Comparative Biochemistry and Physiology, Part B 171 (2014) 12–17

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Molecular characterization of the glucose-regulated protein 78 (GRP78) gene in planarian *Dugesia japonica*

Ke-Xue Ma, Guang-Wen Chen *, Chang-Ying Shi, Fang-Fang Cheng, He Dou, Cheng-Cheng Feng, De-Zeng Liu

College of Life Sciences, Henan Normal University, Xinxiang 453007, PR China

ARTICLE INFO

Article history:
Received 29 November 2013
Received in revised form 2 March 2014
Accepted 4 March 2014
Available online 12 March 2014

Keywords:
Planarian
cDNA cloning
GRP78
Stress
Gene expression

ABSTRACT

GRP78 (78 kDa glucose-regulated protein) has ubiquitously existed in nearly all organisms from yeast to humans, reflecting the central roles it plays in cell survival. In this report, we isolated and sequenced the full-length cDNA of GRP78 (designated DjGRP78) from the planarian *Dugesia japonica*. The cDNA is 2121 bp, including an open reading frame (ORF) of 1983 bp encoding a polypeptide of 660 amino acids with three HSP70 family signatures. DjGRP78 contains signal peptides at the N-terminus and a KTEL peptide motif at the C-terminus, which suggests that it localizes in the endoplasmic reticulum (ER). Fluorescent real time RT-PCR was employed to detect the expression pattern of *Djgrp78* in response to different stressors. Our results show that heat shock and heavy metals (Hg^{2+} and Pb^{2+}) induce *Djgrp78* expression, but starvation does not. Interestingly, we found that *Djgrp78* was up-regulated in planarians with septic tissues, and also verified that it was up-regulated in response to bacterial challenge. Our data indicate that *Djgrp78* may be a multifunctional gene, and play important roles in physiological and pathological stress in planarians.

1. Introduction

The endoplasmic reticulum (ER) is a membranous organelle found in all eukaryotic cells that is crucial for normal cell function and development (Schröder, 2008; Giorgi et al., 2009). It plays a fundamental role in synthesis, folding, sorting, and delivery of proteins to the appropriate cellular destinations. ER function is affected by various intracellular and extracellular stressors, and prolonged ER stress leads to cell death. Cells from all organisms increase the expression of a class of ER stress proteins in response to ER stress, which serve as molecular chaperones and are involved in protein translocation, protein folding and assembly, and the regulation of protein secretion (Kaufman, 1999). Glucose-regulated protein (GRP) 78 (also known as HSPA5 or BiP), is a member of the heat shock protein 70 (HSP70) family of proteins, which are involved in ER stress. GRP78 expression is induced during oxidative stress (Kitamura and Hiramatsu, 2010), chemical toxicity (Falahatpisheh et al., 2007; Stacchiotti et al., 2009), treatment with Ca^{2+} ionophores and inhibitors of glycosylation (Kaufman, 1999). This induction, which is part of the unfolded protein response (UPR), is required to alleviate ER stress, maintain ER function, facilitate protein folding and thus protect cells from the aforementioned toxic insults (Kaufman, 1999; Sherman and Goldberg, 2001). Other reports suggest that GRP78 may protect the host cell against cell death by suppressing oxyradical accumulation and stabilizing mitochondrial function (Yu et al., 1999). GRP78 also has an anti-apoptotic function to prevent ER stress-induced cell death and is used as a biomarker for the onset of the UPR (Lee, 2001). Recent works suggest that GRP78 may play a role in immune defense against foreign pathogens (Luan et al., 2009; Morito and Nagata, 2012).

In spite of numerous investigations into the role of GRP78 in mammals, there has been very limited investigation into it's role in freshwater planarians. Freshwater planarians are unique animals, showing powerful regenerating ability and strong tolerance to prolonged starvation (Bowen et al., 1976; Newmark and Sánchez Alvarado, 2002). As aquatic animals, planarians are easily threatened by water pollution and are often used as test organisms in water environmental toxicology (Pra et al., 2005). We observed that some planarians can survive for several weeks with septic tissues (induced by lysing parts of their bodies). Septic tissues can be easily attacked by a wide array of microbes present in water. Therefore, we are very interested in whether GRP78 is involved in the adaptive stress and innate immune responses in planarians. In the present paper, we cloned the full length cDNA of GRP78 from the planarian *Dugesia japonica* (*Djgrp78*) for the first time, and studied the expression profiles of *Djgrp78* in response to different stressors and bacterial challenge. The results of this study will allow greater understanding of the physiological and immunological roles of GRP78 in planarians.

---

* Corresponding author at: College of Life Sciences, Henan Normal University, No. 46, Jianshe Road, Xinxiang 453007, PR China. Tel.: +86 373 3326190; fax: +86 373 3326162.
E-mail address: Chengw0183@sina.com (G.-W. Chen).

http://dx.doi.org/10.1016/j.cbpb.2014.03.001
1096-4959/

2. Materials and methods

2.1. Animals and treatments

The planarians *D. japonica* used in this study were collected from Tagang Reservoir, Xinxiang City, China. They were cultured in autoclaved tap water in the dark at 18 °C and fed once a week with fresh fish spleen. Animals of similar body size were used for experiments after 7–10 day starvation. For heavy metal stress, planarians (20 animals/group) were exposed to Hg^{2+} ($HgCl_2$, 1, 10, 50, 200 μg/L) and Pb^{2+} [$Pb(NO_3)$, 10, 100, 250, 500 μg/L] respectively, for 48 h. For thermal stress, animals (20 animals/group) were cultured at 25 °C for 2 days, followed by 30 °C for 2 days. A group of control organisms was maintained in parallel for all experiments but not subjected to metal or thermal stress.

Planarians with septic tissues (designated as unhealthy animals) were selected for pathological stress experiments (see Fig. 6B,C). For the bacterial challenge experiments, we cultured the intact and regenerating animals (animals were cut before and after the pharynx) in sterile water containing *Escherichia coli* DH5α (1×10^7 cuf/mL). Samples were collected at 4, 8, 12, 16 and 24 h post-challenge for RNA extraction. Normal regeneration was conducted in sterile water at 18 °C for control experiments.

2.2. RNA extraction and Djgrp78 cDNA cloning

Total RNA was extracted using Trizol reagent (Invitrogen, USA) and 2 μg RNA was used for reverse transcription. A 388 bp EST fragment for *Djgrp78* gene was previously obtained by differential-display RT-PCR in our laboratory. Based on the known EST of *Djgrp78* cDNA, *Djgrp78* 3′-RACE specific primer (5′-ATG ATG CCC AAA GAC AAG CTA CTA GTG-3′) and *Djgrp78* 5′-RACE specific primer (5′-CTC TTT GAT CGA AAT CTT CAC CTC C-3′) were designed for the amplification of cDNA ends (RACE). Both 5′-RACE and 3′-RACE were carried out using a TaKaRa RACE cDNA amplification kit according to the manufacturer's instructions. The PCR products were gel-purified, ligated into the pUCm-T vector, and submitted for sequencing. The resulting sequences were verified and subjected to cluster analysis.

2.3. Amplification of Djgrp78 ORF sequences from genomic DNA

Genomic DNA was extracted using the Takara genomic DNA extraction kit Ver.3.0 according to the manufacturer's instructions. Based on the *Djgrp78* cDNA sequences, we designed a pair of specific primers (forward: 5′-AAC TGT TGT TGA ATC CAT GAA ATC G-3′; reverse: 5′-GGT TTA CAA TTC AGT CTT AGC ATC ATC-3′) to amplify *Djgrp78* ORF sequences from genomic DNA. The PCR program was carried out at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 3 min and a final extension step at 72 °C for 10 min. The PCR products were sequenced.

2.4. Homology analysis

The homology analysis of nucleotide and protein sequences was carried out using blastn and blastp at the National Center of Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast). The deduced amino acid sequence and protein motif features were analyzed with the Expert Protein Analysis System (http://www.expasy.org). Multiple alignments of the GRP78 protein sequences were performed using the software Dnaman 6.0. A phylogenic tree was constructed using the programs Clustal X 1.83 and Mega 3.1 based on the amino acid sequences of DjGRP78 and other known GRP78 sequences. Bootstrap analysis was used with 1000 replicates to test the relative support for the branches produced by neighbor-joining analysis.

2.5. Expression pattern of Djgrp78 in response to different stressors

To assess gene expression of *Djgrp78* in different stress conditions, the transcript levels in stressed animals were compared to those of unstressed animals. Fluorescent real time RT-PCR was performed at least three times with independent RNA samples. Specific sense and antisense primers (forward: 5′-GAG TTA TCG GTG GAG TTG AAG AA-3′; reverse: 5′-CTT GAA TTG TGA CGG TAG GTT GA-3′) were designed to amplify a *Djgrp78* cDNA fragment of 186 bp. SYBR Green chemistry-based RT-PCR was carried out with ABI PRISM 7500 Sequence Detection System (Applied Biosystems). Planarian *elongation factor 2* (*Djef2*) (forward: 5′-TTA ATG ATG GGA AGA TAT GTT G-3′; reverse: 5′-GTA CCA TAG GAT CTG ATT TTG C-3′) was used as the reference gene in all experiments (Pineda et al., 2002). The expression ratios were determined using the method $2^{-\Delta\Delta CT}$, described by Livak and Schmittgen (2001). The data obtained from qRT-PCR analysis for the expression of *Djgrp78* were subjected to one-way analysis of variance (one-way ANOVA). Differences were considered significant at $P < 0.05$.

2.6. Hyperlinking to databases

Nucleotide sequence and protein sequence data are available in the GenBank database under the accession numbers: JN968463 and AET10307, respectively.

3. Results

3.1. Molecular characteristics of the GRP78 gene in the planarian Dugesia japonica

The full-length cDNA of DjGRP78 is 2121 bp, including a 5′-terminal untranslated region (UTR) of 44 bp, a 3′-terminal UTR of 94 bp with a canonical polyadenylation signal sequence AATAAA and a poly (A) tail (Fig. 1), and an ORF of 1983 bp encoding a polypeptide of 660 amino acids with a predicted molecular mass of 72.66 kDa and theoretical isoelectric point of 5.38. The Signal P software analysis indicates that DjGRP78 contains a signal peptide of 18 amino acids (**MKSITVLLISLLVCGIYA**). SMART analysis shows that DjGRP78 displays three conserved heat shock protein 70 (HSP70) family signature motifs: **IDLGTTYS** at the position of 38–45, **VFDLGGGTFDVSLL** at 226–239, and **IVLVGGSTRIPKVQQ** at 363–377. The C terminal of DjGRP78 contains the **KTEL** peptide motif (residues 657–660) (Fig. 1). This motif is essential for retention in the ER lumen (Monro and Pelham, 1987; Persson et al., 2005), which suggests that DjGRP78 localizes in the ER. In addition, we sequenced the ORF sequences from genomic DNA, and found only one intron (44 bp) existed in the *Djgrp78* gene structure. Interestingly, the intron disrupts the codon TG^T at the position of 46 that encodes for cysteine (C). The 5′-putative and 3′-putative splice junctions follow the typical canonical consensus “GT-AG” rule (Fig. 1).

3.2. Homology analysis of DjGRP78

The deduced amino acid sequence of DjGRP78 is highly similar to GRP78s in invertebrates and vertebrates (more than 75% similarity in all matches). There is high similarity to GRP78s of *Hydra magnipapillata* (75.15%), *Caenorhabditis elegans* (76.44%), *Danio rerio* (76.55%), and *Homo sapiens* (76.4%). Based on the nucleotide sequence of DjGRP78, we blasted the *Schmidtea mediterranea* genome database (Robb et al., 2008), and obtained a genome sequence of SmedGRP78 (v31.002536). The genome sequence of SmedGRP78 contains a 46 bp intron, and an ORF of 1983 bp encoding a polypeptide of 660 amino acids with a predicted molecular mass of 72.87 kDa and theoretical isoelectric point of 5.5. SmedGRP78 is highly similar to DjGRP78 (94.07% amino acid identity), especially in HSP70 family signatures and the ER retrieval signal **KTEL** (data not shown).

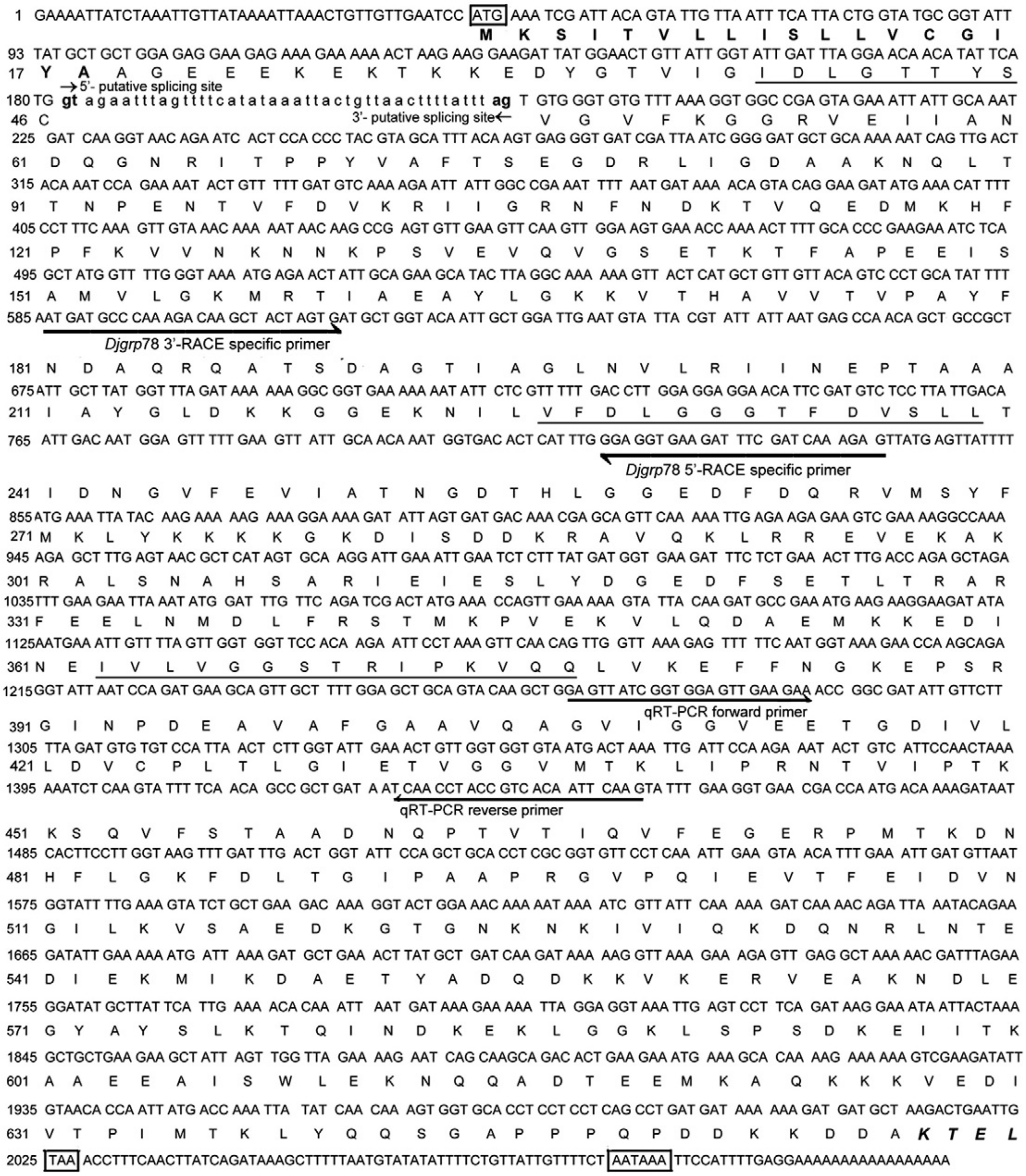

Fig. 1. The nucleotide sequence of the *grp78* gene and the deduced amino acid in the coding region from planarian *Dugesia japonica*. The bold letters mark the signal peptide, and the bold italic letters represent the endoplasmic reticulum retention sequence, KTEL. Three HSP70 protein family signatures are underlined. The start codon, stop codon and the polyadenylation signal (AATAAA) are boxed. The small letters indicate the intron. The intron 5′-putative splicing site and 3′-putative splicing site follow the typical "GT-AG" rule.

Based on the sequences of GRP78 in the above listed and other species, a phylogenetic tree was constructed using the software packages Clustal X 1.83 and Mega 3.1 (Fig. 2). The GRP78 phylogenetic tree shows that animals of Arthropods, Vertebrates, Cnidarians and Mollusks are clustered respectively, with planarians located at the root of the tree. The GRP78 phylogenetic tree does not define the evolutionary relationship of species, rather it shows a current look at the similarities between species based on the similarity of GRP78 homolog sequences. The HSP70 phylogenetic tree reveals that planarians have a closer relationship with vertebrates than flies and nematodes, reflecting the new phylogenetic position of planarians (Ma et al., 2009). The root of the HSP90 phylogenetic tree is unicellular yeast, next to plants, coelenteratas, platyhelminthes, mollusks, arthropods, and vertebrates, which is in agreement with the traditional taxonomy (Ma et al., 2012). This phenomenon suggests that stress proteins in planarians display a divergent evolution.

3.3. Expression pattern of DjGRP78 mRNA in response to different stressors

Studies revealed that GRP78 can be induced by starvation (Lee et al., 2002; Mamadu and Storey, 2006). Therefore, we are greatly interested in understanding whether starvation influences GRP78 expression in planarians. We used fluorescent real-time quantitative PCR to measure the temporal expression of DjGRP78 mRNA in response to prolonged starvation. Unexpectedly, the transcriptional levels of *Djgrp78* did not increase after 30 days of food deprivation, rather, it was reduced slighly (0.65 and 0.69-fold, respectively) relative to controls after 60–90 days of food deprivation, and returned to normal level in the re-fed (RF) animals (Fig. 3).

The expression profile of *Djgrp78* after heat shock treatment is shown in Fig. 4. Planarians were transferred from 18 °C culture conditions to 25 °C for 2 days, the expression levels of *Djgrp78* were approximately 2.2-fold higher than that of the normal levels (Fig. 4A). When

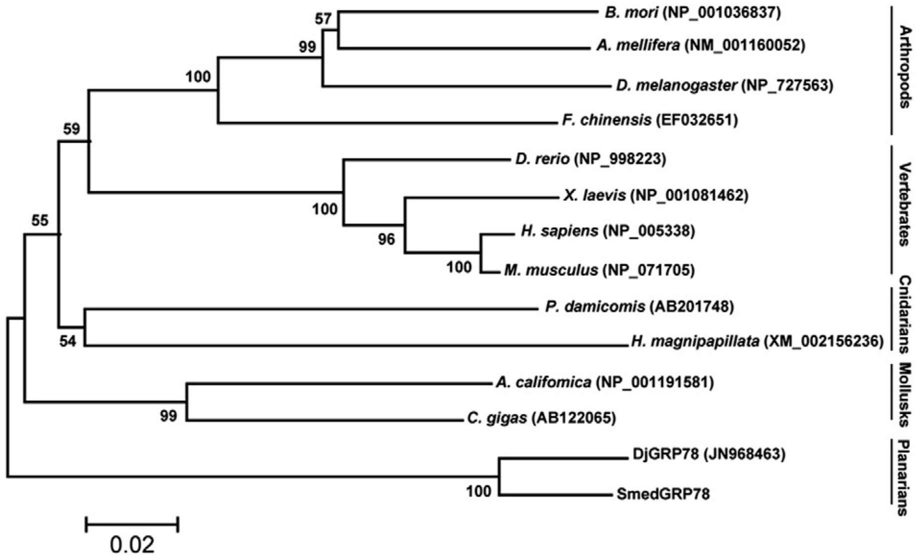

Fig. 2. A phylogenetic tree of GRP78 family members constructed with the neighbor-joining method. The GenBank accession numbers are in brackets. The number at each branch indicates the percentage of times that a node is supported in 1000 bootstraps pseudoreplication by neighbor joining.

elevating the culture temperature to 30 °C for 2 days, the transcripts of *Djgrp78* continually increased to approximately 2.8-fold higher than controls. In order to determine which part of the planarian is most sensitive to thermal stress, we subjected the animals to heat shock at 30 °C for 12 h and then cut the animals into three fragments (head, trunk, and tail), and examined the *Djgrp78* mRNA levels immediately. We found that *Djgrp78* mRNA levels were increased approximately 3.5-fold in the head, 2.2-fold in the tail and 1.5-fold in the trunk (Fig. 4B).

To determine whether *Djgrp78* is induced by heavy metals, we treated the animals with different concentrations of Hg^{2+} and Pb^{2+} for 48 h. The *Djgrp78* mRNA levels increased slightly (approximately 1.5-fold) in 10 μg/L Hg^{2+} treated groups, and increased significantly (approximately 2-fold) in 50 μg/L Hg^{2+} treated groups, but were reduced to 50% of the control expression in 200 μg/L Hg^{2+} treated groups (Fig. 5A). *Djgrp78* expression showed a similar profile in Pb^{2+} treated animals. We found that 100 μg/L and 250 μg/L Pb^{2+} concentrations notably elevated the *Djgrp78* mRNA levels (approximately 1.6-fold and 2.2-fold, respectively), but reduced the expression levels to 0.55-fold in 500 μg/L Pb^{2+} treated groups relative to control samples (Fig. 5B).

Recently, Luan et al. (2009) reported that GRP78 was involved in shrimp immune function which led us to investigate whether DjGRP78 is related to innate immune function in planarians. We measured the DjGRP78 mRNA levels in planarians with septic tissues (unhealthy animals), and found that it was approximately 2.2-fold higher than in control animals (Fig. 6A). To test whether bacteria can stimulate the up-regulation of *Djgrp78* expression, we cultured the intact and regenerating animals in sterile water containing *E. coli* DH5α (1×10^7 cuf/mL). We found that the mRNA levels began to rise at 12 h (1.66-fold), and continued to increase up to 24 h (2.4-fold) following amputation, while it remained unchanged in the intact animals (Fig. 6D). During the normal regeneration, the transcripts of *Djgrp78* increased slightly at 12 h (1.3-fold), then returned to the control levels at 24 h post amputation (Fig. 6D).

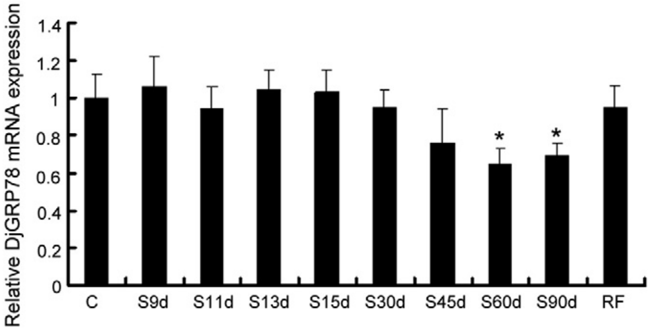

Fig. 3. Expression of *Djgrp78* in response to starvation. Animals starved for 7 days were used as control samples. Values are expressed as mean ± S.D. of three independent samples, collected at each experimental condition and analyzed in triplicate. Asterisks indicate significant differences compared to that of control samples ($^*P < 0.05$).

4. Discussion

In this study, the full-length cDNA of GRP78 gene (*Djgrp78*) was successfully cloned from the planarian *D. japonica*. To our knowledge, this is

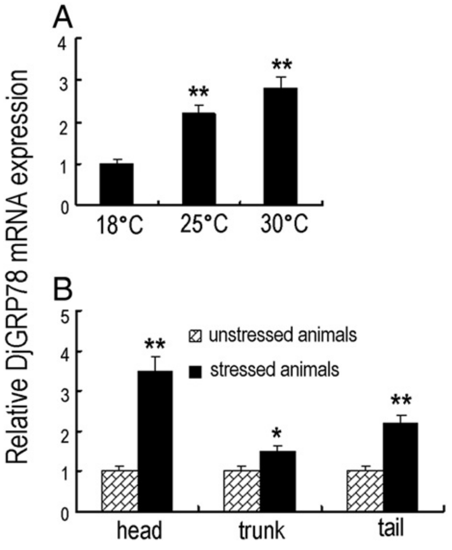

Fig. 4. Expression of *Djgrp78* in response to thermal stress. A) Heat shock treatment at different temperatures. Control animals were cultured in 18 °C; with test groups cultured at 25 °C for 2 days (25 °C) and 25 °C for 2 days, followed by 30 °C for 2 days (30 °C). B) Regional responses of planarians to thermal stress. Animals were subjected to heat shock at 30 °C for 12 h, and then cut into head, trunk and tail fragments, followed immediately by measurement of *Djgrp78* mRNA levels. Unstressed animals were used as controls. Values are expressed as mean ± S.D. of three independent samples, collected at each experimental condition and analyzed in triplicate. Asterisks indicate significant differences compared to control samples ($^*P < 0.05$, $^{**}P < 0.01$).

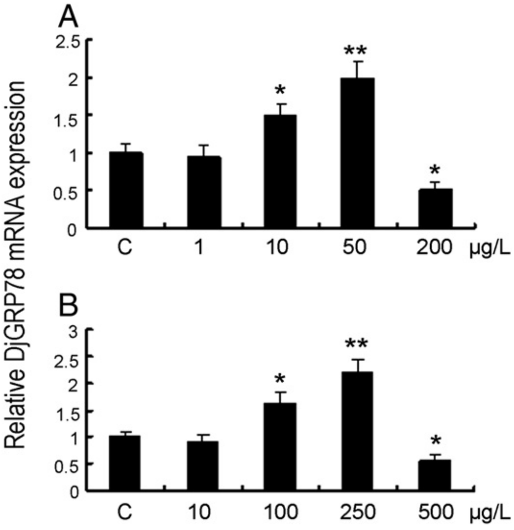

Fig. 5. Expression of *Djgrp78* in response to heavy metal exposure. A) Hg^{2+} treatment; B) Pb^{2+} treatment. Values are expressed as mean ± S.D. of three independent samples, collected at each experimental condition and analyzed in triplicate. Asterisks indicate significant differences compared to control samples ($^{*}P < 0.05$, $^{**}P < 0.01$).

the first report of the GRP78 gene in planarians. Three typical HSP70 protein family signature motifs: **IDLGTTYS** (residues 38–45), **VFDLGGGTFDVSLL** at (residues 226–239), and **IVLVGGSTRIPKVQQ** (residues 363–377) have also been found in the deduced amino acid sequences of the cDNA of DjGRP78 (Fig. 1). The signal peptides at the N terminus and the tetrapeptide **KTEL** at the extreme C terminus of the DjGRP78, indicate that the protein normally resides in the ER. **KTEL** or **KDEL** is a retrieval motif essential for the precise sorting of ER resident proteins along the secretory pathway (Monro and Pelham, 1987; Persson et al., 2005). The molecular characteristics of DjGRP78 indicate that it may have multiple functions in planarians similar to homologous genes reported in other organisms.

GRP78 was first found as a 78 kDa protein which was up-regulated in cells cultured in medium deprived of glucose (Shiu et al., 1977). Subsequently it was reported that GRP78 was induced independently of glucose deprivation (Stoeckle et al., 1988). Several reports of the induction of GRP78 synthesis by starvation were only in mammalian cells (Lee et al., 2002; Mamadu and Storey, 2006). In the study presented here, we found that prolonged starvation cannot induce the up-regulation of DjGRP78 at the mRNA levels, but it can induce the up-regulation of *Djhsp70* (Ma et al., 2009), *Djhsp90* (Conte et al., 2011) and *Djhsp40* (unpublished data) expression. This phenomenon may be related to the role of autophagy under starvation condition in planarians (González-Estevez, 2008). Autophagy not only can degrade and recycle the cellular components to recover energy supplies during nutritional deprivation, but also can remove the unfolded protein aggregates and oxidized proteins, which are the main reason for the induction of GRP78 (Kaushik and Cuervo, 2006; Moore et al., 2006).

The transcriptional up-regulation of ER chaperones is the hallmark of the ER stress response in all eukaryotic organisms (Qian et al., 2001; Liu et al., 2006; Sun et al., 2006; Shinkai et al., 2010). We verified that thermal stress and heavy metals (Hg^{2+} and Pb^{2+}) can induce the up-regulation of *Djgrp78* expression in planarians. This response is especially strong in head tissues under thermal stress perhaps because the head, with the central nervous system, is more sensitive to ER stress. GRP78 accumulation in head tissues may have a neuroprotective role in response to ER stress. This hypothesis is supported by the findings that GRP78 depletion can accelerate cerebellar degeneration in mice (Wang et al., 2010). The molecular mechanisms responsible for the induction of GRP78 expression have been discussed (Rao et al., 2002; Qian and Tiffany-Castiglioni, 2003; Ma and Hendershot, 2004; Schröder, 2008). Pb^{2+} not only induces GRP78 expression (Qian et al., 2001; Falahatpisheh et al., 2007; Stacchiotti et al., 2009; Shinkai et al., 2010), but also specifically binds to it (Qian et al., 2000). GRP78 is thought to associate tightly with unfolded, unassembled, or aberrant proteins and maintain them in a correct folding-competent state as they traverse through the ER. Heat shock or Pb^{2+} binding to GRP78 may disassociate GRP78 from unfolded proteins (Qian and Tiffany-Castiglioni, 2003), which leads to the unfolded protein aggregates and stimulates the ER stress. ER stress signals can be transferred from the ER to the nucleus via IRE1, an ER-resident transmembrane kinase. IRE1 mediates a signaling pathway connecting unfolded protein formation in the ER to the nucleus which controls transcription of genes encoding ER-resident proteins (Qian and Tiffany-Castiglioni, 2003).

Recently, more and more evidences indicate that GRP78 plays diverse roles beyond just the endoplasmic reticulum (Quinones et al., 2008; Gonzalez-Gronow et al., 2009; Morito and Nagata, 2012). Studies have shown that GRP78 is expressed on the cell surface in many tissue types, and is involved in transducing signals from ligands as disparate as activated alpha2-macroglobulin and antibodies (Quinones et al.,

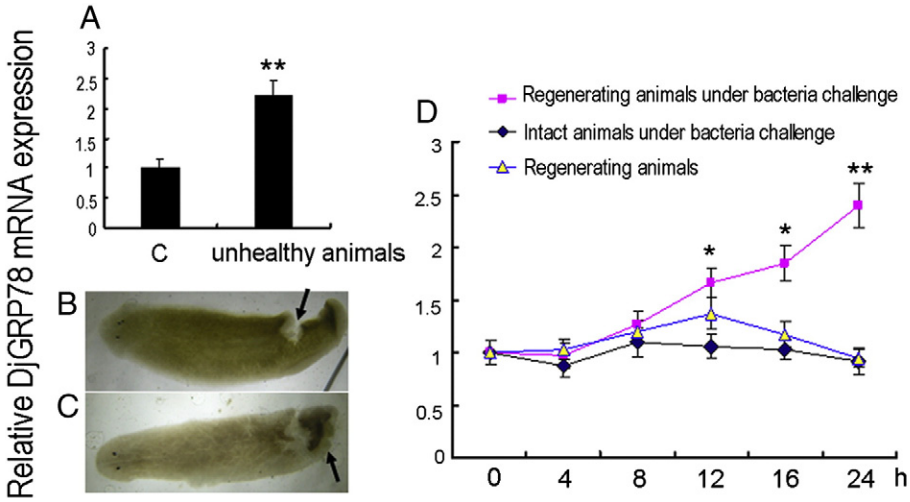

Fig. 6. Expression of *Djgrp78* in response to bacterial challenge. A) Comparison of *Djgrp78* mRNA levels between healthy and unhealthy animals. B) and C) representative images of unhealthy animals with arrows indicating septic tissues. D) Analysis of *Djgrp78* expression at different time points after bacterial challenge. Values are expressed as mean ± S.D. of three independent samples, collected at each experimental condition and analyzed in triplicate. Asterisks indicate significant differences compared to control samples ($^{*}P < 0.05$, $^{**}P < 0.01$).

2008; Gonzalez-Gronow et al., 2009; Zhang et al., 2010; Morito and Nagata, 2012). Luan et al. (2009) reported that the up-regulation of GRP78 was observed in the hepatopancreas and lymphoid organ when shrimps were challenged by white spot syndrome virus, suggesting that GRP78 was involved in shrimp immune reaction. We found that the mRNA levels of DjGRP78 in planarians with septic tissues were approximately 2.2-fold higher than controls (Fig. 6A), and further verified that bacteria can stimulate the up-regulation of *Djgrp78* expression (Fig. 6D). Our data indicate that DjGRP78 may play important roles in physiological and pathological stress in planarians.

In this study, we successfully cloned a full-length *grp78* cDNA from the planarian *D. japonica* for the first time, and analyzed its expression pattern in response to starvation, thermal stress and heavy metal exposure. We also verified that bacteria can stimulate the up-regulation of *Djgrp78* expression. We infer that *Djgrp78* may be a multifunctional gene in planarians.

Acknowledgements

We are grateful to Emily Mills Ko (Immunology Graduate Group, and Department of Neurology at the University of California, Davis) for the critical reading of this manuscript. This work was supported by the National Natural Science Foundation of China (No. 31170357, 30870368, 30670247, 30170119), the Ph.D. Programs Foundation of the Ministry of Education of China (No. 200804760003), the Outstanding Young Scientists Foundation of Henan Province (No. 0312001100), the Innovation Foundation of Henan Province (No. 2005126).

References

Bowen, E.D., Ryder, T.A., Dark, C., 1976. The effects of starvation on the planarian worm *Polycelis tenuis* Iijima. Cell Tissue Res. 169, 193–209.

Conte, M., Isolani, M.E., Deri, P., Mannini, L., Batistoni, R., 2011. Expression of *hsp90* mediates cytoprotective effects in the gastrodermis of planarians. Cell Stress Chaperones 16, 33–39.

Falahatpisheh, H., Nanez, A., Montoya-Durango, D., Qian, Y., Tiffany-Castiglioni, E., Ramos, K.S., 2007. Activation profiles of HSPA5 during the glomerular mesangial cell stress response to chemical injury. Cell Stress Chaperones 12, 209–218.

Giorgi, C., De Stefani, D., Bononi, A., Rizzuto, R., Pinton, P., 2009. Structural and functional link between the mitochondrial network and the endoplasmic reticulum. Int. J. Biochem. Cell Biol. 41, 1817–1827.

González-Estevez, C., 2008. Autophagy in freshwater planarians. Methods Enzymol. 451, 439–465.

Gonzalez-Gronow, M., Selim, M.A., Papalas, J., Pizzo, S.V., 2009. GRP78: a multifunctional receptor on the cell surface. Antioxid. Redox Signal. 11, 2299–2306.

Kaufman, R.J., 1999. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes Dev. 13, 1211–1233.

Kaushik, S., Cuervo, A.M., 2006. Autophagy as a cell-repair mechanism: activation of chaperone-mediated autophagy during oxidative stress. Mol. Asp. Med. 27, 444–454.

Kitamura, M., Hiramatsu, N., 2010. The oxidative stress: endoplasmic reticulum stress axis in cadmium toxicity. Biometals 23, 941–950.

Lee, A.S., 2001. The glucose-regulated proteins: stress induction and clinical application. Trends Biochem. Sci. 26, 504–510.

Lee, M., Choi, I., Park, K., 2002. Activation of stress signaling molecules in bat brain during arousal from hibernation. J. Neurochem. 82, 867–873.

Liu, F., Inageda, K., Nishitai, G., Matsuoka, M., 2006. Cadmium induces the expression of Grp78, an endoplasmic reticulum molecular chaperone, in LLC-PK1 renal epithelial cells. Environ. Health Perspect. 114, 859–864.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-deltadeltaCT method. Methods 25, 402–408.

Luan, W., Li, F., Zhang, J., Wang, B., Xiang, J., 2009. Cloning and expression of glucose regulated protein 78 (GRP78) in *Fenneropenaeus chinensis*. Mol. Biol. Rep. 36, 289–298.

Ma, Y., Hendershot, L.M., 2004. ER chaperone functions during normal and stress conditions. J. Chem. Neuroanat. 28, 51–65.

Ma, K.-X., Chen, G.-W., Lou, H., Fei, L.-N., 2009. Cloning and expression analysis of *hsp70* gene from freshwater planarian *Dugesia japonica*. Biologia 64, 1018–1024.

Ma, K.-X., Chen, G.-W., Liu, D.-Z., 2012. cDNA cloning of heat shock protein 90 gene and protein expression pattern in response to heavy metal exposure and thermal stress in planarian *Dugesia japonica*. Mol. Biol. Rep. 39, 7203–7210.

Mamadu, H., Storey, K.B., 2006. Up-regulation of the endoplastic reticulum molecular chaperone GRP78 during hibernation in thirteen-lined ground squirrels. Mol. Cell. Biochem. 292, 89–98.

Monro, S., Pelham, H.R., 1987. A C-terminal signal prevents secretion of luminal ER proteins. Cell 48, 899–907.

Moore, M.N., Allen, J.I., Somerfield, P.J., 2006. Autophagy: role in surviving environmental stress. Mar. Environ. Res. 62, S420–S425.

Morito, D., Nagata, K., 2012. ER stress proteins in autoimmune and inflammatory diseases. Front. Immunol. 3, 48.

Newmark, P.A., Sánchez Alvarado, A., 2002. Not your father's planarian: a classic model enters the era of functional genomics. Nat. Rev. Genet. 3, 210–219.

Persson, S., Rosenquist, M., Knoblach, B., Khosravi-Far, R., Sommarin, M., Michalak, M., 2005. Diversity of the protein disulfide isomerase family: identification of breast tumor induced Hag2 and Hag3 as novel members of the protein family. Mol. Phylogenet. Evol. 36, 734–744.

Pineda, D., Rossi, L., Batistoni, R., Salvetti, A., Marsal, M., Gremigni, V., Falleni, A., Gonzalez-Linares, J., Deri, P., Saló, E., 2002. The genetic network of prototypic planarian eye regeneration is Pax6 independent. Development 129, 1423–1434.

Pra, D., Lau, A.H., Knakievicz, T., Carneiro, F.R., Erdtmann, B., 2005. Environmental genotoxicity assessment of an urban stream using freshwater planarians. Mutat. Res. 585, 79–85.

Qian, Y., Tiffany-Castiglioni, E., 2003. Lead-induced endoplasmic reticulum (ER) stress responses in the nervous system. Neurochem. Res. 28, 153–162.

Qian, Y., Harris, E.D., Zheng, Y., Tiffany-Castiglioni, E., 2000. Lead targets GRP78, a molecular chaperone, in C6 rat glioma cells. Toxicol. Appl. Pharmacol. 163, 260–266.

Qian, Y., Falahatpisheh, M.H., Zheng, Y., Ramos, K.S., Tiffany-Castiglioni, E., 2001. Induction of 78 kD glucose-regulated protein (GRP78) expression and redox-regulated transcription factor activity by lead and mercury in C6 rat glioma cells. Neurotox. Res. 3, 581–589.

Quinones, Q.J., de Ridder, G.G., Pizzo, S.V., 2008. GRP78: a chaperone with diverse roles beyond the endoplasmic reticulum. Histol. Histopathol. 23, 1409–1416.

Rao, R.V., Peel, A., Logvinova, A., del Rio, G., Hermel, E., Yokota, T., Goldsmith, P.C., Ellerby, L.M., Ellerby, H.M., Bredesen, D.E., 2002. Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. FEBS Lett. 514, 122–128.

Robb, S.M., Ross, E., Sanchez Alvarado, A., 2008. SmedGD: the *Schmidtea mediterranea* genome database. Nucleic Acids Res. 36, D599–D606.

Schröder, M., 2008. Endoplasmic reticulum stress responses. Cell. Mol. Life Sci. 65, 862–894.

Sherman, M.Y., Goldberg, A.L., 2001. Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron 29, 15–32.

Shinkai, Y., Yamamoto, C., Kaji, T., 2010. Lead induces the expression of endoplasmic reticulum chaperones GRP78 and GRP94 in vascular endothelial cells via the JNK-AP-1 pathway. Toxicol. Sci. 114, 378–386.

Shiu, R.P., Pouyssegur, J., Pastan, I., 1977. Glucose depletion accounts for the induction of two transformation-sensitive membrane proteins in Rous sarcoma virus-transformed chick embryo fibroblasts. Proc. Natl. Acad. Sci. U. S. A. 74, 3840–3844.

Stacchiotti, A., Morandini, F., Bettoni, F., Schena, I., Lavazza, A., Grigolato, P.G., Apostoli, P., Rezzani, R., Aleo, M.F., 2009. Stress proteins and oxidative damage in a renal derived cell line exposed to inorganic mercury and lead. Toxicology 264, 215–224.

Stoeckle, M.Y., Sugano, S., Hampe, A., Vashistha, A., Pellman, D., Hanafusa, H., 1988. 78-kilodalton glucose-regulated protein is induced in Rous sarcoma virus-transformed cells independently of glucose deprivation. Mol. Cell. Biol. 8, 2675–2680.

Sun, F.C., Wei, S., Li, C.W., Chang, Y.S., Chao, C.C., Lai, Y.K., 2006. Localization of GRP78 to mitochondria under the unfolded protein response. Biochem. J. 396, 31–39.

Wang, M., Ye, R., Barron, E., Baumeister, P., Mao, C., Luo, S., Fu, Y., Luo, B., Dubeau, L., Hinton, D.R., Lee, A.S., 2010. Essential role of the unfolded protein response regulator GRP78/BiP in protection from neuronal apoptosis. Cell Death Differ. 17, 488–498.

Yu, Z., Luo, H., Fu, W., Mattson, M.P., 1999. The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. Exp. Neurol. 155, 302–314.

Zhang, Y., Liu, R., Ni, M., Gill, P., Lee, A.S., 2010. Cell surface relocalization of the endoplasmic reticulum chaperone and unfolded protein response regulator GRP78/BiP. J. Biol. Chem. 285, 15065–15075.