TNF and FGF Signaling Pathways Participate in the Polyp Bail-out Response in Pocillopora acuta

Po-Shun Chuang[1] and Satoshi Mitarai[1]

[1]Okinawa Institute of Science and Technology Graduate University

May 5, 2020

Abstract

Polyp bail-out is a stress response exhibited by some pocilloporid corals with mechanisms and consequences distinct from those of bleaching. Although induction of polyp bail-out has been demonstrated in the laboratory, molecular mechanisms underlying this response have rarely been discussed. We conducted genetic analyses of Pocillopora acuta during initiation of hyperosmosis-induced polyp bail-out, using both transcriptomic and qPCR techniques. Beyond upregulation of apoptosis and genes related to extracellular matrix (ECM) degradation, corals showed significant activation of tumor necrosis factor and fibroblast growth factor (FGF) signaling pathways throughout a 24-h polyp bail-out induction experiment. A common gene expression profile was found between the FAS and CASP8 genes, which reached their expression peaks at 12 h, whereas a different profile showing significant upregulation up to 18 h was displayed by ECM-degrading proteases and genes in the FGF signaling pathway. These results suggest parallel involvement of an extrinsic apoptotic signaling pathway and FGF-mediated ECM degradation in polyp bail-out. Furthermore, in XIAP, JNK, and NFKB1 genes, we detected a third expression profile showing linear upregulation throughout the 24-h experiment period, indicating activation of anti-apoptotic and cell survival signals during polyp bail-out. Our results provide new insights into signaling pathways inducing polyp bail-out and suggest the feasibility of inducing bail-out by specifically triggering these pathways without exerting lethal stresses on the corals.. This will enable acquisition of viable polyps for possible use in coral reef restoration and in coral research.

Keywords

Polyp bail-out; Apoptosis; ECM degradation; Signaling pathway; Transcriptome

Introduction

Corals in the family Pocilloporidae (Cnidaria: Anthozoa: Scleractinia) are the major reef-building corals in the Indo-Pacific Ocean (Pinzon & LaJeunesse, 2011). Pocilloporid corals generally inhabit shallow water, which renders them susceptible to thermal and osmotic fluctuations (Poquita-Du et al., 2019; Schmidt-Roach, Miller, Lundgren, & Andreakis, 2014). Being representatives of the "robust corals" branch, studies on pocilloporid corals complement our understanding derived from the Acroporidae, the extensively studied corals in the "complex corals" branch, about impacts of anthropogenic climate change on coral reef ecosystems (Traylor-Knowles et al., 2011). Compared to acroporid corals, some pocilloporids have been recognized as being relatively resistant to environmental stresses. However, bleaching, the systematic dissociation of symbiotic zooxanthellae from coral host, can still occur in these corals once stresses reach critical levels (Kvitt, Rosenfeld, Zandbank, & Tchernov, 2011). As with most stony corals, pocilloporids rely on photosynthesis by symbiotic algae as their major energy source and cannot live as heterotrophs for prolonged periods. Extended bleaching can therefore cause mortaility of pocilloporid corals, even though the stressors might not be directly lethal to the coral hosts (Traylor-Knowles et al., 2011).

In recent decades, a novel stress response called "polyp bail-out" has been increasingly reported in pocilloporid corals (Domart-Coulon, Tambutté, Tambutté, & Allemand, 2004; Fordyce, Camp, & Ainsworth, 2017;

Kvitt et al., 2015; Sammarco, 1982; Shapiro, Kramarsky-Winter, Gavish, Stocker, & Vardi, 2016; Wecker et al., 2018). Unlike coral bleaching, polyp bail-out is characterized by dissociation of coral colonies via coenosarc degradation and detachment of zooxanthellate polyps from the calcareous skeletons (Sammarco, 1982). In natural environments, polyp bail-out has been reported in the Great Barrier Reef and reefs along the Pacific coast of Costa Rica, although the triggers are still uncertain (Sammarco, 1982; Wild et al., 2014). Under laboratory conditions, induction of polyp bail-out has been demonstrated with different treatments, including thermal stress, acidification, and hyperosmosis (Domart-Coulon et al., 2004; Fordyce et al., 2017; Kvitt et al., 2015; Serrano, Coma, Inostroza, & Serrano, 2018; Shapiro et al., 2016; Wecker et al., 2018). As detached polyps can be maintained in laboratory conditions for periods of weeks to months (Capel, Migotto, Zilberberg, & Kitahara, 2014; Serrano et al., 2018; Shapiro et al., 2016), these polyps offer a different subject material for *in situ* studies of coral cellular biology and of symbiotic relationships between corals and symbiotic algae. Moreover, since detached polyps are thought to resettle and resume skeletogenesis under favorable conditions, polyp bail-out can be considered as an asexual reproductive method in stony corals and may provide an alternative approach of mass production of coral colonies for reef restoration (Sammarco, 1982; Shapiro et al., 2016).

Identifying signaling pathways participating in polyp bail-out can help to develop methods to induce the response without stressing the corals, which would facilitate survival and resettlement of detached polyps. Furthermore, understanding molecular mechanisms underlying polyp bail-out may help us to better understand its occurrence in nature and its ecological significance. Recently, genomes of some pocilloporid corals have been published (Cunning, Bay, Gillette, Baker, & Traylor-Knowles, 2018; Voolstra et al., 2017), enabling a more thorough understanding of this response from a molecular perspective. A recent transcriptomic study of *Pocillopora damicornis* during polyp bail-out demonstrated overexpression of many caspase-encoding genes in concert with coenosarc degradation, supporting the hypothetical link between polyp bail-out and tissue-specific apoptosis (Kvitt et al., 2015; Wecker et al., 2018). In that study, it was also proposed that proteolytic enzymes, such as cathepsins, trigger degradation of the extracellular matrix (ECM) between coral polyps and the calcareous skeleton, resulting in detachment of individual polyps (Wecker et al., 2018). However, our understanding of signaling pathways activating the apoptotic and proteolytic responses in polyp bail-out is still in its infancy.

In the present study, we applied hyperosmotic stress to induce bail-out in *P. acuta* , a species closely related to *P. damicornis* , according to recent phylogenic classifications (Johnston et al., 2017; Schmidt-Roach et al., 2014). Based on both transcriptomic analysis and qPCR assays, we sought to identify the signaling pathways that lead to polyp bail-out. Results show involvement of multiple signaling pathways in polyp bail-out, including the tumor necrosis factor (TNF) and fibroblast growth factor (FGF) signaling pathways, which likely independently trigger apoptotsis and ECM degradation, respectively.

Materials and Methods

Coral collection and maintenance

In 2018, we purchased six colonies of *Pocillopora acuta* from the Onna Village Fisheries Cooperative in Okinawa, Japan. Coral colonies were transferred to the OIST Marine Science Section at Seragaki, where they were kept in a 3000-L outdoor tank supplied with flowing, sand-filtered natural seawater for over six months before the onset of polyp bail-out experiments.

Polyp bail-out induction and RNA sampling for transcriptomic analysis (Experiment I)

To examine the transcriptomic response during initiation of polyp bail-out, we collected RNA samples at 12-hour intervals during induction of the bail-out response in *P. acuta* , using a hyperosmotic treatment modified from Shapiro et al. (2016). One day prior to the experiments, six coral fragments from a mother colony were clipped off and randomly placed in two 5-L indoor experimental tanks. Artificial seawater (Kaisuimaren, Japan) at 35tanks (three in the treatment group and three in the control group). Light intensity was provided at 300 µmol photons $m^{-2}s^{-1}$ with a 12-hour day-night cycle. Tank temperature was allowed to fluctuate daily in the range of 24 – 26°C. For the treatment group, high-salinity artificial seawater

(48mL/min to create hyperosmotic conditions, while salinity remained unchanged for the control group. Since partial polyp detachment (an indicator of onset of polyp bail-out) was observed in the treatment group at 24 h, we collected both undetached polyps and detached polyps (10 – 15 polyps/sample) separately. The same experiment was repeated three times on different *P. acuta* colonies, with at least one month between experiments (N = 3).

Illumina RNA sequencing and transcriptome assembly

For samples from experiment I, we extracted total RNA following the TRIZOL RNA extraction protocol (Chomczynski, 1993) and checked RNA quality with a Bioanalyzer 2100 (Agilent, Japan). RNA samples with RIN>7 were sent to the DNA Sequencing Section at the Okinawa Institute of Science and Technology (OIST) for library construction and Illumina Hiseq 4000 150 x 150-bp paired-end RNA sequencing. To remove adapters and low-quality sequences, **r** aw reads from Illumina RNA sequencing were trimmed with Trimmomatic v0.36 (Bolger, Lohse, & Usadel, 2014) and were checked for quality using FastQC (Andrews, 2010). To remove RNA reads from *Symbiodinium* and other coral-associated microbiomes, we first mapped trimmed reads (˜877 million read pairs from all sequenced libraries) to a reference *Pocillopora damicornis* genome assembly (GenBank assembly accession: GCA_003704095.1) (Cunning et al., 2018) using Tophat v2.1.1 (Kim et al., 2013). Successfully aligned RNA reads (˜168 million read pairs; successful alignment rate: 19.1%) were then subjected to *de novo* assembly using Trinity v2.8.4 (Grabherr et al., 2011). RNA read quantification was conducted using RSEM v1.3.2 (Li & Dewey, 2011) and reconstructed transcripts shorter than 200 nt or with low coverage (<5 transcripts per million) were discarded. BUSCO analysis was performed using the metazoa_odb9 dataset to examine completeness of the transcriptome assembly (Simao, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015).

Functional annotation, differential expression (DE), and gene ontology (GO) enrichment analyses

We performed functional annotation of the transcriptome assembly by searching the SwissProt eukaryotic protein database (downloaded on 3rd Jul 2019) with blastx, using a threshold of $E=10^{-5}$. Although principle component analysis (PCA) of the annotated transcripts showed some variation among biological replicates (PC2: 14.9%), most of the variation could be attributed to the hyperosmotic treatment (PC1: 57.9%; Fig. 1). We therefore conducted DE analysis on annotated transcripts for pairwise comparisons of S43/S35 (representing response at 12 h) and S46/S35 (response at 24 h), designed to cancel out intra-individual variation and to identify transcriptomic response induced specifically by the treatment. The DE analysis was performed using edgeR (Robinson, McCarthy, & Smyth, 2010) with the criteria of >2-fold absolute change and >5 CPM (counts per million) for at least 3 libraries in a given pairwise comparison. GO enrichment analyses were then performed separately for upregulated and downregulated DEGs at 12 h (S43/S35) and 24 h (S46/S35), using DAVID bioinformatics resources v6.8 (Huang, Sherman, & Lempicki, 2008, 2009) to examine the participation of specific cellular processes and signaling pathways in polyp bail-out.

Validation of transcriptomic results by real-time quantitative PCR (qPCR) (Experiment II)

In order to validate results from the transcriptomic analysis, we conducted a second polyp bail-out experiment in 2019 and collected RNA samples for qPCR analyses. The experimental setup for polyp bail-out induction was the same as in experiment I, except for the lighting conditions (150 μmol photons $m^{-2}s^{-1}$) and the use of filtered natural seawater (34 – 35For a better temporal resolution of the genetic response, coral RNA was sampled at 6-hour intervals through the time-course of polyp bail-out induction (5 time points; N = 6 for treatment group and N = 3 for control group).

cDNA synthesis and qPCR assay

For samples from experiment II, total RNA was extracted as described above and cDNA synthesis was conducted using the SuperScript IV VILO Master Mix (Invitrogen, USA). Synthesized cDNA was subsequently subjected to a qPCR assay comprising 10 stress genes (FAS, CASP8, FGFR2, FGF2, RHO, MMP19, MMP24, JNK, NFKB1, and XIAP; Table 2) on a StepOnePlus Real-Time PCR System (Thermo Fisher Sci-

entific, USA). A β-tubulin gene (TUBB) was selected as the internal control for the qPCR analysis. For each qPCR reaction, 200 μM of each primer and 1 μL of synthesized cDNA (concentration undetermined) were mixed with iQ SYBR Green Supermix (Bio-Rad, USA) to a total volume of 10 μL. qPCR conditions included an initiation step at 95°C for 3 min and 40 cycles of PCR amplification at 95°C for 15 sec and 60°C for 30 sec. A melting curve analysis (MCA) was conducted after qPCR to confirm the specificity of each primer pair. For each target stress gene, the mean [?]CT value ($CT_{\text{control gene}} - CT_{\text{target stress gene}}$) from two technical replicates was used to calculate relative gene expression ($\log_2$-transformed). [?]CT values of the treatment group were then normalized to those of the control group (on average of three replicates) and to the initial conditions (0 h) to determine expression changes during experiments ([?][?][?]CT = [?][?]$CT_{\text{given time point}}$ − [?][?]$CT_{0\ \text{h}}$; [?][?]CT = [?]$CT_{\text{treatment group}}$ − [?]$CT_{\text{control group}}$). Data are presented as means +- SD (standard deviation) for each gene, and statistical significance between time points was tested using Welch's ANOVA with a Games-Howell post-hoc test.

Results

Polyp bail-out induction

To induce the polyp bail-out response in *Pocillopora acuta* , a hyperosmotic environment was established by gradual addition of high-salinity seawater (48increased from 35˜46no obvious signs of a stress response were seen at 12 h in the treatment group. Polyp bail-out was observed at 24 h, at which 30 – 50% of the polyps were found detached from the skeleton (Fig. 2). For the control group (salinity change < 0.5other stress response was observed at any time during the experiment (Fig. 2).

Transcriptome assembly of *P. acuta*

To examine genetic responses of *P. acuta* during polyp bail-out induction, we first constructed a reference transcriptome assembly. After removing RNA reads from coral-associated microbiomes, including those of *Symbiodinium* , a total of 73,903 transcripts were reconstructed (N50 = 1,278 nt; E90N50 = 1,756 nt; GenBank assembly accession: GIDI00000000), with 84% transcriptomic completeness (fragmented, 9.7%; missing, 6.2%) identified by the BUSCO analysis.

Differential expression (DE) analysis

From the transcriptome assembly, 17,908 transcripts were functionally annotated and subjected to DE analysis. As the principle component analysis showed that the majority of variation (57.9%, PC1) comprised a stress response (Fig. 1), DE analysis was designed for pairwise comparisons of S43/S35 and S46/S35, aiming to identify transcriptomic responses induced specific to the hyperosmotic treatment. In the DE analysis, 3,553 DEGs were identified at 12 h (S43/S35), with 1,936 showing upregulation and 1,617 showing downregulation. While at 24 h (S46/S35), a total of 6,618 DEGs were identified, with 2,927 and 3,691 showing upregulation and downregulation, respectively.

Gene ontology (GO) enrichment analysis

To identify molecular mechanisms involved in the polyp bail-out response, GO enrichment analyses were conducted for up- and downregulated DEGs. As most eriched GO terms in our results are likely involved as general responses of corals to osmotic fluctuations, the discussion of which is beyond the scope of this study, we paid specific attention to biological processes and signaling pathways related to apoptosis, ECM degradation, and cell survival, which participate directly in polyp bail-out. Among upregulated DEGs, GO categories such as *apoptotic process* , *extrinsic apoptotic signaling pathway* , *negative regulation of cell death* , *fibroblast growth factor (FGF) receptor signaling pathway* , and *tumor necrosis factor (TNF)-mediated signaling pathway* were significantly enriched at both 12 h and 24 h (p <0.05), while enrichment of *I-κB κιναση/ΝΦ-κΒ σιγναλινγ* was significant at 12 h but not at 24 h (Table 1). In addition, in our transcriptomic data we found remarkable upregulation of eight matrix metalloproteinase (MMP)-encoding transcripts (>10-fold expression change from 0 h to 24 h), although enrichment of the GO category *proteolysis*was insignificant (p = 0.20 at 12 h and 0.14 at 24 h). Among downregulated DEGs, no specific signaling pathway or apoptosis/proteolysis-related GO category showed significant enrichment.

Quantitative PCR of stress genes

To validate involvement of the abovementioned signaling pathways in polyp bail-out, we conducted a second polyp bail-out experiment (experiment II) and collected RNA samples at finer temporal resolution (6-h intervals) for a qPCR assay. The same salinity-changing profile and morphological changes in corals were observed as in experiment I (data not shown). Among the 10 stress genes in our qPCR assay, three expression profiles were identified. For CASP8 and FAS genes, significant upregulation occurred during the first 12 h and remained relatively stable afterward (Fig. 3, profile I). A second expression profile was exhibited by FGF2, FGFR2, RHO, and the two MMPs genes. For these genes, little upregulation was seen in the first 6 h (2.5-fold on average) followed by a significant upregulation of over 40-fold from 6 h to 12 h. Another significant upregulation of 8.4-fold was identified from 12 h to 18 h, after which the gene expression stabilized (Fig. 3, profile II). While for the NFKB1, JNK, and XIAP genes, linear upregulation during the entire experimental period was detected (Fig. 3, profile III).

Discussion

Hyperosmosis induces polyp bail-out in pocilloporid corals

In this study, we applied hyperosmotic stress to *Pocillopora acuta* and observed polyp bail-out after 24 h, when seawater salinity reached ˜46i.e.,*P damicornis, Seriatopora hystrix* , and *Stylophora pistillata,* Shapiro et al. (2016) showed that polyp bail-out can be induced by evaporation-driven hyperosmotic stress. Although the species and salinity-changing profiles are different between this and previous studies, polyp bail-out was observed in both at relatively similar stress levels (10 – 14our preliminary tests on *P. verrucosa* also displayed polyp bail-out under similar conditions and timing (data not shown). This observation implies that polyp bail-out may be a common response in pocilloporid corals and that these corals may experience strong osmotic fluctuations/stresses more frequently than we generally recognize. Although a salinity increase of >10in waters in the subtidal zone (where most pocilloporid corals live), hyperosmotic environments can develop in tide pools and lagoons during low tides, due to evaporation, or when coral fragments get washed ashore occasionally at high tide. Since detached polyps are able to resettle and resume skeletogenesis after the hyperosmosis-induced polyp bail-out (Shapiro et al., 2016), this stress response can be considered as an escape mechanism for "lost" coral polyps to return to the ocean. Additional studies, however, are needed to further illuminate the ecological significance of this stress response.

TNF signaling leads to apoptosis and coenosarc degradation

The significant enrichment of GO terms *apoptotic process* and*extrinsic apoptotic signaling pathway* in our transcriptomic data (Table 1) supports the hypothesis of apoptosis-induced coenosarc degradation proposed in previous studies (Kvitt et al., 2015; Wecker et al., 2018). The extrinsic apoptotic signaling pathway, comprising signals mediated by the TNF receptor family and a caspase cascade initiated by caspase-8, is highly conserved among animals and was proposed in polyp bail-out (Quistad et al., 2014; Wecker et al., 2018). Interestingly, even with significant enrichment of the TNF signaling pathway in our transcriptomic data and upregulation of the FAS receptor gene in the qPCR assay, no overexpression of TNF was found during the polyp bail-out induction in this study, either in the transcriptomic or qPCR analysis. Similar to our finding, in Wecker et al. (2018) TNF overexpression was found with a delayed spike compared to that of caspase and TNF receptor genes. It is possible that signals triggering the TNF pathway during polyp bail-out came from symbiotic microorganisms in the corals, which were filtered out during our data processing step. Since changing the culture environment can substantially alter the composition of coral-associated microbes (Littman, Willis, & Bourne, 2011; Webster et al., 2013), initiation of polyp bail-out may be attributable to shift of chemical signals provided from microbes to the coral host. In fact, it was recently reported that polyp bail-out in*P. damicornis* can be induced by inoculation with the coral pathogen, *Vibrio coralliilyticus* (Gavish, Shapiro, Kramarsky-Winter, & Vardi, 2018). Our results thus add further evidence for involvement of the TNF receptor-mediated extrinsic apoptotic signaling pathway in polyp bail-out and point to a possibly role of symbiotic microbes in this stress response (Fig. 4).

FGF signaling induces ECM degradation and polyp detachment

A previous study of *P. damicornis* suggested participation of ECM degradation in the polyp-detachment step of polyp bail-out (Wecker et al., 2018). Congruent with that study, we found remarkable upregulation of eight MMP-encoding transcripts during the experiments. MMPs are a family of proteases that function in ECM degradation (Birkedal-Hansen et al., 1993; Kojima, Itoh, Matsumoto, Masuho, & Seiki, 2000). Regulation of these MMPs in polyp bail-out is probably linked to the FGF signaling pathway, since expression profiles of FGF2 and FGFR2 matched those of the two MMP genes in our qPCR assay. The MMP-inducing function of the FGF2/FGFR2 signaling pathway has been documented in many mammalian cells for its involvement in cell migration and angiogenesis (Ardi et al., 2009; Birkedal-Hansen et al., 1993; Pintucci et al., 2003). Intracellular signal transduction from FGF receptors to activation of MMPs likely occurs through Rho family GTPases, suggested by the similarity of expression profiles between RHO and the aforementioned genes in our qPCR assay. Furthermore, upon activation, some MMPs have been shown to induce release of FGF2 (Whitelock, Murdoch, Iozzo, & Underwood, 1996). The irreversibility of coral polyp bail-out, and the remarkable upregulation of FGF2 and MMPs from 6 h to 18 h in our qPCR analysis, can thus be explained by formation of a positive feedback loop between MMPs and FGF signals. Based on these results, we hypothesize that the FGF signaling pathway is triggered under environmental stress and the signal is likely relayed by Rho family GTPases to activate MMPs, which in turn initiate ECM degradation and subsequent polyp detachment (Fig. 4).

Survival of polyps after polyp bail-out

Interestingly, in our transcriptomic data, no significant difference in caspase gene expression was found between coenosarc-containing coral tissue (T24-1) and detached solitary polyps (T24-2) collected at the same time. We therefore hypothesize that tissue-specific apoptosis in polyp bail-out is mediated at the post-transcriptional level. In support of this hypothesis, in our transcriptomic data we identified significant enrichment of protein ubiquitination and upregulation of XIAP gene, which both have been shown to regulate caspase activities and cell death (Bader & Steller, 2009; Chen & Qiu, 2013; Deveraux, Takahashi, Salvesen, & Reed, 1997). It was proposed that the anti-apoptotic response is induced by a delayed TNF signal after polyp bail-out (Wecker et al., 2018). However, in our transcriptomic data, GO terms such as *JNK cascade* , *negative regulation of cell death* , and *I-κB κινασε/NΦ-κB σιγναλινγ* were significantly enriched during the experiments (Table 1), indicating earlier activation of anti-apoptotic/cell survival signaling (Karin & Lin, 2002; Roulston, Reinhard, Amiri, & Williams, 1998; Van Antwerp, Martin, Kafri, Green, & Verma, 1996). Our qPCR results further showed concurrent expression profiles of the NFKB1, XIAP, and JNK genes, suggesting possible association of these survival and anti-apoptotic signals in the polyp bail-out repsonse. In mammalian cell models, association of XIAP and activation of the JNK and NF-κB signaling has been illustrated (Lu et al., 2007; Nakano, 2004; Tang et al., 2001). Accordingly, our results suggest that the tissue-specificity of apoptosis in polyp bail-out may be attributed to spatial variation in anti-apoptotic signals, leading to inhibition of caspase activity in coral polyps and to their subsequent survival (Fig. 3). The ultimate trigger of these anti-apoptotic/cell survial signals and their functional involvement in polyp bail-out, however, requires further investigation.

New insights into polyp bail-out and future applications

Based on our results, it appears that the TNF receptor-mediated extrinsic apoptotic pathway, FGF-mediated ECM degradation, and anti-apoptotic/survival signals are involved in polyp bail-out (Fig. 4). This finding suggests the feasibility of inducing polyp bail-out using ligands or agonists specifically targeting these pathways, which, by avoiding exerting systemic stresses on the corals, shall facilitate acquisition of viable polyps. The discreteness of pathways leading to apoptosis and ECM degradation also suggests possible independence of coenosarc degradation and polyp detachment in bail-out. Indeed, separation of these processes has been reported in previous studies using different treatments (Domart-Coulon et al., 2004; Fordyce et al., 2017; Kvitt et al., 2015) and was achieved in this study by subjecting corals to different levels of hyperosmotic stress (Fig. S1). Also, since detachment of polyps is mediated by degradation of ECM, we hypothesize that it might be possible to induce polyp resettlement either by stimulating ECM secretion or by providing suitable ECM components to the polyps. In support of our hypothesis, previous studies have found that fibronectin-

like peptide facilitated attachment of solitary polyps to resettlement substrates, although after resettlement, polyps tended to dissociated into single cells (Domart-Coulon et al., 2004; Puverel et al., 2005). Future studies of resettlement processes are expected to optimize conditions for resettlement and maintenance of coral polyps, potentiating their use for mass production of coral colonies for reef restoration.

Considering that some pocilloporid corals, such as *P. damicornis*and *S. pistillata* , have been widely used as model coral species for both physiological and genetic studies, this study of polyp bail-out mechanisms provides additional information for future coral studies at cellular and molecular levels. Moreover, as pocilloporid corals are dominant reef-building corals in shallow water, these findings may help to understand polyp bail-out in nature and its role in shaping coral reef ecosystems in response to anticipated global climate change.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP18J20226. The authors gratefully acknowledge generous support to the Marine Biophysics Unit from Okinawa Institute of Science and Technology Graduate University (OIST). We thank the OIST DNA Sequencing Section (SQC) for constructing RNA-seq libraries and performing sequencing and the OIST Scientific Computing and Data Analysis Section (SCDA) for providing the high performance computing service for the transcriptome assembly in this study. We appreciate Dr. Steven D. Aird for editing and providing comments on the manuscript.

References

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. In: Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom.

Ardi, V. C., Van den Steen, P. E., Opdenakker, G., Schweighofer, B., Deryugina, E. I., & Quigley, J. P. (2009). Neutrophil MMP-9 proenzyme, unencumbered by TIMP-1, undergoes efficient activation in vivo and catalytically induces angiogenesis via a basic fibroblast growth factor (FGF-2)/FGFR-2 pathway. *Journal of Biological Chemistry, 284* (38), 25854-25866.

Bader, M., & Steller, H. (2009). Regulation of cell death by the ubiquitin–proteasome system. *Current opinion in cell biology, 21* (6), 878-884.

Birkedal-Hansen, H., Moore, W., Bodden, M., Windsor, L., Birkedal-Hansen, B., DeCarlo, A., & Engler, J. (1993). Matrix metalloproteinases: a review. *Critical Reviews in Oral Biology & Medicine, 4* (2), 197-250.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics, 30* (15), 2114-2120.

Capel, K. C. C., Migotto, A., Zilberberg, C., & Kitahara, M. V. (2014). Another tool towards invasion? Polyp "bail-out" in Tubastraeacoccinea.*Coral reefs, 33* (4), 1165-1165.

Chen, Y.-S., & Qiu, X.-B. (2013). Ubiquitin at the crossroad of cell death and survival. *Chinese journal of cancer, 32* (12), 640.

Chomczynski, P. (1993). A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples.*Biotechniques, 15* (3), 532-534, 536-537.

Cunning, R., Bay, R., Gillette, P., Baker, A., & Traylor-Knowles, N. (2018). Comparative analysis of the Pocillopora damicornis genome highlights role of immune system in coral evolution. *Scientific reports, 8* (1), 16134.

Deveraux, Q. L., Takahashi, R., Salvesen, G. S., & Reed, J. C. (1997). X-linked IAP is a direct inhibitor of cell-death proteases.*Nature, 388* (6639), 300-304.

Domart-Coulon, I., Tambutté, S., Tambutté, E., & Allemand, D. (2004). Short term viability of soft tissue detached from the skeleton of reef-building corals. *Journal of Experimental Marine Biology and Ecology, 309* (2), 199-217.

Fordyce, A. J., Camp, E. F., & Ainsworth, T. D. (2017). Polyp bailout in Pocillopora damicornis following thermal stress. *F1000Research, 6* .

Gavish, A. R., Shapiro, O. H., Kramarsky-Winter, E., & Vardi, A. (2018). Microscale tracking of coral disease reveals timeline of infection and heterogeneity of polyp fate. *bioRxiv* , 302778.

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., . . . Zeng, Q. (2011). Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data.*Nature biotechnology, 29* (7), 644.

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2008). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic acids research, 37* (1), 1-13.

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature protocols, 4* (1), 44.

Johnston, E. C., Forsman, Z. H., Flot, J.-F., Schmidt-Roach, S., Pinzón, J. H., Knapp, I. S., & Toonen, R. J. (2017). A genomic glance through the fog of plasticity and diversification in Pocillopora.*Scientific reports, 7* (1), 5991.

Karin, M., & Lin, A. (2002). NF-κB at the crossroads of life and death.*Nature immunology, 3* (3), 221.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., & Salzberg, S. L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome biology, 14* (4), R36.

Kojima, S.-i., Itoh, Y., Matsumoto, S.-i., Masuho, Y., & Seiki, M. (2000). Membrane-type 6 matrix metalloproteinase (MT6-MMP, MMP-25) is the second glycosyl-phosphatidyl inositol (GPI)-anchored MMP. *FEBS letters, 480* (2-3), 142-146.

Kvitt, H., Kramarsky-Winter, E., Maor-Landaw, K., Zandbank, K., Kushmaro, A., Rosenfeld, H., . . . Tchernov, D. (2015). Breakdown of coral colonial form under reduced pH conditions is initiated in polyps and mediated through apoptosis. *Proceedings of the national academy of sciences, 112* (7), 2082-2086.

Kvitt, H., Rosenfeld, H., Zandbank, K., & Tchernov, D. (2011). Regulation of apoptotic pathways by Stylophora pistillata (Anthozoa, Pocilloporidae) to survive thermal stress and bleaching. *PloS one, 6* (12), e28665.

Li, B., & Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics, 12* (1), 323.

Littman, R., Willis, B. L., & Bourne, D. G. (2011). Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environmental Microbiology Reports, 3* (6), 651-660.

Lu, M., Lin, S.-C., Huang, Y., Kang, Y. J., Rich, R., Lo, Y.-C., . . . Wu, H. (2007). XIAP induces NF-κB activation via the BIR1/TAB1 interaction and BIR1 dimerization. *Molecular cell, 26* (5), 689-702.

Nakano, H. (2004). Signaling crosstalk between NF-κB and JNK.*Trends in immunology, 25* (8), 402-405.

Pintucci, G., Yu, P. J., Sharony, R., Baumann, F. G., Saponara, F., Frasca, A., . . . Mignatti, P. (2003). Induction of stromelysin-1 (MMP-3) by fibroblast growth factor-2 (FGF-2) in FGF-2-/- microvascular endothelial cells requires prolonged activation of extracellular signal-regulated kinases-1 and-2 (ERK-1/2). *Journal of cellular biochemistry, 90* (5), 1015-1025.

Pinzon, J. H., & LaJeunesse, T. C. (2011). Species delimitation of common reef corals in the genus Pocillopora using nucleotide sequence phylogenies, population genetics and symbiosis ecology. *Molecular ecology, 20* (2), 311-325.

Poquita-Du, R. C., Quek, Z. B. R., Jain, S. S., Schmidt-Roach, S., Tun, K., Heery, E. C., . . . Huang, D. (2019). Last species standing: loss of Pocilloporidae corals associated with coastal urbanization in a tropical city state. *Marine Biodiversity* , 1-15.

Puverel, S., Tambutte, E., Zoccola, D., Domart-Coulon, I., Bouchot, A., Lotto, S., . . . Tambutte, S. (2005). Antibodies against the organic matrix in scleractinians: a new tool to study coral biomineralization. *Coral reefs, 24* (1), 149-156.

Quistad, S. D., Stotland, A., Barott, K. L., Smurthwaite, C. A., Hilton, B. J., Grasis, J. A., . . . Rohwer, F. L. (2014). Evolution of TNF-induced apoptosis reveals 550 My of functional conservation. *Proceedings of the national academy of sciences* , 201405912.

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics, 26* (1), 139-140.

Roulston, A., Reinhard, C., Amiri, P., & Williams, L. T. (1998). Early activation of c-Jun N-terminal kinase and p38 kinase regulate cell survival in response to tumor necrosis factor α. *Journal of Biological Chemistry, 273* (17), 10232-10239.

Sammarco, P. W. (1982). Polyp bail-out: an escape response to environmental stress and a new means of reproduction in corals. *Marine ecology progress series. Oldendorf, 10* (1), 57-65.

Schmidt-Roach, S., Miller, K. J., Lundgren, P., & Andreakis, N. (2014). With eyes wide open: a revision of species within and closely related to the Pocillopora damicornis species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological Journal of the Linnean Society, 170* (1), 1-33.

Serrano, E., Coma, R., Inostroza, K., & Serrano, O. (2018). Polyp bail-out by the coral Astroides calycularis (Scleractinia, Dendrophylliidae). *Marine Biodiversity, 48* (3), 1661-1665.

Shapiro, O. H., Kramarsky-Winter, E., Gavish, A. R., Stocker, R., & Vardi, A. (2016). A coral-on-a-chip microfluidic platform enabling live-imaging microscopy of reef-building corals. *Nature communications, 7* , 10860.

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics, 31* (19), 3210-3212.

Tang, G., Minemoto, Y., Dibling, B., Purcell, N. H., Li, Z., Karin, M., & Lin, A. (2001). Inhibition of JNK activation through NF-κ B target genes. *Nature, 414* (6861), 313-317.

Traylor-Knowles, N., Granger, B. R., Lubinski, T. J., Parikh, J. R., Garamszegi, S., Xia, Y., . . . Finnerty, J. R. (2011). Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora damicornis. *BMC genomics, 12* (1), 585.

Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R., & Verma, I. M. (1996). Suppression of TNF-α-induced apoptosis by NF-κB. *science, 274* (5288), 787-789.

Voolstra, C. R., Li, Y., Liew, Y. J., Baumgarten, S., Zoccola, D., Flot, J.-F., . . . Aranda, M. (2017). Comparative analysis of the genomes of *Stylophora pistillata* and *Acropora digitifera* provides evidence for extensive differences between species of corals. *Scientific reports, 7* (1), 17583.

Webster, N., Negri, A., Flores, F., Humphrey, C., Soo, R., Botté, E., . . . Uthicke, S. (2013). Near-future ocean acidification causes differences in microbial associations within diverse coral reef taxa. *Environmental Microbiology Reports, 5* (2), 243-251.

Wecker, P., Lecellier, G., Guibert, I., Zhou, Y., Bonnard, I., & Berteaux-Lecellier, V. (2018). Exposure to the environmentally-persistent insecticide chlordecone induces detoxification genes and causes polyp bail-out in the coral P. damicornis. *Chemosphere, 195* , 190-200.

Whitelock, J. M., Murdoch, A. D., Iozzo, R. V., & Underwood, P. A. (1996). The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases. *Journal of Biological Chemistry, 271* (17), 10079-10086.

Wild, C., Rixen, T., Sanchez-Noguera, C., Stuhldreier, I., Jimenez, C., & Merico, A. (2014). Massive coral tissue ablations in reefs of Pacific Costa Rica. *Galaxea, Journal of Coral Reef Studies, 16* (1), 13-14.

Data accessibility statement

The data that support the findings of this study are openly available on NCBI database with the following reference numbers:

RNA-seq raw data: NCBI SRA: SRR10696823-SRR10696829, SRR10708182-SRR10708188, SRR10708225-SRR10708230

Transcriptome assembly: GenBank assembly accession GIDI00000000

Author Contributions

PS. Chuang: Conduction of experiments, design of research, data analysis, manuscript writing

S. Mitarai: Design of research, manuscript writing

Figure 1. Principal component analysis classified RNA-seq libraries by treatments and replicates. The first principal component is concordant with the salinity gradient, while the second principal component reflects variation between biological replicates. Analysis was based on 17,908 functionally annotated transcripts.

Figure 2. *Pocillopora acuta* polyps bail out in response to hyperosmotic stress. Morphological features of polyp bail-out, e.g., coenosarc degradation and polyp detachment, were observed at 24 h. Labels of samples and seawater salinity at specific times (unit: indicated on the photos.

Figure 3. Quantitative PCR (qPCR) of ten stress genes showed three distinct expression profiles. The FAS and CASP8 genes were significantly upregulated during the first 12 h and remained stable afterward (profile I). The FGFR2, FGF2, RHO, MMP19, and MMP24 genes displayed a common expression profile that showed little upregulation for the first 6 h and remarkable upregulation from 6 h to 18 h, after which expression stabilized (profile II). For the JNK, NFKB1, and XIAP genes, linear upregulation was observed during the time-course of the experiment (profile III). Statistical analysis was conducted using Welch's ANOVA with a Games-Howell post-hoc test. Groups with significant differences ($p < 0.05$) are indicated. Data were log2-transformed and are expressed as means +- SD (standard deviation).

Figure 4. Parallel signaling pathways trigger polyp bail-out. Molecular mechanisms leading to polyp bail-out are hypothesized based on our transcriptomic and qPCR data. Environmental stress triggers the TNF and FGF signaling pathways, which activate the caspase (CASP) and matrix metalloproteinase (MMP) cascades, respectively. Activation of caspases leads to apoptosis in the coenosarc and to its subsequent degradation, while activation of MMPs, likely through Rho family GTPases (RHO), results in extracellular matrix (ECM) degradation and detachment of polyps. In polyp bodies, anti-apoptotic and cell survival signals mediated by genes such as JNK, NFKB, and XIAP, are activated to suppress the apoptotic response, which in turn promotes polyp survival.

Figure S1. *Pocillopora acuta* responded differently at different levels of hyperosmotic stress. An acute hyperosmotic stress was created by addition of 48tank at 11.2 mL/min, while a mild stress was created by addition of 48salinity seawater at 4 mL/min for 12 h with unchanging salinity thereafter. Under acute hyperosmotic stress, tentacle retraction was observed at 12 h (salinity: 4624 h (salinity: 48release of both solitary and clustered polyps (upper). Under mild hyperosmotic stress, coenosarc degradation was observed at 48 h, but no detached polyps were seen during the experiment (lower). Seawater salinity is indicated on the side of each photo (unit:

Table 1. Apoptosis and several signaling pathways are activated during polyp bail-out induction. Biological processes and signaling pathways showing significant enrichment at both 12 h and 24 h are indicated in bold ($p<0.05$). P -values and fold enrichment (FE) are indicated for each GO category.

12 h	***p*-value**	**FE**	**24 h**
apoptotic process	8.3E-03	1.38	apoptotic process
regulation of apoptotic process	1.4E-04	2.17	regulation of apoptotic process
positive regulation of apoptotic process	3.8E-03	1.64	positive regulation of apoptotic process
apoptotic signaling pathway	1.2E-02	2.55	apoptotic signaling pathway
extrinsic apoptotic signaling pathway	2.2E-02	3.41	extrinsic apoptotic signaling pathway
negative regulation of cell death	4.6E-02	2.05	negative regulation of cell death
JNK cascade	8.1E-03	2.89	JNK cascade
protein ubiquitination	4.1E-03	1.48	protein ubiquitination
tumor necrosis factor-mediated signaling pathway	2.2E-02	2.69	tumor necrosis factor-mediated signaling pathway
fibroblast growth factor receptor signaling pathway	2.7E-02	1.96	fibroblast growth factor receptor signaling pathway
I-kappaB kinase/NF-kappaB signaling	4.3E-02	2.37	

Table 2. Primers used in the qPCR assay. The reference transcript (Ref), primer positions (Pos), and blastx-based annotation (UniProt ID) are indicated for each gene (GenBank assembly accession: GIDI00000000). Efficiency of qPCR (E) for each gene was tested in a 10x-serial dilution with at least four concentration points (N = 3 at each concentration).

Gene	**UniProt ID**	**Primer (5' –> 3')**	**Ref (Pos)**	**E**
FAS	TNR6_PIG	TNR6_F1: GCCAA-CAACTCAGGA-GACAC TNR6_R1: GCAAAGCAATTACCGCAAAC	4317_c14_g1_i1 (617 – 828)	93.8%
CASP8	CASP8_HUMAN	CASP8_F1: AATGAA-CACTTCCAGGGAAAC CASP8_R1: GGCTAAGCAAGCAGCAAAC	22697_c0_g1_i1 (380 – 587)	95.2%
FGF2	FGF2_BOVIN	FGF2_F1: CTAAACC-CACCAGAAAGT-CATC FGF2_R1: TTGTTTCCAATGCCGTCC	28352_c0_g1_i1 (845 – 1031)	97.2%
FGFR2	FGFR2_XENLA	FGFR2_F1: CCATCTTGGA-CAACACAAAAAC FGFR2_R1: TCCTCTGCTGACCTGATAAC	50055_c4_g1_i1 (540 – 762)	96.6%
RHO	RHO_APLCA	RHO_F1: TCTGCCACGAG-GAAAAAAC RHO_R1: GCCTGTCATAATCTTCCTGTC	53528_c27_g1_i1 (197 – 398)	93.7%

Gene	UniProt ID	Primer (5' –> 3')	Ref (Pos)	E
MMP19	MMP19_HUMAN	MMP19_F1: GGTCTAAAGTA-GAGACCCCAG MMP19_R1: CCAAACGAACATTGCGTCC	3229_c0_g1_i1 (19 – 200)	96.1%
MMP24	MMP24_RAT	MMP24_F1: TCCAATGGAA-GAACGGCAG MMP24_R1: TGAGTAAAGATGCCATGAAGAG	20183_c0_g1_i1 (90 – 308)	98.6%
JNK	JNK_DROME	JNK_F1: TCATCA-GAATCAGACA-GATGATAAG JNK_R1: GAACTGCACGAATTGTTCATTA	33455_c1_g1_i2 (847 – 1013)	92.0%
XIAP	XIAP_HUMAN	XIAP_F1: TTCCAGTCAAA-GACCCCAG XIAP_R1: TCGCCAGTACCCAAGTAATAG	30610_c1_g1_i1 (1637 – 1832)	93.1%
NFKB1	NFKB1_CHICK	NFKB1_F1: CCTTTTG-TACCAGT-GCAGTG NFKB1_R1: TGTCATGAATCTGTTTAGAGGC	27043_c0_g1_i2 (259 – 449)	90.7%
TUBB	TBB_PARLI	TUBB_F2: GCAGTTCACG-GCTATGTTC TUBB_R2: TTTTCACCCTCCTCTTCCTC	36257_c3_g1_i1 (1427 – 1615)	98.4%