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HSP40 gene family in pearl oyster *Pinctada fucata martensii*: Genome-Wide identification and function analysis

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ABSTRACT

HSP40, also called DnaJ, functions as a molecular chaperone by binding to Hsp70 and plays critical roles in the growth, development, and response to heat stress. However, this gene family is rarely reported in pearl oyster. In this study, 31 putative HSP40 genes from *Pinctada fucata martensii* (PmHSP40) were identified through bioinformatics methods and classified into three groups according to the presence of the complete three domains (J, G/F zinc finger domain, and cysteine rich domain). Further analysis showed that the PmHSP40 genes are highly diverse in sequence, domain structure, and tissue and development expression profile, implying diversified functions. In addition, one highly induced PmHSP40 in low-temperature (PmHSP40LT) was cloned, and its function in temperature response was explored. PmHSP40LT has a full length of 1741 bp, containing 1059 bp ORF, 152 bp 5′UTR, and a 507 bp 3′UTR, and encodes 352 amino acids. PmHSP40LT expression was significantly induced at low (17 °C) and high temperature (32 °C) at 6 h, 1 d, and 3 d relative to the control group. Thus, PmHSP40LT possibly participates in response to high and low temperatures in pearl oyster. In conclusion, all these results provide a comprehensive basis for the further analysis of PmHSP40 function in pearl oysters.

1. Introduction

Heat shock proteins (HSPs), also known as heat stress proteins, are a family of proteins produced by cells in response to various stressful conditions, such as toxins, oxidative conditions, hypoxia, glucose deprivation, water deprivation, osmotic pressure, infection, and inflammation [1–4]. The HSP was first discovered from *Drosophila melanogaster* subjected to heat stress [5] and subsequently demonstrated to be ubiquitously and evolutionarily conserved molecular chaperones that are present in all living organisms [2,6,7]. Based on their molecular weights and functions, HSPs can be classified into several families, including HSP100, HSP90, HSP70, HSP60, HSP40, and low molecular mass HSPs [8]. HSP40, also known as DnaJ protein or J-protein [9], generally consists of a J domain, which binds to HSP70. As the co-chaperone of HSP70, HSP40 is capable of tightly regulating ATP hydrolysis, which is necessary for many normal housekeeping and stress-related functions [10]. In addition to the J domain, many HSP40 proteins contain other conserved regions, which are critical to their functions. Based on the difference in these regions, HSP40 proteins can be categorized into three groups according to the differences in these regions. Type I proteins are similar to *Escherichia coli* HSP40 with the J domain, Gly/Phe-rich region (G/F domain), and cysteine-rich domain (CxxCxGxG). Type II proteins possess the J domain and Gly/Phe-rich region, but lack the cysteine repeats, and type III has the J domain alone [11].

To date, more than 40 HSP40 homologs have been identified in mammals [12]. However, few HSP40s have been studied in mollusks. So far, only three HSP40 homologs were cloned from mollusks [13–15]. The pearl oyster *Pinctada fucata martensii* is an important economic shellfish species and mainly cultured for marine pearl production in the southern provinces of China and Japan. *P. f. martensii* is a warm-water shellfish that has a weak endurance capacity to low and high temperatures, thus temperature is an important environmental factor for their distribution [16]. It have been well verified that HSP40 plays important roles in protecting other animals from injury due to extreme temperature [13,15,17]. In *Pomacea canaliculata*, HSP40 was identified to be up-regulated under low temperatures [13]. In *P. f. martensii*, one HSP40 was cloned and demonstrated to be induced under thermal, low salinity, and bacterial challenges [15]. Our previous research showed that HSP40 was highly induced in *P. f. martensii* cultured at low temperatures [16]. Thus, it is meaningful to study the role of HSP40 in the adaptation of *P. f. martensii* to temperature change.

To understand the function of HSP40 in *P. f. martensii* (PmHSP40), we performed comprehensive genomic annotation and transcriptomic

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Table 1
The list of PmHSP40 members identified in *P. f. martensii*.

ID	Length (aa)	PI	Mw (Da)	Locus	Domain	Group	intron number
Pm10002960	162	6.14	17.71	scaffold2691:83514:91236:-	GTPase-J	III	3
Pm10003835	167	6.91	19.6	scaffold4541_M:51776:54405:-	J	III	2
Pm10005310	406	8.46	47.24	scaffold2257:47018:50701: +	J-DUF1992	III	4
Pm10005627	150	7.73	16.49	scaffold3224_M:138766:145042:-	Ras-J	III	2
Pm10007503	401	5.88	45.29	scaffold1942:60182:67573:-	J-G/F-CXXCXGXG	I	4
Pm10007504	401	6.96	45.07	scaffold1942:73634:79905:-	J-G/F-CXXCXGXG	I	4
Pm10009383	233	9.63	27.03	scaffold1962_M:192067:201791:-	J	III	5
Pm10010440	388	4.62	45.7	scaffold1542:70566:81288:-	J-ZF	III	9
Pm10011141	318	8.26	35.66	scaffold1454:199828:208809:-	J-DnaJ_C	III	6
Pm10013879	154	4.92	17.59	scaffold1158:185719:186180:-	J-ZF	III	0
Pm10015652	734	8.37	81.34	scaffold986:280482:302400: +	J-G/F-CXXCXGXG-J-G/F-CXXCXGXG	I	17
Pm10016501	486	9.12	56.25	scaffold914:263037:275652:-	J-TM-SANT	III	10
Pm10016942	286	8.94	33.32	scaffold874:41365:52775: +	J-TM	III	6
Pm10018568	765	8.83	88.45	scaffold737:275865:298873: +	J-G/F-Thioredoxin-TM	III	12
Pm10019362	772	5.57	88.58	scaffold681:184323:202154: +	J-4Thioredoxin	III	19
Pm10020897	370	8.35	43.49	scaffold571:313227:330551: +	J-DUF1977	III	9
Pm10022818	201	5.13	22.79	scaffold448:441666:460013: +	J-G/F	II	6
Pm10023212	627	9	69.29	scaffold420:9781:29712: +	J	III	9
Pm10023459	486	8.39	54.4	scaffold405:399202:437208: +	J	III	12
Pm10025538	184	5.31	20.8	scaffold291:160030:178333: +	J-G/F	II	5
Pm10025601	367	8.85	42.87	scaffold292:392117:394523: +	4TM-J	III	2
Pm10025728	252	9.84	29.03	scaffold284:412362:413117:-	J-TM	III	0
Pm10026120	477	7.82	54.37	scaffold260:49706:60506: +	8TRP-J	III	11
Pm10027462	352	9.09	38.42	scaffold194:350440:351719	J-G/F-DnaJ_C	II	1
Pm10028595	280	9.42	33.04	scaffold141:296489:309523: +	J	III	8
Pm10028765	550	9.16	62.88	scaffold133:551949:574862:-	J	III	10
Pm10028877	2176	6.39	24.73	scaffold126:58756:114226:-	DUF4339-RPT1-J-RPT1	III	51
Pm10031473	694	6.97	80.02	scaffold33:531548:559966: +	J-Thioredoxin-TM	III	18
Pm10032069	444	9.46	50.71	scaffold14:386527:399393: +	J-J-RRM_1	III	12
Pm10032335	252	9.88	29.04	scaffold9:1329026:1329781:-	J-TM	III	0
Pm10032375	765	8.89	88.34	scaffold8:1021203:1036141: +	J-G/F-Thioredoxin-TM	III	12

analysis on a large subset of HSP40 in *P. f. martensii*. Furthermore, we cloned and characterized one HSP40 and analyzed its sequential expression after temperature changes to determine the role of HSP40 genes in temperature adaption. The results could contribute to our understanding of the evolution and function of PmHSP40 in detail.

2. Materials and methods

2.1. Identification of PmHSP40

The annotation information of *P. f. martensii* was obtained from our previous research [18]. The protein domain of all of the identified PmHSP40 genes was re-analyzed and confirmed by Simple Modular Architecture Research Tool (SMART) version 5.1 (http://smart.Embl-heidelberg.de/). Only the genes that were homologous with HSP40 (E-value ≤ 1-e5) and contain the J domains were considered as PmHSP40. The molecular weight and theoretical isoelectric point were analyzed by ProtParam tool (https://web.expasy.org/cgi-bin/protparam/protparam). Based on amino acid sequences, comparison and phylogenetic analysis were performed with ClustalW multiple sequence alignment (http://www.genome.jp/tools-bin/clustalw). Phylogenetic trees were constructed by using MEGA7.0 with the neighbor-joining (NJ) algorithm. Confidence values were obtained with bootstrapping with 1000 replications [19]. Gene structure information for PmHSP40 was obtained from published genome data [18]. The exon and intron structures of PmHSP40s were drew using the Gene Structure Display Server (GSDS, http://gsds.cbi.pku.edu.cn/) [20].

2.2. Expression pattern of PmHSP40 in different tissues and development stages

Transcriptome data from different tissue at different stages were collected from published research [18] and analyzed for the extraction of the expression profiles of PmHSP40 genes. Heatmaps were generated by using the R language package.

2.3. Experimental samples

A total of 150 *P. f. martensii* were collected from Houhong village, Leizhou city, Guangdong province. *P. f. martensii* were randomly divided into three groups and cultured in 300 L barrels. Water temperature was set at 17 °C, 22 °C, and 32 °C. The group in 22 °C was considered as the control group. During the experiment, the same amount of single-cell algae was fed to the cultured shellfish every day, and 50% of the water was changed every 24 h. Eight individuals were randomly selected at 6 h, 1 d, 3 d, and 5 d, and gill tissue were collected and placed into liquid nitrogen, and then stored in −80 °C refrigerator.

2.4. Total RNA extraction and RACE

Trizol (Invitrogen) was used in the extraction of total RNA. SMARTer™ PCR cDNA synthesis kit (Clontech) was then used for reverse transcription. The integrity of the RNA was detected by 1% agarose gel electrophoresis, and the purity and concentration of the RNA were detected with Nano DropND1000 UV spectrophotometer. Then, 5′ RACE and 3′ RACE templates were prepared according to the instructions of SMARTTM RACE cDNA amplification kit. The full length of PmHSP40 was amplified by nested PCR. The PCR product was detected by 1% agarose electrophoresis, and the target gene fragment was selected to connect with the vector pMD18-T, and then transformed into DH5α competent cells. Positive bacteria were selected from the culture medium and sent to Guangzhou Biological Company of China for the sequencing of their cDNAs. The full-length sequence of the gene was obtained by sequencing the resulting 5′-end and 3′-end sequences with DNAMAN. The full-length sequences of the obtained genes were analyzed with BLAST provided by NCBI (http://www.ncbi.nlm.nih.gov/). Open reading frame (ORF) was obtained by using the ORF finder tool (https://www.ncbi.nlm.nih.gov/orffinder/). The primers used for

RACE was listed in Supplement Table 1.

2.5. Quantitative real-time PCR (qRT-PCR) assay

The primer sequences used for qRT-PCR are listed in Supplement Table 1. The qRT-PCR assay was performed on a Roche LightCycler 480 (Roche, Switzerland) with Thermo Scientific DyNAmo Flash SYBR Green qPCR Kit (Thermo Scientific). β-Actin [20] was selected as the reference gene for the verification of gene expression. PCR program was conducted as follows: 5 min at 95 °C and 40 cycles (each cycle was for 30 s at 95 °C, 15 s at 60 °C, and 15 s at 72 °C). Gene expression multiples were calculated using a delta CT method ($2^{\wedge\ (C_T\ \beta\text{-}actin\ -\ C_T\ Target\ gene)}$), and then analyzed by using SPSS 22.0. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Genome-wide identification of PmHSP40 genes in pearl oyster

Homology-based annotation with InterProScan, KEGG, Nr, and manual corrections identified 31 PmHSP40 from *P. f. martensii*. Their classifications, domain structures, genomic locations, and intron numbers are summarized in Table 1. Pm10025538 has been cloned, and its function was identified in previous research [15]. The length of PmHSP40 proteins ranged from 150 (Pm10005627) to 2176 (Pm10028877) amino acids. The predicted pI-values of PmHSP40 proteins ranged from 4.62 (Pm10010440) to 9.88 (Pm10032335). Three (9.7%) PmHSP40 genes had no introns, and four genes (14.47%) had 1-2 intron. Pm10028877, the longest PmHSP40, had 51 introns (Table 1). One tandem duplication event (Pm10007503/Pm10007504) was identified.

3.2. Classification and sequence alignment of PmHSP40 genes

Conserved domain analysis with SMART revealed all of the PmHSP40 shared a conserved J domain comprising about 70 amino acids. Two PmHSP40 (Pm10032069 and Pm10015652) contained two J domains. All of the J domains have the conserved J-box (HPD) sequence (Fig. 1). According to the presence of the complete three domains, the PmHSP40 genes were classified into three types (I, II, III), including 3, 3 and 25 members, respectively. Type I PmHSP40 proteins are characterized by the J domain, G/F domain, and a cysteine-rich domain (CxxCxGxG). The difference of Type II with I is the lack of cysteine repeats domain. Type III PmHSP40 proteins only comprise the J domain. One of the type I PmHSP40 (Pm10015652) comprises two sets of complete three domains (J -G/F-CxxCxGxG-J-G/F-CxxCxGxG), indicating one ancient domain duplication event. Four PmHSP40 (Pm10018568, Pm10019362, Pm10031473 and Pm10032375) proteins contain a thioredoxin domain, suggesting their role in assisting protein folding and quality control in the endoplasmic reticulum. One PmHSP40 (Pm10026120) protein includes eight tetratricopeptide repeat domains (TRPs). Two PmHSP40 (Pm10002960 and Pm10005627) contain one GTPase domain. Taken together, there are 21 different domain combinations among the PmHSP40 proteins, indicating a highly diverse in domain structure.

3.3. Phylogenetic analyses of the J domains from pearl oyster

The phylogenetic analysis of the 33 J domains in the PmHSP40 was performed to provide a better understanding of evolutionary relationships among PmHSP40 proteins. In the phylogenetic tree (Fig. 2), all of the Type I, II, and some of the type III PmHSP40 were clustered together and marked in gray box in Fig. 2. In this group, one-intron-containing gene Pm10027462 was clustered with one 11-intron-containing gene Pm10026120, suggesting Pm10027462 was formed by retrotranscription from Pm10026120. The domain structures of Pm10027462 and Pm10026120 were J-G/F-CT and 8TRP-J, respectively, indicating they have evolved different functions. Intronless Pm10032335 and Pm10025728 were grouped with the intron-containing gene Pm10023459. Pm10032335 and Pm10025728 contain the same domain combination (J-TM), suggesting they evolved from the same ancient gene.

3.4. Expression patterns of PmHSP40 genes in tissues and developmental stages

Analysis of the developmental transcriptomes of *P. f. martensii* indicated that 13 and 4 PmHSP40 genes were highly expressed in eggs

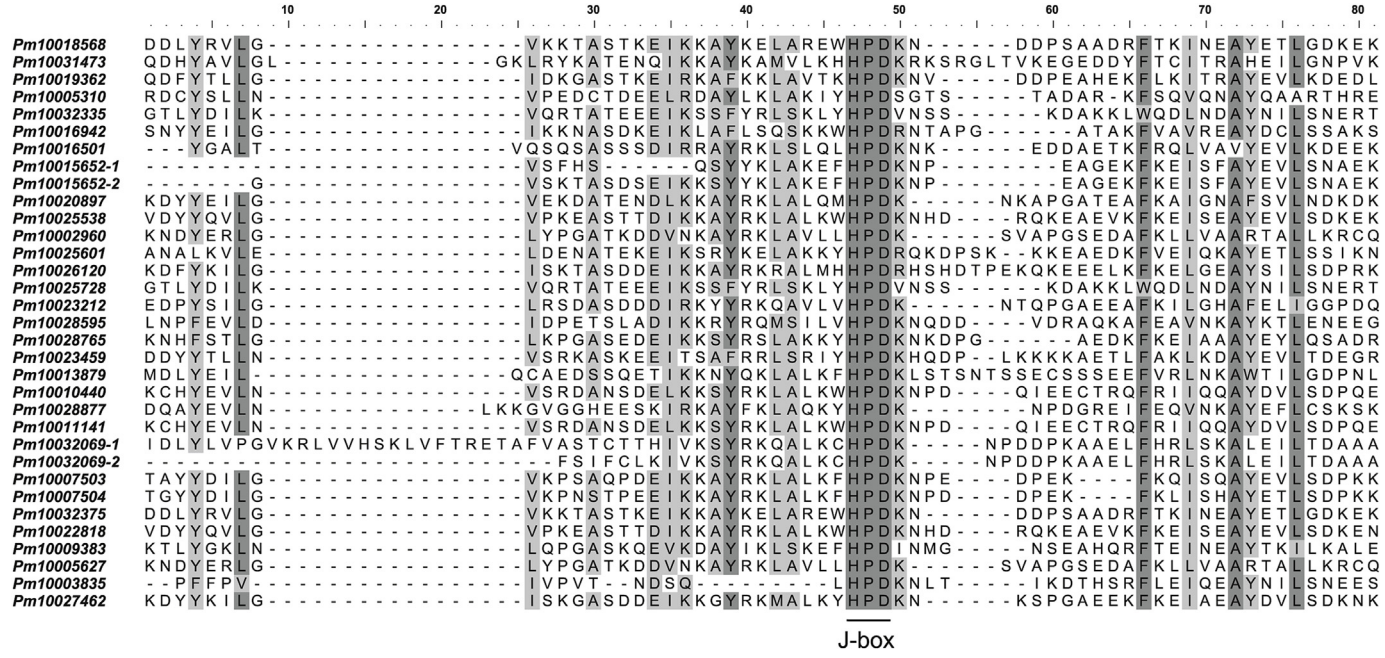

Fig. 1. Multiple sequence alignment of J domain from PmHSP40. Identical amino acids are highlighted in dark gray and similar amino acids are highlighted in light gray.

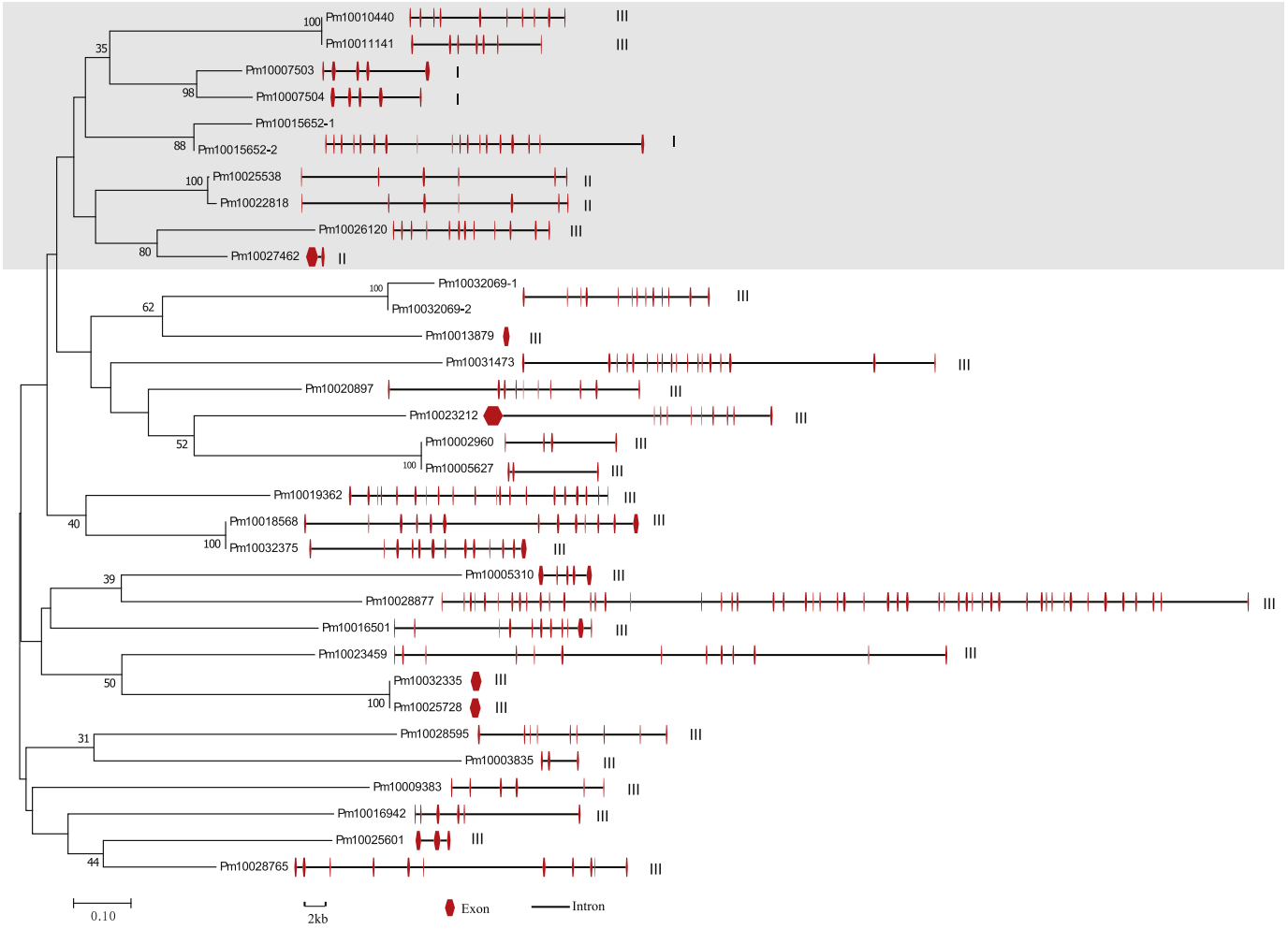

Fig. 2. Phylogenetic tree of the J domain and corresponding gene structure. The numbers (I, II, and III) on the left are the types of PmHSP40s. The genes indicated in gray box are all of the Type I, II, and some of the type III PmHSP40s.

and fertilized eggs, respectively. Pm10011141 was highly expressed in D-stage larvae (RPKM = 1885.77) and accounted for the 68.9% of the total expression of PmHSP40 in D-stage larvae. Three PmHSP40 genes (Pm10022818, Pm10025538, and Pm10028595) were highly expressed in the juveniles, and some PmHSP40 genes were highly expressed at other developmental stages (Fig. 3a). Tissue transcriptomes analysis showed that two PmHSP40 genes (Pm10032335 and 10028765) were over-expressed at the adductor muscle, three PmHSP40 genes (Pm10018568, Pm10028877, and Pm10025728) were highly expressed at hemocytes, and the total expression of PmHSP40 was higher in the gill and gonad than in other tissues (Fig. 3b). The change in temperature also influences the expression of PmHSP40, and five PmHSP40s were induced at the selected line for resistance to low water temperature. The expression of Pm10027462 accounted for 61.92% of the total expression of PmHSP40 at low water temperature, indicating its function in low-temperature adaptation (Fig. 3c).

3.5. Gene cloning of PmHSP40-Pm10027462

We renamed PmHSP40-Pm10027462 to PmHSP40LT as its function in low-temperature adaptation. To further explore its function in temperature adaption, we cloned the PmHSP40LT gene and verified its function. PmHSP40LT cDNA has a full length of 1741 bp and contains 1059 bp of ORF, a 152 bp 5′UTR, and a 507 bp 3′UTR, which encode 352 amino acids (Fig. 4). Domain analysis showed that PmHSP40LT has DnaJ, G/F, and DnaJ_C domains.

3.6. Expression of PmHSP40LT gene at different temperatures

To explore the response of PmHSP40LT gene to the temperature stress, we detected its expression at different temperature and time exposure. The pearl oysters cultured at 22 °C was considered as the control group, 17 °C was the low temperature group, and 32 °C was the high temperature group. After 6 h, 1 d, 3 d, and 5 d exposure, PmHSP40LT expression was detected. The results showed that PmHSP40LT expression was significantly induced at low and high temperatures at 6 h, 1 d, and 3 d compared with control group and down-regulated at 5 d (Fig. 5). These results mean that the PmHSP40LT expression has a significant correlation with environmental temperature.

4. Discussion

HSP40/DnaJ proteins play key roles in assisting protein folding by activating the ATPase domain of HSP70. HSP40 is widely present in the living species, 57 HSP40 were identified from the Channel Catfish [21], 41 J domain containing HSP40 were found in the human genome [11]. In spite of their importance, only few HSP40 genes were characterized from mollusks [13–15]. Systematic analysis of HSP40 genes in mollusks is rarely conducted. In this study, we identified a full set of 31 PmHSP40 genes in the genome of *P. f. martensii*. All of them contain the HPD sequence, which is considered as the hallmark of the J domain, stimulates the ATPase activity of Hsp70, and is crucial for maintaining

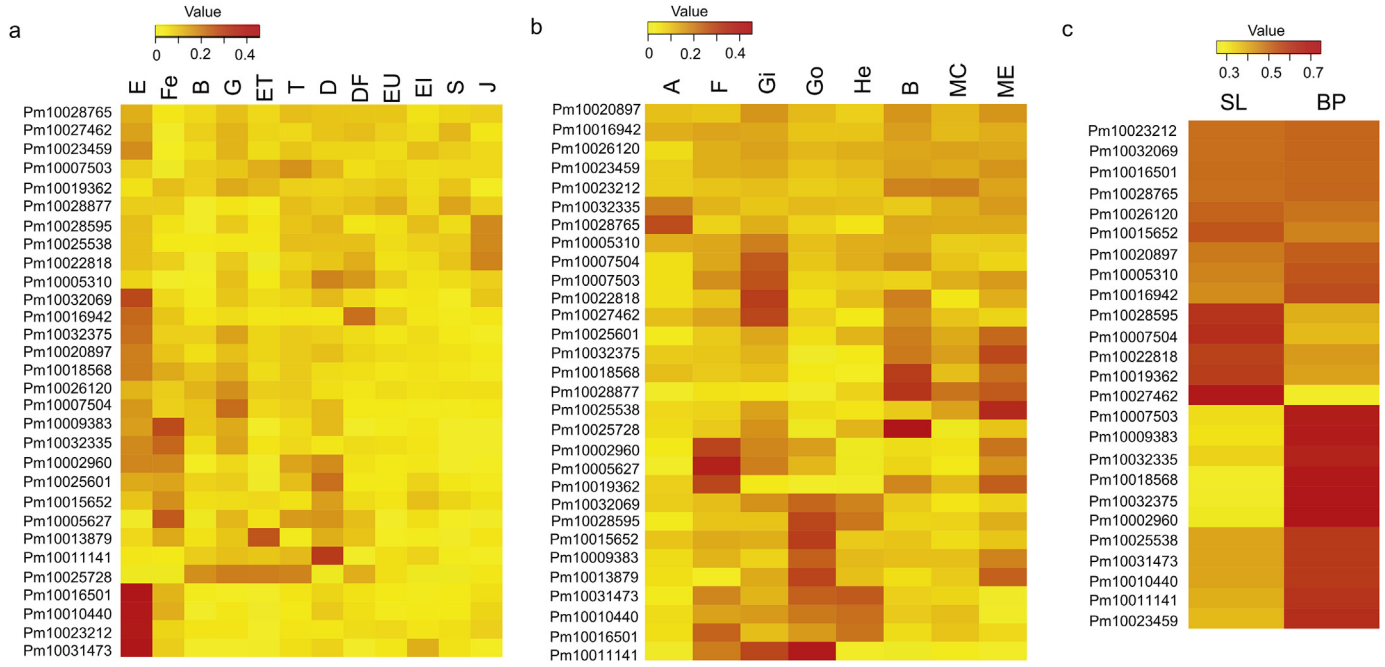

Fig. 3. Diversity in temporal and spatial expression of PmHSP40s in pearl oyster. a. Expression of PmHSP40s in tissues. A: adductor muscle, F: foot, Gi: gill, Go: gonad, He: hepatopancreas, B: haemocytes, MC: mantle central and ME: mantle edge. b. Expression of PmHSP40s in development stages. E: egg; Fe: fertilized egg at 30 min; B: blastula at 5 h 25 min; G: gastrula at 6 h 30 min; ET: early trochophore at 8 h 25 min; T: trochophore at 15 h 45 min; D: D-stage larvae at 19 h 5 min; DF: D-stage larvae before feeding at 4 d; EU: early umbo larvae at 14 d; EL: eyed larvae at 28 d; S: spat at 40 d; J: juveniles at 90 d. c. Expression of PmHSP40s in pearl oysters cultured in the selected line (SL) and based population (BP). The SL were pearl oysters with high survival rate in low water temperature.

HSP40 function, indicating all of these PmHSP40 could function as the co-chaperones of HSP70.

Sequence analysis showed PmHSP40 are diverse in PI, structure, and domain combination. The PI-values of PmHSP40 proteins range from 4.62 to 9.88, indicating acidic and alkaline proteins with different locations and functions in the cell. The intron number of PmHSP40 ranges from 0 to 51. The presence of three intronless PmHSP40s suggested that retrotranscription contributed to the expansion of PmHSP40

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   1 actggtaacttctagaacgactgagagaaatcgggacgtatgcagacagatatataaactgtcaactggagattgatcaacagttgatat
   1                                                                M  G  K  D  Y  Y  K  I  L  G
  91 ttgccagaggtagtgcactctcctaagaagttaaattttatttttagtgtgaggacattcgccATGGGCAAGGATTATTACAAGATCCTTG
  11   I  S  K  G  A  S  D  D  E  I  K  K  G  Y  R  K  M  A  L  K  Y  H  P  D  K  N  K  S  P  G
 181 GAATTTCAAAAGGCGCCAGTGACGATGAAATCAAGAAAGGGTATCGAAAAATGGCTTTGAAATATCACCCGGATAAAAACAAGTCCCCCG
  41   A  E  E  K  F  K  E  I  A  E  A  Y  D  V  L  S  D  K  N  K  R  E  I  Y  D  K  Y  G  E  E
 271 GTGCTGAAGAAAAATTTAAAGAAATTGCAGAAGCCTACGACGTATTGAGTGACAAGAACAAGCGTGAAATTTACGATAAGTATGGTGAGG
  71   G  L  K  N  G  P  S  P  G  G  G  G  G  P  S  G  G  T  Y  H  Y  E  F  H  G  D  P  R  D  T
 361 AGGGTCTGAAAAATGGCCCCTCCCCAGGAGGTGGTGGAGGGCCAAGTGGGGGAACGTATCATTACGAGTTTCATGGAGACCCCAGAGATA
 101   F  R  M  F  F  G  N  D  D  P  F  A  S  F  F  G  G  G  G  G  F  G  G  P  G  V  G  G  S  R
 451 CATTTAGAATGTTTTTTGGCAATGACGATCCTTTCGCTAGTTTTTTCGGAGGTGGTGGCGGCTTTGGTGGCCCAGGTGTAGGTGGTTCAA
 131   H  V  F  T  N  F  G  G  P  G  E  E  H  M  D  V  D  D  D  P  F  G  G  F  G  G  G  G  H  G
 541 GGCATGTGTTCACAAACTTTGGGGGTCCAGGAGAAGAACATATGGATGTTGATGATGATCCGTTCGGTGGATTTGGAGGTGGCGGACATG
 161   M  G  G  G  M  P  R  R  K  R  Q  D  S  A  V  V  R  E  L  P  V  S  L  E  D  I  Y  K  G  T
 631 GAATGGGTGGAGGAATGCCACGGAGAAAACGTCAGGATTCTGCTGTAGTGCGAGAACTGCCAGTATCTTTAGAAGACATTTACAAAGGTA
 191   T  K  K  L  K  I  T  R  R  V  L  N  P  D  G  K  S  T  R  N  E  E  K  I  L  T  I  D  V  K
 721 CAACGAAAAAACTGAAAATCACCCGTAGAGTGCTCAATCCAGATGGAAAATCTACAAGAAACGAAGAGAAAATACTGACAATTGATGTGA
 221   P  G  W  K  A  G  T  K  I  T  F  P  K  E  G  D  Q  S  P  H  S  I  P  A  D  V  I  F  V  I
 811 AACCTGGTTGGAAAGCCGGAACAAAGATAACATTCCCAAAAGAGGGAGACCAAAGTCCTCATAGTATACCAGCCGATGTAATATTTGTGA
 251   K  D  K  P  H  P  K  F  K  R  D  G  S  D  I  K  F  K  A  R  I  S  L  K  E  A  L  C  G  C
 901 TCAAAGACAAACCTCATCCAAAATTCAAAAGAGATGGAAGTGACATAAAATTCAAAGCAAGAATATCACTGAAAGAGGCCTTATGTGGAT
 281   T  L  Q  I  P  T  I  D  G  R  T  I  P  L  K  L  R  E  V  V  K  P  G  V  N  K  R  V  Q  G
 991 GTACACTTCAGATACCAACAATAGATGGGAGAACTATTCCACTAAAACTCAGGGAAGTTGTGAAACCAGGTGTGAATAAGAGAGTTCAAG
 311   E  G  L  P  V  P  K  Q  P  G  K  R  G  D  L  I  I  E  F  D  I  V  F  P  N  H  I  S  N  T
1081 GAGAGGGACTACCTGTGCCCAAACAACCTGGAAAACGTGGTGACCTCATCATAGAATTTGACATTGTATTCCCAAATCATATTTCGAACA
 341   A  K  E  I  L  Q  D  C  L  P  S  S  *
1171 CTGCAAAGGAAATATTACAAGACTGTCTACCTTCATCATAGtatttgtataatacatgtattgttagggtatcatgcagagtaggggaaa
1261 gtggatcgtgatagtgtgtttgagtgaatgtcaagtgtgagtcaccagatgctattaataggaagcaagcaagccctgaagcttactaca
1351 tatacatgtgtcaaacctcatgatcagatacagctgattgcactgtaacagacaacagtaactgtaccttgaaagtgtgccaaatatgaa
1441 aagtcagggcatgaatacctgtcgctgttgtgattttgtagctacctgtataaatgcaatataaatgttttcttacaaagaaatgtaaat
1531 gtttggttgaatgtacaggtataaatgttatgtatgctttataagcctgatgaatgtttttgtttatttttgttgaattttacttggttgt
1621 tatgaaagggtggaacatgtagatatttacatgctcattcaagtgctctttttctaaataaataaatgtgaaaaaaaaaaaaaaaaaaaa
1711 aaaaaaaaa
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Fig. 4. Nucleotide and amino acid sequence of PmHSPLT. Lowercase letters indicate 5′ UTR and 3′ UTR; capital letters indicate amino acid sequences; the underlined amino acid are the DnaJ domain and DnaJ-C domain.

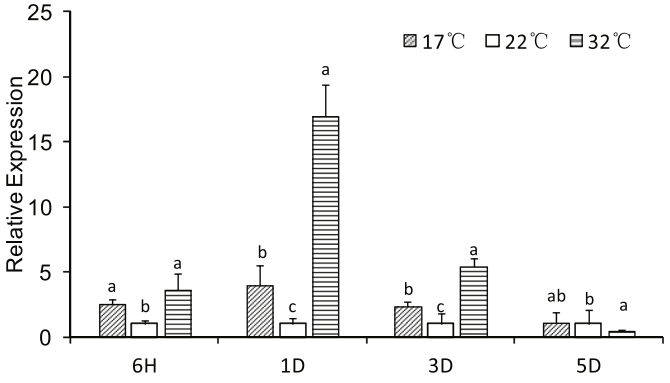

Fig. 5. Temporal expression of PmHSPLT at different temperatures. The pearl oysters cultured at 22 °C was considered as the control group. The same lowercase letters indicate that the relative expression of different temperature groups at the same time point showed no significant difference ($P > 0.05$).

during evolution. The 51-intron-containing PmHSP40 was the longest PmHSP40 and formed by fusing with a domain of unknown function. There are 21 different domain combinations among the identified PmHSP40s. Domain combination causes the diversity of PmHSP40. The J domain is the specific feature that defines a protein as an HSP40 or HSP40-like protein. Apart from the conserved domains, HSP40 combines with additional domains, which may determine the functional diversity of HSP40. For example, the mammalian DnaJ protein ERdj5 contains a J domain and a thioredoxin domain, which help ERdj5 to form appropriate disulphide bonds during the folding of ER proteins [22]. Similar domain combination were also found in PmHSP40, indicating the function and location of PmHSP40 in ER. Two PmHSP40 genes contain Ras/GTPase domains, which was also reported in HSP40 from catfish [21]. Ras/GTPases are a superfamily of molecular switches that regulate cellular proliferation and apoptosis in response to extracellular signals [23,24]. We proposed that Ras/GTPases and HSP70 may be binding together though J domain, and favorable to the regulation of protein folding after sensing extracellular signals. SANT domain were found both in the PmHSP40 and the HSP40 identified from catfish [21,25]. The SANT domain is present in nuclear receptor corepressors and in the subunits of many chromatin-remodeling complexes and has a central role in chromatin remodeling by functioning as a unique histone-interaction module. Thus, a PmHSP40 that contains SANT may be located in the nuclear and function in regulating gene expression by affecting the histone.

The diversity of domain combination causes the functional diversity of PmHSP40. Transcriptomes analysis showed PmHSP40 are widely expressed in the tissues and development stages. In the tissue transcriptomes, PmHSP40 expressed in the gill and gonad accounts for 40% of the total expression in the tissues. In bivalve molluscs, gill is the tissue with the highest exposure to surrounding water and serves as the main interface between the organisms and the environment [26]. Thus, the exceptionally high expression of PmHSP40 in the gill may be an adaptation to dynamic environmental conditions. High expression of PmHSP40 in the gonad corresponds to the high expression of PmHSP40 gene in eggs in the development transcriptomes. Chaperone proteins play a major role in protecting oocytes from thermal shock or other cellular stresses. Good quality eggs contain abundant HSP40 and HSP70 [27]. Thus, highly expressed PmHSP40 may give egg enhanced capacity to handle and respond to stimuli. The highly expressed PmHSP40 in other stage may help pearl oyster cope with stage-specific stimuli or may be involved in stage-specific development or function, which need further studies to elucidate.

Water temperature is a major environmental factor affecting biological survival and growth and causing serious losses in aquaculture [28,29]. As one of the world's most important sea pearly cultured shellfish, *P. f. martensii* mainly lives in tropical and subtropical sea areas and is sensitive to low temperatures [16]. In this study, five PmHSP40 genes were found to be involved in resistance to low water temperature. PmHSP40LT were cloned, and changes in its expression at temperature stress was analyzed. The results showed the expression levels of PmHSP40LT in the low and high temperature group were significantly higher than the expression level in the control group at 6 h, 1 d, and 3 d. Temperature stress causes the misfolding and aggregation of proteins in organisms, and these effects lead to pathological events [30]. The induced PmHSP40LT may bind to misfolded proteins, causing them to be refolded or specifically degraded. These effect is of great significance for the quality of protein synthesis and the control of intracellular environmental stability.

In conclusion, we identified and annotated a full set of 31 HSP40 genes from pearl oyster *P. f. martensii*. Sequence analysis showed that these PmHSP40 are highly diverse in PI, weight, domain structure, and temporal and spatial expression profiles. One highly induced PmHSP40 in low temperature (PmHSP40LT) was cloned, and its function in low and high temperature response was explored. These results would provide a comprehensive basis for further analyzing the function of PmHSP40 and illuminating the evolutionary relationship in pearl oysters.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.08.029.

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