

Characterization of cyclophilin D in freshwater pearl mussel (*Hyriopsis schlegelii*)

Xiu-Xiu Liu¹, Cheng-Yuan Wang¹, Chun Luo¹, Jun-Qing Sheng¹, Di Wu¹, Bei-Juan Hu¹, Jun-Hua Wang¹, Yi-Jiang Hong^{1,2,*}

¹ School of Life Sciences, Nanchang University, Nanchang Jiangxi 330031, China

² Key Laboratory of Aquatic Animals Resources and Utilization of Jiangxi, Nanchang University, Nanchang Jiangxi 330031, China

ABSTRACT

Cyclophilin D (referred to as *HsCypD*) was obtained from the freshwater pearl mussel (*Hyriopsis schlegelii*). The full-length cDNA was 2 671 bp, encoding a protein consisting of 367 amino acids. *HsCypD* was determined to be a hydrophilic intracellular protein with 10 phosphorylation sites and four tetratricopeptide repeat (TPR) domains, but no signal peptide. The core sequence region YKGCIFHRIKDFMVQGG is highly conserved in vertebrates and invertebrates. Phylogenetic tree analysis indicated that CypD from all species had a common origin, and *HsCypD* had the closest phylogenetic relationship with CypD from *Lottia gigantea*. The constitutive mRNA expression levels of *HsCypD* exhibited tissue-specific patterns, with the highest level detected in the intestines, followed by the gonads, and the lowest expression found in the hemocytes.

Keywords: *Hyriopsis schlegelii*; Cyclophilin D; Sequence analysis

INTRODUCTION

Cyclophilin is a type of intracellular receptor of cyclosporin A (CsA). It has a peptidyl-prolyl cis-trans isomerase (PPIase) region and can be combined with CsA (Feng & Xin, 2013). Widely found *in vivo*, cyclophilins have a conserved structure and biological function. Cyclophilin D (CypD) is a mitochondrial matrix protein, and plays a crucial role in protein folding, cell apoptosis, necrosis, and immunosuppression (Thomas et al., 2012). The CypD protein protect cells from death that is induced by oxidative stress and mediated by mitochondria (Basso et al., 2005). It is a key factor in the regulation of the mitochondrial permeability transition pore (MPTP), which plays a role in the release of cytochrome C and other apoptotic factors from mitochondria during cell apoptosis (Forte & Bernardi, 2006). CypD may also interact with mitochondrial adenine nucleotide transporters (ANT, Halestrap et al., 1998;

Hunter & Haworth, 1979) and promote "open" conformation of ANT (Hunter & Haworth, 1979). Moreover, CypD can suppress apoptosis when it is overexpressed (Li et al., 2004; Lin & Lechleiter, 2002; Schubert & Grimm, 2004).

Cyclophilins from the freshwater pearl mussel (*Hyriopsis schlegelii*) (Wang et al., 2016) are related to cell growth and immunity (Luo et al., 2015; Xie et al., 2011). However, whether CypD from *Hyriopsis schlegelii* (*HsCypD*) has a conserved structure remains unclear and its predicted function has yet to be reported. Here, we describe the predicted cDNA sequence and protein structural features of *HsCypD*.

MATERIALS AND METHODS

Experimental animals

Healthy four-year-old *H. schlegelii* individuals ($n=15$), with shell lengths averaging 150.0 ± 10.4 mm, were obtained from the Fuzhou Hongmen Reservoir Exploitation Corporation, Jiangxi Province. They were kept in aerated freshwater at 23 ± 2 °C for one week before the tissues were harvested.

Total RNA extraction, cDNA synthesis, and cloning

Total RNA extraction was performed using TRIzol Reagent (Invitrogen) per the manufacturer's protocols. After the evaluation of RNA quantity, purity, and integrity, RNA from the gonads was used to prepare cDNA with the SMART RACE Kit (Clontech, USA). A cDNA library for *H. schlegelii* was constructed using the SMART cDNA Library Construction Kit (Clontech, USA). The full-length cDNA of *HsCypD* was cloned

Received: 07 November 2016; Accepted: 07 March 2017

Foundation items: This study was supported by the National Natural Science Foundation of China (31660337), Special Aquatic Products Industry Technology System of Jiangxi (JXARS-10), Scientific and Technological Program of Jiangxi Province (KJLD12001, 20152ACF60013 and 150166), and Natural Science Foundation of Jiangxi Province (20122BAB204016)

*Corresponding author, E-mail: yijianghong@126.com

DOI: 10.24272/j.issn.2095-8137.2017.018

by RACE methods, with gene specific primers (Supplementary Table 1) based on the known EST sequence.

Bioinformatics analysis

The cDNA fragments of *HsCypD* were assembled into complete full-length cDNA. The open reading frame was examined using ORF Finder.

Protein molecular weight, isoelectric point (pI), and amino acid composition were analyzed with the Compute pI/Mw function of the ExPASy-ProtParam tool (<http://web.expasy.org/protparam/>). Protein hydrophobicity was analyzed using ExPASy-ProtScale (<http://web.expasy.org/protscale/>). Protein subcellular localization was predicted with PSORT II (<http://www.genscript.com/tools/psort>). Signal peptides were predicted using SignalP 3.0 (<http://www.cbs.dtu.dk/services/SignalP-3.0/>). The protein folding model was predicted using Predict Protein from Columbia University (<https://www.predictprotein.org/>). The protein domains were predicted using the Conserved Domain Database (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). The three-dimensional structures of the protein sequences were predicted using the ExPASy SWISS-MODEL program (<http://swissmodel.expasy.org/>).

Alignment of the amino acid sequences of *HsCypD*, *HsCypC*, and *HsCypH* with other species was performed using ClustalW Multiple Alignment (<http://www.ebi.ac.uk/clustalw/>). A phylogenetic tree was constructed by neighbor-joining (NJ) using MEGA 4.0 (Tamura et al., 2007) with 1 000 bootstrap replicates, based on amino acids alignment.

mRNA expression analysis by quantitative real-time PCR

Real-time PCR was applied to examine the mRNA levels of *HsCypD* in 10 tissues using primer pairs *CypD-F* and *CypD-R* (Supplementary Table 1). Each assay was performed in triplicate with β -actin as the internal reference. Real-time PCR was performed for each cDNA sample on a Mastercycler ep Realplex2 Real-Time Thermal Cycler (Eppendorf) with SYBR Premix Ex TaqTM (TaKaRa). The $2^{-\Delta\Delta CT}$ method (Schmittgen & Livak, 2008) was used to analyze expression levels. All data were relative mRNAs expressed as mean $\pm$ SD ($n=3$), and subjected to Student's *t*-test.

RESULTS

cDNA sequence of *HsCypD* and predicted protein features

The complete cDNA sequence of *HsCypD* was 2 671 bp in length, containing a 5'-untranslated region (UTR, 80 bp), 3'-UTR (1 487 bp), polyadenylation signal (AATAAA), and unstable signal (ATTTTA). The open reading frame (ORF) was 1 104 bp, encoding 367 amino acids (Figure 1). The sequence was deposited in GenBank under accession number KJ747387. The predicted protein of *HsCypD* had a predicted isoelectric point (pI) of 5.43. The largest portion of the *HsCypD* residues was hydrophobic (131 amino acids), followed by uncharged polar amino acids (103 amino acids) (Supplementary Table 2). The hydrophobicity score of *HsCypD* was highest (1.544) at the 24th and 275th sites and lowest (−2.856) at the 260th site (Supplementary Figure 1). Hydrophobicity of *HsCypD* was not

obvious on the atlas, but the strength of hydrophilicity was clear. From the 330th to 360th residues, there was a small area of hydrophilicity that appeared to be very dense (Supplementary Figure 1). Moreover, the grand average of hydropathicity (GRAVY) of *HsCypD* was −0.703, thus showing obvious hydrophilicity. The proportion of hydrophilic amino acids (about 64%) was much larger than that of hydrophobic amino acids (about 36%) (Supplementary Table 2). Therefore, *HsCypD* was predicted to be a hydrophilic protein.

Protein subcellular localization results showed that 69.6%, 8.7%, and 4.3% of the *HsCypD* protein was distributed in the cytoplasm, cell nucleus, and cell membrane, respectively. The distribution of the protein inside the cell was higher than that outside, indicating an intracellular protein. No obvious signal peptide characteristics were observed at the N-terminal (Supplementary Figure 2A,B).

Eight possible folding patterns of *HsCypD* were identified: a N-glycosylation site at 118–122; casein kinase II (CKII) phosphorylation sites at 78–82, 83–87, 199–203, and 257–261; N-myristoylation sites at 74–80, 177–183, 200–206, and 312–318; protein kinase C (PKC) phosphorylation sites at 99–102, 159–162, and 204–207; a cyclophilin peptidyl-proline cis-trans isomerase signal area at 58–76; CAMP and CGMP dependent protein kinase phosphorylation sites at 237–241 and 267–271; a leucine zipper model at 277–299; and a tyrosine kinase phosphorylation site at 354–362 (Supplementary Table 3). The secondary structures observed not only included α -helices, but also β -pleated sheets, β -turns, and random coils. Among them, α -helices (H) accounted for 37.87%, β -turns (E) accounted for 14.44%, and other structures (L) accounted for the remaining 47.68%. Random coils and α -helices were distributed uniformly throughout the protein; however, the α -helices were more obvious at the C terminal.

The *HsCypD* had four tetratricopeptide repeat (TPR) domains that contained TPR-1 and TPR-11, each having two hits. The final specific hit was for a cyclophilin_ABH_like, cyclophilin A, B, and H-like cyclophilin-type peptidylprolyl cis-trans isomerase (PPIase) domain, representing an archetypal cytosolic cyclophilin similar to human cyclophilin A, B, and H.

The predicted three-dimensional structure was mainly composed of three α -helices, eight β -strands, some β -turns, and random coils. It was barrel-shaped, and the top and bottom were a combination of loops and three α -helices, which connected with both ends of the β -strands. The α -helices were more obvious in the C terminal (Supplementary Figure 3).

Phylogenetic relationship of *HsCypD* to homologs of other species

The *HsCypD* was homologous with *CypD* from other species (Supplementary Table 4), and exhibited the highest homology (61%) with *Lottia gigantea*. Multiple alignments revealed that the signature sequence of the *CypD* family could be identified in *HsCypD* (YKGCIFHRIKDFMVQGG), and that the residues involved in CsA binding and PPIase activities were well conserved (Figure 2).

Two major branches of vertebrates and invertebrates were classified on the *CypD* phylogenetic tree. *HsCypD* was found

on the same branch as *Lottia gigantea*. *Homo sapiens* and *Gorilla gorilla* belonged to the same branch, and were the most distant from HsCypD (Figure 3). Furthermore, the phylogenetic tree based on CypD, CypC, and CypH sequences showed that HsCypH and CypDs from most species were grouped together. In particular, the CypC family and most of the CypD family were grouped together on a large branch. CypD from *Lottia gigantea* belonged to a lineage near the CypH group and CypH from *Oryctolagus cuniculus* belonged to a lineage near the CypC

group (Supplementary Figure 4).

Tissue expression profile of HsCypD

The constitutive mRNA expression level of *HsCypD* was examined in 10 different tissues, including hemocytes, gill, mantle, kidney, heart, intestine, hepatopancreas, adductor muscle, gonad, and foot. The highest expression of *HsCypD* was detected in the intestine, with remarkable tissue expression patterns (Figure 4).

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1                               GTGACTCAGATCAAATACCG
21      GCACAATGGCGTCTGTAGAAAATCCGCGTGTCTTCTTTGACGTAGAAATAGGTGGAGACA
81      ATGGCGTCTGTAGAAAATCCGCGTGTCTTCTTTGACGTAGAAATAGGTGGAGACAATGTT
1      M A S V E N P R V F F D V E I G G D N V
141     GGTTCGATTGTCTTTGAGTTGTTCAAAGACAAAGTTCCAAAGACGGCAGAGAATTTCCGA
21      G R I V F E L F K D K V P K T A E N F R
201     GCTCTGTGTACAGGAGAGAAAGGAGAGGGAAAATTTGGCAAGCCATTACATTACAAAGGA
41      A L C T G E K G E G K F G K P L H Y K G
261     TGCATTTTCCACAGGATTATCAAAGACTTCATGGTGCAGGAGGAGATTTCACCAATGCT
61      C I F H R I I K D F M V Q G G D F T N A
321     GATGGCACTGGAGGAGAAAGCATCTATGGAGAAAAATTTGAGGATGAAGACTTCACGCAA
81      D G T G G E S I Y G E K F E D E D F T Q
381     AAACATGATGTCCTGGCTTACTTAGTATGGCCAATGCTGGTCCAAACACAAATGGTTCC
101     K H D V P G L L S M A N A G P N T N G S
441     CAGTTCTTCATTACAACAGTTCCAACCTCCACCTAGATGGGAAACACGTGGTCTTTGGA
121     Q F F I T T V P T P H L D G K H V V F G
501     AGGGTCTGAAGGGCATGGATGTTGTTAGAACTCTTGAGAATGAGGAAGTCAAAAGTGAA
141     R V L K G M D V V R T L E N E E V K S E
561     AAGCCTGTGAAGGAATGCAAAATCGTTGATTGTGGGGAATTCCTCCAGGGGCTGATGAT
161     K P V K E C K I V D C G E I P P G A D D
621     GGTGTACCAAGTGGACGATGGAACAGGTGACAAATATGCTGAAAATCCTGATGATTCTGGT
181     G V P V D D G T G D K Y A E N P D D S G
681     CTTGACTTTACTGATAAAAACAGTGTCAACAGTGAATCAATGTTATTGATGAGATACGC
201     L D F T D K N S V N S V I N V I D E I R
741     AAGATAGGAAATAATTTGTTTAAAGACAAGGAAGTATTGAAGGCTAAGAAAAAGTATTCA
221     K I G N N L F K D K E V L K A K K K Y S
801     AAGGCACTCAGATATGTTGAGAAATGAAAAATGATTGGAACTTGGTTCAGATGAAGAG
241     K A L R Y V E K L K N D L E L G S D E E
861     GATGATGATGAGATAGATAAGAAACAGACATTGTCAATTACCTTAATTTAGCTGCTTGC
261     D D D E I D K K Q T L S I Y L N L A A C
921     AACATACAGCTACAGAAATATGATGAGGCTTTAAACATTGTAACAAGGCTCTAGATATT
281     N I Q L Q K Y D E A L K H C N K A L D I
981     GATGAGAAGATGTGAAGCTTTGTTTCAGACGAGGCCAGACCATAATGCAATGAAGAAC
301     D E K N V K A L F R R G Q T H N A M K N
1041    TGGGAAGAGGCATTGGGAGATCTTCAGAGAGCAGCAGAACTAGAACCAATGACAAGGCT
321    W E E A L G D L Q R A A E L E P N D K G
1101    ATAAGAAAGGAGAAAGTGAAGGAGGAGAGATGCTTACAAAACCTGAAGAGAAAAACTA
341    I R K E K V K K A R D A Y K T E E K K L
1161    TATGCTAAAATGTTCTCAGCATGATAGTGCCATTGTGCTACTAATCATTATTCTCTCATG
361    Y A K M F S A *
1221    TATATGTATATGTGGTTAGTTTTTATATGTAAGCAACTGTGAATTTATTGCATTTCTCGT
1281    ACTGTCATTTATCATTATTTTGTAAACGTCATGATCATTGTTTACCAGCAATTTATTCAT
1341    TTATAGACATATATTATGGACCAGGTTTTCTGCTGCCACTATAATGAAGTACTTTGTCCA
1401    TATATATATATATACAAAAATAACTTGCTCTCAGTAGATAATTTCTGGAATGCCAAGA

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1461 GCTGACTCTTCTACCTGACTCTTGCATTAGCAAATGTAAATGCTTGCCTTAATGGTAC
 1521 AAAGATGTCCAGATTAACATATCAGATGTAAGTTTGTCTGAGCCACCCTGAGACTTTC
 1581 CTTCTCGGTACAAGAATATCCAATCTGAAAAATTATTAGTAATTTGTTTTAAACCACAT
 1641 TTGAGAACTACATTGATGTAGGAAATGGGTCAACTCTAAGTTCTTGCAAACAGTAGTT
 1701 GTAGGAACAACAAATAATCTGGTAATAAATATTATTTGTCTGAATTTAGTGAATAATG
 1761 TGTGGTGTGTTTGGTGAACCTCGGCAGCCTGTTGAGTGTACATGCATTTCACCTATTTC
 1821 TTTAATGATCCAAGGATTGGGAAAAATTTATTTTGTCTGAATAGCTTCTTAGGCATTTT
 1881 CTATATTATGTTTTGTTTGAATCCGAAGAATACCTGTAAGTCTCTGTCCAGTCCCAA
 1941 TTTACAGTCTTGTAAATAATGCTTATTAATGTGAAGAGTGCCTCAATGGCCTCTTGAT
 2001 CCAAACCTTTTGTGAGTCTTGCAAATCACCTCAAATTAAGTGTGAAGGATTATTGTTAAC
 2061 AATACTACTAGCATGGAATGTTAGAAAGATGCAAGCTATCCCAGTGAAAAATATCGGCTT
 2121 TTGTGACAATATTCAAGTATGAAAACCATCATTAGCAAAAGCGTAAATATGATGGGATTG
 2181 ATCAGTACGTGCTAATAGCACTGATGTAGAGTTATCCAGGAAAGGAGTTTGTACTATGGG
 2241 AGTGTTTTGGAAAAATGTCGGTAAATAACACAGCGAACTGTAAGTGTGATGTCATTGAT
 2301 GATATTTTCTTACCAGATAATATCTCGCTTGTCTGAGTTTGAATGTGATAAGTAAATGA
 2361 GAGCATTCTGGTGATAATTATTTGGTCTTCTTAATATAAGAAGTTGCCAATACTGTCC
 2421 ATTCAAAAATTAGAACATTAATCGGTTGACAATCTTTTATTAGTAGGAAAAATGTTTA
 2481 GGTTTTCTACTGTAGAACTGATAAGATATTTCTTGGGAATGTTGAGATCATTGTGGCAT
 2541 AAATGTGTATTGACACATTAATCATTAAAGTTGATGTAATGTATGAAAAACGAATTTT
 2601 ATGTAATGGTATGCTGTCCATTTATAATAAATCTGAAAAAAAAAAAAAAAAAAAAA
 2661 AAAAAAAAAA

Figure 1 cDNA and deduced amino acid sequence of HsCypD

*: Stop codon. Initiation codon (ATG), stop codon (TGA), unstable signal ATTTTGA, poly(A), and poly adenosine signal (AATAAA) are in bold. Conserved amino acid residues are shadowed. Framed area is proline cis-trans isomerase signal area.

<i>Oncorhynchus mykiss</i>	TTGKPLHFKGCPFHRIIKQFMIQGGDFSNQNGTGEGEYGEKFEDEFHYKHDKEGLLSM
<i>Salmo salar</i>	TTGKPLHFKGCPFHRIIKQFMIQGGDFSNQNGTGGESEYGEKFEDEFHYKHDKEGLLSM
<i>Danio rerio</i>	STGKPLHFKGCPFHRIIKSFMIQGGDFSNQNGTGGESEYGDKEFEDEFHYKHDREGLLSM
<i>Zonotrichia albicollis</i>	TTGKPLHYKGC PFHRIIKQFMIQGGDFSNQNGTGGESEYGEKFEDEFHYQHDKPGLLSM
<i>Taeniopygia guttata</i>	TTGKPLHYKGC PFHRIIKQFMIQGGDFSNQNGTGGESEYGEKFEDEFHYKHDKPGLLSM
<i>Gallus gallus</i>	TTGKPLHYKGC PFHRIIKQFMIQGGDFSNQNGTGGESEYGEKFEDEFHYKHDKPGLLSM
<i>Homo sapiens</i>	TTGKPLHFKGCPFHRIIKKFMQGGDFSNQNGTGGESEYGEKFEDEFHYKHDREGLLSM
<i>Gorilla gorilla</i>	TTGKPLHFKGCPFHRIIKKFMQGGDFSNQNGTGGESEYGEKFEDEFHYKHDQEGLLSM
<i>Mus musculus</i>	TTGKPLHFKGCPFHRIIKKFMQGGDFSNQNGTGGESEYGEKFEDEFHYKHDREGLLSM
<i>Ochotona princeps</i>	TTGKPLHFKGCPFHRIIKKFMQGGDFSNQNGTGGESEYGEKFEDEFHYKHDREGLLSM
<i>Canis lupus familiaris</i>	TTGKPLHFKGCPFHRIIKKFMQGGDFSNQNGTGGESEYGEKFEDEFHYKHDREGLLSM
<i>Sus scrofa</i>	TTGKPLHFKGCPFHRIIKKFMQGGDFSNQNGTGGESEYGEKFEDEFHYKHDKEGLLSM
<i>Xenopus laevis</i>	STGKPLHFKGCPFHRIIKKFMQGGDFSNQNGTGGESEYGEKFEDEFHYKHDKEGLLSM
<i>Branchiostoma floridae</i>	TTGKPLHFKGCPFHRIIKDFMIQGGDFSNQNGTGGESEYGEKFEDEFHYKHDKEGLLSM
<i>Hyriopsis schlegelii</i>	-FGKPLHYKGCIFHRIIKDFMIQGGDFSNQNGTGGESEYGEKFEDEFYKHDVPGLLSM
<i>Lottia gigantea</i>	-SGKPLHYKGC PFHRIIKDFMIQGGDFTAGDG TGGESEYGEKFEDEFHYKHERPGLLSM
<i>Crassostrea gigas</i>	STGCPLHFKKCPFHRIIKDFMIQGGDFSNKNGTGGESEYGEKFEDEGFYTHDKPGLLSM

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Figure 2 Identification of a highly-conserved region across CypD amino acid sequences and their homologs

Sequences were aligned using ClustalW alignment. (*) single fully-conserved residue; (:) conservation of strong group; (.) conservation of weak group. Boxes show the well-conserved amino acid regions (18 aa residues) of all analyzed CypDs. The sequences used in the analyses are listed in Supplementary Table 4.

DISCUSSION

HsCypD was identified and characterized in the freshwater pearl mussel *Hyriopsis schlegelii*. It comprised a cyclophilin-type peptidyl-prolyl cis-trans isomerase (PPIase) region, four

significant TPR domains, and specific tertiary structure, as shared by other Cyps families (Blackburn et al., 2015; Ottiger et al., 1997; Wang et al., 2009). The PPIase region is highly-conserved in cyclophilins in vertebrates and invertebrates (Wang et al., 2009). The TPR domain connects to the single N-terminal cyclophilin domain of Cyp40 by a 30-amino acid linker

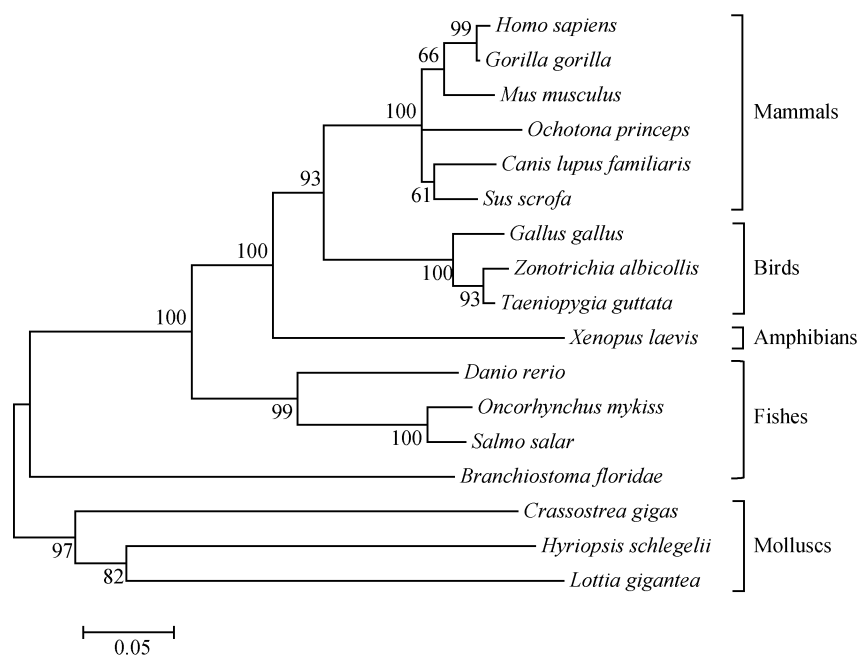


Figure 3 Phylogenetic tree (neighbor-joining) of CypD sequences including *Hyriopsis schlegelii* and 16 other species constructed using MEGA 4.0

The values at the nodes indicate the percentage of trees in which this grouping occurred after bootstrapping (1 000 replicates; shown only when >60%). The sequences used in the analyses are listed in Supplementary Table 4.

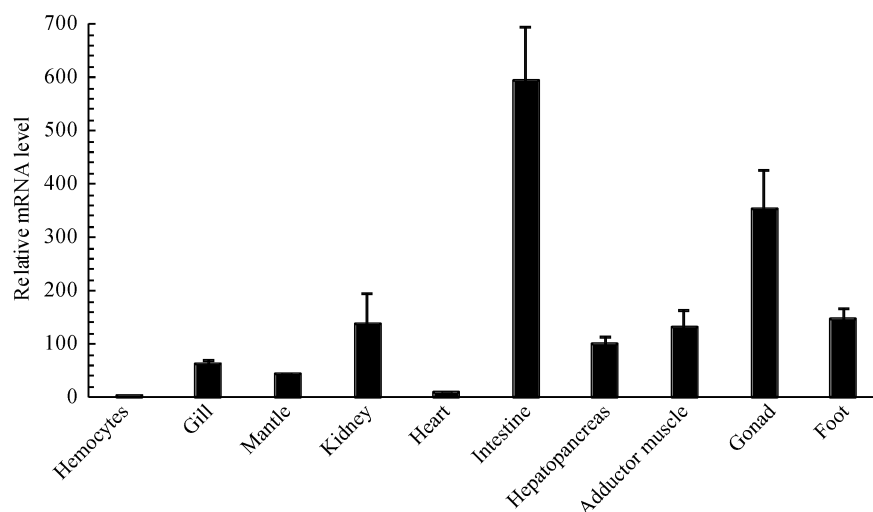


Figure 4 *HsCypD* mRNA expression levels in different tissues of *H. schlegelii*

HsCypD transcript levels in hemocytes, gill, mantle, kidney, heart, intestine, hepatopancreas, adductor muscle, gonad, and foot were normalized to that of hemocytes. Three individuals were used in each experiment for tissue collection and the experiment was repeated three times. Vertical bars represent mean $\pm$ SD ($n=3$).

(Taylor et al., 2001). Similar to the tertiary structure of human CypA and CypB (Mikol et al., 1994; Ottiger et al., 1997), *HsCypD* exhibited a right-handed barrel structure formed by eight β -strands. The top and bottom of this structure combined loops and three α -helices connected with both ends of the β -strands. *HsCypD* showed high conservativeness in these

domains and tertiary structure.

The phylogenetic tree confirmed that *HsCypD* was more distantly related to CypDs from vertebrates than from invertebrates, similar to the evolutionary structure of CypA from *V. philippinarum* (Chen et al., 2011). Generally, the same types of cyclophilins (e.g., CypA and CypD) but isolated from different

species are more closely related to each other than different types of cyclophilins from the same species (Lee et al., 2002), although there are exceptions (Chen et al., 2011). In this study, HsCypH belonged to a lineage near the CypD group on the phylogenetic tree, with CypD from *L. gigantea* grouped with CypH and CypH from *O. cuniculus* grouped with CypC (Supplementary Figure 4). These findings demonstrate the close phylogenetic relationship of cyclophilins and suggest that this family likely has a common origin and is highly conserved.

N-myristoylation is a lipid anchor modification of some proteins targeting them to membrane locations, thus transforming the function of the modified proteins, and plays a significant role in many cellular pathways, such as apoptosis, signal transduction, and alternative extracellular protein export (Borgese et al., 1996; Maurer-Stroh et al., 2002). PKC is an important neurotransmitter in intracellular signal transduction and participates in transmembrane signaling (Nishizuka, 1984). Another protein kinase, CKII, a highly conserved serine/threonine kinase of eukaryotic cells, is responsible for responding to growth factors (Marais et al., 1992). Tyrosine kinase is a key molecule in signal transduction and growth control (Cheng et al., 1993). The TPR domain can bind competitively to Hsp90 or Hsp70 and thus serve as co-chaperones (Young et al., 1998). The predicted HsCypD possessed these binding sites and domains. Thus, we speculated that HsCypD might have the ability to anchor to membranes, and might be involved in specific transfer processes of signal transduction and growth of cells, as well as performing as a chaperone.

Cyps are widely distributed in various tissues (Danielson et al., 1988; Qiu et al., 2009). The high expression of Cyps in tissues is related to certain functional mechanisms (Qiu et al., 2009; Watashi et al., 2005). In this study, the highest mRNA expression level of *HsCypD* was detected in the intestine. We speculated that HsCypD was very active in the intestine and might be involved in specific transfer processes of signal transduction and cytoprotection (Hausenloy et al., 2012; Tavecchio et al., 2013). Further systematic research is currently underway to characterize the functions and regulatory mechanisms of HsCypD.

REFERENCES

- Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. 2005. Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *Journal of Biological Chemistry*, **280**(19): 18558-18561.
- Blackburn EA, Wear MA, Landré V, Narayan V, Ning J, Erman B, Ball KL, Walkinshaw MD. 2015. Cyclophilin40 isomerase activity is regulated by a temperature-dependent allosteric interaction with Hsp90. *Bioscience Reports*, **35**(5): e00258.
- Borgese N, Aggujaro D, Carrera P, Pietrini G, Bassetti M. 1996. A role for N-myristoylation in protein targeting: NADH-cytochrome b5 reductase requires myristic acid for association with outer mitochondrial but not ER membranes. *The Journal of Cell Biology*, **135**(6): 1501-1513.
- Chen LL, Mu CK, Zhao JM, Wang CL. 2011. Molecular cloning and characterization of two isoforms of cyclophilin A gene from *Venerupis philippinarum*. *Fish & Shellfish Immunology*, **31**(6): 1218-1223.
- Cheng HC, Matsuura I, Wang JH. 1993. *In vitro* substrate specificity of protein tyrosine kinases. In: Khandelwal RL, Wang JH. Reversible Protein Phosphorylation in Cell Regulation. US: Springer, 103-112.
- Danielson PE, Forss-Petter S, Brow MA, Calavetta L, Douglass J, Milner RJ, Sutcliffe JG. 1988. pI B15: a cDNA clone of the rat mRNA encoding cyclophilin. *DNA*, **7**(4): 261-267.
- Feng WH, Xin Y. 2013. Progress in research on cyclophilin A and malignant tumors. *Tumor*, **33**(10): 928-934. (in Chinese)
- Forte M, Bernardi P. 2006. The permeability transition and BCL-2 family proteins in apoptosis: co-conspirators or independent agents? *Cell Death & Differentiation*, **13**(8): 1287-1290.
- Halestrap AP, Kerr PM, Javadov S, Woodfield KY. 1998. Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, **1366**(1-2): 79-94.
- Hausenloy DJ, Boston-Griffiths EA, Yellon DM. 2012. Cyclosporin A and cardioprotection: from investigative tool to therapeutic agent. *British Journal of Pharmacology*, **165**(5): 1235-1245.
- Hunter DR, Haworth RA. 1979. The Ca²⁺-induced membrane transition in mitochondria: I. The protective mechanisms. *Archives of Biochemistry and Biophysics*, **195**(2): 453-459.
- Kyte J, Doolittle RF. 1982. A simple method for displaying the hydropathic character of a protein. *Journal of Molecular Biology*, **157**(1): 105-132.
- Lee YK, Hong CB, Suh Y, Lee IK. 2002. A cDNA clone for cyclophilin from *Griffithsia japonica* and phylogenetic analysis of cyclophilins. *Molecules and Cells*, **13**(1): 12-20.
- Li YM, Johnson N, Capano M, Edwards M, Crompton M. 2004. Cyclophilin-D promotes the mitochondrial permeability transition but has opposite effects on apoptosis and necrosis. *Biochemical Journal*, **383**(1): 101-109.
- Lin DT, Lechleiter JD. 2002. Mitochondrial targeted cyclophilin D protects cells from cell death by peptidyl prolyl isomerization. *Journal of Biological Chemistry*, **277**(34): 31134-31141.
- Luo C, Xu L, He J, Shi JW, Sheng JQ, Peng K, Wang JH, Huang B, Hong YJ. 2015. The expression of cyclophilin A (CyPA) in *Hyriopsis schlegelii* in response to pathogens and its effect on the growth of hela cells. *Acta Hydrobiologica Sinica*, **39**(3): 475-481. (in Chinese)
- Marais RM, Hsuan JJ, McGuigan C, Wynne J, Treisman R. 1992. Casein kinase II phosphorylation increases the rate of serum response factor-binding site exchange. *The EMBO Journal*, **11**(1): 97-105.
- Maurer-Stroh S, Eisenhaber B, Eisenhaber F. 2002. N-terminal N-myristoylation of proteins: refinement of the sequence motif and its taxon-specific differences. *Journal of Molecular Biology*, **317**(4): 523-540.
- Mikol V, Kallen J, Walkinshaw MD. 1994. X-ray structure of a cyclophilin B/cyclosporin complex: comparison with cyclophilin A and delineation of its calcineurin-binding domain. *Proceedings of the National Academy of Sciences of the United States of America*, **91**(11): 5183-5186.
- Nishizuka Y. 1984. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature*, **308**(5961): 693-698.
- Ottiger M, Zerbe O, Güntert P, Wüthrich K. 1997. The NMR solution conformation of unligated human cyclophilin A. *Journal of Molecular Biology*, **272**(1): 64-81.
- Qiu LH, Jiang SG, Huang JH, Wang WF, Zhu CY, Su TF. 2009. Molecular cloning and mRNA expression of cyclophilin A gene in black tiger shrimp (*Penaeus monodon*). *Fish & Shellfish Immunology*, **26**(1): 115-121.

- Schmittgen TD, Livak KJ. 2008. Analyzing real-time PCR data by the comparative C_T method. *Nature Protocols*, **3**(6): 1101-1108.
- Schubert A, Grimm S. 2004. Cyclophilin D, a component of the permeability transition-pore, is an apoptosis repressor. *Cancer Research*, **64**(1): 85-93.
- Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**(8): 1596-1599.
- Tavecchio M, Lisanti S, Lam A, Ghosh JC, Martin NM, O'Connell M, Weeraratna AT, Kossenkov AV, Showe LC, Altieri DC. 2013. Cyclophilin D extramitochondrial signaling controls cell cycle progression and chemokine-directed cell motility. *Journal of Biological Chemistry*, **288**(8): 5553-5561.
- Taylor P, Dornan J, Carrello A, Minchin RF, Ratajczak T, Walkinshaw MD. 2001. Two structures of cyclophilin 40: folding and fidelity in the TPR domains. *Structure*, **9**(5): 431-438.
- Thomas B, Banerjee R, Starkova NN, Zhang SF, Calingasan NY, Yang LC, Wille E, Lorenzo BJ, Ho DJ, Beal MF, Starkov A. 2012. Mitochondrial permeability transition pore component cyclophilin D distinguishes nigrostriatal dopaminergic death paradigms in the MPTP mouse model of Parkinson's disease. *Antioxidants & Redox Signaling*, **16**(9): 855-868.
- Wang CY, Sheng JQ, Hong YJ, Peng K, Wang JH, Wu D, Shi JW, Hu BJ. 2016. Molecular characterization and expression of metallothionein from freshwater pearl mussel, *Hyriopsis schlegelii*. *Bioscience, Biotechnology, and Biochemistry*, **80**(7): 1327-1335.
- Wang QF, Wang FQ, Ge HL. 2009. The progress of cyclophilin A in cancer research. *International Journal of Immunology*, **32**(2): 99-103. (in Chinese)
- Watashi K, Ishii N, Hijikata M, Inoue D, Murata T, Miyanari Y, Shimotohno K. 2005. Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. *Molecular Cell*, **19**(1): 111-122.
- Xie K, Xu L, Sheng JQ, Zeng LG, Wang JH, Hong YJ. 2011. The full-length cDNA library of hemocyte induced by *Aeromonas hydrophila* and molecular characteristics of cyclophilin a from *Hyriopsis schlegelii*. *Acta Hydrobiologica Sinica*, **35**(5): 783-789. (in Chinese)
- Young JC, Obermann WMJ, Hartl FU. 1998. Specific binding of tetratricopeptide repeat proteins to the C-terminal 12-kDa domain of hsp90. *Journal of Biological Chemistry*, **273**(29): 18007-18010.