Genomic basis for coral resilience to climate change

Daniel J. Barshis[1,2], Jason T. Ladner, Thomas A. Oliver, François O. Seneca, Nikki Traylor-Knowles, and Stephen R. Palumbi

Department of Biology, Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950

Recent advances in DNA-sequencing technologies now allow for in-depth characterization of the genomic stress responses of many organisms beyond model taxa. They are especially appropriate for organisms such as reef-building corals, for which dramatic declines in abundance are expected to worsen as anthropogenic climate change intensifies. Different corals differ substantially in physiological resilience to environmental stress, but the molecular mechanisms behind enhanced coral resilience remain unclear. Here, we compare transcriptome-wide gene expression (via RNA-Seq using Illumina sequencing) among conspecific thermally sensitive and thermally resilient corals to identify the molecular pathways contributing to coral resilience. Under simulated bleaching stress, sensitive and resilient corals change expression of hundreds of genes, but the resilient corals had higher expression under control conditions across 60 of these genes. These "frontloaded" transcripts were less up-regulated in resilient corals during heat stress and included thermal tolerance genes such as heat shock proteins and antioxidant enzymes, as well as a broad array of genes involved in apoptosis regulation, tumor suppression, innate immune response, and cell adhesion. We propose that constitutive frontloading enables an individual to maintain physiological resilience during frequently encountered environmental stress, an idea that has strong parallels in model systems such as yeast. Our study provides broad insight into the fundamental cellular processes responsible for enhanced stress tolerances that may enable some organisms to better persist into the future in an era of global climate change.

acquired stress tolerance | *Acropora hyacinthus* | thermal stress | transcriptomics | Cnidarian

Many species have evolved to tolerate a multitude of stressful environmental changes (1). Comparisons across plants (2) and animals (3–5) have shown some species and populations to be far more resilient than others, with marked influences on growth, survival, disease resistance, and ultimately evolutionary fitness (4, 6, 7). At the species level, physiological stress regimes are known to set biogeographic limits, determine microhabitat preferences, and generate ecological patterns such as intertidal zonation (3, 8–10). The advent of climate change has heightened the need to understand stress responses (10, 11), especially for species such as terrestrial plants and many sedentary marine taxa that cannot easily migrate to new environmental optima (12–14).

At the genomic scale, some species show a rapid, widespread stress response across thousands of genes. In cells of the budding yeast *Saccharomyces cerevisiae*, this environmental stress response (ESR) is broadly consistent across a profusion of external stressors (15). In nonmodel organisms in natural habitats, such as reef-building corals, the genome-wide responses to environmental stress are only just beginning to be described (16). However, in the current era of a rapidly changing climate, it is imperative to understand the mechanisms of the stress response, particularly those that may confer enhanced tolerance of changing environmental conditions (10, 11, 14).

Reef-building corals, the foundation of tropical coastal marine resources, are exceptionally vulnerable to climate change (e.g., refs. 17 and 18). In recent decades, the increasing frequency and severity of catastrophic coral bleaching (the dissociation of the coral host and its endosymbiotic algae *Symbiodinium sp.*) and

bleaching-induced mortality (19–22) has called into question whether corals have the capacity to acclimatize or adapt to global climate change (19, 20). However, during mass coral bleaching events, survival of scattered coral colonies suggests that some groups of corals may possess inherent physiological tolerance to environmental stress (23, 24). In addition, some high-temperature environments naturally retain healthy, growing coral populations (25–27), and these corals can show elevated bleaching tolerances (e.g., refs. 18 and 28). These thermotolerant corals are among the most likely to cope with future climate change (*sensu* ref. 24) and, thus, represent an essential source of information about mechanisms underlying observed differences in coral physiological resilience (defined here as the capacity for an organism to experience relative environmental extremes and either resist cellular stress or rapidly recover from it; *sensu* ref. 29).

At the molecular level, recent evidence suggests that differential regulation of apoptosis (i.e., programmed cell death) may be essential to postbleaching survival of resilient corals (30). Alternatively, enhanced thermotolerance in other marine organisms (e.g., intertidal limpets, mussels, sea cucumbers, and amphipods) has been linked to higher constitutive expression of heat shock proteins (Hsps) (31–34). A growing number of studies find that the basic coral heat stress response involves a wide array of cellular processes, akin to the ESR in yeast (15). These include induction of molecular chaperones (e.g., Hsps) and antioxidant enzymes but also involve Ca^{2+} homeostasis disruption, cytoskeletal reorganization, and altered cell signaling and transcriptional regulation (e.g., refs. 35–37). As a result, differences in physiological resilience could be caused by regulation of many molecular processes.

In this study, we report transcriptome-wide gene expression patterns underlying marked differences in thermal resilience between two populations of the common reef-building coral *Acropora hyacinthus* on Ofu Island, American Samoa. The back-reef environment in Ofu is composed of distinct pools that experience variable levels of temperature, pH, and oxygen driven by tidal fluctuations (26, 38). The most variable of these pools reach ≥34 °C during summer low tides and exhibits daily thermal fluctuations up to 6 °C (26, 38). Corals in the more variable pools show higher stress protein biomarker levels (39), more heat-tolerant *Symbiodinium* genotypes (27), faster growth rates (38, 40), and enhanced thermal tolerance (28). These studies demonstrate that more physically challenging areas of the back reef harbor

some of the most thermotolerant corals in the region, but the key molecular mechanisms involved are entirely unknown.

To identify potential mechanisms behind physiological resilience, we conducted a simulated bleaching experiment on *A. hyacinthus* from a previously characterized thermally tolerant coral population [highly variable (HV) pool] and a more sensitive neighboring population [moderately variable (MV) pool] (pools 300 and 400, respectively, from ref. 28). Replicate fragments of *A. hyacinthus* were sampled from the two populations ($n = 6$ individuals from the HV pool and $n = 5$ individuals from the MV pool). Samples of each colony were exposed to control/ ambient (mean, 29.2 °C) and heated (mean, 32.9 °C) temperatures in outdoor, flow-through aquaria for 72 h. We used the RNA-Seq method [Illumina platform (41)] to measure gene expression differences between heated corals and corals under normal conditions from both pools (see *Materials and Methods* for further details). To control for tank and transplant effects, our experiments compared gene expression profiles of heated corals to genetically identical fragments exposed to normal temperature conditions. Overall, we identified hundreds of transcripts that respond to heat stress; however, heat-tolerant and heat-sensitive corals showed different patterns of gene expression. In particular, 60 of the genes up-regulated in response to heat stress in the sensitive coral population show a reduced response and a higher constitutive level of expression in tolerant corals (i.e., they are already up-regulated under control conditions in tolerant corals). We hypothesize that this transcriptional "frontloading" of stress-related genes may be a primary mechanism of thermal tolerance of high temperatures in reef-building corals.

Results

Shared Response to Thermal Exposure. At the onset of bleaching (after 72 h), expression across 33,496 reference contigs (i.e., contiguous gene sequences) showed heat-related differences in both the tolerant (HV) and susceptible (MV) populations [principal components analysis (PCA) and hierarchical clustering of gene expression; Fig. 1 and Figs. S1 and S2].

The largest numbers of differentially expressed genes were found in the comparisons of control and heated treatments [484, 247, and 159 for all, MV, and HV samples, respectively; 5% false-discovery rate (FDR) correction; Fig. 2]. Among these three comparisons, 574 unique contigs were identified comprising 404 unique Uniprot matches (Table S1). These genes had an average fold change of 3.58 (range, 1.73–14.02) for up-regulated and −3.61 (range, −2.01 to −27.24) for down-regulated contigs (Dataset S1*A*).
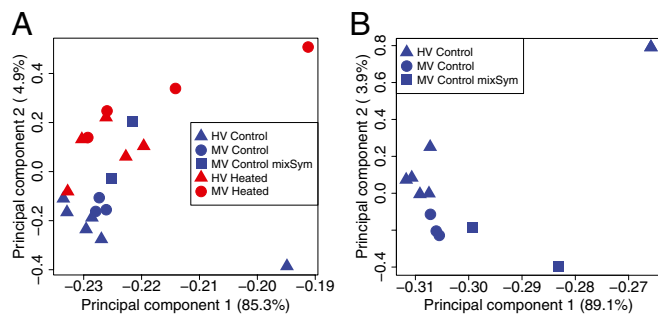
Fig. 1. PCA components 1 and 2 (*x* and *y* axis, respectively) of expression values for all 33,496 contigs in the reference assembly for all samples (*A*) and control coral samples (*B*). The numbers in parentheses represent the proportion of variance explained by that principal component. Specific colors reflect treatments and shapes reflect sample populations as shown in each legend (mixSym represents those colonies where <95% of a single *Symbiodinium* clade type was found). PCA was computed in R using the princomp function and a correlation matrix.
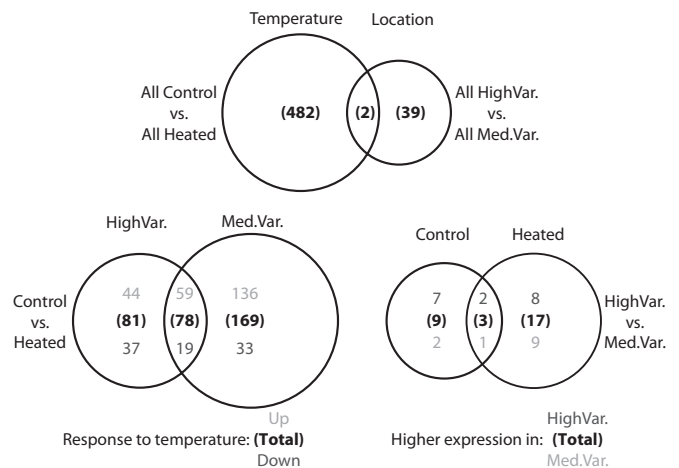
Fig. 2. Venn diagram showing the number of differentially expressed genes detected during analysis based on temperature, location, within-location temperature response, and within-treatment location differences. Bold numbers in parentheses represent totals and respective shades of gray denote up- vs. down-regulated or higher in HV vs. MV, respectively.

There were 78 genes that responded identically to heat stress in the HV and MV groups: 59 were up-regulated and 19 down-regulated (Fig. 2). However, the MV corals showed 55% more differentially expressed genes than the HV corals (247 vs. 159; Fig. 2). The most highly up-regulated of the 78 genes found in both the MV and HV control vs. heated comparisons was a tumor necrosis factor receptor-associated factor 3 homolog (*TRAF3*) (contig180146_147773; Dataset S1 *B* and *C*), which can play roles in apoptosis, negative regulation of NF-κB/Nfkb1 transcription factor activity, and immune regulation (42, 43). Another member of the tumor necrosis factor family (TNF receptor superfamily member 27; contig147815) was also significantly up-regulated in heated corals (Dataset S1 *A–C*). Conspicuously absent in these shared-response genes are other common coral stress response transcripts such as Hsps, other molecular chaperones, and antioxidants.

The most highly down-regulated gene in control-heated colony comparisons (27.2 fold reduction) was a mannose-binding lectin (contig600050; Dataset S1 *A and C*), with putative roles in positive immune regulation, positive regulation of nitric-oxide synthase activity, and positive regulation of nitric oxide synthase biosynthetic process.

Categorical classification of genes with altered expression during heat stress identified the highest number of genes relating to (*i*) the components of membranes (168 contigs), (*ii*) maintenance of calcium ion homeostasis (64 contigs), and (*iii*) involvement in transcription and DNA replication (61 and 54 contigs, respectively; Table S1). Cell–cell adhesion proteins (37 contigs) and genes involved in apoptosis and apoptosis regulation (24 contigs) were also numerous and showed mixed up- and down-regulation responses. Four out of five genes associated with coral calcification were down-regulated under heat stress (Table S1).

Population-Specific Gene Expression Within-Treatment. Even without heat stress, control corals from the HV and MV pools differed significantly in gene expression across 12 genes [9 higher in HV corals (mean fold change, 8.12); and 3 higher in MV corals (mean fold change, 6.92); Fig. 2 and Dataset S1*E*]. HV controls up-regulated genes in 19 gene ontology (GO) categories comprised primarily of genes related to collagen and extracellular matrix components (Dataset S2*E*). After heat treatment, HV and MV corals differed at 20 genes [10 higher in HV corals (mean fold change, 21.50); and 10 higher in MV corals (mean

fold change, 12.15); Fig. 2 and Dataset S1E]. Three of these genes were shared with the set of 12 differentially expressed genes among control samples. HV heated corals remained significantly up-regulated in collagen and extracellular matrix proteins compared with MV heateds (Dataset S2E). By contrast, MV heateds up-regulated a number of cytochrome P450 family members, which are preliminarily annotated as iron or tetra-pyrrole binding (Dataset S2E).

Population-Specific Response to Temperature. Thermally resilient (HV) corals showed strikingly different transcriptomic response to heat exposure than more sensitive (MV) corals in several important ways. In HV corals, 81 genes reacted significantly to heat stress but did not significantly change in the MV corals (Fig. 2). Further inspection of the data showed that in MV corals, these 81 genes exhibited similar mean fold changes across MV and HV groups (Fig. S3). However, 64 of these 81 (79%) genes did not pass our standard deviation filter in the MV comparison because of high intercolony variability in MV corals. Thus, most genes that responded significantly in HV corals also responded in a similar fashion in MV corals, although significance was precluded by more variable responses among individuals.

By contrast, we found 169 genes that reacted to heat stress in MV corals but not in HV corals. All but one of these 169 genes showed the same direction of change in HV and MV corals. In most of these cases (87%), however, lack of significant change in HV corals is a result of a reduced magnitude of response (i.e., lower fold change) of these genes in HV corals, not higher variance; all 169 genes passed the SD filter in the HV analysis. This pattern (Fig. 3) was apparent for down-regulated contigs (30 out of 33 showed greater decrease in MV corals; χ^2 test; $P < 2.2e^{-16}$), as well as up-regulated contigs (117 out of 136 genes showed greater increase in MV corals; χ^2 test; $P < 2.6e^{-6}$).

Across all 117 genes with reduced up-regulation in HV corals, many showed higher expression in HV versus MV controls (60 of

117). Furthermore, reduced up-regulation in HV (measured as each gene's ratio of HV to MV fold change upon heat stress) is associated with higher constitutive expression in HV (measured as each gene's ratio of HV to MV control expression; Fig. 4). This relationship shows that genes reacting less during heat stress in HV corals may start off with a higher level of expression before heat stress. A similar pattern, although reversed, is true for down-regulated genes: most genes with reduced down-regulation in HV corals show lower expression levels of these genes in HV controls (21 of 30). In other words, these data show that many up-regulated genes in the thermally sensitive MV corals are already expressed at higher levels in thermally tolerant HV corals and that many down-regulated genes are at lower constitutive levels (i.e., turned off under control conditions) in HV corals.

Transcripts at higher constitutive expression under ambient conditions and lower reactivity to heat stress in HV corals include canonical heat stress genes such as Hsp70, TNF, peroxidasin, and zinc metalloproteases (Dataset S3). Genes with lower constitutive expression and lower response to heat stress in HV corals include a carbonic anhydrase, multiple lectins, and a suite of transcription factors (Dataset S3). These genes represent candidates potentially playing key roles in coral resilience to increased environmental temperatures.

Discussion

Coral Analog to the Yeast ESR? Elevated temperature exposure elicited a significant change in gene expression profiles among corals (Figs. 1 and 2). Many of the differences we identified correspond to specific genes or functional categories reported previously to respond to thermal stress in corals, including Hsps, antioxidants/oxidative stress enzymes, cell–cell adhesion molecules, apoptosis regulators, and proteins involved in calcium ion homeostasis (Table S1 and Dataset S1; e.g., refs. 36, 37, and 44–46). These previous studies were conducted across a range of life stages, coral species, exposure conditions, and durations, suggesting that many aspects of the coral heat-stress response may be highly conserved.

Conceptually, this multigene response in corals is similar to the genomic ESR in yeast (15), wherein a broad suite of genes and molecular processes respond in a coordinated fashion to a multitude of exogenous stressors (47). Thermal stress has received the most attention in corals, although substantial conservation in the pathways responding to stress has also been shown following darkness (48) and low pH (49) exposures. Although additional studies, standardization in technologies, and testing of various stress exposures are required to match the breadth of experimentation for model systems such as yeasts, evidence to date supports a potential coral analog to the yeast ESR.

Reduced Reaction in Physiologically Resilient Corals. Although components of the coral stress response are conserved, PCA and differential gene expression analysis also revealed substantial differences between the tolerant and sensitive corals in their reaction to heat (Figs. 2–4) and in baseline expression under control conditions (Figs. 1 and 4 and Figs. S1 and S2). Many of the genes that respond differently to heat stress in our two populations show a striking pattern of consistently reduced response in corals from the more resilient HV population (Fig. 3). Up-regulated genes are less up-regulated in 117 of 136 cases, whereas down-regulated genes are less down-regulated in 30 of 33 cases (Fig. 3).

A reduced heat stress reaction in the more tolerant coral population may result from two potential gene regulatory phenomena. First, some of these genes could have reduced reaction because they begin at a higher constitutive level in resilient corals under control conditions. These "frontloaded" genes may confer resilience through faster reaction at the protein level during
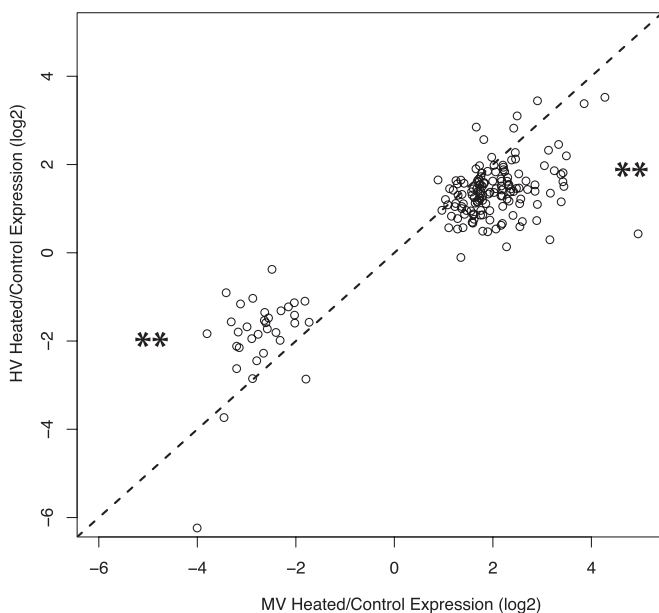
Fig. 3. Scatterplot of the log2 fold changes in gene expression in response to heat stress in the MV corals vs. the HV corals for the 169 genes that were unique to the MV control vs. heated comparison. Each open circle represents an individual contig, the dashed line is a 1:1 line, ** denotes a highly significant departure ($P < 1e^{-15}$ and $1e^{-5}$ for up- and down-regulated contigs, respectively) from a 50/50 null expectation of distribution around the 1:1 line (χ^2 test for goodness of fit).

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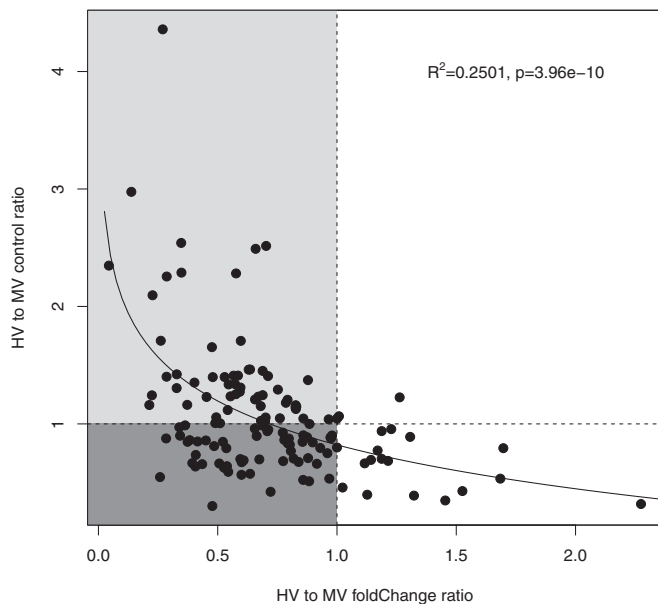**Fig. 4.** Scatterplot comparing the relative ratio of heat-to-control fold changes in expression between HV and MV corals (on the *x* axis) to the HV to MV control expression ratio (on the *y* axis) across the 135 up-regulated genes unique to the MV comparison set to examine whether HV controls show higher constitutive expression (points that are >1 on *y* axis) relative to MV controls for those genes with reduced response to heat stress in HV corals (points that are <1 on *x* axis). The lighter and darker portions of the graph represent the genes that are potentially frontloaded or stress indicators in expression, respectively. The trend line was calculated using a logarithmic regression, and associated R^2 and *P* values are shown in the plot.

transient heat stress. As a result, frontloading may prepare an individual for frequently encountered stress.

Alternatively, a reduced response may be a result of resilience, rather than a cause. The more heat-tolerant HV corals may have experienced lower levels of physiological stress during our experiment and, therefore, may exhibit smaller expression changes in these "stress-indicator" genes as a result. We present these categories of frontloaded and stress-indicator genes as a useful conceptual framework from which to explore the potential mechanisms that may distinguish reactions among stress tolerant and stress sensitive populations.

Frontloaded Genes. We find 60 genes that are potentially frontloaded in heat-tolerant corals (Fig. 4, *Upper Left*, light shading). One well-known gene in this frontloaded subset is Hsp70 (contig81180 matches Hsp70/HSPA5; Dataset S3*A*). Much of the early work on gene expression and environmental tolerances focused on relative levels of Hsp expression (see refs. 50 and 51 for reviews). Hsp70 often has higher constitutive gene expression levels in more thermally tolerant populations or populations from more thermally extreme habitats, as has been shown in a diversity of metazoan taxa including crustaceans, insects, echinoderms, and molluscs (e.g., refs. 32–34 and 52). Our expression results broaden the scope of this phenomenon beyond Hsps and suggest that a number of genes in the more physiologically resilient HV corals follow a similar pattern of frontloading.

Beyond the Hsps, a set of genes involved in cell death signaling and/or immunity also shows a clear pattern of frontloading. Seven of the 12 most strongly frontloaded genes (>70% higher expression in HV vs. MV controls) fall in these categories, including a TNF receptor, two hemicentins in the immunoglobin superfamily, a serine protease precursor (plasminogen), a serine/threonine protein kinase, and two zinc metalloproteinases (Dataset S3*A*). These genes may play a role in shifting the set points at

which apoptosis or immune system activation occur in the presence of environmental stress and change the way coral cells commit to apoptosis, immune response, or cell repair (53).

A different sort of frontloading may occur among down-regulated genes. We find that a majority (21 of 30; 70%; Dataset S3*B*) of the down-regulated, reduced reaction genes exhibit lower constitutive expression in HV than MV controls. The role of reduced expression of these genes in thermal resilience is unclear but reduced expression of some of these genes [e.g., transcription factors HES1, SP5 (54)] could have direct effects on expression of other coral genes.

Mild stress exposures are known to elicit subsequent increases in stress tolerance in a broad suite of organisms [e.g., humans (55), plants (56), bacteria (57), fungi (58), corals (59), fish (60)]. In yeast, such acquired stress resistance is strongest when the mild or primary stress is the same as the more severe or secondary stress [e.g., mild heat stress, followed by severe heat shock (58)]. In intertidal sculpins, fish survived an osmotic or hypoxic shock better if they had had pre-exposure to a moderate heat stress 8–48 h prior (60). These examples are analogous to the increased stress tolerance seen in HV corals, except that in our case, the acquired stress resistance appears to be a result of natural exposure to the extremes of temperature, flow, pH, or other conditions in the HV pool (26, 28, 38), rather than exposure to a single mild primary stress in the laboratory.

Yeast cells that had acquired stress resistance showed smaller changes in gene expression across a large subset of ESR genes in subsequent stress treatments (58). Similarly, the genes that show a reduced reaction to heat stress in the tolerant corals are comprised of many genes and molecular pathways previously shown to change in expression during heat stress exposure (35–37, 44–46). However, our analysis goes a step further to reveal that many of these genes are frontloaded under control conditions in HV corals, suggesting that constitutive transcriptional activity may be altered by natural environmental exposures in the more tolerant population. Additional research into the temporal nature of the frontloading response, as well as the direct role of exposure to environmental variability in acquired stress tolerance in corals, could provide further insight into the mechanistic link between frontloading and the enhanced resilience of the HV coral population.

Linked Cell Death and Immune Response. Included among the frontloaded genes are the TNF receptors (TNFRs). Members of this gene family, along with the similar TNF receptor-associated factors (TRAFs) also show significantly increased expression under heat stress in all comparisons (Datasets S1 *A–C* and S3). The TNFRs and TRAFs are important regulators of the apoptosis cascade because they initiate signal transduction pathways that can result in caspase activation and apoptosis (e.g., ref. 43). Several recent studies have focused on the role of apoptosis regulation in coral bleaching and differential bleaching tolerance (30, 53). Additionally, four other frontloaded genes have functional annotations as oncogenes or protooncogenes (e.g., oncoprotein induced transcript 3, *RET* protooncogene; Dataset S3). Although implicated in a diversity of functions such as uric acid reabsorption and extracellular signaling, each of these can play an important role in regulating apoptosis/cell death (61, 62).

However, the role of the TNFRs and TRAFs goes beyond apoptosis in other organisms and their reaction to heat stress and frontloading may signify a very different cellular cascade. These genes are also involved in the regulation of the immune system through activation of nuclear factor-κB (NF-κB/Nfkb1) and c-Jun N-terminal kinase (JNK/MAPK8) (43). Thus, the differential expression of these genes, combined with the implication of apoptosis regulation in enhanced coral stress tolerance and the role of innate immunity in coral disease resistance (e.g., ref. 63), makes this particular protein family a prime candidate for

involvement in multiple pathways related to coral health and stress tolerance.

Stress-Indicator Genes. The second category of genes that respond less to heat stress in HV corals may contain those that are less up- or down-regulated because of lower levels of intracellular stress in more heat-tolerant colonies. In yeast, reduced transcription during secondary stress was partly attributed to the increased availability of stress-reducing proteins generated during a primary stress (58). In our dataset, these genes would show equal or lower expression in HV controls compared with MV controls and reduced reaction (i.e., lower fold change) during heat stress in tolerant corals (Fig. 4, *Lower Left*, dark shading). Approximately 39% (57 up-regulated and 9 down-regulated) of the 169 reduced-reaction genes fall into this category. These include two genes with blast matches to small Hsps (contig214198 and contig182527 matching Hsp23/HSPB1 and Hsp16.2, respectively), one gene with a match to the cell–cell adhesion protein Sushi, von Willebrand factor (CSMD1; contig77844), and one gene with a match to the antioxidant Cu-Zn superoxide dismutase (Cu-Zn SOD) (contig212121_178489; Dataset S3). Cu-Zn SOD is involved in reducing damage from oxygen radicals thought to be produced by heat-stressed symbionts (64), and the reduced expression changes in this case may signify less need for this process in tolerant corals.

Symbiodinium and Stress. An additional layer of complexity in the coral stress response is the contribution of different *Symbiodinium* types to coral stress resistance. In particular, association with *Symbiodinium* clade D leads to reduced levels of bleaching and greater maintenance of photosynthetic efficiency during heat stress in multiple species including *A. hyacinthus* (28, 65). However, the molecular linkages between clade D and enhanced coral tolerance limits are almost completely unknown. In our experiments, all corals from the more thermally tolerant HV population hosted >94% clade D (Fig. S4 and *SI Materials and Methods*), whereas MV corals were largely clade C (only two of five individuals hosting 19% and 23% clade D in control fragments; Fig. S4). Thus, the expression patterns observed here potentially represent insight into the machinery behind *Symbiodinium* influence on differential coral thermal tolerance limits.

Prior research has shown only a subtle effect of *Symbiodinium* genotype on coral gene expression patterns (66). The mixed corals (76–81% clade C and 19–23% clade D; Fig. S4) in our dataset show slightly different position in PCAs (Fig. 1*B* and Fig. S1), but they are not intermediate between the two groups. Along principal component axis 2, the major axis distinguishing MV and HV corals, mixed corals are more distant from corals with clade D *Symbiodinium* than are corals with predominantly clade C (Fig. 1*B*). These data suggest that corals with different *Symbiodinium* types might express stress genes slightly differently but that the major transcription differences in MV and HV corals may not be attributable to symbiont-type. However, this trend is not conclusive and future studies might focus on clades C and D in a common garden or common host (*sensu* ref. 67) to fully characterize the potential linkages between *Symbiodinium* type, host gene expression patterns, and enhanced coral bleaching resilience.

Conclusions

Corals respond to their environment in a complex fashion, as befits a long-lived organism with little isolation between external physical and internal cellular environments. Our transcriptome data focus on conspecific corals with differing degrees of bleaching resilience and suggest that, in addition to the large number of genes involved in acute heat stress, coral resilience may involve the constitutive, frontloaded expression of several genes that are important in the reaction to environmental stress. Other genes that are different between resilient and sensitive

corals may be stress indicators and reflect lower states of physiological stress in corals with cellular mechanics tuned for high temperatures. These concepts parallel emerging data from model systems, and yet represent further insight into what genomic mechanisms may be responsible for naturally occurring elevated resilience in organisms that are consistently exposed to variable environmental conditions. Additional research will be required to elucidate which frontloaded genes are absolutely required for enhanced thermotolerance, what long-term costs frontloading may have, and whether this phenomenon is acclimatory or adaptive.

Our division of genes into frontloaded and stress-indicator categories represents a mechanistic hypothesis about the way corals respond to temperature, which might serve to explain other differences in coral bleaching thresholds such as latitudinal variation (18). Such mechanistic information about the links between climate and coral health is critical for predicting future impacts of global climate change.

Materials and Methods

Sample Collection and Stress Exposure. Small branchlets (~2 cm³) of *A. hyacinthus* were collected from 16 different coral colonies from two back reef pools on the south side of Ofu Island, American Samoa (14°11′S, 169°36′W). Ten colonies of *A. hyacinthus* were sampled from a larger MV pool, and six colonies were sampled from a smaller HV pool (pools 400 and 300, respectively; for temperature profiles, see ref. 28). Replicate samples (*n* = 2) of the same colony were randomly placed in one of two experimental tanks per condition. The ambient/control condition ranged from 26.8–34.5 °C (mean = 29.2 °C, *n* = 2 tanks), whereas the heat stress condition was elevated by ~2.7 °C over ambient conditions (27–37.6 °C; mean, 31.9 °C; *n* = 2 tanks). Coral health was monitored every 6–12 h, and samples were taken at 1200 hours after 72 h of exposure to the experimental conditions. The 72-h time point was chosen based on our previous study: a 72-h exposure to elevated temperatures induced initial mortality in the MV samples, whereas HV samples remained alive and appeared resilient (figure 5 from ref. 28). All samples were preserved in RNAlater (Life Technologies) and stored at −80 °C until subsequent analysis.

RNA Isolation and mRNA Sequencing. Total RNA was extracted from each sample using a modified TRIzol (GibcoBRL/Invitrogen) protocol (*SI Materials and Methods*). A total of 31 libraries were constructed and sequenced using the Illumina Genome Analyzer II (Illumina) at three different sequencing facilities (for full details, see *SI Materials and Methods*). One of the elevated temperature samples from the HV pool (colony 3) was not sequenced because of poor RNA extraction. The reference transcriptome [33,496 contigs; National Center for Biotechnology Information (NCBI) Transcriptome Shotgun Assembly database under BioProject PRJNA177515 and the Dryad data repository (dx.doi.org/10.5061/dryad.bc0v0)] was generated using CLC Genomics Workbench (Version 4; CLC Bio) incorporating all high-quality reads (Table S2; for full details, see *SI Materials and Methods*).

Gene Expression Analysis. A total of 31 libraries representing 35 individual Illumina lanes were aligned to the reference transcriptome (Table S2 and *SI Materials and Methods*). After quality check (*SI Materials and Methods*), a total of 11 lanes (6 control and 5 heated; *n* = 6 individuals) from the HV population and 9 lanes (5 control and 4 heated; *n* = 5 individuals) from the MV population were used for gene expression analyses. Read counts were analyzed using the package *DESeq* (68) in the statistical environment R (www.CRAN.R-project.org). Low-expression (average normalized expression, <5) contiguous sequences (i.e., "contigs") were excluded from analyses to avoid potential artifact caused by assembly and/or sequencing errors, and high-interindividual variability contigs (within group mean, <1 SD) were also excluded, so that statistical comparisons would not be overly influenced by outlier individuals. The FDR was controlled at 5% according to the method of Benjamini and Hochberg (ref. 69; p.adjust in R). A total of six pairwise comparisons were performed on the dataset to investigate differences in gene expression patterns in response to temperature and based on locations: (*i*) all controls vs. all heateds; (*ii*) all HV vs. all MV corals; (*iii*) HV controls vs. HV heateds; (*iv*) MV controls vs. MV heateds; (*v*) HV controls vs. MV controls; and (*vi*) HV heateds vs. MV heateds.

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