Identification and expression analysis of a heat-shock protein 70 gene in *Polycelis* sp.

Fangfang Cheng¹ · Zimei Dong¹ · Yanping Dong¹ · Yingxu Sima¹ ·
Jing Chen¹ · Xiaoyan Li¹ · Guangwen Chen¹ · Dezeng Liu¹

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Abstract Heat-shock protein 70 (HSP70) is ubiquitously found in a variety of organisms and plays an important role in cytoprotection, environmental monitoring, and disease resistance. In this study, the full-length complementary DNA (cDNA) of *hsp70* from planarian *Polycelis* sp. was first cloned using rapid amplification of cDNA ends (RACE). The expression levels of *Pyhsp70* were analyzed in the presence of various stressors by real-time PCR, and its temporal-spatial expression patterns were also examined in both intact and regenerative animals by whole-mount in situ hybridization. The results show that (1) the deduced amino acid sequence of *Pyhsp70* includes three typical HSP70 family signature motifs and is highly conserved during evolution; (2) *Pyhsp70* expression is induced by prolonged starvation, tissue damage, and ionic liquid but inhibited by high or low temperatures; and (3) *Pyhsp70* mRNA is mainly expressed in the head peripheral region and in the regenerating blastema during regeneration. These results suggest that the highly expressed *Pyhsp70* gene may contribute to enhance cytoprotection and tolerance against stress-induced molecular damage, and the migration of neoblasts to the wound, which might also be involved in the proliferation and differentiation of neoblasts. Our work provides basic data for the study of stress responses and regenerative mechanism in freshwater planarians.

Keywords Planarian · *Hsp70* · Stress response · Regeneration

Introduction

Heat-shock proteins (HSPs) are ubiquitously found in all domains of life from bacteria to humans and function as multiple chaperones such as in protein homeostasis, in preventing the aggregation of damaging proteins, and in promoting proper folding, assembly, refolding, and repairing of proteins (Freeman et al. 2000; Daugaard et al. 2007; Kalmar and Greensmith 2009; Heikkilä 2010). Up to now, HSPs have been classified into several major categories, for instance, low molecular mass HSPs, HSP40, HSP60, HSP70, HSP90, and HSP100 based on the molecular weights, amino acid sequences, and the degree of homology (Heikkilä 2010). Among these, HSP70 is an important member of the heat-shock protein family; studies on *hsp70* gene expression and function in a variety of organisms have highlighted their crucial involvement in cellular homeostasis, cytoprotection, environmental monitoring, and disease resistance, especially in cancer treatments and prognoses (Henderson and Pockley 2010; Jenei et al. 2013; Beaman et al. 2014; Nadin et al. 2014). Thus, the levels of *hsp70* are usually used as a parameter of stress from environmental pollution, noxious stimuli, and the therapeutic effects of malignant cancers and neurodegenerative diseases (Ciocca and Calderwood 2005; Ciocca et al. 2013).

Freshwater planarians are free-living members of the phylum Platyhelminthes, with simple structure, quick regeneration, and higher identity to vertebrates in certain genes (Sánchez Alvarado 2000; Salo and Baguna 2002), and they are an emerging model for studying regeneration, development, and evolution in animals. *Polycelis* sp. is a freshwater planarian, characterized by more than a hundred eyes in the

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Fangfang Cheng and Zimei Dong contributed equally to this work.

✉ Guangwen Chen
chengw0183@sina.com

¹ College of Life Science, Henan Normal University, Xinxiang 453007, Henan, China

head (Aikawa and Shimozawa 1991). Compared with the two most commonly studied species of freshwater planarians, *Dugesia japonica* and *Schmidtea mediterranea*, it is more susceptible to various environmental stresses such as temperature variation, heavy metals, and toxic substance pollution; therefore, it is more suitable to be used as test organisms in water environmental toxicology (Li 2008; Knakiewicz and Ferreira 2008; Gleixner et al. 2014; Yang et al. 2014).

Here, we identified a full-length *hsp70* gene from *Polycelis* sp. and investigated its temporal and spatial expression pattern in intact and regenerating animals. At the same time, the expression pattern of *Pyhsp70* was examined in response to prolonged starvation, high and low temperatures, tissue damage, and ionic liquid stressors. Our work provides basic data to aid in the elucidation of *hsp70* gene function and the molecular mechanism of stress responses and regeneration in planarians.

Materials and methods

Animals and treatments

The animals used in the study, *Polycelis* sp., were collected from Yangcheng County, Shanxi Province, China (elevation 1658 m, 112° 09.133' E, 35° 16.398' N, water temperature 5.5–10.5 °C, pH 6.0). The worms were cultured in autoclaved tap water at 8 °C, fed once a week with fresh fish spleen, and starved for at least 2 weeks prior to use in experiments. Regenerating fragments were obtained by transverse amputation at the post-auricle level from intact worms (Fig. 4a).

For stress response experiments, 190 worms were evenly divided into 19 groups (10 animals per group) and exposed to different gradient stressors according to the pre-experiments. For starvation stress, the worms were starved for 15 (the control), 30, 60, and 15 days after being re-fed (starved after 60 days). For thermal stress, environments at 4, 8 (the control), 12, 16, and 20 °C were designed, and worms were cultured under different temperature conditions for 40 min. For tissue damage stress, the regenerative fragments at 0, 0.5, and 1 day after amputation were pooled, and intact animals were used as the control. To induce stress with chemicals, 0, 10, 20, 30, 40, and 50 mg/L 1-octyl-3-methylimidazolium bromide ([C₈mim]Br) were designed as treated groups, and worms were exposed to [C₈mim]Br in the dark at 10 °C for 3 days.

Isolation of the planarian *Pyhsp70* gene

Total RNA was extracted using Trizol reagent (TaKaRa, China) following the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized from 1 µg total RNA using oligo dT primers and M-MLV reverse transcriptase (TaKaRa, China) according to the manufacturer's

instructions. All primers used in this study are shown in Table 1. Based on *Djhsp70* gene (GenBank accession number: ABY83101.1), a *Pyhsp70* cDNA fragment of 211 bp was first amplified with a set of specific primers: Pyhsp70-F and Pyhsp70-R (Table 1). Based on the EST of *Pyhsp70* cDNA, the 5' gene-specific primers (Pyhsp70 5'GSP1 and GSP2) and 3' gene-specific primers (Pyhsp70 3'GSP1 and GSP2) were designed. The corresponding full-length transcripts were amplified by rapid amplification of complementary DNA (cDNA) ends (RACE) using both the 5' and 3'-Full RACE kits (TaKaRa, China) according to the manufacturer's instructions. The PCR products were gel-purified and then ligated into the pMD19-T vector, which was then sequenced. The sequence reported here has been deposited into the GenBank database (GenBank accession number: KP027180).

Sequence analysis and phylogenetic tree construction

The similarity analysis of nucleotide and protein sequences was carried out by using BLASTn and BLASTp at Web servers of the National Center of Biotechnology Information (<http://www.ncbi.nlm.nih.gov/blast>). Pairwise protein and nucleotide sequence identities were calculated using the MegAlign program in DNASTar software package, respectively. Multiple HSP70 protein sequences were aligned using Clustal 1.83 (<http://www.clustal.org/>). A phylogenetic tree analysis was carried out from the amino acid sequence alignments by the neighbor-joining (NJ) method using Mega 4.0 program (<http://www.megasoftware.net/>). *Pocillopora damicornis* and *Sycon ciliatum* were used for the out-group. Statistical support was provided by 1000 bootstrap replications.

Quantitative real-time PCR

Quantitative real-time PCR was carried out as described previously (Dong et al. 2012). *Pyhsp70* 151 bp (*Pyhsp70-r*) was

Table 1 Primers used in this study

Primers	Sequences (5'→3')
Pyhsp70-F	CGTCAAGCAACAAAGGATGC
Pyhsp70-R	TCCTGCTGTCGATTTCACATC
Pyhsp70 3' GSP1	TCAATCCTTTCAATAGAAGATGG
Pyhsp70 3' GSP2	AGAAGATTTTGACAACCGTATGG
Pyhsp70 5' GSP1	ATAAATCTTTCTTGCTTCCTCT
Pyhsp70 5'GSP2	TCTATTGAAAGGATTGATACATCG
Pyhsp70-rF	CCTGACGAAGCAGTTGCCTATG
Pyhsp70-rR	GTTTGCTTGGTGGGAATAGTGC
Pyβ-actin-rF	GCCGGTCGAGATTGACTGACTAT
Pyβ-actin-rR	CTGGAAGAGAGCCTCGGGACACCT

used for real-time PCR; a *Pyβ-actin* gene 249 bp (*Pβ-actin-r*) was used as the internal control (accession number: KP134530). Primers (Table 1) were designed using Oligo 6.0 software, and all primers generated a single PCR band of the expected size. PCR products were verified by DNA sequencing.

Whole-mount in situ hybridization

Sense and antisense digoxigenin-labelled probes were designed according to a *Pyhsp70* fragment (from position 415 to 1153). The probes were synthesized using the RNA in vitro labeling kit (Roche). Whole-mount in situ hybridizations (WISH) were performed with intact and regenerating worms (0, 0.5, 1, 3, 7, 11, and 15 days after being decapitated) as described previously (Pearson et al. 2005; Dong et al. 2014). In brief, planarians were treated with 2 % HCl to kill and remove mucus and immediately fixed with 4 % paraformaldehyde at 4 °C for 4 h. Samples were dehydrated through a graded series of methanol. Bleaching was performed for 12 h in 6 % H₂O₂ in methanol under UV light. Then samples were hydrated by a graded series of methanol, followed by proteinase K treatment (20 mg/mL) for between 10 and 20 min depending on the size. Hybridization was performed at 60 °C for 28 h in a solution containing 50 % formamide, 5× SSC, 50× Dehardt's, 0.1 % Tween 20, 0.1 mg/mL heparan sulfate, 1 mg/mL yeast tRNA, and 10 % dextran sulfate with digoxigenin-labelled RNA probes; washed in phosphate buffer; and incubated with anti-digoxigenin antibody in a blocking solution. Samples were then rinsed in maleic buffer, and color was developed with NBT/BCIP in maleic buffer for 3–6 h until a signal was visible. Samples were observed with a Leica DMLB stereomicroscope, and images were captured with a Leica DFC300FX camera.

Statistical analysis of data

Statistical analysis was performed using SPSS 13.0 software. Significant interactions with diverse stressors and changes of the regeneration stage were determined by one-way ANOVA followed by post-hoc multiple comparisons using the LSD test. Three biological replicates were used, and differences were considered statistically significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results

Characterization of *Pyhsp70* gene in *Polycelis* sp.

A full-length cDNA of 1941 bp was identified by RACE-PCR from both ends. Sequence analysis of *Pyhsp70* cDNA revealed that: (1) a 36 bp of 5' untranslated region (5'-UTR)

located upstream of a start codon (ATG); (2) an open reading frame (ORF) of 1848 bp encoding a polypeptide of 615 amino acids, with a calculated molecular mass of 67.61 kDa and an isoelectric point of 5.61; and (3) a 57 bp 3'-UTR with a canonical polyadenylation signal sequence AATAAA and a poly (A) tail. The deduced amino acid sequence of *Pyhsp70* included three typical HSP70 family signature motifs: IDLGTTFSCV at the position of 9–18, IFDLGGGTFDVSIL at the position of 197–210, and IVLVGGSTRIPKIQK at the position of 334–348. The putative adenosine triphosphate/guanosine triphosphate (ATP/GTP)-binding site (AEAYLG) is located at residues 131–136 (Fig. 1).

Homology analysis of PYHSP70

Comparison of the PYHSP70 with those of published HSP70 proteins showed that PYHSP70 displayed high identity with those from *Mus musculus* (AAC84168.1, 74.8 %), *Meleagris gallopavo* (XP003212746.1, 75.1 %), *Xenopus tropicalis* (XP002937574.2, 75.4 %), *Paracentrotus lividus* (Q06248.1, 67.0 %), *Marsupenaeus japonicus* (ABK76338.1, 74.5 %), *Drosophila auraria* (CAA55168.1, 75.1 %), *Paphia undulate* (JX885711, 73.2 %), *Perna viridis* (ABJ98722.1, 75.1 %), *D. japonica* (ABY83101.1, 74.8 %), and *Hymenolepis microstoma* (CDJ09463.1, 74.6 %).

To shed light on the evolutionary position of *Pyhsp70*, a phylogenetic tree was constructed using the amino acid sequences of *Pyhsp70* and those of other invertebrates and vertebrates by the NJ method. In phylogenetic trees (Fig. 2), all vertebrate HSP70s clustered together and laid at the terminal of the phylogenetic tree, while for invertebrates HSP70s (except PYHSP70), all animals from the same phylum clustered together. Interestingly, PYHSP70 laid at the base of the tree and did not cluster with those of other Platyhelminthes (such as *D. japonica*, *Fasciola gigantica*, and *Echinococcus granulosus*).

Expression pattern of *Pyhsp70* in response to different stressors

In order to determine whether external stressors could induce *Pyhsp70* expression in planarians, expression patterns of *Pyhsp70* in response to diverse external stressors were examined by quantitative real-time PCR. For starvation stress, the expression value of *Pyhsp70* decreased slightly after 30 days of food deprivation. However, when worms were starved for 60 days, the expression levels significantly increased and then decreased in the re-fed (RF) animals compared with the controls (Fig. 3a). For thermal stress, the expression value of *Pyhsp70* significantly decreased when worms were cultured at 4, 12, 16, and 20 °C compared to the control (8 °C) (Fig. 3b). For tissue damage stress, the expression value of *Pyhsp70* exhibited a gradual upregulation on days 0, 0.5, and


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11  GAAACTTATCACTCATCACTTCAAAATTTAAAGTATGCTCTCATCTCTCTCAATTGATCTCTGAAACACCTTCTCTGCTC
1  M S H T P A I G I D L G T T F S C V
91  GGTGATTTTCAACATGTTAAAGTTGAAATT ATCGTAAACGATCAAGGTAACGAACTACTCCATCGTACGTTGCTTCCACGACAAACGAA
19  G V F Q H G K V E I I G N D Q G N R T T P S Y V A S N D N E
181  AGAATGATGAGACGCTGCTAAGAAATCAAGCTGCAATGAAATCCATCAAAACACAGCTCTTGTATGCTAAGGCTGATGAGACGCAAAATTT
49  R M I G D A A K N Q A A M N P S N T V F D A K R L M G R K F
271  GCGGATCCTTCAGTTCAATCAGATATGAAACATTTCTCATACAACGTCATCGACGTAGACGCGCAAGCCGAAAGATTGAGGTCGAAATTCAG
79  A D P S V Q S D M K H F S Y N V I D V D G K P K I E V E F K
361  GGAATGAAACAAGATCTTCTCACCAGAGAGATTTTCATCATGTTTCTCATAAAAATGAAGGAGACCGCAGAGGCTTACCTTGGAGGAAAG
109  G V N K I F S P E E I S S M V L I K M K E T A E A Y L G G K
451  GTCACAGACGCTGTTATAACTGTACCTGCTATTTCAAGATAGCCAGGTCAGCAACCAAGGATGCGGCTTATGCTGTTTAAAT
139  V T D A V I T V P A Y F N D S Q R Q A T K D A G A I A G L N
541  GTTCTTCAATAATTAATGAACCAACAGCAGCAGCAATGCTATGCTCTGACAAAAAGACTGTAGCGCAAGAAATATGCTTATCTTT
169  V L R I I N E P T A A A I A Y G L D K K T V A A K N I L I F
631  GATCTTGGAGGAGTACATGATGATCAATCTTCAATGAAGATGATGCTTGGAGTTAAATCAACTGACGAGGACACTCATCTC
199  D L G G G T F D V S I L S I E D G I F E V K S T A G D T H L
721  GGTGGAGAGATTTTGAACCGTATGTTGATCACTTGTACAGAGTTCAAGAGGAGCACAAGAAAGATTTATCTGGAATAAAGAA
229  G G E D F D N R M V D H F V Q E F K R K H K K D L S G N K R
811  TCTCTCCGCACTAAGAACTGATGTGAAGAGCAAGAGAACCTCTCTTCAAGCGCCCTTGCATCAGTTGAGGTTGATCTACTGTT
259  S L R L R T A C E R A K R T L S S S A L A S V E V D S L F
901  GATGATGCTGCTTCTATCTCAATCACTGACGACGCTTTTGAAGAACTTTGCGCTGATTTGTTAAGTCTACTATGATCCGCTTGA
289  D G I D F Y T S I T R A R F E E L C A D L F K S T M D P V E
991  AAGTCTCTCAGGATGCTAAATGGAACAAAAAGCTATCAATGAATGATGCTTGGATGATCACTAGAAATCTTAAGATCAGAAA
319  K S L K D A K M D K K A I N E I V L V G G S T R I P K I Q K
1081  CTCTACAAGACTTTTCAATGCGAGAGATTTGAACAAATCCATCAACCTGACGAAAGCAGTTGCTATGTTGCGGAGTACAGGCTGCA
349  L L Q D F F N G R E L N K S I N P D E A V A Y G A A V Q A A
1171  ATCTTGAATGATACGAGGCTGATCAAGACTTGTATTAGTTGACGTAGCACTTTGAGTCTTGTATGAGCTGATGAGTGGC
379  I L T G D T S E A V Q D L L L V D V A P L S L G I E T V G G
1261  GCTATGTCAGTTATCATCAAGAGAACTGCACTATTTCCACCAAGCAACCAAGTATTCACCACTCATGATGATTAATCAACATGTTGTC
409  A M S V I I K R N C T I P T K Q T Q V F T T H D D N Q T C V
1351  AAGATGTTGTTGTCAGGCTGAGAGAGCATGATCAAGGACAATAACAGCTAGGAGAAATCGACTTGACCGTATTTCCCGCAGCACA
439  K I V V C E G E R A M I K D N N K L G E F D L T G I P P A P
1441  CGAGGAGCGCACAGATTGAAGTACTTTCGATCAACGCAACGCTATCTTAATGTCCTCGCGCTGAGAAAGGCGAGCAATCGTGTG
469  R G A A Q I E V T F D I N A N G G I L N V S A V E K G S N R V
1531  AGCAAAATCAACATTAAGCAACGATAAGGCGCACTATCAAGAGGATATTGAGAAATGAGTTGCTGATGCGCAGAAATTTAGAAAGAG
499  S K I T I S N D K G R L S K E D I E K M V A D A E K F R N E
1621  GATGAGAAACAGAAAGAAATTTCTGATGCAAGAACTTTGAGACTTATGCTTTCAGGTTAAGAGACCCCTTGAAGACGAAAGAGTG
529  D E K Q K E I L D A K N S L E T Y A F Q V K T T L E D E R V
1711  CAGATGAAGCTTTCAAGGAGATAAGTACTTAGTCAATGACAAGTGTGACGAGGTAATCTCTTGGATTGAGTGCACAGAGTGTAGC
559  Q M K L S K E D K Y L V N D K C D E V I S W I E C N Q S A S
1801  AAGATGAGTTCGATGATGATGAAGAGGACCTCGAGAGCCTACCTACCGCTCATGTGCAAGCTCTATGTTGCGAAACGATATGCTGCA
589  K D E F S M K K D L E S A C L P V M S K L Y G R K R *
1891  ATTAGGAAAGTAAATATTATATAATATGATCAATACAAAAA

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Fig. 1 Nucleotide and deduced amino acid sequence of *Pyhsp70*. The nucleotide sequence is displayed in the 5'-3' directions and numbered to the left. The start codon, stop codon, and the putative polyadenylation

signal (AATAAA) are boxed. Three HSP70 protein family signatures are underlined. A putative ATP-GTP binding site is boxed and shown in *italics*

1 after amputation (Fig. 3c). For ionic liquid-induced stress, the expression of *Pyhsp70* exhibited a significant upregulation in a dose-dependent manner (Fig. 3d).

Expression pattern of *Pyhsp70* in intact and regenerating planarians

The expression pattern of *Pyhsp70* was analyzed by whole-mount in situ hybridization in intact and regenerative planarians. In intact worms, the *Pyhsp70* transcripts were widespread; strong signals were mainly located in the head peripheral region and intestinal branches (Fig. 4h). In regenerating worms, the transcript signals were strongly expressed in the blastema whether in the fragments regenerating a head (anterior regeneration) or a tail (posterior regeneration) and throughout the regeneration processes (Fig. 4b–g, i–o). Interestingly, *Pyhsp70* expression extended to the post-blastema at 15 days of posterior regeneration (Fig. 4g) and at 11 days of

anterior regeneration (Fig. 4n–o). No signal was observed in the control (Fig. 4a).

Discussion

The *hsp70* family is a multi-gene family with specific functions in different cell compartments and cellular contexts (Mayer and Bukau 2005). In this study, a cytosolic member of the *hsp70* family, *Pyhsp70*, was first cloned and characterized from planarian *Polycelis* sp. Multiple sequence alignment revealed that PYHSP70 shared a high similarity in amino acid sequence with those from other animals; moreover, the deduced amino acid sequence of *Pyhsp70* possesses several highly conserved functional motifs characteristic of the HSP70 family (Shim et al. 2006). Phylogenetic analysis supported that PYHSP70 laid at the most basal position of the tree, more ancestral than those of other HSP70s from phylum

Fig. 2 Phylogenetic analysis of HSP70 sequences. The tree was constructed by neighbor-joining method. The PYHSP70 is marked by the *asterisk*. The bootstrap confidence values were calculated from 1000 replications

Platyhelminthes. In summary, we suggest that PYHSP70 may have conserved functions in planarian *Polycelis* sp.

HSP70 can be induced in response to a wide variety of physiological and environmental insults (Rios-Arana et al.

Fig. 3 RT-PCR analysis of *Pyhsp70* in response to external stressors. **a** Starvation stress. **b** Thermal stress. **c** Tissue damage stress. **d** Ionic liquid stress. *Pyβ-actin* is used as the internal control. Vertical bars represent the

mean±SD ($N=3$). Asterisks indicate statistical differences (* $P < 0.05$; ** $P < 0.01$). *d* days after fed, *RF* re-fed

Fig. 4 Expression of *Pyhsp70* mRNA in intact and regenerating planarians, *Polycelis* sp. **a** The control. **b–g** Dorsal view in the posterior regeneration fragments, 0, 0.5, 1, 3, 7, and 15 days after amputation. **h** Expression of *Pyhsp70* mRNA in intact planarian. **i–o** Dorsal view of anterior regeneration fragments, followed by 0, 0.5, 1, 3, 7, 11, and 15 days after decapitation (showing the auricle by arrow). In all images, anterior is to the top. Scale bars 0.5 mm

2005; Seo et al. 2006; Ventura et al. 2007) and enhances cytoprotection and tolerance against stress-induced molecular damage. In this study, *Pyhsp70* is induced by long-term starvation and tissue trauma. At the same time, *Pyhsp70* is also induced by ionic liquids, which are composed entirely of ions and are a category of molten salts with popularization and

application in various fields (Ranke et al. 2004; Lee et al. 2005). Currently, they are seen as promising green substitutes for traditional volatile organic compounds in manufacturing, processing, and cleaning technologies (Keskin et al. 2007; Li et al. 2010). [C8mim]Br, being one of the most commonly encountered imidazolium and increasing attention, has been

paid to the toxic bioassay and environmental risk assessment to aquatic organisms (Landry et al. 2005). These results are consistent with those of previous studies (Ma et al. 2009; Navarro et al. 2009; Senf et al. 2013; Akerfelt et al. 2010). For example, in planarians *D. japonica* and *Schmidtea polychroa*, HSP70 is induced by various stress factors (Ma et al. 2009; Navarro et al. 2009). In vertebrates, HSP70, as a marker for tissue damage, can activate both neutrophils and macrophages to infiltrate damaged muscle and modulate regeneration following injury (Ortega et al. 2009; Kovalchin et al. 2006; Tashiro 2009). At the same time, HSP70, as a crucial molecule chaperone, can protect cells to survive and adapt to all kinds of environmental stresses (Akerfelt et al. 2010). Based on this, we suggest that the biochemical function of *Pyhsp70* is conserved in planarians; moreover, *Polycelis* sp., as aquatic animals, are more suitable to be used as test organisms in water environmental toxicology by the change of the expression levels of the *Pyhsp70*.

Interestingly, the expression value of *Pyhsp70* displayed downregulation as the culture temperature was increased and decreased, and this result was unexpected in light of the results obtained above and at odds with previous studies in planarians *D. japonica* and rotifera (Ma et al. 2009; Yang et al. 2014). The most favorable living temperature for planarian *Polycelis* sp. is 6–10 °C, but when the culture temperature is elevated to 20 °C, they lyse easily. This is likely caused by the destruction of thermolabile elements of the transcription system similar to other animals when the temperature exceeds their limit (Diller 2006). However, the specific reasons remain to be determined and more work will need to be done in the future.

The spatial expression pattern of *Pyhsp70* was analyzed by whole-mount in situ hybridization experiments. In regenerating worms, the transcript signals were strongly expressed in the blastema and extended to the post-blastema with regeneration process. Regeneration in planarians relies upon a population of stem cells, called neoblasts that proliferate in response to injury and differentiate to form the missing tissues (Reddien and Sánchez Alvarado 2004; Sánchez Alvarado 2006; Saló 2006). Planarian pluripotent stem cells are present throughout the mesenchymal space and migrate to the organ rudiments or blastema in the process of regeneration, and this migration is stimulated by a cut or a wound (Baguna 2012). HSP70 overexpression has been involved in tumor growth and metastatic potential in rodent tumor models (Garrido et al. 1998). HSP70 also promoted cell migration in hepatocellular carcinoma (Liu et al. 2014) and was considered as a target drug for cancer therapy (Wang 2011; Evans et al. 2010). Thus, we hypothesize that PYHSP70 may promote neoblasts' migration to the wound and also play a major role in the proliferation and differentiation of neoblast cells similar to the roles of HSP70 in tumor growth (Garrido et al. 1998).

In intact worms, the *Pyhsp70* transcripts were widespread, but the signals were stronger in the head peripheral region in

which the chemoreceptor and tactile sensor (auricle) are located (Newmark and Sánchez Alvarado 2002). Furthermore, the hybridization signal of *Pyhsp70* was observed in the intestinal branches of some samples. In oysters, Hsp70-positive cells were also observed in epithelial cells of the gills and intestinal tissues, and the mechanism was not clear (Moraga et al. 2005). At present, we are not certain of the cause of *Pyhsp70* in planarians' intestinal branches; previous studies have shown that the non-hybridized probe easily accumulated in the intestinal vaults, from which it cannot be properly rinsed away, thereby generating background noise (Cardona et al. 2005). The specific reasons are still far from clear, and further study will be needed.

Conclusions

A full-length *Pyhsp70* cDNA from *Polycelis* sp. was successfully cloned, and its expression pattern in intact and regenerating worms was analyzed. The results suggest that *Pyhsp70* may contribute to enhance cytoprotection and tolerance against stress-induced molecular damage; it was also involved in the proliferation, migration, and differentiation of neoblasts in the process of regeneration. To date, the particular roles of *Pyhsp70* proteins in planarians are still far from clear; further study on *hsp70* function in simple invertebrate models such as planarians will be needed to provide a new insight into the role played by *hsp70s* in more complex vertebrates.

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References

- Aikawa M, Shimozaawa A (1991) The multiple eyes of *Polycelis*. 1. Relation between the number of eyes and body length. *Hydrobiologia* 227:257–262
- Akerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. *Nat Rev Mol Cell Biol* 11:545–555
- Baguna J (2012) The planarian neoblast: the rambling history of its origin and some current black boxes. *Int J Dev Biol* 56:19–37
- Beaman GM, Dennison SR, Chatfield LK, Phoenix DA (2014) Reliability of HSP70 (HSPA) expression as a prognostic marker in glioma. *Mol Cell Biochem* 393:301–307

- Cardona A, Fernández J, Solana J, Romero R (2005) An in situ hybridization protocol for planarian embryos: monitoring myosin heavy chain gene expression. *Dev Genes Evol* 215:482–488
- Ciocca DR, Calderwood SK (2005) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cells Stress Chaperones* 10:86–103
- Ciocca DR, Arrigo AP, Calderwood SK (2013) Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. *Arch Toxicol* 87:19–48
- Daugaard M, Rohde M, Jaattela M (2007) The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett* 581(19):3702–3710. doi:10.1016/j.febslet.2007.05.039
- Diller KR (2006) Stress protein expression kinetics. *Annu Rev Biomed Eng* 8:403–424
- Dong ZM, Shi CY, Zhang HX, Dou H, Cheng FF, Chen GW, Liu DZ (2014) The characteristics of *Sox* gene in *Dugesia japonica*. *Gene* 544:177–183
- Dong ZM, Yuwen YQ, Wang QH, Chen GW, Liu DZ (2012) Eight genes expression patterns during visual system regeneration in *Dugesia japonica*. *Gene Expr Patterns* 12:1–6
- Evans CG, Chang L, Gestwicki JE (2010) Heat shock protein 70 (hsp70) as an emerging drug target. *J Med Chem* 53(12):4585–4602
- Freeman BC, Michels A, Song J, Kampinga HH, Morimoto RI (2000) Analysis of molecular chaperone activities using in vitro and in vivo approaches. *Methods Mol Biol* 99:393–419
- Garrido C, Fromentin A, Bonnotte B, Favre N, Moutet M, Arrigo AP, Mehlen P, Solary E (1998) Heat shock protein 27 enhances the tumorigenicity of immunogenic rat colon carcinoma cells. *Cancer Res* 58:5495–5499
- Gleixner AM, Pulugulla SH, Pant DB, Posimo JM, Crum TS, Leak RK (2014) Impact of aging on heat shock protein expression in the substantia nigra and striatum of the female rat. *Cell Tissue Res* 357:43–54
- Heikkilä JJ (2010) Heat shock protein gene expression and function in amphibian model systems. *Comp Biochem Physiol A Mol Integr Physiol* 156:19–33
- Henderson B, Pockley AG (2010) Molecular chaperones and protein-folding catalysts as intercellular signaling regulators in immunity and inflammation. *J Leukoc Biol* 88(3):445–462
- Jenei ZM, Gombos T, Föhrhész Z, Pozsonyi Z, Karádi I, Jánoskúti L, Prohászka Z (2013) Elevated extracellular HSP70 (HSPA1A) level as an independent prognostic marker of mortality in patients with heart failure. *Cell Stress Chaperones* 18:809–813
- Kalmar B, Greensmith L (2009) Induction of heat shock proteins for protection against oxidative stress. *Adv Drug Deliv Rev* 61:310–318
- Keskin S, Kayrak-Talay D, Akman U, Hortaşu Ö (2007) A review of ionic liquids towards supercritical fluid applications. *J Supercrit Fluids* 43:150–180
- Knakievicz T, Ferreira HB (2008) Evaluation of copper effects upon *Girardia tigrina* freshwater planarians based on a set of biomarkers. *Chemosphere* 71:419–428
- Kovalchin JT, Wang R, Wagh MS, Azoulay J, Sanders M (2006) In vivo delivery of heat shock protein 70 accelerates wound healing by up-regulating macrophage-mediated phagocytosis. *Wound Repair Regen* 14:129–137
- Landry TD, Brooks K, Poche D, Woolhiser M (2005) Acute toxicity profile of 1-butyl-3-methylimidazolium chloride. *Bull Environ Contam Toxicol* 74:559–565
- Lee SM, Chang WJ, Choi AR, Koo YM (2005) Influence of ionic liquids on the growth of *Escherichia coli*. *Korean J Chem Eng* 22(5):687–690
- Li MH (2008) Effects of nonionic and ionic surfactants on survival, oxidative stress, and cholinesterase activity of planarian. *Chemosphere* 70:1796–1803
- Li XY, Luo YR, Yun MX, Wang J, Wang JJ (2010) Effects of 1-octyl-3-methylimidazolium bromide on the anti-oxidant system of earthworm. *Chemosphere* 78:853–858
- Liu CC, Jan YJ, Ko BS, Wu YM, Liang SM, Chen SC, Lee YM, Liu TA, Chang TC, Wang J, Shyue SK, Sung LY, Liou JY (2014) 14-3-3 σ induces heat shock protein 70 expression in hepatocellular carcinoma. *BMC Cancer* 14:425
- Ma KX, Chen GX, Lou H, Fei LN (2009) Cloning and expression analysis of hsp70 gene from freshwater planarian *Dugesia japonica*. *Biologia* 64:1018–1024
- Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci* 62:670–684
- Moraga D, Meistertzheim AL, Tanguy-Royer S, Boutet I, Tanguy A, Donval A (2005) Stress response in Cu²⁺ and Cd²⁺ exposed oysters (*Crassostrea gigas*): an immunohistochemical approach. *Comp Biochem Physiol C Toxicol Pharmacol* 141:151–156
- Nadin SB, Sottile ML, Montt-Guevara MM, Gauna GV, Daguerre P, Leuzzi M, Gago FE, Ibarra J, Cuello-Carrión FD, Ciocca DR, Vargas-Roig LM (2014) Prognostic implication of HSPA (HSP70) in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. *Cell Stress Chaperones* 19:493–505
- Navarro BS, Michiels NK, Köhler HR, D'Souza TG (2009) Differential expression of heat shock protein 70 in relation to stress type in the flatworm *Schmidtea polychroa*. *Hydrobiologia* 636:393–400
- Newmark PA, Sánchez Alvarado A (2002) Not your father's planarian: a classic model enters the era of functional genomics. *Nat Rev Genet* 3:210–219
- Ortega E, Hinchado MD, Martín-Cordero L, Asea A (2009) The effect of stress inducible extracellular Hsp72 on human neutrophil chemotaxis: a role during acute intense exercise. *Stress* 12:240–249
- Pearson JC, Lemons D, McGinnis W (2005) Modulating Hox gene functions during animal body patterning. *Nat Rev Genet* 6:893–904
- Ranke J, Mölter K, Stock F, Bottin-Weber U, Poczbütt J, Hoffmann J, Ondruschka B, Filser J, Jastorff B (2004) Biological effects of imidazolium ionic liquids with varying chain lengths in acute *Vibrio fischeri* and WST-1 cell viability assays. *Ecotoxicol Environ Saf* 58:396–404
- Reddien PW, Sánchez Alvarado A (2004) Fundamentals of planarian regeneration. *Annu Rev Cell Dev Biol* 20:725–757
- Rios-Arana JV, Gardea-Torresdey J, Webb R, Walsh EJ (2005) Heat shock protein 60 (HSP60) response of *Platynotus patulus* (Rotifera: Monogononta) to combined exposures of arsenic and heavy metals. *Hydrobiologia* 546:577–585
- Saló E (2006) The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes). *Bioessays* 28:546–559
- Salo E, Baguna J (2002) Regeneration in planarians and other worms: new findings, new tools, and new perspectives. *J Exp Zool* 292:528–539
- Sánchez Alvarado A (2000) Regeneration in the metazoans: why does it happen? *Bioessays* 22:578–590
- Sánchez Alvarado A (2006) Planarian regeneration: its end is its beginning. *Cell* 124:241–245
- Senf SM, Howard TM, Ahn B, Ferreira LF, Judge AR (2013) Loss of the inducible Hsp70 delays the inflammatory response to skeletal muscle injury and severely impairs muscle regeneration. *PLoS One* 8(4), e62687
- Seo J, Park TJ, Lee YM, Park H, Yoon YD, Lee JS (2006) Small heat shock protein 20 gene (Hsp20) of the intertidal copepod *Tigriopus japonicus* as a possible biomarker for exposure to endocrine disruptors. *Bull Environ Contam Toxicol* 76:566–572
- Shim JK, Jung DO, Park JW, Kim DW, Ha DM, Lee KY (2006) Molecular cloning of the heat-shock cognate 70 (Hsc70) gene from the two-spotted spider mite, *Tetranychus urticae*, and its expression in response to heat shock and starvation. *Comp Biochem Physiol B* 145:288–295

- Tashiro S (2009) Mechanism of liver regeneration after liver resection and portal vein embolization (ligation) is different? J Hepato-Biliary-Pancreat Surg 16:292–299
- Ventura M, Canchaya C, Zhang Z, Fitzgerald GF, Van Sinderen D (2007) Molecular characterization of hsp20, encoding a small heat shock protein of *Bifidobacterium breve* UCC2003. Appl Environ Microbiol 73:4695–4703
- Wang RE (2011) Targeting heat shock proteins 70/90 and proteasome for cancer therapy. Curr Med Chem 18(27):4250–4264
- Yang JH, Mu YW, Dong SM, Jiang QC, Yang JX (2014) Changes in the expression of four heat shock proteins during the aging process in *Brachionus calyciflorus* (rotifera). Cell Stress Chaperones 19:33–52