)**. For further details, please see **Table 3** and **Supplementary Tables 3, 5.**

rab11 in particular has implicated it in endocytic recycling (Lock and Stow, 2005), whereby membrane components internalized in endosomes are transported to (and then recycled at) the plasma membrane (Montealegre and van Endert, 2010). This protein has also been linked to the establishment and maintenance of the anemone-dinoflagellate endosymbiosis (Chen et al., 2003). Specifically, rab11 is normally kept away from the symbiosome, but high concentrations are documented around phagosomes containing dying endosymbionts at high temperatures (Chen et al., 2005a,b). Later, Downs et al. (2009) demonstrated that other rab proteins (e.g., rab7) are involved in symbiophagy, whereas Weston et al. (2015) took the large number of rab proteins uncovered in corals exposed to high temperatures for several hours to be indicative of dinoflagellate cell exocytosis. However, as was the case herein, their sampling regimen did not explicitly allow for the uncovering of proteins that *enact* bleaching, merely those *associated with* it. Whether up-regulation of rab11 and other rab proteins reflects the possibility of symbiophagy, dinoflagellate exocytosis, or simply an increased need to traffic membrane components to the plasma membrane during periods of elevated temperature remains to be determined. Furthermore, given that a many corals switched symbionts over the duration of the study (**Figure 6**), differential rab protein levels may simply be a testament to the need to resheath newly acquired dinoflagellate cells with symbiosome membranes.

Prior works on endosymbiotic anthozoans have yielded conflicting results with respect to the relative importance of canonical eukaryotic stress response (CSR) proteins

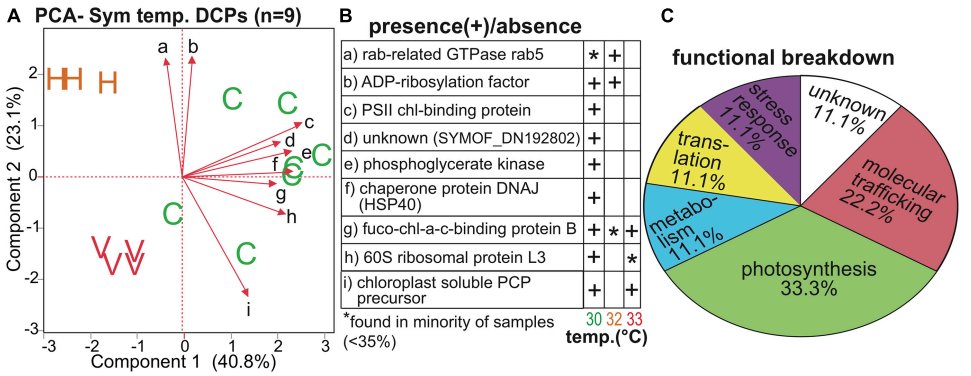

FIGURE 5 | Temperature-sensitive Symbiodiniaceae proteins. A PCA with the nine proteins whose concentrations differed significantly across temperatures **(A)** in which "C," "H," and "V" correspond to **c**ontrol (30°C), **h**igh (32°C), and **v**ery high (33°C) temperatures, respectively. The black, small-font, lower-case letters adjacent to the biplot rays correspond to the row where the respective data for each protein have been displayed in **(B)**. A functional breakdown of these proteins is depicted in **(C)**. chl = chlorophyll. Fuco-chl-a-c binding = fucoxanthin-chl-a-c binding protein B. PCP = peridinin chl-a-binding protein. PS = photosystem II. For details on these nine proteins, please see **Table 4** and **Supplementary Tables 3, 5**.

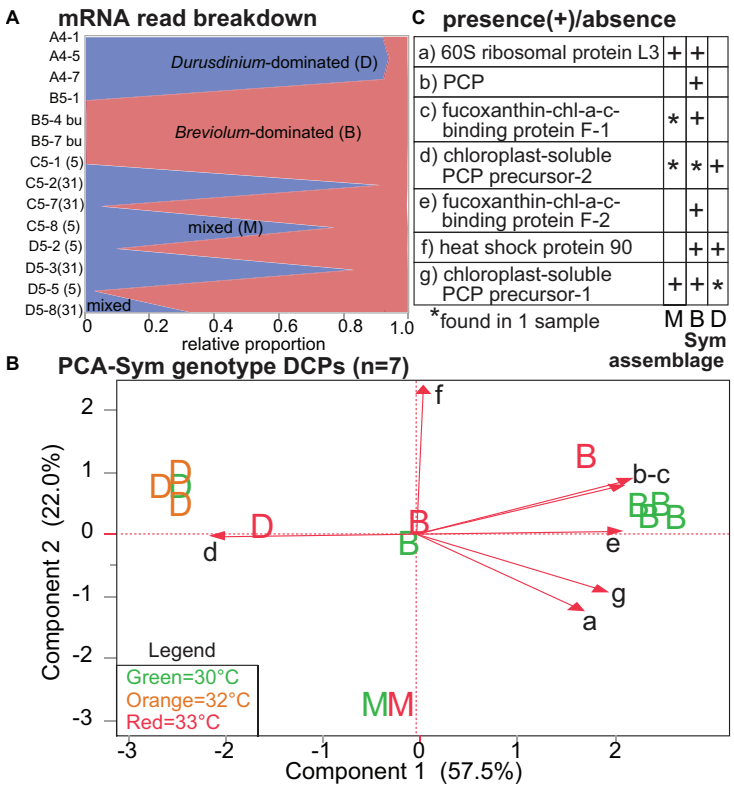

FIGURE 6 | Proteomic differences across Symbiodiniaceae (Sym) lineages. In the mRNA breakdown of the 14 samples analyzed by shotgun proteomics **(A)**, the blue and red shading reflect the proportions of *Durusdinium* spp. ("D") and *Breviolum* spp. ("B") dinoflagellates, respectively. Samples for which no genus comprised >80% of the mapped mRNA reads were deemed "mixed-lineage" ("M"). The numbers in parentheses following the sample codes for the two genotypes that underwent endosymbiont shuffling, C5 [black(a) from Little Conch (offshore)] and D5 [grey60 from Cheeca Rocks (inshore)], represent sampling days. A PCA with seven proteins whose concentrations differed significantly across Sym assemblages **(B)**; letters correspond to Sym assemblages, and the colors correspond to temperatures (see inset legend.) The black, small-font, lower-case letters adjacent to the biplot rays correspond to the row where the respective data for each protein have been displayed in **(C)**. Five, one, and one of these proteins were involved in photosynthesis (71.4%), the stress response (14.3%), and translation (14.3%), respectively. chl = chlorophyll. PCP = peridinin chl-a binding protein. For details on these nine proteins, please see **Table 4** and **Supplementary Tables 3, 5**.

TABLE 5 | Select stress response "housekeeping" proteins, stress-sensitive proteins involved in the canonical eukaryotic stress response (CSR), and anthozoan-dinoflagellate bleaching-associated proteins identified in prior works.

Accession	Compartment	Protein name	Prior mRNA/protein trend	mRNA/protein reference(s)	Prior trend corroborated?
HOUSEKEEPING CSR PROTEINS					
OFAVBQ_DN205531_c1_g1_i1	Coral host	78 kDa glucose-regulated protein (GRP78)	Stress-sensitive	Aranda et al. (2011) Lõhelaid et al. (2014)[a]	No No
OFAVBQ_DN217505_c2_g1_i3	Coral host	Endoplasmin-like isoform X1	Stress-sensitive	Ruiz-Jones and Palumbi (2017)	No
OFAVBQ_DN202907_c9_g3_i1	Coral host	Glutathione S-transferase	Constitutively maintained	**Whalen et al. (2010)[a]**	Yes
OFAVBQ_DN215553_c1_g3_i1	Coral host	Heat shock cognate 71 kDa protein	Constitutively maintained	**Oakley et al. (2017)[b]** Poli et al. (2017)	Yes Yes[c]
OFAVBQ_DN214823_c0_g2_i2	Coral host	HSP60	Stress-sensitive	**Chow et al. (2009)**	No
OFAVBQ_DN218952_c1_g1_i5	Coral host	HSP90-beta	Constitutively maintained	Nakamura et al. (2012)	Yes
OFAVBQ_DN225001_c3_g1_i2	Coral host	Peroxiredoxin-5	Stress-sensitive	Levy et al. (2016)	No
OFAVBQ_DN208085_c0_g2_i1	Coral host	Peroxiredoxin-6	Stress-sensitive	**Mayfield et al. (2018b)**	No
SYMBOF_DN199915_c1_g4_i1.p1	Endosymbiont	HSP70	Constitutively maintained	Putnam et al. (2013)	Yes
SYMBOF_DN200180_c0_g1_i1.p1	Endosymbiont	HSP70	Constitutively maintained	Mayfield et al. (2011)	Yes
SYMBOF_DN197301_c4_g6_i3.p1	Endosymbiont	HSP90[d]	Constitutively maintained	Mayfield et al. (2017a,b,c)	Yes No
SYMBOF_DN217254_c0_g1_i1.p1	Endosymbiont	HSP90	Stress-sensitive	Rosic et al. (2011)[e]	
SYMBOF_DN198813_c2_g3_i2.p1	Endosymbiont	HSP90	Stress-sensitive	Lin et al. (2019)	No
ANTHOZOAN-DINOFLAGELLATE STRESS RESPONSE AND BLEACHING-ASSOCIATED PROTEINS IDENTIFIED IN PRIOR WORKS					
OFAVBQ_DN172775_c0_g1_i1	Coral host	Beta-gamma crystallin	Stress-sensitive	Downs et al. (2002)	No
comp97680_c0_seq1	Coral host	Calmodulin	Stress-sensitive	Ricaurte et al. (2016)	No
SYMBOF_DN174588_c0_g1_i1.p1	Endosymbiont	Calmodulin	Stress-sensitive	Weston et al. (2015)	No
OFAVBQ_DN223119_c2_g2_i5.p1	Coral host	Carbonic anhydrase	Stress-sensitive	Weston et al. (2015)	No
OFAVBQ_DN214557_c1_g2_i5	Coral host	Caspase	Stress-sensitive	Tchernov et al. (2011)	No
SYMBOF_DN215553_c1_g1_i2.p1	Endosymbiont	Chaperone protein DNAK	Stress-sensitive	Weston et al. (2015)	No
SYMBOF_DN182054_c0_g1_i1.p1	Endosymbiont	E3 ubiquitin-protein ligase	Stress-sensitive	Mayfield et al. (2018b)	No
OFAVBQ_DN194262_c1_g1_i1	Coral host	HSP70	Stress-sensitive	Seveso et al. (2017)	No
SYMBOF_DN204865_c1_g2_i1.p1	Endosymbiont	HSP90	Stress-sensitive	Ross (2014)	No
SYMBOF_DN165260_c0_g1_i2.p1	Endosymbiont	Glutathione S-transferase	Stress-sensitive	Weston et al. (2015)	No
SYMBOF_DN239554_c0_g1_i1.p1	Endosymbiont	Peptidylprolyl isomerase D	Stress-sensitive	Mayfield et al. (2018b)	Yes
SYMBOF_DN228106_c0_g1_i1.p1	Endosymbiont	Peroxiredoxin-1	Stress-sensitive	Weston et al. (2015)	No
SYMBOF_DN205301_c4_g1_i1.p1	Endosymbiont	Peroxiredoxin-6[e]	Stress-sensitive	Mayfield et al. (2018b)	No
OFAVBQ_DN213774_c4_g1_i3	Coral host	Superoxide dismutase	Constitutively maintained Stress-sensitive	Coles and Brown (2003) Oakley et al. (2017)	No No
OFAVBQ_DN199726_c0_g2_i1	Coral host	Thioredoxin	Constitutively maintained Stress-sensitive	Cziesielski et al. (2018) Ricaurte et al. (2016)	No No
Contig14748	Coral host	(poly)ubiquitin-B	Stress-sensitive	Coles and Brown (2003)	No
SYMBOF_DN214550_c0_g1_i1.p1	Endosymbiont	Ubiquitin[f]	Stress-sensitive	Barshis et al. (2010)	No

Please note that a more detailed version of this table featuring additional proteins can be found in the ***Supplementary Material*** *(****Supplementary Table 6****). In the "Prior mRNA/protein trend" column, molecules have either been deemed "stress-sensitive" or "constitutively maintained" based on results of prior gene expression (reference in regular font) or protein-based (****bold font****) studies. In the second half of the table, we instead looked at CSR and bleaching-associated proteins identified in prior proteomic works of anthozoans to determine whether trends were congruent. Please note that this does not represent an exhaustive list of all proteins involved in the CSR (Kultz, 2005), and some heat shock proteins (HSPs) found previously at the mRNA level to be stress sensitive (e.g., hsp32 in Seveso et al., 2020) were not sequenced herein. One CSR protein uncovered herein, an endosymbiont HSP40 (SYMBOF_DN201284_c0_g2_i5.p1), was down-regulated in bleaching corals.*

[a] *Octocoral study.* [b] *Sea anemone study.* [c] *Subtle depth effects noted.* [d] *Note that one Symbiodiniaceae HSP90 differed in concentration across Symbiodiniaceae assemblages.* [e] *Affected by infection status in Sproles et al. (2019), with another isoform (peroxiredoxin-1) up-regulated at high temperatures in Weston et al. (2015).* [f] *See* ***Table 4****.*

in thermo-acclimation (**Table 5** and **Supplementary Tables 4–6**). Whereas both corals and Symbiodiniaceae from upwelling reefs constitutively express mRNAs encoding heat shock proteins (HSPs; Mayfield et al., 2011, 2012), others have observed a more traditional heat shock response at the gene (Rosic et al., 2011) and protein (Coles and Brown, 2003) levels in corals from stable temperature environments. Herein numerous CSR proteins in both the host and dinoflagellate compartments were among the most abundant housekeeping proteins found in all samples, including controls (**Table 5** and **OSDF**). Although one HSP90 was a DCP, it only differed in concentration across endosymbiont assemblages. Furthermore, an endosymbiont HSP40 (chaperone protein DNAj) was only sequenced from controls, and another endosymbiont molecular chaperone, DNAK, was only up-regulated at high temperatures in one host genotype (grey60; **Supplementary Figure 7** and **OSDF**). As an additional example, peroxiredoxins, which are important in the stress responses of both corals (Weston et al., 2015) and fish (Kultz et al., 2007), were instead constitutively maintained in the corals sampled herein (**Table 5**).

As a notable exception, one CSR protein, peptidylprolyl isomerase (Kim et al., 2017), was found only in stressed endosymbiont communities in Mayfield et al. (2018b), and, when profiling the bleaching response on a genotype-to-genotype basis herein (**Supplementary Material**), this antioxidant was also up-regulated at high temperatures in endosymbiont communities within two of the three inshore genotypes (skyblue and grey60). This remains, at present, the lone protein that both represents a CSR protein *and* has been found to be up-regulated at high temperature in more than one study (**Table 5**). The peptidylprolyl isomerase is also critical in the cellular response of sea squirts (Lopez et al., 2017), and even marine fish (Kultz et al., 2007), to environmental stress. With the exception of this molecule, our results obfuscate efforts to use CSR mRNA or protein concentration levels as biomarkers for coral health/resilience given their constitutively high abundance. Rab protein levels, as also recommended by Weston et al. (2015), appear to be better candidates for gauging levels of coral heat dosing.

Constitutive activation of the CSR is not a common strategy for most life forms (Hochachka and Somero, 2002) given the high metabolic burden it poses (Sokolova et al., 2012). Specifically, the energy associated with re-establishing homeostasis could detract from growth and reproduction. Perhaps the excess energy from endosymbiont photosynthesis permits sustained periods of engaged CSR. It is also possible that the constitutive maintenance of a large number of CSR and antioxidant proteins could simply be indicative of the high protein turnover and metabolic rate associated with these corals in summer; the low frequencies of oxidative stress and apoptosis markers (**Table 5** and **OSDF**) further signify that the control corals may not have actually been exhibiting a CSR. In the future, we will analyze the proteomes of field biopsies from these same coral colonies collected during different seasons to determine whether (1) the high control coral levels of certain CSR proteins documented herein reflect acclimation to a relatively high summer mean of 30°C or (2) they are presented year-round (*sensu* Mayfield et al., 2019). It will also be worthwhile repeating the experiment in winter (when ambient temperatures are only 21–22°C) to determine whether high-temperature-challenged corals acclimated to colder temperatures exhibit a more traditional CSR upon heating.

It was hypothesized over 20 years ago that protein turnover is the cellular process that ultimately dictates whether corals will effectively acclimatize (Gates and Edmunds, 1999), with highest turnover rates generally associated with the more thermotolerant massive corals like *O. faveolata*. If high concentrations of CSR and protein turnover-associated proteins are not necessarily indicative of stress, but instead high protein turnover rates, then the observation that the majority of the temperature-responsive proteins were involved in host coral lipid trafficking and metabolism and Symbiodiniaceae lipid trafficking and photosynthesis (see **Supplementary Material**.) could signify that these energy mobilization processes are modulated in a way that ensures that there is sufficient cellular ATP to maintain the high metabolic rate and turnover needed to continually break down proteins, refold denatured ones, and otherwise maintain proteostasis under elevated temperatures (beyond those already high protein turnover rates characteristic of controls). This could also explain the up-regulation of a key regulator of protein turnover, calumenin, in bleaching samples (herein and in Oakley et al., 2017); indeed, this protein is up-regulated upon chronic high-temperature exposure in other marine invertebrates (Sleight et al., 2018). Although signaling molecules went largely undetected by our proteomic approach, it is possible that lipid-signaling molecules generated by the lipid trafficking proteins uncovered served as the molecular "intermediaries" through which proteostasis and lipid metabolism were linked. Whether or not constitutively high protein turnover, or even an active CSR, is an energetically sustainable strategy over longer-term timescales remains to be determined, but, at the time of writing, the inshore corals continue to persist and reproduce annually, even as those around them succumb to bleaching and/or SCTLD.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories, as well as in the **Online Supplementary Data File**. The names of the repository/repositories and accession numbers can be found in the article and its **Supplementary Material**

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because this study was carried out with reef corals (see **Supplementary Material** for permitting information).

AUTHOR CONTRIBUTIONS

AM, CA, and GK collected the samples, ran the experiments, and carried out the molecular benchwork. AM analyzed the data, created the figures, and wrote the manuscript. IE and DM secured funding and mobilized boat and laboratory support.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.660153/full#supplementary-material

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