bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

BI-1 mediated cascade improves redox homeostasis during thermal stress and prevents oxidative damage in a preconditioned stony coral

Eva Majerová*, Crawford Drury

Hawai�i Institute of Marine Biology, University of Hawai�i at Mānoa, Kāne�ohe, HI 96744

*Correspondence:

Eva Majerová

46-007 Lilipuna Rd, Kaneohe HI, 96744

majerova@hawaii.edu

808-236-7455

Preprint: BioRxiv 10.1101/2021.03.15.435543

Keywords: Coral Bleaching, Oxidative Stress, siRNA gene knockdown, Acclimatization, Antioxidants

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Abstract

Global coral reef decline is driven in large part by the breakdown of the coral-algal symbiosis during temperature stress. Corals can acclimatize to higher temperatures, but the cellular processes underlying this ability are poorly understood. We show that preconditioning-based improvements in thermal tolerance in *Pocillopora acuta* are accompanied by increases in host glutathione reductase (GR) activity and expression, which prevents DNA damage. We found a strong correlation between *GR* and *BI-1* expression in heat-stressed preconditioned corals and discovered an antioxidant response element (ARE) in the *GR* promoter, suggesting BI-1 could regulate *GR* expression through the Nrf2/ARE pathway. To fortify this link, we developed an siRNA-mediated gene knockdown protocol and targeted the coral BI-1 gene. BI-1 knock-down decreased glutathione reductase expression and activity, and increased oxidative DNA damage in heat-stressed preconditioned corals, showing that enhanced regulation of antioxidant response during acute heat stress is a key mechanism that prevents oxidative DNA damage after preconditioning.

Teaser

Preconditioning improves redox homeostasis and prevents oxidative stress in a thermally stressed reef-building coral

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Introduction

Healthy coral reefs support nearly one-third of marine species and provide shelter, nursery habitat and coastal protection across the tropical oceans [1–3]. The foundation of this ecosystem is the coral-algal symbiosis, which is disrupted by thermal stress and is critically threatened by climate change. Climate induced mass bleaching has impacted most of the world's reefs and is predicted to increase in frequency and intensity, threatening the long-term persistence of these ecosystems [4–6]. A deeper understanding of the cellular and molecular mechanisms underlying coral-algal symbiosis maintenance is critical for modern conservation and management [7,8].

Corals are energetically dependent on their intracellular algal symbionts (family *Symbiodiniaceae*), which provide photosynthetically synthesized sugars and receive shelter and a supply of inorganic molecules from the host. During thermal stress, symbionts release increased reactive oxygen species (ROS), which are believed to trigger molecular cascades resulting in coral bleaching and can lead to the eventual death of the coral host organism [9–17]; however, definitive proofs for this hypothesis are still missing [18].

Under normal conditions, ROS are scavenged by antioxidant systems in the host and symbionts to reduce damage to cell membranes, lipids and nucleic acids [19,20]. Under increased temperatures, ROS concentrations become elevated and the coral holobiont activates first-line enzymatic antioxidants such as catalase or superoxide dismutase and maintain a reducing intracellular environment via the glutathione redox cycle [10,12,14,16,21–24]. These systems are well-studied in *Symbiodiniaceae*, where genera with different thermal resilience vary in antioxidant gene expression [12,16,23] and thermally resilient algae generally produce more antioxidants,

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

which are able to better maintain cellular homeostasis. Conversely, the role of host-derived antioxidants in bleaching and thermal resilience remains disputed. While pioneering studies show antioxidant activation in adult coral host tissue and larvae under heat stress [16,25–29], more recent experiments have failed to find this pattern [10,22]. However, the coral host control of the level of oxygen radicals and ROS is likely a major component in the dynamics of coral-algal symbiosis maintenance [18,30].

Intra-generational flexibility in coping with stress is critical for the long-term persistence of coral reefs, 'buying time' for adaptive change [31]. Corals are sessile invertebrates unable to avoid stressful environmental change, highlighting the importance of acclimatization. Corals can improve their thermal tolerance after pre-exposure to sublethal temperatures [32–39], but the molecular triggers and consequences of this process remain poorly understood.

In this work, we show that preconditioned *P. acuta* prevents heat-induced oxidative DNA damage via increased activity of glutathione reductase. This glutathione reductase activity is highly correlated with coral BI-1 (Bax-inhibitor 1) gene expression in heat-stressed corals. We developed and conducted siRNA-mediated gene knockdown experiments in living adult corals to investigate the link. BI-1 downregulation leads to a decreased glutathione reductase expression and decreased glutathione reductase activity and results in an accumulation of oxidative DNA damage in coral tissue upon acute heat stress. The evidence from vertebrate models and the discovery of an antioxidant response element (ARE) in the coral glutathione reductase gene putative promoter region suggest that BI-1 likely impacts coral antioxidant system through the Nrf2/ARE signaling pathway.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Results

Thermal preconditioning improves bleaching susceptibility in P. acuta

This study uses samples from the same coral colonies described in the preconditioning experiment of Majerova et al. [39]. When exposed to acute heat stress (32°C), preconditioned (PC) corals showed substantially increased symbiosis stability when compared to non-preconditioned (NPC) corals (Fig. 1B). After 3 days of heat stress, NPC corals were visibly bleached while PC corals resembled control, non-treated corals. This difference was more pronounced after 5 days of heat stress. However, PC corals still lost symbionts, but significantly more slowly than in NPC corals ($p < 0.001$) [39].

Preconditioning selectively increases the activity of host-derived glutathione reductase

The activity of glutathione reductase in *P. acuta* is influenced by acute heat stress (ANOVA, activity~treatment*time + (1|coral); p(time) = 0.0011) and was constitutively higher in PC corals than NPC corals ($p_{(treatment)} < 0.001$, Fig. 2A). Interestingly, the activity of peroxide-scavenging antioxidants was dynamic ($p_{(time)} = 0.0086$) but did not differ between PC and NPC corals. Increased activities of two different peroxidases - catalase and glutathione peroxidase – have been detected in stressed corals [16,21,25,27], but the catalase activity kit used here may detect any enzyme with peroxidase activity (Bioassay Systems, personal communication). Thus, we separately tested the activity of glutathione peroxidase with a specific kit (Bioassays). We were not able to detect any glutathione peroxidase activity in our samples so we assume that

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

the peroxidase activity is primarily catalase, although we cannot conclusively exclude technical issues with the assay.

To examine if the observed increase in glutathione reductase activity was connected to the stimulation of the gene expression, and if such response is common for both partners or is host-specific, we analyzed the expression of coral host and symbiont gene in PC and NPC corals upon heat stress (Fig. 2B). Host glutathione reductase expression changed over time and between treatments (ANOVA, expression~treatment*time + (1|coral), $p_{(time)} < 0.001$, $p_{(treatment)} = 0.0098$). We observed an increase in PC corals shortly after the beginning of the heat stress ($p_{(1h)} = 0.0736$) that peaked at 3 hours, when the expression was ~ 2-fold higher compared to NPC corals ($p_{(3h)} = 0.0015$).

Symbiont glutathione reductase expression was not significantly different between treatments ($p = 0.8099$) and did not change over time ($p = 0.1485$). This suggests that glutathione reductase dynamics are driven by the coral host; however, there was no significant correlation between protein activity and gene expression for either host or symbiont cells (Fig S1).

Increased activity of antioxidants protects DNA from oxidative damage

Glutathione reductase helps stabilize the reducing environment of the cell, thus enhancing its ROS scavenging ability and preventing cellular stress such as oxidative DNA damage [19,40]. To clarify whether the increase in glutathione reductase activity improves ROS protection in heat-stressed corals, we analyzed the level of oxidized guanine species (8-OHdG),

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

markers of oxidative DNA damage [19]. There was a clear difference between PC and NPC corals in time (Two-Way ANOVA, 8-OHdG~conditioning*time with Tukey post-hoc testing, $p_{(time:conditioning)} = 0.0098$, Fig. 3). Unlike in PC corals, we observed an accumulation of 8-OHdG in NPC corals after 24 hours of the acute heat stress ($p_{(NPC)} = 0.0991$, $p_{(PC)} = 0.4082$, $p_{(NPC-PC,\ 24h)} = 0.0310$, p(NPC – PC, 0h) = 0.7449). Surprisingly, in PC corals, the level of 8-OHdG decreased slightly, but not significantly during heat stress.

To fortify the connection between antioxidants and oxidative DNA damage in corals, we treated NPC corals with 10mM mannitol during acute heat stress. Mannitol protects plants and algae from ROS and is considered a non-enzymatic antioxidant [41] that was shown to prevent general DNA damage in coral tissue aggregates [20]. Again, NPC corals accumulated marks of oxidative DNA damage during heat stress (One-way ANOVA, $p_{(treatment)} = 0.0044$), but the addition of 10mM mannitol eliminated this increase (One-Way ANOVA, $p_{(no\ treatment:mannitol)} = 0.0298$, $p_{(control:mannitol)} = 0.6869$).

pa-BI-1 controls the expression of glutathione reductase in preconditioned corals.

BI-1 (BAX inhibitor 1) is an anti-apoptotic protein that – among others – promotes cell survival by increasing the production of antioxidants through the activation of Nrf2 transcription factor in human cells [42,43]. We previously showed that PC corals increase the expression of pa-*BI-1* during acute heat stress compared to NPC corals [39]. Since these observations were made on the same set of samples as our measurements of the expression of glutathione reductase (pa-*GR*), we examined the correlation between the gene expressions of pa-*BI-1* and pa-*GR*. Surprisingly, we observed a strong positive correlation in PC (Pearson =

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

0.948, $p_{(lm)} = 0.004$) but not NPC (Pearson = 0.126, $p_{(lm)} = 0.4679$) corals (Fig. S2). The strongest correlation occurred during the first 3 hours of the heat stress, where we observed a significant overexpression of both genes in PC but not NPC corals ([39] and Fig. 2), suggesting coral BI-1 can regulate the expression of antioxidant genes upon stress conditions.

To confirm this hypothesis, we developed a protocol for siRNA-mediated gene knockdown in living adult corals and inhibited the expression of pa-*BI-1* in heat-stressed PC corals (Fig. 4). We optimized the timing between siRNA administration, the beginning of a heat stress and coral sampling to reach the most significant knock-down of pa-BI-1 during the first hours of the acute heat stress when it was most strongly overexpressed in PC corals [39]. We successfully inhibited pa-*BI-1* expression in 8 out of 17 corals (we set a threshold of 86% gene expression as a successful knock-down) ranging from 13% to 86% expression compared to siNTC – corals treated with control siRNA (52.02% ± 26.23, mean ± SD). siNTC was used in all experiments to exclude the effect of the siRNA treatment itself on the studied pathways. For all further analyses, we chose only the 8 corals with a successful knock-down and disregarded the corals with no pa-*BI-1* knock-down.

As expected, we observed an overexpression of both pa-*BI-1* and pa-*GR* in heat-stressed PC corals (Fig. 4A, siNTC) when compared to control corals (PC corals at ambient temperature). After siBI-1 knockdown (siBI-1), the expression of both genes decreased (Fig. 4A, Wilcoxon test, $p_{(BI-1)} = 0.0078$, $p_{(GR)} = 0.03125$) but was still significantly higher than in control corals (Wilcoxon test, $p_{(BI-1)} = 0.03906$, $p_{(GR)} = 0.02344$). There was a strong correlation between pa-*BI-1* expression and pa-*GR* expression following the gene knockdown (lm, GR~BI-1, p = 0.0011, r^2 = 0.51), indicating the inhibition of pa-*BI-1* expression leads to a decrease in pa-*GR* expression.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

To exclude non-specific effects of the siRNA treatment, we analyzed the correlation of pa-*BI-1* expression with 4 genes (pa-*HSP70*, pa-*Bcl-2*, pa-*BAK*, pa-*BAX*) which follow a similar expression patterns as pa-*BI-1* in PC and NPC corals upon heat stress, and with pa-*NFKBI* (NFkB inhibitor) that was differentially expressed ([39], Fig S3A). *pa-BAX* and *pa-Bcl-2* showed strong correlation with *pa-BI-1* gene expression in PC corals (Fig S3B) ($r_{(Pearson)}$ = 0.856 and 0.726; $p_{(lm)} < 0.001$) and a weaker but still considerable correlation in NPC corals ($r_{(Pearson)}$ = 0.642 and 0.531; $p_{(lm)} < 0.001$). *pa-BAK* and *pa-HSP70* showed a moderate correlation with pa-BI-1 in PC corals ($r_{(Pearson)}$ = 0.446 and 0.438; $p_{(lm)}$ = 0.013 and 0.0023) but – as expected – the expression of *pa-NFKBI* did not correlate with the expression of *pa-BI-1* in any corals ($r_{(Pearson)}$ = 0.27 and 0.203 (for PC and NPC corals, respectively); $p_{(lm)}$ = 0.12 and 0.18).

We hypothesized that if the siRNA treatment is not specific to the *pa-BI-1* gene or impacts the whole bleaching pathway, we would observe a shift in multiple genes involved in the coral bleaching cascade. Upon siBI-1 treatment, the expression of none of these genes changed significantly (Fig S3B), supporting the specificity of the siRNA treatment to siBI-1 gene expression.

Decrease in glutathione reductase gene expression results in a decrease in enzyme activity

Since changes in gene expression are not always directly mirrored in the protein level and/or activity, we measured the activity of glutathione reductase in coral host tissue after siBI-1 gene knockdown. The decrease in pa-*GR* expression after 3 hours of heat stress is followed by a significant decrease in the enzymatic activity at 24 hours post stress (Paired t-test, $p_{(activity)}$ = 0.0391, Fig. 4B). There was a rapid decrease in the glutathione reductase activity in 5 corals,

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

and a very slight increase in 2 corals (Fig. 4B dotted lines connecting siNTC-siBI-1 pairs), suggesting that decreased pa-*GR* expression does result in decreased pa-GR activity, but there may be other genotype-specific effects.

Corals with inhibited expression of pa-BI-1 are more prone to oxidative DNA damage

To examine the connection between antioxidant system and oxidative DNA damage in heat-stressed corals, we analyzed the level of oxidative DNA damage using an 8-OHdG marker in corals with pa-*BI-1* knockdown and subsequent decrease in glutathione reductase activity. As shown in Fig. 4C, siBI-1 treated corals accumulate significantly more oxidized guanines in DNA during acute heat stress (24 hours at 32 °C, Paired t-test, $p = 0.0181$) when compared to the PC corals treated with control siRNA. This observation supports the hypothesis that during heat stress, corals use antioxidants to protect important cellular structures from oxidative damage.

An antioxidant responsive element (ARE) lies within the promotor of coral glutathione reductase gene

In mammals, BI-1 can activate Nrf2 transcription factor that in turn regulates gene expressions through the *cis*-acting elements in the Nrf2 target gene promoters called antioxidant responsive elements (ARE) [42,44]. Nrf2 gene has not yet been described in reef-building corals but the *Nematostella vectensis* putative Nrf2 protein sequence (GenBank KU746947.1), returns blast hits (tblastn) for several uncharacterized loci in stony corals (e.g., *Orbicella faveolata* LOC110060612, 93 % query cover, 29.89% identity and 1e-43 E-value, or

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Pocillopora damicornis, LOC113687044, 53% query cover, 30.94% identity and 1e-43 E-value) suggesting a protein with a Nrf2-like function may be present in stony corals.

We searched the promoter region (lies within NW_020843386.1) of the glutathione reductase gene (XM_027196629.1) in *Pocillopora damicornis* [45] and found an ARE-similar sequence 5'-TGACTTAGC-3' [44,46] 557 bp upstream of the predicted beginning of the gene ORF. This ARE was first discovered and described in the promoter of glutathione peroxidase in human liver carcinoma cells (HepG2) at positions -76 and -387 with respect to +1 transcription start site [46]. This striking resemblance suggests that the Nrf2/ARE pathway might be evolutionary more conserved than has been previously thought.

Discussion

Like most organisms, corals have the ability to acclimatize to stress conditions after pre-exposure and recent studies have confirmed that acclimatization reduces the severity of bleaching and mortality [32–36,39,48]. However, this natural phenomenon may be genotype-specific [37] and could be lost under future climate-change scenarios [38]. One of the main obstacles to the application of this strategy for conservation [7,8,31] is our limited understanding of molecular and cellular mechanisms behind acclimatization and/or adaptation to increased temperatures. Here we show that preconditioning-based acclimatization is mediated by the interaction of the pro-life gene *BI-1* and antioxidant response, which impacts cellular phenotypes such as DNA damage.

Transcriptomic studies in acclimatized corals shed light on the main gene families and cellular pathways that play a role in the bleaching process, but functional studies have been

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

largely missing in reef-building corals [32,33,35,49]. Recently, we showed that preconditioning in *Pocillopora acuta* leads to improved thermal tolerance due to modulations in the programmed cell death pathway (PCD), most likely via autophagy/symbiophagy [39]. However, the primary signals or molecular consequences of such a prolonged symbiosis maintenance under thermal stress are unclear. During heat stress, the coral host and symbionts release increased reactive oxygen species (ROS) [10,11,13,14,18,24,50] which can activate an array of regulatory pathways, often depending on the level of ROS accumulation [51,52]. For example, in model animals, low doses of ROS activate cell survival signaling pathways such as UPR (unfolded protein response) or Nrf2, while high doses of ROS activate PCD [52]. We thus hypothesized that after preconditioning, the level of ROS signaling molecules in heat-stressed corals was reduced, which resulted in changes in PCD signaling.

We find that preconditioned (PC) corals with higher tolerance to thermal stress and reduced bleaching rate [39] have higher activity of glutathione reductase but not peroxide-scavenging enzymes in the host tissue when compared to non-preconditioned (NPC) corals (Fig. 2A). Gene expression analysis suggests that the observed increase in glutathione reductase activity derives from the host cells but is probably dependent on more factors than just expression rate (Fig. 2B). While we observe a difference in host-derived glutathione reductase (pa-GR) gene expression between NPC and PC corals at one hour after the beginning of the heat stress, peaking at three hours and then returning to the same level as NPC corals, the activity of the antioxidant is constantly higher in PC corals even in ambient conditions (time 0). The level of mRNA does not always correlate with the cellular protein levels and the relationship between the two strongly varies during dynamic transitions such as short-term adaptation

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

(reviewed in [53]). Discrepancies between mRNA levels and antioxidant activities were previously observed for example in heat-stressed *Symbiodiniaceae* [54]. Based on our transcriptomic and protein analyses, we hypothesize that while posttranscriptional and posttranslational modifications may positively impact the activity, turnover rate, or localization of the antioxidant in PC corals in ambient conditions, the rapid induction of pa-*GR* gene expression in PC corals is potentially a major contributor to the observed activity differences upon acute heat stress.

Glutathione is a non-enzymatic antioxidant that exists in reduced (GSH) and oxidized (GSSG) form in the cell (reviewed in 30). GSH neutralizes ROS while being oxidized to glutathione disulfide (GSSG); this oxidized state is converted back to the reduced state by glutathione reductase (GR). Under normal conditions, over 90 % of the glutathione pool is maintained as GSH by GR activity, so GR is directly responsible for maintaining the reducing environment of the cell and for ROS scavenging. The level of GSH and/or the activity of enzymes involved in the glutathione redox cycle are inversely associated with the oxidative DNA damage [55,56]. Moreover, heat exposure induces oxidative stress and DNA damage in mice, humans, plants and fish [57–62], and heat acclimation led to a decreased accumulation of 8-OHdG markers in blood cells of navy boiler tenders exposed to high heat during work [63]. Finally, increased DNA damage was observed in coral tissue explants exposed to elevated temperatures or to direct sunshine [20,64].

We tested the level of oxidized guanine species, markers of oxidative DNA damage, in PC and NPC corals after 24 hours of heat stress and found that while NPC corals accumulate these markers, PC corals do not (Figure 3). Surprisingly, we found that during heat stress, the level of 8-OHdG in PC corals slightly but not significantly decreases which could be explained by

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

– for example – more efficient DNA damage repair procedures that act in parallel with the enhanced antioxidant system in corals after preconditioning. However, this speculation would have to be further tested. Previous experiments showed that the addition of exogeneous antioxidant mannitol can reduce DNA damage in heat-stressed coral cell aggregates [20], so we used this antioxidant on a new set of NPC corals to solidify the link between antioxidant system and DNA damage in the whole adult coral organism. NPC corals suffered DNA damage upon heat stress unless 10mM mannitol was added (Fig. 3), clearly implicating the antioxidant system as the major contributor in the differences in DNA damage between PC and NPC corals.

DNA damage is – besides ROS signaling – another trigger of diverse PCD pathways, including autophagy [65], supporting our previous results showing preconditioning improves coral thermal tolerance via modulations in the autophagy pathway [39]. BI-1 (BAX-1 inhibitor), a pro-survival PCD gene involved in the regulation of PCD pathways, was shown to reduce ROS accumulation in vertebrates and to activate Nrf2, which is a transcription factor of various antioxidants, including glutathione reductase [42,66]. Interestingly, we saw an upregulation of pa-*BI-1* in PC corals upon heat stress, peaking at 3 h after the stress start [67], which parallels host glutathione reductase expression (Fig. 1B). We compared the expression rates of pa-*BI-1* and pa-*GR* in PC and NPC corals and strikingly, they are highly correlated in PC corals in the early phase of the heat stress response (< 6hours), but not in NPC corals (Fig. S2). We hypothesize that preconditioning may enable pa-BI-1 to effectively regulate the expression of pa-*GR* through epigenetic modifications of the pa-*GR* regulatory elements. DNA methylation patterns vary between corals living in different environments and is dynamic over time in corals exposed to environmental changes, very likely enabling gene expression fine tuning in response to

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

various conditions [68–71]. Future experiments should investigate this link between preconditioning, epigenetic modifications, and expression of particular genes in corals.

To fortify the functional correlation between pa-BI-1 and pa-GR, we developed a protocol for siRNA-mediated gene knockdown in living adult corals and inhibited pa-*BI-1* gene expression (Fig. 4A). We manipulated the gene in 8 out of 17 corals and hypothesize that the mucus that corals excrete when disturbed likely interfered with the siRNA transfection in some individuals. In the 8 corals with efficient pa-*BI-1* knockdown, pa-*GR* expression decreased, leading to a decline in pa-*GR* activity (Fig. 4B). This proves that pa-BI-1 can regulate gene expression of pa-GR in preconditioned corals. In vertebrate models, BI-1 was found to regulate expression of genes coding for antioxidants through Nrf-2 transcription factor [42,43]. Although Nrf2 has not been described in reef-building corals, a homolog of the Nrf-2 gene was identified and annotated in *Nematostella vectensis* (GenBank KU746947.1, [72]), where the Nrf-2 mediated oxidative stress response pathway was activated in its symbiotic but not apo-symbiotic morph during thermal stress [73]. Nrf2 regulates the expression of antioxidant genes via binding to the so-called ARE (antioxidant response element) *cis*-elements located upstream of the transcription start site [44]. In the promoter of coral glutathione reductase gene, we found an ARE similar to one described in the promoter of the glutathione peroxidase gene in human liver cells [46], suggesting the Nrf2/ARE pathway is conserved in Cnidarians, where it controls the expression of antioxidant genes during environmental stress response. We thus propose this pathway could also connect BI-1 and glutathione reductase in *Pocillopora acuta*.

The decreased expression of pa-*GR* was mirrored in the lower activity of glutathione reductase antioxidant in siBI-1 corals, and these corals accumulated more oxidative damage

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

than corals treated with a control siRNA (siRNA with no known target in *P. acuta*) (Fig. 4B, C), supporting the hypothesis that antioxidants prevent cellular oxidative damage in corals.

In summary, we propose a model where in non-preconditioned heat-stressed corals, the amount of ROS exceeds the ability of the corals to scavenge it, which leads to oxidative cellular damage, activation of programmed cell death pathway (autophagy/symbiophagy), symbiosis disruption and coral bleaching. After preconditioning, heat-stressed corals improve the ability to maintain cellular redox homeostasis through BI-1-mediated glutathione reductase overexpression which prevents the accumulation of oxidative stress markers, avoids the activation of programmed cell death and results in prolongated coral-algal symbiosis. This work describes the cellular and molecular principles of coral symbiosis maintenance under heat stress and how it is modulated during acclimatization. We also show that prolongated symbiosis maintenance during heat stress in preconditioned corals does not impair genome stability in short-term period, but the long-term consequences still need to be analyzed.

Materials and Methods

Collections, experimental setup, and preconditioning

Seven colonies of *P. acuta* were collected in spring 2018 at different sites and depths ranging between 1 to 4m across Kāneʻohe Bay, Hawaiʻi to maximize genetic diversity. Experimental treatments followed in Majerova et al. [39]. Briefly, corals were fragmented and allocated into preconditioning (PC) and control (NPC) treatments and PC corals were exposed to a 29°C for 72h before returning to ambient while NPC corals were maintained at 26°C (Fig 1A).

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Fragments were then clipped into 5cm nubbins and reallocated into heat stress treatments. After two weeks at 26°C, corals were exposed to a 32°C treatment or control and sampled at 0, 1, 3, 6, 12, and 24 hours. Fragments were stored immediately at -80°C. Bleaching rate was assessed as the shift in symbiont-to-host signal ratio with time-lapse confocal microscopy (Zeiss LSM-710) as described in Majerova et al.[67].

Antioxidant activity assays

Samples previously stored at -80°C were homogenized in ice cold extraction buffer (100mM Tris-HCl, 20mM EDTA, pH 7.5) in Qiagen TissueLyser ($30s^{-1}$ for 20s) with acid-washed glass beads (Sigma). The symbiont and host cells were separated with low-speed centrifugation (800g, 5min, 4°C) and the supernatant was then sonicated for 3 mins. Cell debris were pelleted by centrifugation (14,000g, 10min, 4°C) and whole cell protein extract concentration was measured using Qubit Protein Assay Kit (Thermo Fisher). Protein extracts were immediately used for EnzyChrom Catalase Assay Kit and EnzyChrom Glutathione Reductase Assay Kit (BioAssay Systems), respectively. The working protein concentration was optimized prior to the experiment to ensure the measured activity was within the range of kit detection limits. We used 1340ng and 300 ng of the whole cell protein extract in Catalase Assay Kit and Glutathione Reductase Assay Kit, respectively, to normalize the enzymatic activity to total protein. Each analysis included one sample per timepoint per colony (n=7).

The Catalase Assay Kit is not specific to catalase and measures activity of all peroxide-scavenging antioxidants, so we used the EnzyChrom Glutathione Peroxidase Assay Kit (BioAssay

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Systems) to distinguish activity of different peroxide-scavenging antioxidants. All assays (total protein 2 – 10μg) were below the detection limit of the kit.

Gene expression analysis

Gene expression was analyzed as described in Majerova et al.[39]. Briefly, RNA was extracted via RiboZol RNA Extraction Reagent (VWR Life Science) with DNase I step between phenol-chloroform extraction and ethanol precipitation. 1μg of RNA was reversely transcribed with High-Capacity cDNA reverse transcription kit (ThermoFisher Scientific). Reverse transcription quantitative PCR (RT-qPCR) reactions were run in 12 μl with PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) for 40 cycles. Profiles of four genes for each organism (host and symbiont) under different treatments were compared with Best Keeper software and pa-*EF-1* (elongation factor 1a, *P. acuta*), and s*SAM* (S-adenosyl L-methionine synthetase, *Symbiodinium sp.)* showed the highest expression stability upon heat stress and were chosen as the reference genes. All primer sequences are listed in Table S1. Expression of target genes was calculated relative to the non-preconditioned coral at time 0 with ΔΔCt method.

$$\Delta\Delta\mathrm{Ct} = 2^{-[(Ct_{target,treated} - Ct_{ref,treated}) - (Ct_{target,control} - Ct_{ref,control}]}$$

Each analysis included one sample per timepoint per colony (n=7).

Oxidative DNA damage assay

To assess the impact of oxidative stress on DNA, we analyzed the level of 8-Hydroxy-2'-deoxyguanosine (8-OHdG) in PC and NPC corals as a proxy for cumulative oxidative DNA

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

damage. 2 fragments from 6 corals in each treatment were sampled prior to and after 24 hours of thermal stress (as described above), frozen and kept at -80°C.

We used a custom extraction protocol to extract DNA from host cells after symbiont and host cells were separated as described above. After the low-speed centrifugation, SDS (0.5% final concentration) was added to the supernatant containing host cells and nuclei and incubated at 58°C for 15 min. Then, proteinase K (0.5 mg/ml final concentration) was added and incubated at 58°C for 2h. After incubation, samples were cooled to room temperature and KAc was added to a final concentration 0.5M. Samples were centrifuged (14,000g, 10min, 4°C) and the supernatant was precipitated by isopropanol (1:0.7 ratio). After precipitation, the DNA pellet was resuspended in water and the sample was treated by RNAse A for 15 min. DNA was purified by phenol-chloroform extraction followed by ethanol precipitation (O/N, -20°C) and resuspended in water. 500ng of total genomic DNA in 20µl was denatured at 95°C for 5 min, rapidly cooled down on ice, digested to single nucleotides by 20U of Nuclease P1 (NEB) at 37°C for 30 min and dephosphorylated by 1U of Shrimp Alkaline Phosphatase (NEB) for 30 min at 37°C. All the enzymes were inactivated at 75°C for 10 min. DNA was then diluted 1:5 in 1x Assay buffer and processed according to the kit instructions (DNA Damage Competitive ELISA Kit, Invitrogen). The initial DNA concentration used in the assay was optimized to ensure the resulting values fall within the optimum range of kit detection limits (between Std3 and Std6). We used MyCurveFit.com software to model the standard curve and to predict result values. The analysis was conducted in 2 samples per timepoint per colony using 6 colonies in total. The results from biological replicates were averaged before statistical analysis.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Due to the results indicating that corals with increased glutathione reductase activity are less prone to oxidative DNA damage accumulation, we decided to test the impact of exogenous antioxidants to heat-stressed non-preconditioned corals. For this analysis, a new set of corals was collected from Kāne'ohe Bay in spring 2020 and prepared as describe above. This new set of corals was used for the following mannitol experiment and all the siRNA-involved experiments (see below).

To analyze the impact of exogenous antioxidant to oxidative DNA damage, we treated NPC nubbins with 10mM mannitol [20] or a seawater control for the duration of the heat stress experiment (6 NPC and 6 PC corals, 2 nubbins per colony per treatment). All coral fragments were sampled after 24h of acute heat stress (32°C), frozen and kept at -80°C. DNA damage assay was conducted as described above.

siRNA-mediated knockdown

BLOCK-iT™ RNAi Designer (ThermoFisher Scientific) was used to design siRNA for the *P. damicornis* BI-1 mRNA sequence (XM_027189407.1 , [45]). We chose the highest-ranking siRNA sequence containing BCD Tuschl's patterns and targeting region 194- 212 of the 1437pb long mRNA sequence. Control siRNA (siNTC) was designed to contain the same nucleotide composition but having no known target in the *P. damicornis* mRNA sequence database. Both siRNA molecules were synthesized by Gene Link, Inc.

PC coral fragments (~ 3 cm long) were placed into 20 ml cultivation vials, completely submerged into flow-through tanks with ambient temperature seawater and left to acclimatize overnight. The next day, vials were moved into precise temperature-controlled water bath, the

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

seawater was carefully removed without disturbing corals and the siRNA transfection was carried out according to the manufacturer's instructions (INTERFERin® transfection reagent, Polyplus). siRNA transfection mix (20 µl of 10nM siRNA and 16 µl INTERFERin® reagent in 250 µl 0.2nm filtered seawater) was pipetted directly on the exposed coral and after ~ 2 minutes, 5 ml of 0.2 nm filtered seawater was added to fully cover the nubbin and coral was incubated at ambient temperature for 6 hours. After this time, vials were fully submerged into a flow-through tank with ambient temperature seawater for two days. 48 hours after the beginning of the siRNA transfection, corals were exposed to an acute heat stress (32°C, ramping speed of ~1°C per 10 mins). At 3 hours post-stress, each coral was sampled for the gene expression analysis and at 24h hours post-stress for glutathione reductase activity and DNA damage assay. This sampling consumed the fragment.

Statistical analyses

All statistical analyses were run in RStudio on data assembled in Microsoft Excel. Datasets were tested for normality (histogram plot) and heteroscedascity (Levene test). Where needed, data were normalized to logarithmic scale or using BestNormalize function in R (package BestNormalize).

We analyzed antioxidant activity after normalizing data to the initial control activity (NPC, time 0) for each colony. We used a generalized mixed model with treatment and time as main effects and colony as a random effect with a Tukey post-hoc testing for each timepoint.

We analyzed gene expression after normalizing data to the initial control expression for each colony. We used a generalized mixed model to examine normalized expression of each

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

gene separately, using conditioning treatment and time as main effects and colony as a random effect. We estimated least-squares means and compared between treatments for each timepoint using bonferroni adjusted p-values.

To analyze DNA damage, we ran a two-way ANOVA to compare the level of 8-OHdG (pg per µl DNA) of PC and NPC corals with time and conditioning as main effects. The differences between timepoints and conditioning were tested using Tukey post-hoc analysis. The effect of mannitol treatment to DNA damage was tested by one-way ANOVA with treatment (control, heat stress, heat stress with mannitol) as variable followed by a Tukey post-hoc testing.

We calculated Pearson correlation coefficients and variance explained using linear regression for the relationships between a) gene expression and enzymatic activity and b) BI-1 and glutathione reductase expression.

The effect of siRNA-mediated knockdown was analyzed using paired samples Wilcoxon test on data normalized to control (ambient temperature, non-treated) coral samples. Antioxidant activity data were also first normalized to control coral samples and then subjected to paired T-test. DNA damage analysis in siRNA-treated corals was analyzed with paired t-test on raw data.

Conflict of Interest

The authors declare no conflict of interest.

Data availability statement

All data needed to evaluate the conclusions in the paper are present in the paper and the Supplementary Materials. The data can be provided by Eva Majerova pending scientific review

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

and a completed material agreement. Requests for the data should be submitted to majerova@hawaii.edu

Acknowledgements

We would like to thank Shayle Matsuda and our interns Fiona C. Carey, Filip Blaštík, and Vojtěch Prokůpek for their help with coral collection and siRNA knock-down optimization tests, and Tomáš Buryška for advice on execution of enzymatic assays. We thank the To-Bo Lab (HIMB) for access to instruments. We dedicate this manuscript to Ruth Gates, who mentored us and inspired us to use molecular tools to understand the coral bleaching crisis. This work was funded by the Paul G. Allen Family Foundation and the Annenberg Foundation. Corals were collected under SAP-2020-25 to HIMB. This is HIMB contribution xx and SOEST contribution xx.

Author Contributions

EM conceived the experiments, conducted research, analyzed data, and wrote the manuscript. **CD** analyzed data and wrote the manuscript. Both authors approved the final version.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1. Vercelloni, J. *et al.* Using virtual reality to estimate aesthetic values of coral reefs. *R. Soc. Open Sci.* **5**, (2018).
2. Fisher, R. *et al.* Species Richness on Coral Reefs and the Pursuit of Convergent Global Estimates. *Curr. Biol.* **25**, 500–505 (2015).
3. Knowlton, N. *et al.* Coral Reef Biodiversity. in *Life in the World's Oceans* (ed. McIntyre, A. D.) 65–78 (Wiley-Blackwell, 2010). doi:10.1002/9781444325508.ch4.
4. Eakin, C. M., Sweatman, H. P. A. & Brainard, R. E. The 2014–2017 global-scale coral bleaching event: insights and impacts. *Coral Reefs* **38**, 539–545 (2019).
5. Hughes, T. P. *et al.* Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science* **359**, 80–83 (2018).
6. van Hooidonk, R., Maynard, J. A. & Planes, S. Temporary refugia for coral reefs in a warming world. *Nat. Clim. Change* **3**, 508–511 (2013).
7. van Oppen, M. J. H., Oliver, J. K., Putnam, H. M. & Gates, R. D. Building coral reef resilience through assisted evolution. *Proc. Natl. Acad. Sci.* **112**, 2307–2313 (2015).
8. Committee on Interventions to Increase the Resilience of Coral Reefs, Ocean Studies Board, Board on Life Sciences, Division on Earth and Life Studies, & National Academies of Sciences, Engineering, and Medicine. *A Research Review of Interventions to Increase the Persistence and Resilience of Coral Reefs*. (National Academies Press, 2019). doi:10.17226/25279.
9. Oakley, C. A. & Davy, S. K. Cell Biology of Coral Bleaching. in *Coral Bleaching* (eds. van Oppen, M. J. H. & Lough, J. M.) vol. 233 189–211 (Springer International Publishing, 2018).
10. Nielsen, D. A., Petrou, K. & Gates, R. D. Coral bleaching from a single cell perspective. *ISME J.* **12**, 1558–1567 (2018).
11. Suggett, D. J. *et al.* PHOTOSYNTHESIS AND PRODUCTION OF HYDROGEN PEROXIDE BY *SYMBIODINIUM* (PYRRHOPHYTA) PHYLOTYPES WITH DIFFERENT THERMAL TOLERANCES [1]. *J. Phycol.* **44**, 948–956 (2008).
12. Levin, R. A. *et al.* Sex, Scavengers, and Chaperones: Transcriptome Secrets of Divergent *Symbiodinium* Thermal Tolerances. *Mol. Biol. Evol.* **33**, 2201–2215 (2016).
13. Gardner, S. G., Raina, J.-B., Ralph, P. J. & Petrou, K. Reactive oxygen species (ROS) and dimethylated sulphur compounds in coral explants under acute thermal stress. *J. Exp. Biol.* **220**, 1787–1791 (2017).
14. Roberty, S., Fransolet, D., Cardol, P., Plumier, J.-C. & Franck, F. Imbalance between oxygen photoreduction and antioxidant capacities in Symbiodinium cells exposed to combined heat and high light stress. *Coral Reefs* **34**, 1063–1073 (2015).
15. Saragosti, E., Tchernov, D., Katsir, A. & Shaked, Y. Extracellular Production and Degradation of Superoxide in the Coral Stylophora pistillata and Cultured Symbiodinium. *PLoS ONE* **5**, e12508 (2010).
16. Krueger, T. *et al.* Differential coral bleaching—Contrasting the activity and response of enzymatic antioxidants in symbiotic partners under thermal stress. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **190**, 15–25 (2015).
17. Lesser, M. P. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in ymbiotic dinoflagellates. *Limnol. Oceanogr.* **41**, 271–283 (1996).
18. Szabó, M., Larkum, A. W. D. & Vass, I. A Review: The Role of Reactive Oxygen Species in Mass Coral Bleaching. in *Photosynthesis in Algae: Biochemical and Physiological*

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Mechanisms (eds. Larkum, A. W. D., Grossman, A. R. & Raven, J. A.) vol. 45 459–488 (Springer International Publishing, 2020).

19. Halliwell, B. & Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*. (Oxford University Press, 2015). doi:10.1093/acprof:oso/9780198717478.001.0001.
20. Nesa, B. & Hidaka, M. Thermal stress increases oxidative DNA damage in coral cell aggregates. *Proc. 11th Int. Coral Reef Symp.* **Session number 5**, (2008).
21. Bielmyer-Fraser, G. K., Patel, P., Capo, T. & Grosell, M. Physiological responses of corals to ocean acidification and copper exposure. *Mar. Pollut. Bull.* **133**, 781–790 (2018).
22. Lopes, A. R. *et al.* Physiological resilience of a temperate soft coral to ocean warming and acidification. *Cell Stress Chaperones* **23**, 1093–1100 (2018).
23. Krueger, T. *et al.* Antioxidant plasticity and thermal sensitivity in four types of *Symbiodinium* sp. *J. Phycol.* **50**, 1035–1047 (2014).
24. Diaz, J. M. *et al.* Species-specific control of external superoxide levels by the coral holobiont during a natural bleaching event. *Nat. Commun.* **7**, 13801 (2016).
25. Ross, C., Ritson-Williams, R., Olsen, K. & Paul, V. J. Short-term and latent post-settlement effects associated with elevated temperature and oxidative stress on larvae from the coral Porites astreoides. *Coral Reefs* **32**, 71–79 (2013).
26. Yakovleva, I. *et al.* Algal symbionts increase oxidative damage and death in coral larvae at high temperatures. *Mar. Ecol. Prog. Ser.* **378**, 105–112 (2009).
27. Yakovleva, I., Bhagooli, R., Takemura, A. & Hidaka, M. Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **139**, 721–730 (2004).
28. Lesser, M. P., Stochaj, W. R., Tapley, D. W. & Shick, J. M. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. *Coral Reefs* **8**, 225–232 (1990).
29. Hillyer, K. E. *et al.* Metabolite profiling of symbiont and host during thermal stress and bleaching in the coral Acropora aspera. *Coral Reefs* **36**, 105–118 (2017).
30. Hughes, D. J. *et al.* Coral reef survival under accelerating ocean deoxygenation. *Nat. Clim. Change* **10**, 296–307 (2020).
31. Drury, C. Resilience in reef-building corals: The ecological and evolutionary importance of the host response to thermal stress. *Mol. Ecol.* **29**, 448–465 (2020).
32. Bay, R. A. & Palumbi, S. R. Rapid Acclimation Ability Mediated by Transcriptome Changes in Reef-Building Corals. *Genome Biol. Evol.* **7**, 1602–1612 (2015).
33. Bellantuono, A. J., Granados-Cifuentes, C., Miller, D. J., Hoegh-Guldberg, O. & Rodriguez-Lanetty, M. Coral Thermal Tolerance: Tuning Gene Expression to Resist Thermal Stress. *PLoS ONE* **7**, e50685 (2012).
34. Middlebrook, R., Hoegh-Guldberg, O. & Leggat, W. The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *J. Exp. Biol.* **211**, 1050–1056 (2008).
35. Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N. & Bay, R. A. Mechanisms of reef coral resistance to future climate change. *Science* **344**, 895–898 (2014).
36. Schoepf, V. *et al.* Stress-resistant corals may not acclimatize to ocean warming but maintain heat tolerance under cooler temperatures. *Nat. Commun.* **10**, 4031 (2019).
37. Dilworth, J., Caruso, C., Kahkejian, V. A., Baker, A. C. & Drury, C. Host genotype and stable differences in algal symbiont communities explain patterns of thermal stress response of

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Montipora capitata following thermal pre-exposure and across multiple bleaching events. *Coral Reefs* **40**, 151–163 (2021).
38. Ainsworth, T. D. *et al.* Climate change disables coral bleaching protection on the Great Barrier Reef. *Science* **352**, 338–342 (2016).
39. Majerova, E., Carey, F. C., Drury, C. & Gates, R. D. Preconditioning improves bleaching tolerance in the reef-building coral *Pocillopora acuta* through modulations in the programmed cell death pathways. *Mol. Ecol.* **30**, 3560–3574 (2021).
40. Couto, N., Wood, J. & Barber, J. The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. *Free Radic. Biol. Med.* **95**, 27–42 (2016).
41. Patel, T. K. & Williamson, J. D. Mannitol in Plants, Fungi, and Plant–Fungal Interactions. *Trends Plant Sci.* **21**, 486–497 (2016).
42. Lee, G.-H. *et al.* Bax Inhibitor-1 Regulates Endoplasmic Reticulum Stress-associated Reactive Oxygen Species and Heme Oxygenase-1 Expression. *J. Biol. Chem.* **282**, 21618–21628 (2007).
43. Harvey, C. J. *et al.* Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. *Free Radic. Biol. Med.* **46**, 443–453 (2009).
44. Raghunath, A. *et al.* Antioxidant response elements: Discovery, classes, regulation and potential applications. *Redox Biol.* **17**, 297–314 (2018).
45. Cunning, R., Bay, R. A., Gillette, P., Baker, A. C. & Traylor-Knowles, N. Comparative analysis of the Pocillopora damicornis genome highlights role of immune system in coral evolution. *Sci. Rep.* **8**, 16134 (2018).
46. Banning, A., Deubel, S., Kluth, D., Zhou, Z. & Brigelius-Flohé, R. The GI-GPx Gene Is a Target for Nrf2. *Mol. Cell. Biol.* **25**, 4914–4923 (2005).
47. Collier, R. J., Baumgard, L. H., Zimbelman, R. B. & Xiao, Y. Heat stress: physiology of acclimation and adaptation. *Anim. Front.* **9**, 12–19 (2019).
48. Foo, S. A. & Byrne, M. Acclimatization and Adaptive Capacity of Marine Species in a Changing Ocean. in *Advances in Marine Biology* vol. 74 69–116 (Elsevier, 2016).
49. Thomas, L. *et al.* Mechanisms of Thermal Tolerance in Reef-Building Corals across a Fine-Grained Environmental Mosaic: Lessons from Ofu, American Samoa. *Front. Mar. Sci.* **4**, (2018).
50. Armoza-Zvuloni, R. & Shaked, Y. Release of hydrogen peroxide and antioxidants by the coral <i>Stylophora pistillata</i> to its external <i>milieu</i>. *Biogeosciences* **11**, 4587–4598 (2014).
51. Schieber, M. & Chandel, N. S. ROS Function in Redox Signaling and Oxidative Stress. *Curr. Biol.* **24**, R453–R462 (2014).
52. Redza-Dutordoir, M. & Averill-Bates, D. A. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1863**, 2977–2992 (2016).
53. Liu, Y., Beyer, A. & Aebersold, R. On the Dependency of Cellular Protein Levels on mRNA Abundance. *Cell* **165**, 535–550 (2016).
54. Krueger, T. *et al.* Transcriptomic characterization of the enzymatic antioxidants FeSOD, MnSOD, APX and KatG in the dinoflagellate genus Symbiodinium. *BMC Evol. Biol.* **15**, 48 (2015).

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

55. Lenton, K. J., Therriault, H., Fülöp, T., Payette, H. & Wagner, J. R. Glutathione and ascorbate are negatively correlated with oxidative DNA damage in human lymphocytes. *Carcinogenesis* **20**, 607–613 (1999).
56. Dannenmann, B. *et al.* High Glutathione and Glutathione Peroxidase-2 Levels Mediate Cell-Type-Specific DNA Damage Protection in Human Induced Pluripotent Stem Cells. *Stem Cell Rep.* **4**, 886–898 (2015).
57. Purschke, M., Laubach, H.-J., Rox Anderson, R. & Manstein, D. Thermal Injury Causes DNA Damage and Lethality in Unheated Surrounding Cells: Active Thermal Bystander Effect. *J. Invest. Dermatol.* **130**, 86–92 (2010).
58. Kantidze, O. L., Velichko, A. K., Luzhin, A. V. & Razin, S. V. Heat Stress-Induced DNA Damage. *Acta Naturae* **8**, 75–78 (2016).
59. Houston, B. J. *et al.* Heat exposure induces oxidative stress and DNA damage in the male germ line†. *Biol. Reprod.* **98**, 593–606 (2018).
60. Heise, K. Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout Zoarces viviparus L. *J. Exp. Biol.* **209**, 353–363 (2006).
61. Liu, F.-W., Liu, F.-C., Wang, Y.-R., Tsai, H.-I. & Yu, H.-P. Aloin Protects Skin Fibroblasts from Heat Stress-Induced Oxidative Stress Damage by Regulating the Oxidative Defense System. *PLOS ONE* **10**, e0143528 (2015).
62. Cvjetko, P. *et al.* Dynamics of heat-shock induced DNA damage and repair in senescent tobacco plants. *Biol. Plant.* **58**, 71–79 (2014).
63. Huang, Y.-K. *et al.* Heat acclimation decreased oxidative DNA damage resulting from exposure to high heat in an occupational setting. *Eur. J. Appl. Physiol.* **112**, 4119–4126 (2012).
64. Nesa, B., Baird, A. H., Harii, S., Yakovleva, I. & Hidaka, M. Algal Symbionts Increase DNA Damage in Coral Planulae Exposed to Sunlight. *Zool. Stud.* **51.**, 12–17 (2012).
65. Surova, O. & Zhivotovsky, B. Various modes of cell death induced by DNA damage. *Oncogene* **32**, 3789–3797 (2013).
66. Robinson, K. S., Clements, A., Williams, A. C., Berger, C. N. & Frankel, G. Bax Inhibitor 1 in apoptosis and disease. *Oncogene* **30**, 2391–2400 (2011).
67. Majerova, E., Carey, F., Drury, C. & Gates, R. *Preconditioning improves bleaching tolerance in the reef-building coral Pocillopora acuta through modulations in the autophagy pathway*.
68. Rodríguez-Casariego, J. A. *et al.* Genome-Wide DNA Methylation Analysis Reveals a Conserved Epigenetic Response to Seasonal Environmental Variation in the Staghorn Coral Acropora cervicornis. *Front. Mar. Sci.* **7**, 560424 (2020).
69. Putnam, H. M., Davidson, J. M. & Gates, R. D. Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evol. Appl.* **9**, 1165–1178 (2016).
70. Liew, Y. J. *et al.* Intergenerational epigenetic inheritance in reef-building corals. *Nat. Clim. Change* **10**, 254–259 (2020).
71. Liew, Y. J. *et al.* Epigenome-associated phenotypic acclimatization to ocean acidification in a reef-building coral. *Sci. Adv.* **4**, eaar8028 (2018).
72. Layden, M. J. *et al.* MAPK signaling is necessary for neurogenesis in Nematostella vectensis. *BMC Biol.* **14**, 61 (2016).

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

73. Weizman, E. & Levy, O. The role of chromatin dynamics under global warming response in the symbiotic coral model Aiptasia. *Commun. Biol.* **2**, 282 (2019).

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

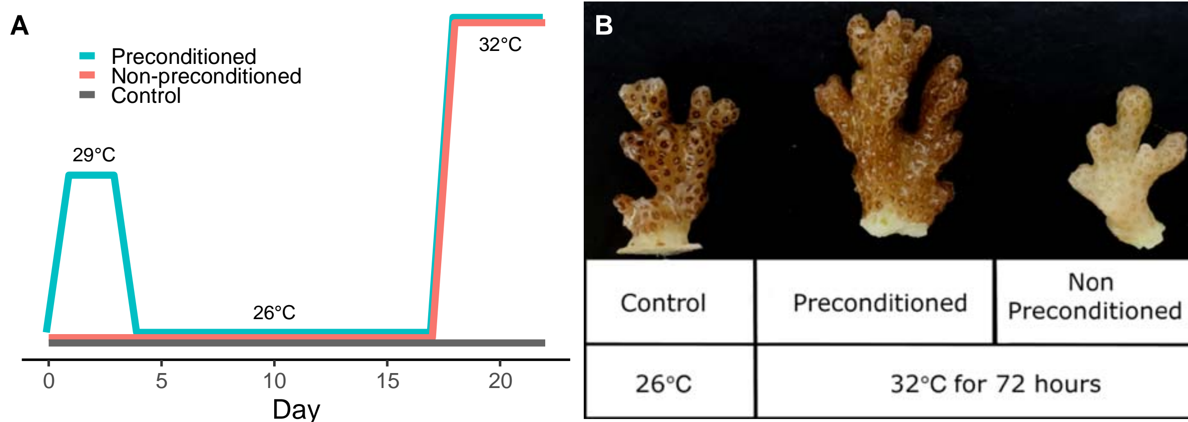

Figure 1 – Experimental design. A) Preconditioning profile. Preconditioned corals (PC) were exposed to sublethal 29°C for 72 hours and returned to 26°C for additional two weeks before undergoing acute thermal stress (32°C). Non-preconditioned (NPC) corals were exposed to acute thermal stress with no previous preconditioning. B) Different response to acute heat stress (32°C for 72 hours) in preconditioned (PC) and non-preconditioned (NPC) corals (right). While PC corals resembled control corals, NPC corals were visibly bleached.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

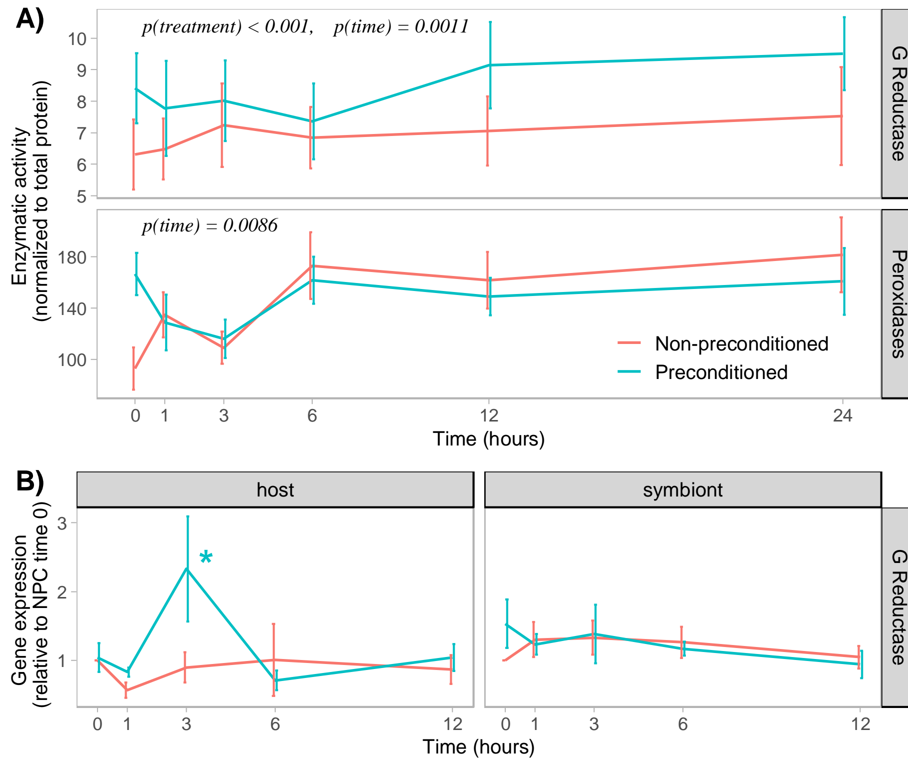

Figure 2 – *Enzymatic activity and gene expression during thermal stress*

A) Thermally *PC* corals *have higher* activity of glutathione *reductase than NPC,* but do not differ in the activity of peroxide-scavenging enzymes. Activity was measured in host cell extracts. *B)* Gene expression pattern of glutathione reductase in host and symbiont cells. Gene expression of host-derived glutathione reductase significantly increased at 3 hours of acute heat stress in *PC corals, but not NPC* corals. Graphs depict means with standard errors, n=6.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Figure 3

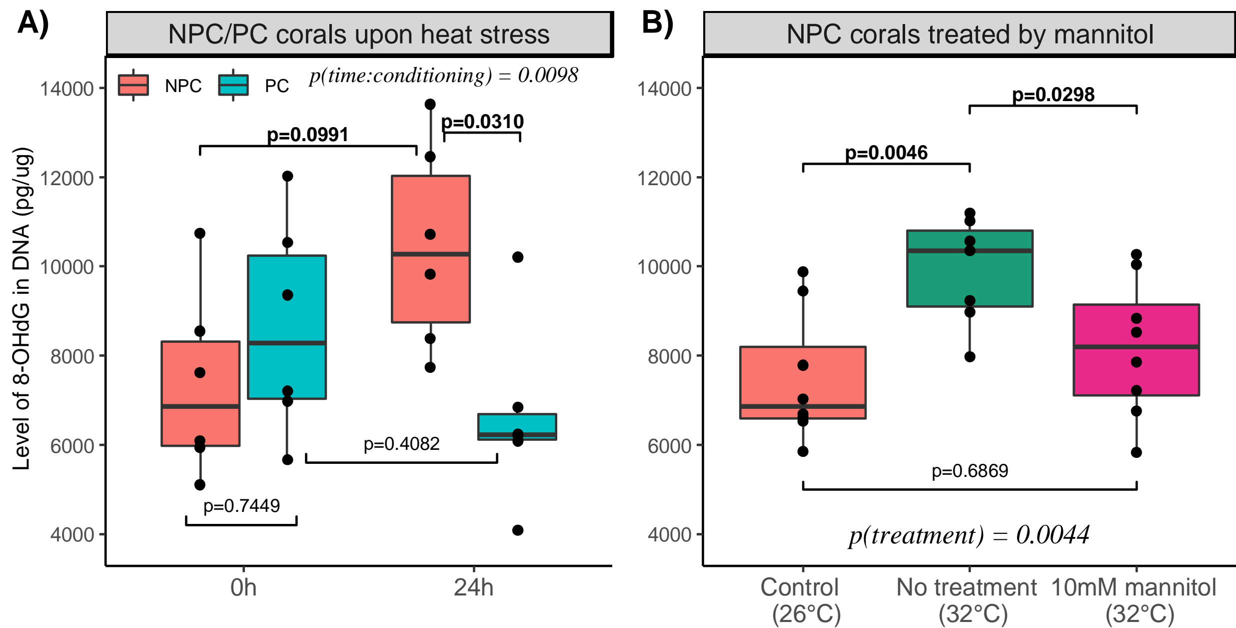

Figure 3 – Oxidative DNA damage during heat stress.
A) Non-preconditioned corals accumulate DNA damage (the oxidized guanine species, 8-OHdG) but preconditioned corals do not. After 24 hours of the stress, there is a significant difference in the level of 8-OHdG between the treatments. n = 6 B) The accumulation of 8-OHdG in non-preconditioned corals during acute heat stress is prevented by the addition of 10 mM mannitol, a non-enzymatic antioxidant. n = 8. The boxplots show median of the data, first and third quartile and respective datapoints.

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.15.435543; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Figure 4

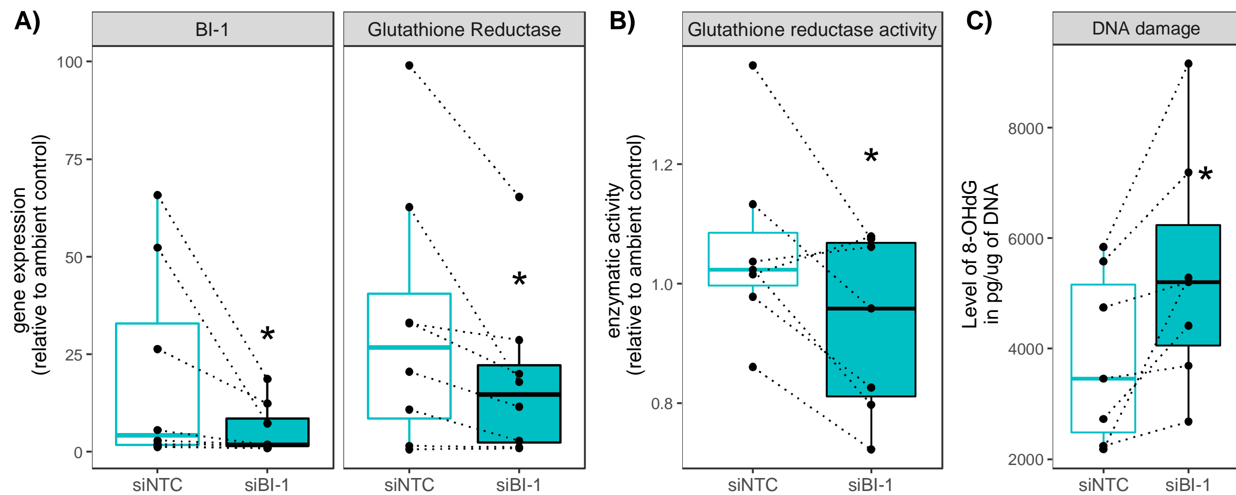

Figure 4 – BI-1 overexpression in heat-stressed preconditioned corals improves DNA damage protection via regulation of glutathione reductase activity. A) Expression of BI-1 and glutathione reductase genes in heat-stressed PC corals is efficiently reduced after siRNA-mediated gene knockdown (siBI-1). siNTC represents PC corals treated with a negative control siRNA. Data are normalized to untreated corals at ambient temperature, n=8 B) The activity of glutathione reductase decreases upon siBI-1 knockdown in PC corals. Data are normalized to untreated corals at ambient temperature, n=7. C) The level of oxidized guanine in coral DNA during heat stress increases after siBI-1 knockdown in PC corals (n = 7). Dotted lines connect paired samples from the same colony. The boxplots show median of the data, first and third quartile and respective datapoints.