Fish and Shellfish Immunology 95 (2019) 383–390

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

Time course analysis of immunity-related gene expression in the sea cucumber *Apostichopus japonicus* during exposure to thermal and hypoxic stress

Da Huo[a,b,c,d], Lina Sun[a,b,c,d,**], Libin Zhang[a,b,c,d], Hongsheng Yang[a,b,c,d,*], Shilin Liu[a,b,d], Jingchun Sun[a,b,d], Fang Su[a,b,c,d]

[a] *CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China*
[b] *Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, 266237, China*
[c] *University of Chinese Academy of Sciences, Beijing, 100049, China*
[d] *Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, 266071, China*

ARTICLE INFO

Keywords:
Anoxia
Heat stress
Global climate change
Holothurian
Echinoderm
Immune system
High temperature
Immune-associated genes
Stress response
Aquaculture

ABSTRACT

Temperature and dissolved oxygen concentration are important abiotic factors that can limit the growth and survival of sea cucumbers by affecting their immune systems. As global warming intensifies, sea cucumbers are increasingly exposed to adverse environmental conditions, which can cause severe economic losses and limit the sustainable development of sea cucumber aquaculture. It is therefore important to better understand how sea cucumbers respond to environmental stress, especially with regard to its effects on immunity. In the present study, the time series of immunity-related gene expression in sea cucumbers under thermal and hypoxic stresses were analyzed separately. The expression trends of 17 genes related to the nuclear factor κB (NF-κB) pathway, the protease family, the complement system, heat shock proteins (HSPs) and the transferrin family during exposure to two stresses at eight time points were concluded. These genes have interconnected roles in stress defense. The expression levels of genes relating to the NF-κB pathways and HSPs were strongly affected in the sea cucumber thermal stress response, while *melanotransferrin (Mtf)*, *ferritin (Ft)* and *mannan-binding C-type lectin (MBCL)* were affected by hypoxia. In contrast, *complement factor B (Bf)*, *myosin V (Mys)* and *serine protease inhibitor (SPI)* were not that sensitive during the initial period of environmental stress. Similar expression patterns under both thermal and hypoxic stress for certain genes, including an increase in *Hsp90* and decreases in *lysozyme (Lys)*, *major yolk protein (MYP)* and *cathepsin C (CTLC)* were observed in sea cucumbers. Conversely, *NF-κB* and *Hsp70* were differentially affected by the two stress treatments. Lysozyme-induced immune defense was inconstant in sea cucumbers coping with stress. A gene ontology (GO) analysis of the selected genes revealed that the most co-involved terms related to immunity and iron ion. Our analysis suggests that sea cucumbers demonstrate complex and varied immune responses to different types of stresses. This dynamic image of the immune responses and stress tolerance of sea cucumbers provides new insights into the adaptive strategies of holothurians in adverse environments.

1. Introduction

Global climate change has caused elevated sea temperatures and thus has a direct impact on marine organisms. Additionally, because high water temperatures reduce oxygen solubility, increase stratification and reduce ventilation, thermal stress is commonly accompanied by hypoxic stress [1]. As a nutrient-rich aquaculture species, the sea cucumber *Apostichopus japonicus* is widely farmed in Asia, including in China, Japan, Russia and Korea. In 2018, although the area of sea cucumber aquaculture increased 8.68% in China, the harvest reduced 20.72% compared with the previous year [2]. The primary causes of this severe economic and resource loss were environmental,

---

[*] Corresponding author. CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao, 266071, Shandong, China.

[**] Corresponding author. CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, Shandong, China.

E-mail addresses: sunlina@qdio.ac.cn (L. Sun), hshyang@ms.qdio.ac.cn (H. Yang).

https://doi.org/10.1016/j.fsi.2019.09.073
Received 31 July 2019; Received in revised form 27 September 2019; Accepted 30 September 2019
Available online 01 October 2019
1050-4648/

Abbreviation list

Actb	β-actin
NF-κB	nuclear factor κB
IκB	inhibitor of NF-κB
Lys	lysozyme
SPI	serine protease inhibitor
CTLC	cathepsin C
AjC3-2	complement component C3-2
AjC3	complement component C3
Bf	complement factor B
Hsp70	heat shock protein 70
Hsp90	heat shock protein 90
gp96	heat shock protein gp96
Ft	ferritins
Mtf	melanotransferrin
MYP	major yolk protein
MBCL	mannan-binding C-type lectin
Thy	thymosin β
Mys	myosin V
TLR	toll like receptors
PRR	pattern recognition receptor
GO	gene ontology
CC	cellular component
MF	molecular function
BP	biological process

specifically, oxygen deficiency and elevated temperature. This is concerning, because as global warming intensifies, it is likely that the environmental conditions for sea cucumbers will become more unsuitable and productivity will further decline.

Exposure to high temperatures and low concentrations of dissolved oxygen can cause severe thermal and hypoxic stress to marine organisms, and such stresses affect many biological processes, especially immunity. For example, previous research has examined the expression of *Hsp70* in *Ruditapes philippinarum* and *Larimichthys crocea* under hypoxia [3,4] and in *Crassostrea gigas* under thermal stress [5]. Immune-related genes linked to the Rel/NF-κB signaling pathway have been studied in *Haliotis diversicolor* under thermal and hypoxic stresses [6]; as has the *ferritin* gene in *Haliotis rufescens* under thermal stress [7]. However, limited research has addressed these immune response of sea cucumbers exposed to thermal and hypoxic stresses. In a previous study, the behavior, physiology, histomorphology, enzyme activity and molecular characteristics of *A. japonicus* were shown to be influenced by thermal and hypoxic stresses [8,9]. Furthermore, immune-related pathways were identified as being affected by thermal and hypoxic stresses in *A. japonicus* from the aspect of proteins and metabolites [10,11]. Nonetheless, most studies on the effects of heat and hypoxic stress on sea cucumbers have considered a single time point, and a serial analysis of gene expression over time is lacking. Therefore, in the present study, we have analyzed immune gene expression in sea cucumbers under environmental stress over a three-day time course. Our results clarified key immune gene expression and were helpful to understand the different defense mechanisms deployed by echinoderms under diverse environmental stresses.

2. Material and methods

2.1. Animals

Around 110 healthy sea cucumbers (*A. japonicas*), each weighing around 100 g, were collected from the coast of Weihai, China. Before the experiment, sea cucumbers were acclimated in tanks containing aerated sand-filtered seawater at 16 ± 0.5 °C and with a dissolved oxygen concentration of 8 ± 1 mg/L for at least one week, and were fed once a day (in the morning). Any uneaten feed was removed daily during both the acclimation and experimental periods. Following acclimation, active sea cucumbers were randomly allocated into one of the two environmental stress treatments and cultured separately. The thermal stress treatment was conducted with a high temperature of 26 °C and a sufficient concentration of dissolved oxygen (constant aeration); the hypoxic stress treatment was conducted with a low dissolved oxygen concentration of 2 mg/L and at a suitable temperature of 16 °C. A heating rod and an on-line oxygen dissolving control system were used to maintain the temperature and dissolved oxygen level, respectively [9]. The intestine is a sensitive organ that can be partly eviscerated when sea cucumbers are exposed to adverse environments, thus it was selected as the target tissue for our analysis of gene expression. After exposure to stress for 0 h, 2 h, 4 h, 6 h, 12 h, 24 h, 48 h and 72 h, six individuals from each treatment were promptly dissected and their intestines were preserved in liquid nitrogen and stored at −80 °C. Because *A. japonicus* is not an endangered or protected species, no permission was needed for animal collection, and an ethics statement is not applicable.

2.2. Data acquisition and analysis

Based on previous research on sea cucumbers immunity, 17 immunity-related genes were selected for RT-qPCR analysis [12,13]. Total RNA was extracted from intestines using the MiniBEST Universal RNA Extraction Kit (Takara, Shiga, Japan) following the manufacturer's instructions and a NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure its quality and concentration. RNA samples from three sea cucumbers per treatment at each timepoint, with RNA integrity numbers ≥ 7, OD260/OD280 = 2.0 ± 0.2 and OD260/OD230 = 2.0 ± 0.2, were selected for the further experiments. First-strand cDNA was synthesized from each RNA sample using reverse transcriptase (Takara) and used as a template for RT-qPCR. Gene expression levels were examined using SYBR Green® real-time PCR assays (SYBR PrimeScrip ™ RT-PCR Kit II, Takara) with an Eppendorf Mastercycler® ep realplex (Eppendorf, Hamburg, Germany); *β-actin* (*Actb*) was used as a reference gene for internal standardization.

RT-qPCR primers (Table S1) were designed for optimal performance using the primer3 software [14] and according to sequence information from the *A. japonicus* transcriptome database. The total volume of the RT-qPCR amplification mix was 20 μl, which comprised 10 μl of SYBR Green Master Mix (Takara), 8 μl of RNase-free water, 1 μl of diluted cDNA, and 0.5 μl (each) of the forward and reverse primers (10 mM). Thermal cycling was performed according to the following procedure: 95 °C for 5 s, followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s and 72 °C for 30 s. The specificity of the amplification products was examined by melting curve analysis. The $2^{-\triangle\triangle CT}$ method was used to analyse the comparative mRNA expression levels of the selected genes [15]; this analysis was performed using the SPSS19 software (IBM Corp., Armonk, NY, USA). All data are displayed as means ± standard deviation. A significance analysis of each gene between the different time points compared with 0 h was performed using one-way ANOVA with Tukey's test. In Figs. 1 and 2, means that do not share a given letter (like a and b) differ significantly ($P < 0.05$). The heatmap in Fig. 3 was made using TBtools and is clustered by row scale [16]. A gene ontology (GO: http://www.geneontology.org/) analysis was used to classify and group the selected genes and the GO network was built using Cytoscape software (The Cytoscape Consortium, San Diego, CA, USA) [17].

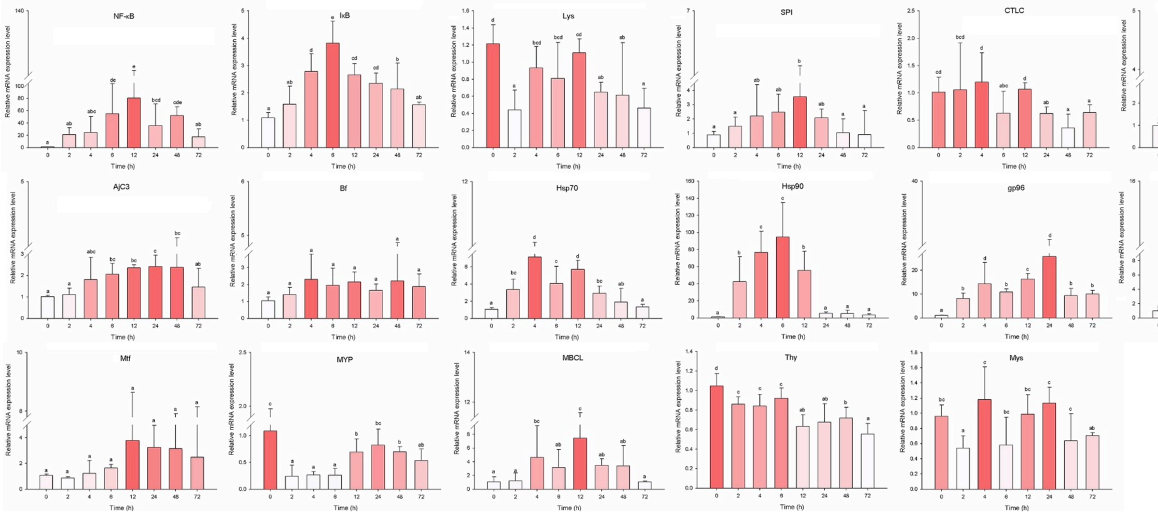

Fig. 1. Expression of key immune genes in sea cucumbers under thermal stress.

3. Results and discussion

In *A. japonicus* aquaculture, water temperature and dissolved oxygen are two of the most important limiting factors [11]. Environmental stresses—including heat, hypoxia and low salinity—have negative effects on marine invertebrate immune systems, decreasing resistance to disease and leading to death. Therefore, it is important to understand how immune genes are affected by stress exposure and the roles they play in defense mechanisms. In this study, the expression dynamics of key genes from the nuclear factor κB (NF-κB) pathway, the protease family, the complement system, the heat shock proteins and the transferrin family—along with other genes involved in immunity—were observed in *A. japonicus* under thermal stress or hypoxic stress (Figs. 1–2). For discussion, we have divided our experimental environmental stress exposure into three phases: an early exposure stage (2 h–12 h), a middle exposure stage (12 h–48 h) and a late exposure stage (48 h–72 h). Furthermore, all descriptions of increased or decreased gene expression are based on a comparison to the expression levels at 0 h, and a heatmap of gene expression over time is shown in Fig. 3. Overall, it seems that over half of the selected immunity-related gene expression showed a changed tendency within the first two days of exposure (Fig. 3), and then returned to their original expression states by 72 h.

3.1. NF-κB pathway-related gene expression

NF-κB and *inhibitor of NF-κB (IκB)* are genes vital to the NF-κB pathway. In a previous study of the abalone exposed to thermal and hypoxic stress, they both showed increased expression [6,18]. In our study, the expression of *NF-κB* and *IκB* showed similar increased expression affected by thermal stress, being increased in the early stage of stress exposure, peaking at 12 h and 6 h, respectively, and then decreased to initial levels in the late stage (at 72 h) of thermal stress (Fig. 1). In contrast, under hypoxic stress, *NF-κB* showed significantly lower expression in the early and middle stages (from 2 h to 48 h), recovering to original levels at 72 h. *IκB* did not appear to be consistently changed by hypoxic stress, being significantly increased at 2 h and 6 h, and significantly decreased at 24 h and 72 h (Fig. 2). Actually, activated NF-kb induces rapid and high-level expression of IκBα, and then attenuate on-going NF-kb activation [19]. A possible explanation for the discrepant results may be that *NF-κB* and *IκB* may have different response speeds towards different type of environmental stress. In addition, NF-κB has also been found to be sensitive to hypoxic stimulation independent of the activity of hypoxia inducible factor—a key transcription factor that regulates oxygen homeostasis [20,21]. Therefore, it may also be because that some other genes were simultaneously taking part in the regulation, like *HIF-1α*, etc [22].

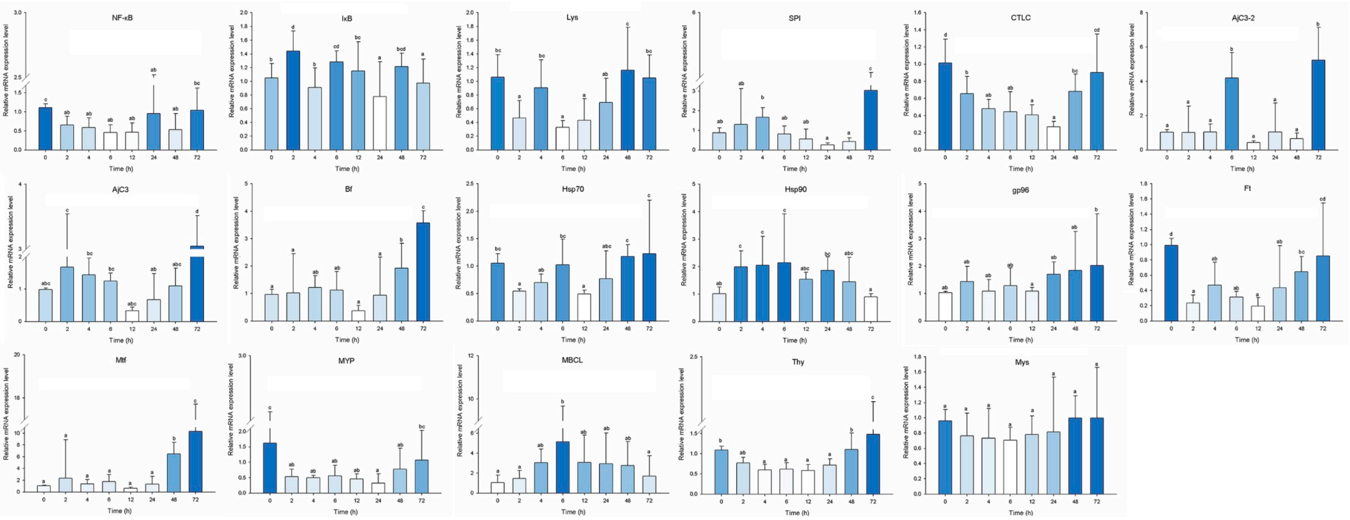

Fig. 2. Expression of key immune genes in sea cucumbers under hypoxic stress.

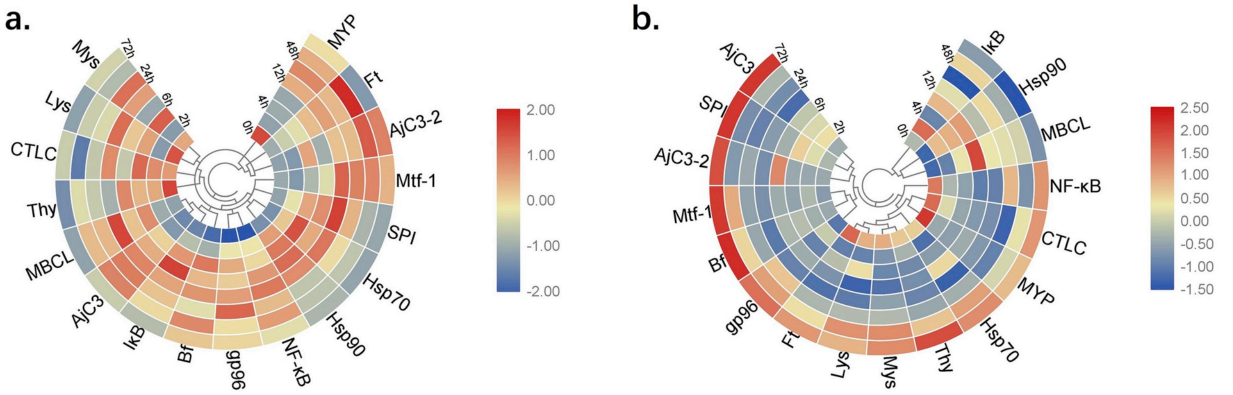

Fig. 3. Heatmap of immune gene expression over time. (a) Under thermal stress; (b) under hypoxic stress.

3.2. Protease-related gene expression

Lysozyme (Lys) plays an important role in immunity modulation and anti-inflammatory activities, because it damages bacterial cell walls and hydrolyses fibrin to safeguard against infection [23,24]. We found that *Lys* gene expression decreased during environmental stress exposure. Specifically, *Lys* expression showed a significant decrease at 2 h, 24 h, 48 h and 72 h under thermal stress (Fig. 1), and in the early stage of hypoxic stress at 2 h, 6 h and 12 h (Fig. 2). Because these changes in *Lys* expression were not dependent on time- or dosage, this may indicate that lysozyme-induced immune defense is flexible in sea cucumbers coping with stress. Serine protease inhibitor (SPI) can provide defense against pathogens or parasites, and acts by inhibiting fungal or bacterial proteinases [25]. Here, *SPI* showed increased expression, reaching a peak at 12 h and then decreasing in the middle and late stages of thermal stress (Fig. 1). Under hypoxic stress, *SPI* showed significantly increased expression in the late stage at 72 h (Fig. 2).

As a family of lysosomal cysteine proteases, cathepsins could be distinguished by substrate specificity, and can degrade polypeptides [26]. Among them, cathepsin C (CTLC) has been identified as an activator of serine protease and is involved in the mammalian immune system [27,28]. In our study, *CTLC* expression significantly decreased in the middle and late stages (from 24 h until 72 h) under thermal stress. Moreover, *CTLC* was also significantly decreased in the early and middle stages (from 2 h to 48 h) under hypoxic stress, but return to initial levels in the late stage at 72 h. In a previous study on heat stress in sea cucumbers, the cathepsin family member, cathepsin B and cathepsin D, also showed decreased expression due to heat stress [29]. These results suggest that serine proteases may be a dominant mediator of key biological progresses during thermal stress.

3.3. Complement system-related gene expression

The complement system contributes to the innate immune response in invertebrates, and echinoderms possess a simplified complement system [30]. Complement component C3 is involved in the three pathways of the complement system, i.e. the classical, alternative and lectin pathways. In this study, *complement component C3 (AjC3)* expression was significantly increased in the early and middle stages (from 6 h to 48 h), and then returned to initial levels at 72 h under thermal stress (Fig. 1). However, under hypoxic stress, *AjC3* only showed significantly increased expression at 72 h (Fig. 2). *Complement component C3-2 (AjC3-2)* showed no significant changes in expression during the first day of exposure to thermal stress, but then the expression significantly increased in the late stage (at 48 h and 72 h). Under hypoxic stress, *AjC3-2* expression was significantly increased at 6 h and 72 h compared with 0 h. Complement factor B (Bf) can activate C3 and is involved in the alternative pathway [31]. *Bf* showed no significant expression changes when exposed to thermal stress, but was significantly increased in the late stage at 48 h and 72 h under hypoxic stress. These data suggest that the alternative pathway is less affected by thermal stress than hypoxic stress.

3.4. Heat shock protein-related gene expression

Environmental stress factors such as heat shock [32], osmotic stress [33] and ultraviolet light [34] can result in the production of heat shock proteins (HSPs) by the cell. Subgroups of HSPs range in molecular mass from 10 to 200 kDa and include heat shock protein (Hsp)70, Hsp90 and gp96. These molecules could play important roles in signal transduction, cell cycle control and protein refolding and degradation [35]. Hsp70 aids protein import, translocation processes, proteolytic degradation and aggregation prevention [36] and protection against apoptosis [37]. In our study, thermal stress caused a significant increase in *Hsp70* during the early and middle stages (from 2 h to 24 h), but expression returned to initial levels after 48 h (Fig. 1). In contrast, *Hsp70* expression showed a significant decrease in the early stage (at 2 h, 4 h and 12 h) under hypoxic stress (Fig. 2). In a previous study, a moderate expansion of the Hsp70 subfamily was found in sea cucumbers [38]. It is tempting to infer that this expansion may underlie a strategy that enables sea cucumbers to adapt to fluctuating environmental conditions, especially in response to thermal stress.

Hsp90 manages protein folding and degradation, and plays a role in signal transduction, stress adaptation [35,39] and proteasome assembly and maintenance [40]. It has also previously been identified as very sensitive to heat shock and hypoxia in the Chinese shrimp *Fenneropenaeus chinensis* [41]. Here, the expression of *Hsp90* significantly increased in sea cucumbers during the early stage of thermal stress (from 2 h to 12 h), but during the middle and late stages (from 24 h to 72 h), it showed no significant change. Similarly, when exposed to hypoxic stress, *Hsp90* expression significantly increased in sea cucumbers during 2 h–6 h, but showed no significant changes between 12 h and 72 h. *Heat shock protein gp96 (gp96)* was also sensitive to thermal stress, showing significantly increased expression levels throughout the early, middle and late stages; however, it showed no significant changes in expression during hypoxic stress. *gp96* gene can potently induce T-cell mediated innate immunity through toll like receptors (TLRs), and adaptive immunity through antigen presentation [42]. The rapid accumulation of HSPs observed in our study could ensure cell homeostasis by refolding denatured proteins, degrading unstable or misfolded proteins, and preventing proteins from aggregation and restoring, thereby helping sea cucumbers survive under conditions of environmental stress [43].

3.5. Expression of transferrin family members and other immune-related genes

Melanotransferrin (Mtf) belongs to the transferrin-family proteins, and plays important roles in cell migration, angiogenesis, invasion and plasminogen activation [44–46]. In our study, the *Mtf* gene showed no significant expression changes during three days of thermal stress, nor in the early and middle stages (from 2 h to 24 h) of hypoxic stress. However, in the late stage (from 48 h to 72 h), *Mtf* showed significantly increased expression during hypoxic stress. Ferritins (Fts) are the main iron storage proteins and are related to innate immune responses [26]. In our study, expression levels of the *Ft* gene were only significantly increased at 48 h under thermal stress (Fig. 1). Under hypoxic stress, *Ft* expression was significantly decreased in the early and middle stages from 2 h to 48 h, and then return to initial levels in the late stage, at 72 h (Fig. 2). This decreased *ferritin* expression might result in a greater availability of free iron in the environment, which may protect cells from oxidative stress [47]. Lectins recognize specific carbohydrate moieties, and are involved in immune defense [26]. As a type of the pattern recognition receptors (PRRs), C-type lectins can also induce cell aggregation [48]. In our analysis, *Mannan-binding C-type lectin (MBCL)* showed significant upregulation at 4 h and 12 h under thermal stress, and at 6 h under hypoxic stress (Fig. 2). Thus, cell aggregation and an immune response are likely to occur promptly in sea cucumbers under stress. Thymosin β (Thy) has immunoregulatory and anti-inflammatory properties and links the actin cytoskeleton to key immune and cell growth-signaling cascades [49,50]. Here, the *Thy* gene showed significantly lowered expression throughout the early, middle and late stages (from 2 h to 72 h) under thermal stress, and from 4 h to 24 h under hypoxic stress. *Myosin V (Mys)* only showed significant downregulation at 2 h, after which its expression recovered to initial levels until the end of the thermal stress treatment. Notably, *Mys* showed no significant change in expression during three days of hypoxic stress. Major yolk protein (MYP) is a transferrin superfamily member with iron-binding ability. In our analysis, the *MYP* gene showed significantly downregulated expression within two days of hypoxic stress (Fig. 2) and within three days of thermal stress, except at the 24 h time point (Fig. 1). A previous study has reported a similar expression pattern of *MYP* in sea cucumbers under heat stress [29]. This decreased expression of *MYP* gene under environmental stress might be related with the depressed transduction and participation in iron adjustment to maintain basic metabolism to survive.

3.6. Immune system responded to environmental stress

A strong relationship between immune response and environmental stress has been reported in the many publications. A gene ontology (GO) analysis was conducted to explore the functions of key immunity genes in our study (Fig. 4), and include representatives from the “cellular component” (CC), “molecular function” (MF) and “biological process” (BP). Among these GO terms, the most co-involved among all the GO terms are related with immune and iron ion, including inflammatory response (GO: 0006954), innate immune response (GO: 0045087), ion transport (GO: 0006811) and iron ion homeostasis (GO: 0055072). Notably, these two terms—immunity and iron ion—were also shown to be significantly enriched in a previously reported dataset of differentially expressed proteins in *A. japonicus* under thermal and hypoxic stress [10]. Because sea cucumbers lack an adaptive immune system, they depend on innate immunity to respond to stress, and both humoral and cellular responses occur when sea cucumbers are exposed to sub-optimal environmental conditions. Ions are involved in

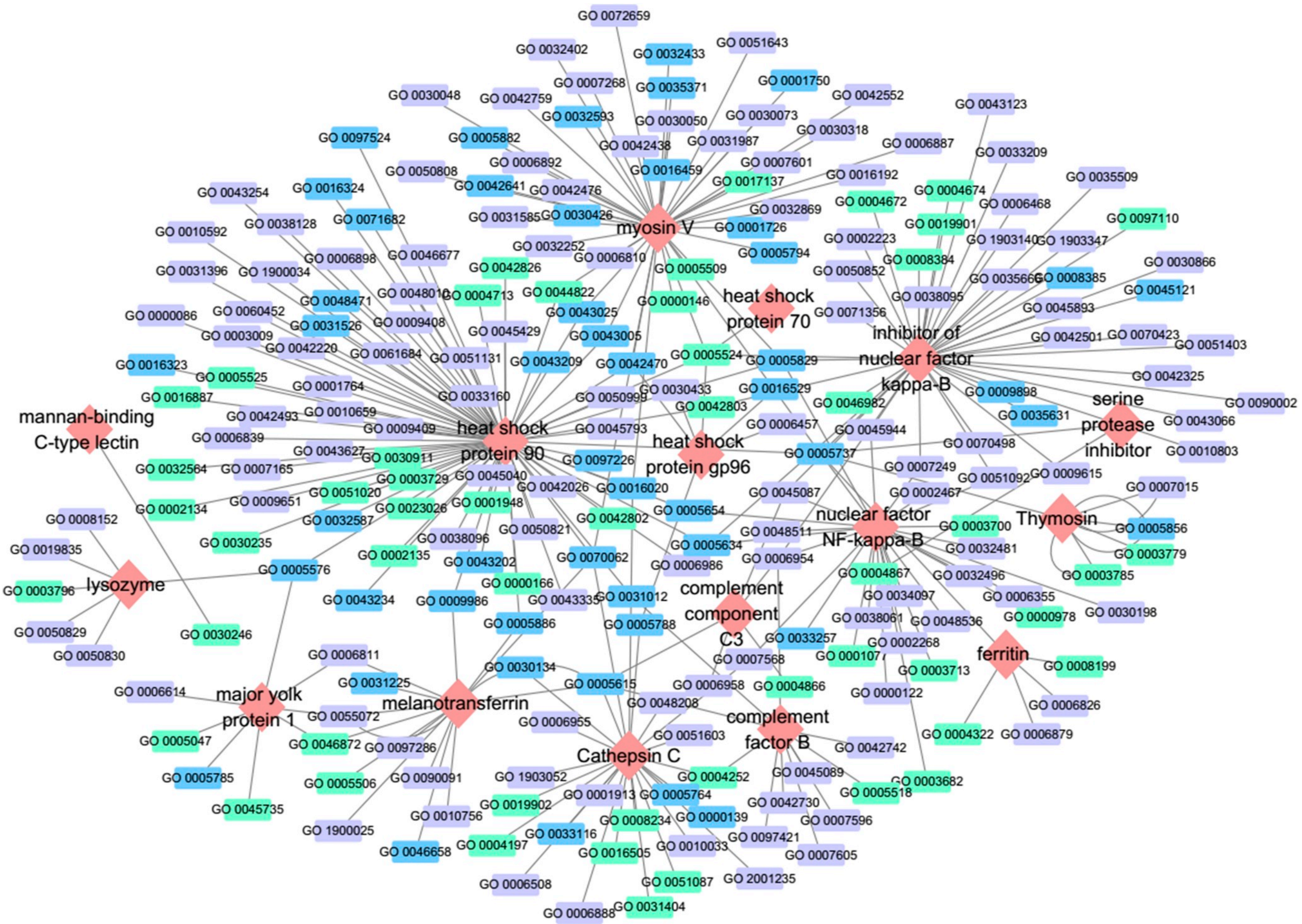

Fig. 4. GO term analysis of selected genes (red rhombus, key gene; blue rectangle, CC terms; purple rectangle, BP terms; green rectangle, MF terms). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

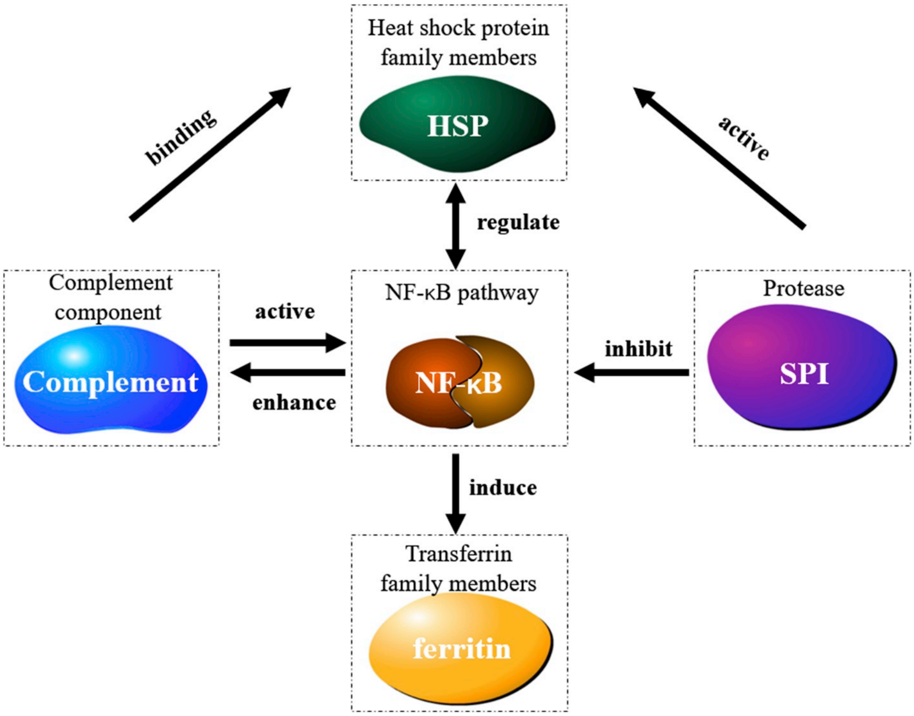

Fig. 5. Key immune processes involved in defense against environmental stress.

regulating osmotic pressure and substance transport, while iron promotes free radical generation and cellular oxidative stress [29]. Hypoxic stress can cause some sea cucumbers to become edematous with sea water and slowly die [9]. This highlights the importance of ionic responses for sea cucumber survival, and is consistent with the changes we observed in ion-related pathways in sea cucumbers under stress.

Immune molecules from the NF-κB pathway, the protease family, the complement system, the heat shock protein family and the transferrin family participate in the innate immune system of sea cucumbers, and thus facilitating their response to environmental stress (Fig. 5). These genes have interconnected roles in stress defense. For example, Hsp90 and Hsp70 act as part of a multi-chaperone network and cooperate with other molecular co-chaperones [43]; Hsp70 may also direct a new mechanism of NF-κB regulation under thermal stress [51]. Moreover, anti-inflammatory effects of HSPs have been proposed based on their inhibition of NF-κB [52,53], while SPI can activate the DNA-binding activity of heat shock transcription factor at concentrations that inhibit NF-κB [54]. The expression of the complement receptor 2 gene can be enhanced by NF-κB [55], and the induction of complement genes can activate the NF-κB pathway and induce further inflammatory factors [56,57]. Furthermore, the complement acts as an important agent in the clearance of damaged tissues through opsonization by binding to Hsps [58]. As a transcriptional activator, NF-κB serves to limit damage from radicals by inducing ferritin [59], and MYP and heat shock cognate protein 70 play an essential role in activating the TLR pathway via interacting with myeloid differentiation factor 88 in *A. japonicus* [60]. These complex interactions might explain the dynamic and diverse expression of these immune-related genes in sea cucumbers under thermal or hypoxic stress.

4. Conclusions

Deteriorating environmental conditions, especially the recent increase in thermal and hypoxic stresses, are threatening the sea cucumber aquaculture industry. Our findings reveal that the *A. japonicus* immune system responds rapidly to environmental stress. We observed a dynamic immune response, with immune-related genes being differentially expressed over time after exposure of *A. japonicus* to environmental stress, and these genes and molecules connect as a coefficient network to play a role in stress defense in sea cucumber. As they changed over time, sea cucumbers develop specific strategy to defense. In summary, *MYP* was repressed by both stresses while *Mys* was not significantly changed either in most time points within three days. Complementary genes and *NF-κB* were induced by thermal stress, while *Thy* gene expression was repressed. *Mtf* showed less sensitivity to thermal stress than to hypoxia, which might be because more irons are needed to sustain oxygen homeostasis when sea cucumbers are under hypoxia. Certain HSP family members were less sensitive to hypoxic stress than thermal stress, which may be due to proteins being more easily denatured by heat than hypoxia. These results provide information about the innate immunity of *A. japonicus* and represent a key immune index of a marine organism under environmental stress. The findings in this study facilitate an understanding of the adaptive strategies of holothurians in coping with global climate changes.

CRediT authorship contribution statement

Da Huo: Investigation, Data curation, Methodology, Validation, Writing - original draft, Writing - review & editing. **Lina Sun:** Formal analysis, Funding acquisition, Writing - review & editing. **Libin Zhang:** Project administration, Writing - review & editing. **Hongsheng Yang:** Supervision, Funding acquisition, Writing - review & editing. **Shilin Liu:** Resources, Writing - review & editing. **Jingchun Sun:** Project administration, Writing - review & editing. **Fang Su:** Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by The National Key Research and Development Program of China (2018YFD0901602), the National

Natural Science Foundation of China (grant No. 41776161, 41776162), the Agricultural Seed Project of Shandong Province (grant No. 2016LZGC032, 2017LZGC010), The Youth Talent Support Program of the Laboratory for Marine Ecology and Environmental Science, Pilot National Laboratory for Marine Science and Technology (Qingdao; grant No. LMEES-YTSP-2018-01-09). Supported by the Taishan Scholars Program (Distinguished Taishan Scholars) and Youth Innovation Promotion Association CAS (2019209).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.09.073.

References

[1] J.L. Sarmiento, T.M. Hughes, R.J. Stouffer, S. Manabe, Simulated response of the ocean carbon cycle to anthropogenic climate warming, Nature 393 (6682) (1998) 245.

[2] Ministry of Agriculture, China Fishery Statistical Yearbook, China Agriculture Press, Beijing, 2018.

[3] H. Nie, L. Liu, H. Wang, Z. Huo, X. Yan, Stress levels over time in *Ruditapes philippinarum*: the effects of hypoxia and cold stress on hsp70 gene expression, Aquacult. Rep. 12 (2018) 1–4.

[4] Q.F. Wang, W.L. Shen, C.C. Hou, C. Liu, X.F. Wu, J.Q. Zhu, Physiological responses and changes in gene expression in the large yellow croaker *Larimichthys crocea* following exposure to hypoxia, Chemosphere 169 (2017) 418–427.

[5] A.L. Meistertzheim, A. Tanguy, D. Moraga, M.T. Thébault, Identification of differentially expressed genes of the Pacific oyster *Crassostrea gigas* exposed to prolonged thermal stress, FEBS J. 274 (24) (2007) 6392–6402.

[6] X. Zhang, Y. Huang, X. Cai, Z. Zou, G. Wang, S. Wang, Y. Wang, Z. Zhang, Identification and expression analysis of immune-related genes linked to Rel/NF-κB signaling pathway under stresses and bacterial challenge from the small abalone *Haliotis diversicolor*, Fish Shellfish Immunol. 41 (2) (2014) 200–208.

[7] K. Salinas-Clarot, A.P. Gutiérrez, G. Núñez-Acuña, C. Gallardo-Escárate, Molecular characterization and gene expression of ferritin in red abalone (*Haliotis rufescens*), Fish Shellfish Immunol. 30 (1) (2011) 430–433.

[8] D. Huo, L. Sun, X. Li, X. Ru, S. Liu, L. Zhang, L. Xing, H. Yang, Differential Expression of miRNAs in the Respiratory Tree of the Sea Cucumber (*Apostichopus japonicus*) under Hypoxia Stress G3 Genes|Genomes|Genetics, 2017.

[9] D. Huo, L. Sun, X. Ru, L. Zhang, C. Lin, S. Liu, X. Xin, H. Yang, Impact of hypoxia stress on the physiological responses of sea cucumber *Apostichopus japonicus*: respiration, digestion, immunity and oxidative damage, Peerj 6 (2018) e4651.

[10] D. Huo, L. Sun, L. Zhang, X. Ru, S. Liu, X. Yang, H. Yang, Global-warming-caused changes of temperature and oxygen alter the proteomic profile of sea cucumber *Apostichopus japonicus*, J. Proteom. 193 (2019) 27–43.

[11] D. Huo, L. Sun, L. Zhang, X. Ru, S. Liu, H. Yang, Metabolome responses of the sea cucumber *Apostichopus japonicus* to multiple environmental stresses: heat and hypoxia, Mar. Pollut. Bull. 138 (2019) 407–420.

[12] Z. Xue, H. Li, X. Wang, X. Li, Y. Liu, J. Sun, C. Liu, A review of the immune molecules in the sea cucumber, Fish Shellfish Immunol. 44 (1) (2015) 1–11.

[13] A. Yang, Z. Zhou, Y. Dong, B. Jiang, X. Wang, Z. Chen, X. Guan, B. Wang, D. Sun, Expression of immune-related genes in embryos and larvae of sea cucumber *Apostichopus japonicus*, Fish Shellfish Immunol. 29 (5) (2010) 839–845.

[14] A. Untergasser, I. Cutcutache, T. Koressaar, J. Ye, B.C. Faircloth, M. Remm, S.G. Rozen, Primer3—new capabilities and interfaces, Nucleic Acids Res. 40 (15) (2012) e115-e115.

[15] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative CT method, Nat. Protoc. 3 (6) (2008) 1101–1108.

[16] C. Chen, R. Xia, H. Chen, Y. He, TBtools, a Toolkit for Biologists Integrating Various Biological Data Handling Tools with a User-Friendly Interface, (2018), p. 289660 BioRxiv.

[17] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, N. Amin, B. Schwikowski, T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genome Res. 13 (11) (2003) 2498–2504.

[18] L. He, X. Zhang, Y. Huang, H. Yang, Y. Wang, Z. Zhang, The characterization of RHEB gene and its responses to hypoxia and thermal stresses in the small abalone *Haliotis diversicolor*, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 210 (2017) 48–54.

[19] V. Malhotra, H.R. Wong, Interactions between the heat shock response and the nuclear factor-κB signaling pathway, Crit. Care Med. 30 (1) (2002) S89–S95.

[20] M.M. Baze, K. Hunter, J.P. Hayes, Chronic hypoxia stimulates an enhanced response to immune challenge without evidence of an energetic tradeoff, J. Exp. Biol. 214 (19) (2011) 3255–3268.

[21] G.L. Semenza, Regulation of oxygen homeostasis by hypoxia-inducible factor 1, Physiology 24 (2) (2009) 97–106.

[22] M. Tafani, A. Russo, M. Di Vito, P. Sale, L. Pellegrini, L. Schito, S. Gentileschi, R. Bracaglia, F. Marandino, E. Garaci, Up-regulation of pro-inflammatory genes as adaptation to hypoxia in MCF-7 cells and in human mammary invasive carcinoma microenvironment, Cancer Sci. 101 (4) (2010) 1014–1023.

[23] X. Yang, L. Cong, M. Lu, H. Liu, B. Zhu, Characterization and Structure Analysis of a Gene Encoding I-type Lysozyme from Sea Cucumber *Stichopus Japonicus*, (2007), pp. 542–547.

[24] T. Wang, Y. Sun, L. Jin, Y. Xu, L. Wang, T. Ren, K. Wang, Enhancement of non-specific immune response in sea cucumber (*Apostichopus japonicus*) by Astragalus membranaceus and its polysaccharides, Fish Shellfish Immunol. 27 (6) (2009) 757–762.

[25] M. Laskowski Jr., I. Kato, Protein inhibitors of proteinases, Annu. Rev. Biochem. 49 (1) (1980) 593–626.

[26] F. Ramírez-Gómez, P.A. Ortíz-Pineda, C. Rojas-Cartagena, E.C. Suárez-Castillo, J.E. García-Ararrás, Immune-related genes associated with intestinal tissue in the sea cucumber *Holothuria glaberrima*, Immunogenetics 60 (1) (2008) 57.

[27] C.T. Pham, T.J. Ley, Dipeptidyl peptidase I is required for the processing and activation of granzymes A and B in vivo, Proc. Natl. Acad. Sci. 96 (15) (1999) 8627–8632.

[28] P.J. Wolters, C.T. Pham, D.J. Muilenburg, T.J. Ley, G.H. Caughey, Dipeptidyl peptidase I is essential for activation of mast cell chymases, but not tryptases, in mice, J. Biol. Chem. 276 (21) (2001) 18551–18556.

[29] D. Xu, S. Zhou, L. Sun, RNA-seq based transcriptional analysis reveals dynamic genes expression profiles and immune-associated regulation under heat stress in *Apostichopus japonicus*, Fish Shellfish Immunol. 78 (2018) 169–176.

[30] L.C. Smith, J. Ghosh, K.M. Buckley, L.A. Clow, N.M. Dheilly, T. Haug, J.H. Henson, C. Li, C.M. Lun, A.J. Majeske, Echinoderm Immunity, Invertebrate Immunity, Springer, 2010, pp. 260–301.

[31] J. Volanakis, Participation of C3 and its Ligands in Complement Activation, the Third Component of Complement, Springer, 1990, pp. 1–21.

[32] A. Barat, P.K. Sahoo, R. Kumar, C. Goel, A.K. Singh, Transcriptional response to heat shock in liver of snow trout (*Schizothorax richardsonii*)—a vulnerable Himalayan Cyprinid fish, Funct. Integr. Genom. 16 (2) (2016) 203–213.

[33] L. Zhang, Q. Feng, L. Sun, K. Ding, D. Huo, Y. Fang, T. Zhang, H. Yang, Differential gene expression in the intestine of sea cucumber (*Apostichopus japonicus*) under low and high salinity conditions, Comp. Biochem. Physiol. Genom. Proteonom. 25 (2018) 34–41.

[34] Y. Cao, N. Ohwatari, T. Matsumoto, M. Kosaka, A. Ohtsuru, S. Yamashita, TGF-β1 mediates 70-kDa heat shock protein induction due to ultraviolet irradiation in human skin fibroblasts, Pflüg. Arch. 438 (3) (1999) 239–244.

[35] W. Wang, B. Vinocur, O. Shoseyov, A. Altman, Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response, Trends Plant Sci. 9 (5) (2004) 244–252.

[36] F.U. Hartl, Molecular chaperones in cellular protein folding, Nature 381 (6583) (1996) 571.

[37] D.D. Mosser, A.W. Caron, L. Bourget, C. Denis-Larose, B. Massie, Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis, Mol. Cell. Biol. 17 (9) (1997) 5317–5327.

[38] L. Gao, Z. Yuan, S. Yu, Y. Yang, Y. Li, C. He, Genome-wide identification of HSP70/110 genes in sea cucumber *Apostichopus japonicus* and comparative analysis of their involvement in aestivation, Comp. Biochem. Physiol. Genom. Proteonom. 28 (2018) 162–171.

[39] C. Queitsch, T.A. Sangster, S. Lindquist, Hsp90 as a capacitor of phenotypic variation, Nature 417 (6889) (2002) 618.

[40] J. Imai, M. Maruya, H. Yashiroda, I. Yahara, K. Tanaka, The molecular chaperone Hsp90 plays a role in the assembly and maintenance of the 26S proteasome, EMBO J. 22 (14) (2003) 3557–3567.

[41] F. Li, W. Luan, C. Zhang, J. Zhang, B. Wang, Y. Xie, S. Li, J. Xiang, Cloning of cytoplasmic heat shock protein 90 (FcHSP90) from *Fenneropenaeus chinensis* and its expression response to heat shock and hypoxia, Cell Stress Chaperones 14 (2) (2009) 161–172.

[42] W. Liu, M. Chen, X. Li, B. Zhao, J. Hou, H. Zheng, L. Qiu, Z. Li, S. Meng, Interaction of toll-like receptors with the molecular chaperone gp96 is essential for its activation of cytotoxic t lymphocyte response, PLoS One 11 (5) (2016) e0155202.

[43] B. Huang, C. Xu, Identification and characterization of proteins associated with plant tolerance to heat stress, J. Integr. Plant Biol. 50 (10) (2008) 1230–1237.

[44] R. Sala, W.A. Jefferies, B. Walker, J. Yang, J. Tiong, S.A. Law, M.F. Carlevaro, E. Di Marco, A. Vacca, R. Cancedda, The human melanoma associated protein melanotransferrin promotes endothelial cell migration and angiogenesis in vivo, Eur. J. Cell Biol. 81 (11) (2002) 599–607.

[45] M. Demeule, Y. Bertrand, J. Michaud-Levesque, J. Jodoin, Y. Rolland, R. Gabathuler, R. Béliveau, Regulation of plasminogen activation: a role for melanotransferrin (p97) in cell migration, Blood 102 (5) (2003) 1723–1731.

[46] T.M. Rose, G.D. Plowman, D.B. Teplow, W.J. Dreyer, K.E. Hellström, J.P. Brown, Primary structure of the human melanoma-associated antigen p97 (melanotransferrin) deduced from the mRNA sequence, Proc. Natl. Acad. Sci. 83 (5) (1986) 1261–1265.

[47] K. Larade, K.B. Storey, Accumulation and translation of ferritin heavy chain transcripts following anoxia exposure in a marine invertebrate, J. Exp. Biol. 207 (8) (2004) 1353–1360.

[48] J. Matsumoto, C. Nakamoto, S. Fujiwara, T. Yubisui, K. Kawamura, A novel C-type lectin regulating cell growth, cell adhesion and cell differentiation of the multipotent epithelium in budding tunicates, Development 128 (17) (2001) 3339–3347.

[49] C.N. Baxevanis, G.J. Reclos, S. Perez, D. Kokkinopoulos, M. Papamichail, Immunoregulatory effects of fraction 5 thymus peptides. I. Thymosin α1 enhances while thymosin β4 suppresses the human autologous and allogeneic mixed lymphocyte reaction, Immunopharmacology 13 (2) (1987) 133–141.

[50] J. Young, A. Lawrence, A. MacLean, B. Leung, I. McInnes, B. Canas, D. Pappin, R. Stevenson, Thymosin β 4 sulfoxide is an anti-inflammatory agent generated by monocytes in the presence of glucocorticoids, Nat. Med. 5 (12) (1999) 1424.

[51] I.V. Guzhova, Z.A. Darieva, A.R. Melo, B.A. Margulis, Major stress protein Hsp70 interacts with NF-kB regulatory complex in human T-lymphoma cells, Cell Stress Chaperones 2 (2) (1997) 132.

[52] K.K. Meldrum, A.L. Burnett, X. Meng, R. Misseri, M.B. Shaw, J.P. Gearhart, D.R. Meldrum, Liposomal delivery of heat shock protein 72 into renal tubular cells blocks nuclear factor-κB activation, tumor necrosis factor-α production, and subsequent ischemia-induced apoptosis, Circ. Res. 92 (3) (2003) 293–299.

[53] C.G. Yoo, S. Lee, C.T. Lee, Y.W. Kim, S.K. Han, Y.S. Shim, Anti-inflammatory effect of heat shock protein induction is related to stabilization of IκBα through preventing IκB kinase activation in respiratory epithelial cells, J. Immunol. 164 (10) (2000) 5416–5423.

[54] A. Rossi, G. Elia, M.G. Santoro, Activation of the heat shock factor 1 by serine protease inhibitors an effect associated with nuclear FACTOR-κB inhibition, J. Biol. Chem. 273 (26) (1998) 16446–16452.

[55] L.A. Vereshchagina, M. Tolnay, G.C. Tsokos, Multiple transcription factors regulate the inducible expression of the human complement receptor 2 promoter, J. Immunol. 166 (10) (2001) 6156–6163.

[56] M. Michalski, A.S. Swierzko, J. Lukasiewicz, A. Man-Kupisinska, I. Karwaciak, P. Przygodzka, M. Cedzynski, Ficolin-3 activity towards the opportunistic pathogen, Hafnia alvei, Immunobiology 220 (1) (2015) 117–123.

[57] Y.F. Yang, Y.D. Zhou, J.C. Hu, F.L. Luo, Y. Xie, Y.Y. Shen, W.X. Bian, Z.N. Yin, H.L. Li, X.L. Zhang, Ficolin-A/2, acting as a new regulator of macrophage polarization, mediates the inflammatory response in experimental mouse colitis, Immunology 151 (4) (2017) 433–450.

[58] Z. Prohászka, M. Singh, K. Nagy, E. Kiss, G. Lakos, J. Duba, G. Füst, Heat shock protein 70 is a potent activator of the human complement system, Cell Stress Chaperones 7 (1) (2002) 17.

[59] E.L. Kwak, D.A. Larochelle, C. Beaumont, S.V. Torti, F.M. Torti, Role for NF-κB in the regulation of ferritin H by tumor necrosis factor-α, J. Biol. Chem. 270 (25) (1995) 15285–15293.

[60] Z. Lv, C. Li, M. Guo, Y. Shao, W. Zhang, X. Zhao, Major yolk protein and HSC70 are essential for the activation of the TLR pathway via interacting with MyD88 in *Apostichopus japonicus*, Arch. Biochem. Biophys. 665 (2019) 57–68.