蚤状溞(Daphnia pulex)热休克蛋白 Hsp90 基因的克 降与表达分析^{*}

 陈 苹¹ 邱成功¹ 邹 秀¹ 周健恺¹ 徐善良¹ 王春琳¹

 王丹丽¹ 赵云龙²

(1. 宁波大学海洋学院 宁波 315211; 2. 华东师范大学生命科学学院 上海 200062)

提要 利用 RACE 技术从蚤状溞(*Daphnia pulex*)中克隆到 Hsp90 基因 cDNA 全长为 2568bp, 开放 阅读框为 2155bp, 编码 718 个氨基酸残基, Hsp90 蛋白中存在 GxxGxG、LxxLL 模块(亮氨酸拉链)和 C 末端的 MEEVD 序列。同源性比对结果显示蚤状溞 Hsp90 基因与日本对虾和刀额新对虾的同源性 最高为 85%, 与其它甲壳纲物种的同源性保持在 79%及以上。进化分析发现, 蚤状溞 Hsp90 基因与 剑水蚤、日本沼虾、红螯相手蟹等甲壳纲的亲缘关系最近。用 Real Time PCR 技术, 检测了 Hsp90 mRNA 在蚤状溞不同生殖状态下的表达水平: Hsp90 mRNA 在两性溞(带冬卵)中的表达量明显高于孤 雌溞(带夏卵)(*P*<0.05), 且在冬卵中的表达量最低。推测 Hsp90 可能参与了蚤状溞的生殖转化调控。 Hsp90 mRNA 在雄溞中的表达量是孤雌溞的 2.4 倍, 说明 Hsp90 可能参与了精子的形成过程。 关键词 蚤状溞; Hsp90; Real time-PCR; 生殖转化

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蚤状溞(Daphnia pulex)为一种常见的小型浮游动物,属节肢动物门(Arthropoda)、甲壳纲(Crustacea)、鳃足亚纲(Branchiopoda)、枝角亚目(Cladocera)。枝角类营养丰富,易培养,繁殖周期短且繁殖量大,是许多水生动物的优良天然活饵料。枝角类有两种生殖方式,即孤雌生殖(parthenogenesis)和两性生殖(sexual reproduction)。外界条件适宜时枝角类行孤雌生殖(无性生殖),当外界环境条件恶化时行两性生殖(有性生殖)。孤雌生殖有助于其种群的迅速发展,而两性生殖形成的休眠卵(冬卵)能确保其渡过恶劣环境,以维持种群的存在与延续,但种群数量会在短期内迅速下降(Jiang et al, 1979)。蚤状溞等枝角类能灵敏准确的判断外界环境的优良,选择适应的生殖状态,从而避免种群灭绝的危机。

热休克蛋白(heat shock protein, Hsp)是一类在生物进化过程中高度保守并广泛存在于原核及真核生

物中的蛋白。它是机体在应激情况下细胞内迅速合成 的一组蛋白质,由 Ritassa(1964)在 1962 年首次从果 蝇中发现。到了 1974 年, Tisseres 等(1974)发现热休 克反应中可以转录合成一组特殊蛋白,而且伴随着 这类蛋白的合成,细胞的其它蛋白合成却受到抑制, 而且昆虫体内热激蛋白的表达量越高,其耐热性就 越强(Le et al, 2001; Murphy et al, 2003)。除了高热之 外,多种应激原如重金属、饥饿、缺氧、缺血等都可 以诱导 Hsp 的表达。根据分子重量和同源程度, 热休 克蛋白分为 Hsp90s (83—99kDa)、Hsp70s (68— 80kDa)、Hsp60s, 以及小 Hsp (25-28kDa) (Lindquist et al, 1988), 其中 Hsp90 家族是较为重要的一族, 目 前研究较多(徐明波等, 1991)。Hsp90 是一种 ATP 依 赖的伴侣蛋白,是真核生物中含量最丰富的胞质蛋 白。Hsp90 广泛参与细胞的信号转导、激素应答及转 录调控过程,对细胞在生理、病理及应激条件下的生

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存发挥了重要作用。在细胞发生应激反应时, Hsp90 可以和那些由于环境刺激而使自身构象发生改变的 蛋白相互作用、保证蛋白进行适当的折叠并防止蛋 白非特异性聚集,所以Hsp90有可能通过环境的改变 参与了枝角类的生殖转化调控。罗文等(2012)也推测 到一些化学感应基因(CSP)和热激蛋白(HSP)基因在 枝角类孤雌生殖中起到非常重要的作用。目前已经有 多种甲壳纲动物包括虾(Wu et al, 2008)、龙虾(Chang et al, 1999; Spees et al, 2002; Chang, 2005)、水蚤(Bond et al, 1993; Kotov et al, 2006; Soetaert et al, 2006)、陆 生十足类(Gusev et al, 2006)对 Hsp90 基因进行研究, 而且有研究报道表明 Hsp90 参与甲壳纲的生理发育 调解,如对刀额新对虾(Metapenaeus ensis)Hsp90 的 研究显示 Hsp90 能调解雌性激素的信号转导,进而调 解卵黄蛋白原的合成(Wu et al, 2008)。对美洲海螯虾 (Homarus americanus)进行渗透胁迫研究,发现 Hsp90 mRNA 在腹部肌肉中的表达水平显著增加 (Spees et al, 2002); 对大型溞(Daphnia magna)的研究 显示 Hsp90 是幼体发育的上调基因, 在大型溞的发育 中起到积极作用(Soetaert et al, 2006)。但是人们对于 蚤状溞 Hsp90 基因知之甚少。本研究以蚤状溞为研究 对象,采用同源克隆和RACE-PCR的方法,克隆了蚤 状溞 Hsp90 的 cDNA 全长,利用 Real-Time PCR 技术 分析了 Hsp90 mRNA 在蚤状溞不同生殖状态下的表 达水平, 旨在探索枝角类生殖转化的规律和分子机 理。

1 材料与方法

1.1 实验材料

试验用蚤状溞由本实验室单克隆培养获得,体 长(3.2±0.8)mm。挑选健康活力强的个体暂养于实验 室玻璃培养箱内,培养温度(25±1)°C,光周期为 14h 光照和 10h 黑暗。培养于"Banta 粪土培养液"(1.5g 兔子粪 + 2g 干稻草 + 10g 土壤 + 950mL 自来水经 煮沸后取上清液),并交叉投喂小球藻等单细胞藻类, 每天投喂饵料 1 次。雄溞的获得:培养两周后,当溞 密度达到 3000 只/L 以上时,缸内溞的生殖方式逐渐 转为有性生殖,雄溞出现。

提取总 RNA 的试剂盒为 Axygen 公司产品。反 转录试剂盒(PrimeScript RT Master Mix Perfect Real Time Kit)为 TaKaRa 公司; Realtime-PCR 采用 TaKaRa 公司的 *Premix Ex Taq*TM Hot Start Version 试剂盒,逆 转录酶及其它主要试剂为 TaKaRa 公司产品。 3'RACE、5'RACE PCR 扩增使用 Clontech 公司的 SMARTer[™] RACE cDNA Amplification 试剂盒。PCR 的扩增引物由上海桑尼生物公司合成, PCR 产物由上 海 Invitrogen 生物公司克隆测序。

1.2 引物设计

从 GenBank 中获得已注册的迷糊酢蚤(Daphnia ambigua, ABI35828.1)、山形水蚤(Daphnia sp. Yamagata, ABI35815.1)、光滑水蚤(Daphnia laevis, ABI35823.1) 和纹水蚤(Daphnia dubia, ABI35824.1)基因, 根据此 序列, 运用 Vector NTI 11.0 软件找到 Hsp90 基因的保 守区域,在保守区域内设计出蚤状溞 Hsp90 基因的兼 并引物 Hsp90-F/R, 进行 PCR 扩增, 获得一条长约 250bp 核心的片段, 再根据此部分片段设计 5'RACE 和 3'RACE 特异性引物 HSP-5'RACE 和 HSP-3'RACE 扩增出DpHsp90全长cDNA序列,进一步设计出实时 荧光定量 PCR 引物 Hsp90-YF 和 Hsp90-YR。基于蚤 状溞内参基因 18S(AF014011.1)的 cDNA 序列设计内 参基因序列引物 18S-F 和 18S-R, 用 1 对内参基因引 物 18S-F/R 和 1 对特异性引物 Hsp90-YF/YR 来检测 蚤状溞不同生殖状态下 Hsp90 mRNA 的表达情况(引 物见表 1)。

表 1 试验中用到的引物名称及序列 Tab.1 Oligonucleotide primers used in the experiments

6	1 1
Primer name	Primer sequence $(5'-3')$
HSP-F	ACCCYAGCAAGTTGGACAG
HSP-R	AACCAGGTADGCMGAGTAGAA
HSP-5'RACE	CCAGGTAGGGAGAGTAGAAACCCACAC
HSP-3'RACE1	TGTCCCCAACAAGAATGACCGTACC
HSP-3'RACE2	ATCTGGACCCGCAATCCCGATGAC
HSP CDS 验证 F	AAATGGAAGCCGAAGCCGAG
HSP CDS 验证 R	GGGAGCAACGTAATGAAGTGAAG
Hsp90-YF	CGACGATCAAGAGCCCAATAAG
Hsp90-YR	TGAACCCGAAGCAGTTTGTGTG
18S-F	TTCACGGGTTGGTTGCCTG
18S-R	CGACGACCGAATAACAATAGAGC

M=A or C, K= G or T, R=A or G

1.3 蚤状溞总 RNA 的提取及 cDNA 第一链的合成

采用 RNA 提取试剂盒(RNA Extraction Kit, Axygen)分别提取孤雌幼溞、孤雌溞(带夏卵)、两性溞 (带冬卵)、雄溞和休眠卵的总 RNA。用反转录试剂盒 (PrimeScript RT Master Mix Perfect Real Time Kit)进 行上述 RNA 反转录以得到 cDNA。37°C 反转录 15min, 85°C 5s 灭活反转录酶。合成的 cDNA 产物于 -20°C保存或直接用于 PCR。

1.4 Hsp90 基因 cDNA 片段的克隆

以蚤状溞两性溞(带冬卵)cDNA 为模板,采用兼 并引物 HSP-F 和 HSP-R 进行 PCR 扩增, PCR 反应体系 为 50μL,优化后扩增条件:94°C 预变性 3min;94°C 变 性 30s,53°C 退火 30s,72°C 延伸 1min,35 个循环;72°C 延伸 10min;4°C 保存。PCR 产物于 1.5%琼脂糖凝胶 电泳检测,送上海 Invitrogen 生物公司克隆测序。

1.5 Hsp90 基因 cDNA 全长序列的获得

测序结果在 NCBI 数据库(http://www.ncbi. nlm.nih.gov)中进行 BlastX 分析比对,以上实验所得 Hsp90 部分序列与其它物种的 Hsp90 同源,则根据测 得的序列再设计 5'RACE 和 3'RACE 特异性引物 HSP-5'RACE 和 HSP-3'RACE 用于 RACE 扩增。采用 SMART[™] RACE cDNA 试剂盒(Clontech)进行 5' RACE 和 3' RACE 获得全长 cDNA。

1.6 生物信息学分析

用 Protparam 软件 (http://au.expasy.org/tools/ protparam.html)进行蛋白理化特性预测,核酸和蛋白 序列相似性比较利用 http://www.ncbi.nlm.nih.gov 网 站上的 BlastX 工具进行比对分析;通过 NCBI 的 ORF Finder 进行开放阅读框分析并预测氨基酸序列; SignalP 程序分析信号肽;采用 Clustal W 软件进行多 序列比对分析。用 MEGA5.1 软件中的 N-Jn (eighborjoining)构建进化树。用自展法(Bootstrap)进行 1000 次重复检验。

1.7 实时荧光定量 PCR 分析

实验室设置 3 个 10L 玻璃钢,每只缸接种密度 50 只/L 孤雌溞,进行充气培养,其它培养条件一样。当 培养密度达到 3000 只/L 以上时进行采样,从每只缸 分别采孤雌幼溞、孤雌溞(带夏卵)、两性溞(带冬卵)、 雄溞和休眠卵 5 种样本,每种样本 3 个平行组。

首先优化模板浓度,将各类模板稀释成10、10⁻¹、 10⁻²、10⁻³、10⁻⁴、10⁻⁵、10⁻⁶、10⁻⁷ μ mol/L 八个浓度梯 度,分别用引物进行扩增,以确定最佳模板浓度。另外 对引物退火温度进行优化,优化后反应条件如下: 95°C预变性30s;95°C变性5s,57°C退火30s,72°C30s, 40个循环。每种生殖状态的样品设3次重复。反应结 束后确定 Real-time PCR 的扩增曲线和溶解曲线,数据 采集和处理在 ABI StepOnePlusTM Instrument 上进行。

每种生殖状态下溞的样品进行 3 次重复。试验所 得数据以平均值(Mean, M) ± 标准差(Stdeva, SD)表示, 所测数据以 SPSS 14.0 软件进行数据统计分析, 采用 One-Way ANOVA 法进行显著性检验,并用 Duncan 检验法进行多重比较。本实验根据 Livak 等(2001)的 方法进行引物的效率检测, PCR 产物的溶解曲线没有 杂峰,显示产物特异性好。采用 2^{-Ct}法分析处理 qRT-PCR 结果, Ct 定义为内标 18S Ct 值与目的基 因(Hsp90)Ct 值的差值,以孤雌幼溞对照组的表达量 为 1。

2 结果

2.1 蚤状溞 Hsp90 基因 cDNA 全长的克隆

以蚤状溞 cDNA 全长为模版,用兼并引物进行 PCR扩增,获得一条 250bp 片段,经 Blast X 分析,显 示与已登录的迷糊酢蚤 D. ambigua (ABI35828.1)、山 形水蚤 D. sp. Yamagata (ABI35815.1)、光滑水蚤 D. laevis (ABI35823.1)和纹水蚤 D. dubia (ABI35824.1) Hsp90 基因的同源性都达到了 97%以上,表明该片段 是 Hsp90 基因的一部分。以这段序列设计 5'RACE 和 3'RACE 引物分别进行 5'-RACE 和 3'-RACE,测序后 所得序列通过比对拼接得到了一条 2568bp 的全长蚤 状溞 Hsp90 序列(GenBank 登录号: KC845247)(图 1)。 为验证序列的可靠性,重新设计 HSP-CDS-F、HSP CDS-R克隆 Dptra 全长序列,结果与拼接结果一致,证 明完整 cDNA 克隆成功。将该溞 Hsp90 基因的全长 cDNA 序列命名为 Daphnia pulex Hsp90 (DpHsp90)。

2.2 蚤状溞 DpHsp90 蛋白序列特征分析

蚤状溞 Hsp90 全长核苷酸序列和推导出的氨基 酸序列如图 1 所示, cDNA 全长为 2568bp, 包含了一 个 164bp 的 5'-UTR 区域和一个 249bp 的 3'-UTR(非 编码区)区域,其中包括一个终止密码子(TGA)、多聚 腺苷酸信号序列(ATTAAA)和 polyA 尾。2155bp 的开 放阅读框(ORF)编码了 718 个氨基酸, 含有多个磷酸化 位点(Casein kinase II phosphorylation site)和 N-糖基化 位点(N-glycosylation site)。结构域分析显示 HSP90 在 编码区存在 5 个该蛋白家族的特有的保守信号区: NKEIFLRELISNSSDALDKIR (34-54aa): LGTIAKSGT (101 — 109aa); IGQFGVGFYSPYLVTD (125 — 140aa); IKLYVRRVF (346-354aa); GVVDSEDLP LNISRE (372—386 aa); 一个GxxGxG序列, 一个LxxLL模块, 同时在 HSP90 羧基末端存在一个保守的 MEEVD 序 列(714—718 aa), 这与已知的所有昆虫 HSP90 基因结 构一致(Peter et al, 1998), 与细胞质 HSP90 羧基末端 的保守基序相同。

TGCAAACAATCTTTGTGCTTTTGTTAAATTATCTTCTAAAGCAAA $\frac{ATG}{M}$ CCTGAAGAAGTTC M P E E V 0 AAATGGAAGCCGAAGCCGAGACTTTCGCATTCCAGGCCGAGATTGCTCAGCTTATGAGCT M E A E A E T F A F Q A E I A Q L M S L TGATCATCAACACCTTCTACTCAAACAAGGAAATCTTCTTGCGTGAGTTGATCTCCAACT DI F T ΚI V P N K N DR Т ттбатастботатсбосатбассаловсстватттовтталсалсттовотастаттбсса I G M T K A D L V D T G I G M T K A D L V N N L G T I A K AGTCTGGAACTAAAGCTTTCATGGAAGCCCCTCTCTGCCGGAACTGACATCTCCATGATTG

 S
 G
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 I
 G

 GTCAGTTTGGTGTGGGGTGTCACTCACCTACCTGGTTACCGACAAGGTGACAGTTCACTT
 Q
 F
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 H
 S

 CCĂAGCACĂATGATĞATĞAGCAGTATĜTTŤGGĞAATCGŤCAĞCTĜGTĠGTŤCTŤTCĂCTĂ K H N D D E Q Y V W E S S A G G S F T I TCAAGCCTGATCATGGAGAACCCATGGGTAGAGGAACCAAGATTGTCCTCCATTTGAAGG P MGR DHG Е G ΤK L Н L AAGATCAAATGGATTACCTAGAGGAGAAGAAGAAAATCAAAGAGGTTGTCAAGAAGCACTCAC D Q M D Y L E E K K I K E V V K K H S Q AGTTCATTGGCTACCCAATCAAGCTTTTGGTTGAGAAGGAGCGCGACAAGGAAGTGAGCG F I G Y P I K L L V E K E R D K E V S D F I G Y P I K L L V E K E R D K E V S D ACGATGAAGCTGAGGAGGAAGAAAGAAGGACGAGAATGAGGAGAAACCAAAGGTCGAAG D E AEEEKKDENEEKP K V D ATGTTGGTGAGGATGAGGAAGCCGACAAGGAGGGCTGGCAAGAAGAAGAAGAAGAAGACCATCA EADK E D Е E A G Κ K K Κ Κ AGGAGAAATACTCTGTTGATGAGGAACTCAACAAGACCAAGCCCATCTGGACCCGCAATC E K Y S V D E E L N K T K P I W T R N P CCGATGACATCAGCCAGGAAGAATACGGAGGAGTATCAAGTCATTGACCAATGACTAGG Q Е Е G Е F K AGGATCATCTTGCCGTCAAGCATTTCTCTATTGAAGGCCAGCTCGAGTTCCGTGCTCTCC D Н LAVKHESTEGQLEERAL I TCTTCGTCCCACGCCGTGCTCCTTTCGATCTGTTTGAGĂACCGCAAGCAGAAGAACCACĂ $\begin{array}{cccccc} TCTTCGTCCCACGCCGTGCTCCTTTCGALCTGTTTGAGACGCGCTGTCCCCGGTGCTCTCATCGACGGAGGCCCCCCCGGTGTCTCATCGACGACGCGAGGAGCTCCCCCCGGTCK L V V R V F I M D N C E E L I P E Y ACCTCAACTTCATGAAGGGGAGTCGTCGGCGCTCTGAGGATCTTCCTCTCAACATTTCTCGTG L N F M K G V V D S E D L P L N I S R E AAATGCTCCAGCAAACAAGATCTGGAGGTCATCGCAAGAACTGGTCAAGAAATGCA M I O O N K I L K V I R K N L V K K C M$ TGGAACTTTTCGAGGAGTTGGCTGAGGACAAGGAGAACTTTAAGAAGTTCTACGAACAAT E L F E E L A E D K E N F K K F Y E Q F TCAGCAAGAACTTGAAGATTGGGAGTCGATGAAGATCGCCG S K N L K L G V H E D S T N R K K I A D ACCTCATCCGTTACCACACTTCCGCTTCTGGAGAAGATCAGGTTTCCTTCAAGGAATACG L I R Y H T S A S G E D Q V S F K E Y V TTTCTCGCATGAAGGAGAACCAGAAGCACATCTACATCACCGGGTGAGAACAGAGATC S R M K E N Q K H I Y Y I T G E N R D Q AAGTCAGCAACTCCTCCTTCGTCGAGCGTGTCAAGAAGCGCGGATTGGAGGTGATCTTCA V S N S S F V E R V K K R G L E V I F M TGACTGAGCCCATTGATGAGTACGTCGTCCAGCAACTCAAGGAATACGATGGCAAGCAGC T E P I D E Y V V Q Q L K E Y D G K Q L TCGTCTCAGTCACCAAGGAGGGTCTGGAACTCCCCGAAGATGATGAAGAAAACCAAGAAAA V S V T K E G L E L P E D D E E T K K R GCGAGAGCGACAAGGCTAAATTCGAGGGTCTCTGCAAAATCATGAAGGATATCTTGGACA K F E G L C DK A M K D ΚI Т AGAAAGTCGAGAAGGTTGTTGTTTCCAACCGTCTGGTCGAGTCTCCCTGCTGTATCGTCA K V E K V V V S N R L V E S P C C I V T CCTCCCAGTACGGCTGGACAGCCAACATGGAGCGTATCATGAAGGCTCAGGCATTGAGGG S Q Y G W T A N M E R I M K A Q A L R D ATACCTCCACCATGGGTTACATGGCCGCCAAGAAGCACTTGGAAATCAACCCCGACCATC T S T M G Y M A A K K H L E I N P D H P CCATCATCGAAGCCCTGCGCGTCAAAGCCGAGGCTGACAAGAACGACAAAGCCGTCAAGG L R V K A E A D K N D K А ΕA ATTTGGTCATGTTGCTGTTTGAAACCTCTCTGCTGTCATCTGGTTTCTCCCTGGAAGAGC <u>L V M L L</u> F E T S L L S S G F S L E E P CAGCCGTCCATGCTTCTCGCATCTACCGCATGATCAAATTGGGTTTGGGCATCGATGAAG A V H A S R I Y R M I K L G L G I D E D D V P A G G E E A K A E E E M P P L E N ATGACGAAGAAGATGCTTCTCGCATGGAGGAAGTCGAT<u>TAA</u>ACGTGTCAATTCCTGTT<u>AT</u> D E D A S R <u>M E E V D</u> \boxed{TAAA} ACACAAACTCAGTTTCTCTCAATTGTTCTTAATTCAGCGTTTTTGCGAACTGGAAT

61

121

181 241

421 87

481

107 541 127

601

147 661

2341 2401

2461

2521

图 1 蚤状溞 Hsp90 基因的 cDNA 全长及推断氨基酸

Fig.1 Nucleotide and deduced amino acid sequences of D. pulex Hsp90 cDNA

CGTTGCTCCCTCATTCCTGGTATTGTATAACTACGTGCTAAGTGTTGGAATTCAATACAA

方框分别表示起始密码子(ATG)、终止密码子(TAA)和多聚腺苷酸信号序列(ATTAAA)。浅灰色阴影表示 Hsp90 的 5 个信号序列, 加

粗下划线表示 GxxGxG、LxxLL 和 MEEVD 序列

2.3 蚤状溞 Hsp90 结构分析

DNAMAN 预测的 Hsp90 蛋白的等电点是 5.01, 相对分子质量为 82552.61。Hsp90 蛋白含有一个 HATPase_c 结构域(残基 36—149 位)、一个 Hsp90 蛋 白结构域(残基 190—718 位)和一个 Mg²⁺结合位点(残 基 45 位-天冬酰胺)(图 2)。



图 2 蚤状溞 Hsp90 蛋白结构域示意图 Fig.2 Hsp90 protein structure domain of *D. pulex*

2.4 蚤状溞 Hsp90 氨基酸序列同源性分析

利用 ClustalW 对蚤状溞 Hsp90 和 NCBI 中其它 甲壳纲动物(共 14 种)Hsp90 的多序列比对见图 3。分 析表明:这些比对的序列之间的同源性较高。蚤状溞 Hsp90 与日本对虾(Marsupenaeus japonicus)和刀额新 对虾(Metapenaeus ensis)的同源性最高为 85%,与其 它 11 个种类:中华绒螯蟹(Eriocheir sinensis)、斑节 对虾(Penaeus monodon)、凡纳滨对虾(Litopenaeus vannamei)、中国对虾(Fenneropenaeus chinensis)、矮 小拟镖剑水蚤(Paracyclopina nana)、拟穴青蟹(Scylla paramamosain)、真宽水蚤(Eurytemora affinis)、日本 沼虾(Macrobrachium nipponense)、剑水蚤(Tigriopus japonicus)、三疣梭子蟹(Portunus trituberculatus)、脊 尾白虾(Exopalaemon carinicauda)、红螯相手蟹 (Chiromantes haematocheir)的同源性都在 79%以上。

利用 MEGA5.1 软件对 NCBI 中部分节肢动物的 Hsp90 基因序列进行了分子系统学分析,在构建系统 发生树的基础上研究了蚤状溞和其它种类 Hsp90 蛋 白之间的进化关系(见图 4)。从进化树中,可以看出 蚤状溞 Hsp90 首先与剑水蚤(*T. japonicus*)、日本沼虾 (*M. nipponense*)、红螯相手蟹(*C. haematocheir*)、拟穴 青蟹(*S. paramamosain*)、中华绒螯蟹(*E. sinensis*)等甲 壳纲聚为一类,然后再与蛛形纲的肩突硬蜱(*Ixodes scapularis*)、龙骨三色蝎(*Opistophthalmus carinatus*) 聚为一亚群。昆虫纲的印度跳蚁(*Harpegnathos saltator*)、刺柚釉小蜂(*Quadrastichus erythrinae*)、褐 飞虱(*Nilaparvata lugens*)、白背飞虱(*Sogatella furcifera*)、中华稻蝗(*Oxya chinensis*)、豆荚草盲蝽 (*Lygus hesperus*)等聚为另一亚群。

2.5 Hsp90 mRNA 在蚤状溞各生殖状态下的表达差异 Hsp90 基因在孤雌幼溞、孤雌溞(带夏卵)、两性 溞(带冬卵)、雄溞和休眠卵(冬卵)中的表达差异如图 5

所示。从图 5 中可以看出: Hsp90 mRNA 在雄溞体中 表达量最高, 其次为两性溞(带冬卵), 孤雌溞(带夏卵) 次之, 在休眠卵中的表达量最低。Hsp90 mRNA 在两 性溞(带冬卵)和雄溞中的表达量显著高于孤雌溞(带 夏卵)和孤雌幼溞(*P*<0.05)。

3 讨论

Hsp90 蛋白家族是一类在生物进化过程中高度 保守并广泛存在的分子伴侣,在非应激和正常生理 状态下占细胞蛋白总量的 1%-2%, 其具体功能尚未 明确、它能够识别并调节胞内基质的活性并在环境 胁迫时发挥功能。为进一步了解 HSP90 基因特点,本 文获得蚤状溞 HSP90 cDNA 的完整序列, 2155bp 的开 放阅读框(ORF)编码了 718 个氨基酸, 在蚤状溞基因 组中定位于 scaffold 173:115844—118680 (GenBank accession: GL732695.1), 编码 718 氨基酸。通过氨基 酸序列比对, 作者发现蚤状溞 Hsp90 与已知的甲壳纲 具有共同的序列特征、包含HSP90家族的5个特征信 号序列(NKEIFLRELISNSSDALDKIR、 IGQFGVGFYSAFLVAD, LGTIAKSGT, IKLYVRRVFI 和 GVVDSEDLP LNISRE)(见图 1), 有研究表明, 植 物、动物和真菌的 HSP90 家族中均存在这 5 条特征 序列(Gupta, 1995), 大多数生物的这 5 条特征序列几 乎完全相同(Farcy et al, 2007; Li et al, 2009), 仅有少 数生物存在个别氨基酸差异(Brunt et al, 2004)。这些 信号特征可被 HOP (HSP70 and HSP90 organizing protein)的 TPR (Tetra-tricopeptide repeat)区段识别, 进而与热休克蛋白 70 构成分子伴侣复合体(Chen et al, 2005)。在同源性比对结果中还发现 GxxGxG、 LxxLL 模块。GxxGxG 模块是 Hsp90 与 ATP 结合的 结构基础(Prodromou et al, 1997), 它的存在对 Hsp90 功能的正常发挥具有重要作用; LxxLL 模块在 Hsp90

_ ropenaeus vannamei	mfelimselvelfafqaeiaqimsliintfysn Mveeimseevetfafqaeiaqimsliintfysn	KEIFLRELISNSSDA 48	Fenneropenaeus_chinensis	DLPLNISREMLQQNKILKVIRKNLVKKILELFEEIVDKESY DLPLNISREMLQQNKILKVIRKNLVKKILELFEEIVDKESY	KKFYENFS
Aarsupenaeus japonicus	MVEEIMSEEVEIFAFQAEIAQLMSLIINIFYSN MVEEIMIEEVEIFAFQAEIAQLMSLIINIFYSN	KEIFLRELISNSSDA 48 KEIFLRELISNSSDA 48	Marsupenaeus_japonicus	DIPLNISREMLQQNKILKVIRKILVKKILELFEEIVEDKESI	KKFYENFS
letapenaeus_ensis	MVEETMTEEVETFAFQAEIAQLMSLIINTFYSN	KEIFLREIISNSSDA 48	Metapenaeus_ensis	DL PLNISREMLQQNKILKVIRKNLVKKTLELFEELIDDKESY DL PLNISREMLQQNKILKVIRKNLVKKTLELFEELIDDKESY	KKFYENFS WWFYFNFS
ortunus_trituberculatus	MPEDAAMEDVETFAFQAEIAQLMSLIINTFYSN	KEIFLREIISNSSDA 48	Portunus_trituberculatus	DLPLNISREMLQQNKILKVIRKNLVKKAMELFEELVEDKDNY	KKFHENFS
riocheir_sinensis	MPEEAIMEEVETFAFQAEIAQLMSLIINTFYSN MPEEAIMEDVETFAFQAEIAQLMSLIINTFYSN	KEIFLRELISNSSDA 48	Eriocheir_sinensis Chiromantes haematocheir	DLPLNISREMLQQNKILKVIRKNLVKKALELFEELIEDKDNY. DLPLNISREMLQQNKILKVIRKNLVKKALELFEELIEDKDNY.	KRFYENFS KKFYENFS
acrobrachium_nipponense	MAADETQIAEEVETFAFQAEIAQLMSLIINTFYSN	KEIFLRELISNSSDA 50	Macrobrachium_nipponense	DLPLNISREMLQQNKILKVIRKNLVKKSMELFEELAEDKENY	KKFYESFA
kopaleamon_carinicauda aphnia_pulex	-MPEEVQMEAEAETFAFQAEIAQLMSLIINIFYSN -MPEEVQMEAEAETFAFQAEIAQLMSLIINIFYSN	KEIFLRELISNSSDA 49	Daphnia_pulex	DLPLNISREMLQQNKILKVIRKNLVKKCMELFEELAEDKENF	KKFYEQFS
griopus_japonicus	MIDPAGGDCETFAFQAEIAQLMSLIINTFYSN	KEIFLRELISNSSDA 47	Tigriopus_japonicus Paracyclonina_nana	DLPLNI SREMLQQNKI LKVIRKNLVKKCMDL FEELAEDKDNY. DLPLNI SREMLOONKI LKVIRKNLVKKCMEL FDELAEDKDAY	KKYYEQFS KKFYEOFA
urytemora_affinis	MSDEIETFAFQAEIAQLMSLIINTFYSN	KEIFLRELISNASDA 43	Eurytemora_affinis	DLPLNISREMLQQNKILKVIRKNIVKKVMDVIEEISEDKDNY	KKFYEOFG
enaeus_monodom	LDKIRYESLIDPSKLESGKDLFIKLVPNKDDRILI	IIDSGIGMIKADLVN 98	Penaeus_monodom	KNLKLGIHEDSTNRKKLAEFLRYHTSASGDEMSSLKEYVSRM	KENQKHIY
enneropenaeus_chinensis itopenaeus vannamei	LDKIRYESLIDPSKLENGKDLFIKLVPNKDDRILI LDKIRYESLIDPSKIESGKDLFIKLVPNKDDRILI	IIDSGIGMIKADLVN 98 TIDSGIGMIKADLVN 98	Litopenaeus_vannamei	KNLKLGIHEDSINKKKLAEFLKIHISASGDEMSSLKEIVSKI KNLKLGIHEDSINKKKLAEFLRYHISASGDEMSSLKEYVSKI	KENQKHIY
arsupenaeus_japonicus	LDKIRVESLIDPSKLDLGKDLFIKLVPNKDDRILT I DKIPVESI I DPSKI DSGKDI FIKLVPNKE DRILT	IIDSGIGMTKADLVN 98	Marsupenaeus_japonicus Metapenaeus ensis	KNLKLGIHEDSTNRKKLAEFLRYHISASGDDMSSLKECVSRM KNLKLGIHEDSTNRKKLAEFLRYHISASGDEMSSLKEYVSRM	KENQKHIY KENOKHIY
cylla_paramamosain	LDKIRYESLTDPSKLESGKELFIKLIPDKNDRTLT	IIDSGIGMIKADLVN 98	Scylla_paramamosain	KNIKLGIHEDSTNRKKLAEFLRYHTSASGDEMSSLKDYVSRM	KENŐKOIY
ortunus_trituberculatus	LDKIRYESLIDPSKLESGKELFIKLIPDKNDRILI LDKIRYESLIDPSKLESGKDLFIKLVPNKNDRILI	IIDSGIGMIKADLVN 98 IIDSGIGMIKADLVN 98	Eriocheir_sinensis	KNIKLGIHEDSINKKKLAEFLKIHISASGDEMSSLKDIVSKM KNIKLGIHEDSINKKKLAEFLRYHISASGDEMSSLKDYVSRM	KENQKQII
hiromantes_haematocheir	LDKIRYESLIDPSKLESGKELFIKLVPNKNDRILI I DKIRYESI I DRSKI DAGKELFIKL I DNDDDRIJI	IIDSGVGMIKADLVN 98	Chiromantes_haematocheir Macrobrachium nipponense	KNIKLGIHEDSTNRKKLAEFLRYHTSASGDEMSSLKDYVSRM KNLKLGIHEDATNRKKLAELLRYHTSSTGDEMCSLKDYISRM	KENQKQIY KENOKHIY
xopaleamon_carinicauda	LDKIRYESLIDPSKLENVKDLYIRIEVDKNDRSLT	IYDIGIGMIKADLVN 97	Exopaleamon_carinicauda	KNIKLGIHEDSTNRKKLSELLRFYTSASGDEMSSLKDYVSRM	KENŐKQIY
aphnia_pulex griopus japonicus	LDKIRYESLIDPSKLDSGKDLEIKIVPNKNDRILI LDKIRYESLIDPSKLDSQKELFIKIVPDKDAKILI	IQDSGIGMIKADLIN 97	Tigriopus_japonicus	KNIKLGIHEDSINKKKIADLIRINISASGDEMCSFADYVSRM	KENQKDIY
aracyclopina_nana urytemora_affinis	LDKIRYESLIDPSKLEIQKELFIKIVPDKENKILI LDKIRYESLIDASKLEGQKDLYIKLIPDVDAKILI	IIDSGVGMTKADLIN 93 IIDSGIGMTKADMIN 93	Paracyclopina_nana Eurytemora_affinis	KNIKLGIHEDSINRKKLASLLRFYISASGDEPCSFGDV/SRM KNIKLGIHEDSINRKKLAGHLRFYISASGDEMCSLGDV/SRM	KEIQKDVY KEIQKDVY
anaeus monodom	NIGTIAKSCIKA FMFALOAGADISMIGOFGUGFYS	AVIVADEVTVVSENN 148	Penaeus monodom	FIIGEIREQVQNSAFVERVKKRGFEVIYMIEPIDEYCVQQLK	EYDGKQLV
enneropenaeus_chinensis	NLGTIAKSGTKAFMEALQAGADISMIGQFGVGFYS	AYLVADKVTVVSRNN 148	Fenneropenaeus_chinensis	FIIGETREQUONSAFVERVKRGFEVIYMTEPIDEYCVOOLK	EYDGKQLV
topenaeus_vannamei larsupenaeus japonicus	NLGTIAKSGTKAFMEALQAGADISMIGQFGVGFYS NLGTIAKSGTKAFMEALQAGADISMIGOFGVGFYS	AYLVADKVIVVSKNN 148 AYLVADKVIVVSKNN 148	Litopenaeus_vannamei Marsupenaeus_japonicus	FIIGEIREQVQNSAFVERVKKRGFEVIYMIEFIDEYCVQQLK FIIGEIREQVQNSAFVERVKKRGFEVIYMIEFIDEYCVQQLK	EYDGKQLV EYDGKQLV
etapenaeus_ensis	NLGTIAKSGTKAFMEALQAGADISMIGQFGVGFYS	AYLVADKVTVVSKNN 148	Metapenaeus_ensis Scylla paramamossin	FITGETREQVQNSAFVERVKKRGFEVIYMTEPIDEYCVQQLK YITGESREOVHNSAFVERVKKRGFEVIYMVEPIDEYCVQQLK	EYDGKOLV
.yna_paramamosain ortunus_trituberculatus	NLGTIAKSGIKAFMEALQAGADISMIGQFGVGFYS NLGTIAKSGIKAFMEALQAGADISMIGQFGVGFYS	AYLVADKVIVVSKNN 148 AYLVADKVIVVSKNN 148	Portunus_trituberculatus	YIIGESREQVHNSAFVERVKKRGFEVVYMVEPIDEYCVQQLK	EYDGKQLV
riocheir_sinensis hiromantes haematocheir	NLGTIAKSGTKAFMEALQAGADISMIGOFGVGFYS NLGTIAKSGTKAFMEALOAGADISMIGOFGVGFYS	AYLVADKVTVISKNN 148 AYLVADKVTVVSRNN 148	Eriocheir_sinensis Chiromantes_haematocheir	<pre>11GESREQVHNSAFVERVKKRGFEVVYMTEPIDEYCVQQLK VIIGESGSRCTIAAFVERVKKRGFEVVYMVEPIDEYCVQQLK</pre>	e ydgrolv E yggrolv
lacrobrachium_nipponense	NLGTIAKSGTKAFMEALQAGADISMIGQFGVGFYS	AYLIADKVTVVSRNN 150	Macrobrachium_nipponense	YITGESREQVENSAFVERVKKRGFEVVYMTEPIDEYCVQQLK	EFDGKQLV
xopaleamon_carinicauda aphnia pulex	NLGTIAKSGIKAFMEALQAGADISMIGQFGVGFYS NLGTIAKSGIKAFMEALSAGADISMIGQFGVGFYS	AILVADRVVVÍSKNN 147 PYLVIDKVIVHSKHN 149	Exopaleamon_carinicauda Daphnia_pulex	YIIGENRDQVSNSSFVERVKKRGLEVIFMIEFIDEYVVQQLK	EYDGKQLI
igriopus_japonicus	NIGIIAKSGTKAFMEALQAGADISMIGQFGVGFYS	AYLIADKVIVISKHN 147 AYLVADRVVVISKHN 142	Tigriopus_japonicus Paracyclopina_nana	YITGESKEVVAASAFVERLKKRGLEVVYMTEPIDEYVVQQLK YITGESREVVAASAFVERLKKRGFEVVYMTEPIDEYVVOOLK	EYDGKNLV EYDGKNLV
urytemora_affinis	NLGTIAKSGIKAFMEALQAGADISMIGQFGVGFYS	AYLVADKVVVISKHN 143	Eurytemora_affinis	YITGESKEVVAISSFVERLKKRGLEVVYMTEPIDEYVVQQLK	EFDGKNLV
anaeus_monodom	DDEQYIWESSAGGSFTVRHDT-GEPIGRGTKITLH	LKEDQTEYLEERRVK 197	Penaeus_monodom	SVIKEGLELPEDEEEKKKFEEQKIKFENLCKVMKDILDKRVE	KVVVSNRL
enneropenaeus_chinensis topenaeus_vannamei	DDEQYIWESSAGGSFIVRHDT-GEPIGRGTKITLH DDEQYIWESSAGGSFIVRHDT-GEPIGRGTKITLH	LKEDQIEYLEERRVK 197 LKEDQIEYLEERRVK 197	Litopenaeus_vannamei	SVIKEGLELPEDEEEKKKYEEQKIKFENLCKVMKDILDKCVE	KVVVSNRL
larsupenaeus_japonicus	DDEQYIWESSAGGSFTVRHDI-GEPIGRGTKITLH	LKEDQTEYLEERRVK 197	Marsupenaeus_japonicus Metapenaeus ensis	SVTKEGLELPEDEEEKKKYEEQKTKFENLCKVMKDILDKRVE SVTKEGLELPEDEEEKKKFEEQKTKFENLCKVMKDIJ.DKRVF	KVVVSNRL KVVVSNRI
cylla_paramamosain	DDEQYVWESSAGGSFIVRIDH-GEPIGRGIRIILH	LKEDQTEYLEERRVR 197	Scylla_paramamosain	SVTKEGLELPEDEDEKKKLEEQKTKFENLCKVVKDILDKRVE	KVVVSNRL
ortunus_trituberculatus riocheir sinensis	DDEQYVWESSAGGSFIVRIDH-GEPLGRGTRIILH DDEOYVWESSAGGSFIVRIDH-GEPLGRGTOIILH	LKEDQTEYLEERRIR 197 LKEDQTEYLEERRIK 197	Portunus_trituberculatus Eriocheir_sinensis	SVIREGLELPEDEDERRRLEEDRIRFENLCKVVRDILDRRVE SVIREGLELPEDEDERKKFEEDRSKFENLCKVVRDILDRRVE	KVVVSNRL
hiromantes_haematocheir	DDEQYVWESSAGGSFIVRIDH-GEPVGRGTRIILH	LKEDQTEYLEERRIK 197	Chiromantes_haematocheir Macrobrachium_nipponense	SVTKEGLELPEDDDEKKKLEEQKAKFENLCKVVKDILDKRVE SVTKEGLELPEDDDEKKKEDEOKSKEENLCKVMEDILDKRVE	KVVVSNRL KVVVSNRL
copaleamon_carinicauda	DDEQYIWESAAGGSFIIVRFDM-GEFIGRGIKIILH DDEQYIWESAAGGSFIIKFDK-GQPLQRGIKIILY	LKEDQVEYLEERRIK 199	Exopaleamon_carinicauda	SVTKEGLDLPEDEEQKKKSEEQKQRLENLCKIMKDILDKRVE	KVVVSNRL
aphnia_pulex griopus japonicus	DDEQYVWESSAGGSFTIKPDH-GEPMGRGTKIVLH DDEQYIWESSAGGSFTIRTDP-GPPTGRGTOTVTH	LKEDQMDYLEEKKIK 198 LKEDQTEYLEERRIK 196	Daphnia_pulex Tigriopus_iaponicus	SVIKEGLELPEDDEETKKRESDKAKFEGLCKIMKDILDKKVE SVIKEGLELPEDECKKKFEEAKIKFEGLCKVMKDILDKKVE	KVVVSNRL KVIVSNRL
aracyclopina_nana urytemora_affinis	DDEQYIWESSAGGSFTIKTDTISEPLGRGTKIVLH DDEQYIWESSAGGSFTIKTDS-GEPMGRGTKIVLH	LKEDQMEYCEERRVK 193 MKEDQCEYIEEKKIK 192	Paracyclopina_nana Eurytemora_affinis	SVIKEGLELPEDEEEKKKREEDKAKFENLCKVMKDILDKKVE SVIKEGLELPEDEEEKKKREEDVKKFEPLCKVMKDILDKKVE	KVIVSNRL KVVVSSRL
langeus manade	***** ***:*******: * . *: ***:*.*:	:**** :* **::::	Penaeus monodom	VTSPCCIVTSOYGWIANMERTMKACALRDTSTMGYMALWWH:	EINPDHST
enneropenaeus_chinensis	EIVKKHSQFIGYFIKLVEKERDKEVSDDEEEEKE	EKEEEAEE 240	Fenneropenaeus_chinensis	VTSPCCIVTSQYGWTANMERIMKAQALRDTSTMGYMAAKKHL	EINPDHSI
itopenaeus_vannamei larsupenaeus janonicus	EIVKKHSQFIGYPIKLLVEKERDKEVSDDEEEEKE EIVKKHSQFIGYPIKLLVEKERDKEVSDDEEEEKE	EKEEEAEE 241 EKEEKEKEEGEE 244	Litopenaeus_vannamei Marsupenaeus_japonicus	VISPCCIVISQIGWIANMERIMKAQALEDISIMGYMAAKKHL VISPCCIVISQYGWIANMERIMKAQALEDISIMGYMAAKKHL	EINPDHSI
letapenaeus_ensis	EIVKKHSQFIGYPIRLLVEKERDKEVSDDEEEEKE	EKEEEAEE 240	Metapenaeus_ensis Scylla_paramamossin	VTSPCCIVTSQYGWIANMERIMKAQALRDISIMGYMAAKKHL VTSPCCIVTSOYGWIANMERIMKAQALRDISIMGYMAAKKHL	EINPDHSI EINPDHST
cysa_paramamosain 'ortunus_trituberculatus	EIVKKHSQFIGIFIKLLVEKERDKEVSDDEEEEKE	EEKEKKEEED 242	Portunus_trituberculatus	VTSPCCIVTSQYGWTANMERIMKAQALRDTSTMGYMAAKKHL	EINPDHSI
riocheir_sinensis	EVVKKHSQFIGYPIKLLVEKERDKEVSDDEEEEKE EIVKKHSQFIGYPIKLLVEKFRDKEVSDDEEEEKE	EEKKDEEE 240 EEEKDEFF 240	Eriocheir_sinensis Chiromantes haematocheir	VISPCCIVISQYGWIANMERIMKAQALRDISIMGYMAAKKHL VISPCCIVISQYGWIANMERIMKAQ-LRDISIMGYMAAKKHL	£INPDHSI EINPDHSI
lacrobrachium_nipponense	EIVKKHSQFIGYPIKLLVEKERDKEVSDDEEEEKE	EEEKKEGEEKKEGEE 249	Macrobrachium_nipponense	VISPCCIVISQYGWSANMERIMKAQALRDTATMGYMAAKKHL VISPCCIVISOYGWTANMERIMKAQALRDTATMGYMAAKKHL	EINPDHSI
xopaleamon_carinicauda aphnia pulex	EVVKKHSQFIGYPIRLLVEKEREKQVPDDDEEEEQ EVVKKHSQFIGYPIKLLVEKERDKEVSDDEAEEEE	KDEDISKAIG 241 KKDENE 239	Exopaleamon_cannicauda Daphnia_pulex	VESPCCIVISQIGWIAUMERIMKAQALRDASINGIMAAKKHL VESPCCIVISQYGWIANMERIMKAQALRDISINGYMAAKKHL	EINPDHPI
igriopus_japonicus	EVVKKHSÖFIGYPIKLLVEKERDKEISDDEADDED ETVKKHSÖFIGYPIKLIVEKERDKEISDDEADDED	KKEDEEKKEG 241	Tigriopus_japonicus Paracyclopina_pana	VNSPCCIVTSQYGWTANMERIMKAQALRDTSTMGYMAAKKQL VNSPCCIVTSOYGWTANMERIMKAQALRDTSTMGYMAAKKHL	EINPEHSI
aracyclopina_nana urytemora_affinis	EIVKKHSQFIGIFIKLLVQKEREKEVSDEAEVEE	PKEG 234	Eurytemora_affinis	VSSPCCIVTSQYGWTANMERIMKAQALRDTSTMGYMAAKKHL	EINPDHSI
enaeus_monodom	DKPKIEDVGEDEEADKEKGEDKKKKKTVKEK	YTEDEELNKTKPLWT 286	Penaeus_monodom	IETLROKADADKNDKSVKDLVMLLFESSLLSSGFSLEDPGVH	ASRIYRMI
anneropenaeus_chinensis topenaeus vannamei	DKPKIEDVGEDEEADKEKGEDKKKKKKTVKEK DKPKIEDVGEDEDADKEKGDDKKKKKKTVKEK	YTEDEELNKTKPLWT 286 YTEDEELNKTKPLWT 286	renneropenaeus_chinensis Litopenaeus_vannamei	IEILRQKADADKUDKSVKDLVKLLFESSLLSSGFSLEDPGVH IEILRQKADADKUDKSVKDLVKLLFESSLLSSGFSLEDPGVH	ASRI1RMI ASRI1RMI
arsupenaeus_japonicus	EKPKIE DVGE DE DADKE KGDDKKKKKTVKEK	YTEDEELNKTKPLWT 290	Marsupenaeus_japonicus Metapenaeus_ensie	IETLKOKADADKNDKSVKDLVMLLFESSLLSSGFSLEDPGVH IETLROKADADKNDKSVKDLVMLLFESSLLSSGFSLEDPGVH	ASRIYRMI ASRIYRMT
etapenaeus_ensis cylla_paramamosain	DDKPKIEDVGEDEDADK-KEGDKKKKKIVKEK	YTEDEELNKIKPLWI 288	Scylla_paramamosain	IETLRQKADADKNDKSVKDLVMLLFESALLSSGFTLEDPGVH	AGRIYRMI
artunue trituberculatue	DEKPKIEDVGEDEDADK-KDGDKKKKKTVKEK DEKPKIEDVGEDEDADK-KEGGKKKK-TVKEN	YIEDEELNKIKPLWI 288 YSEDEELNKIKPLWI 285	Portunus_trituberculatus Eriocheir_sinensis	IEILRQKADADKNDKSVKDLVMLLFESALLSSGFTLEDPGVH IETLRQKADADKNDKSVKDLVMLLFESALLSSGFTLEDPGVH	AGRIYRMI AGRIYRMI
iocheir sinensie		VIEDEFINKTKRINT 285	Chiromantes_haematocheir	IETLROKADADKIDKSVKDLVMLLFESALLSSGFTLEDPGVH. IETLROKADADKIDKSVKDLVMLLFESSLLSSGFSLEDPAVE	AGRIYRM
iocheir_sinensis hiromantes_haematocheir	DEKPKIEDVGEDEDADK-KEGGKKKK-TVKEK		Macrobrachium ninnenene-	and any concerning the second s	Constrain.
iocheir_sinensis niromantes_haematocheir acrobrachium_nipponense topaleamon_carinicauda	DEKPKIEDVGEDEDADK-KEGGKKKK-IVKEK DKDKEKPKIEDVGEDEDADK-KDDSKKKK-IVKEK DKPEVEDVGADEGADGVEKAKKMKTIKVK	YTEDEELNKTKPLWT 297 YTEDEELNKTKPLWT 285	Macrobrachium_nipponense Exopaleamon_carinicauda	IETLROKADADKNDKSVKDLVLLLFETALLASGFNLEDPGVH	AARIYRM.
hirocheir_sinensis hiromantes_haematocheir acrobrachium_nipponense kopaleamon_carinicauda aphnia_pulex oricous_iaponicre	DEKFKIEDVGEDEDADK-XEGGKKKK-TVKEK DKDKEKFKIEDVGEDEDADK-KDGKKKK-TVKEK DKFEVEDVGADEGADGVEKAKKKKIIKK ED-ADEKKIEDVGEDEADA-KAKKKKIIKK ED-ADEKKIEDVGEDEDADS-KAK-KKKKTIVF	YTEDEELNKTKPLWT 297 YTEDEELNKTKPLWT 285 YSVDEELNKTKPLWT 283 YTEDEELNKTKPLWT 289	Macrobrachium_nipponense Exopaleamon_carinicauda Daphnia_pulex Tigrlopus japonicus	IETLRQKADADKNDKSVKDLVLLLFETALLASGFNLEDPGVH. IEALRVKAEADKNDKAVKDLVMLLFETSLLSSGFSLEEPAVH. VENLRQKAEADKNDKSVKDLVLLLFETALLSSGFSLEDPAVH	AARIYRMI ASRIYRMI SQRIHRMI
nonico-Indoetratados niromantes_haematocheir acrobrachium_nipponense copaleamon carinicauda aphnia pulex griopus_japonicus aracyclopina_nana nytemora_affinis	DEKPKIEDVOEDEDADK-KGGKKKK-TVKEK DEVEKEKEIEDVOEDEDADK-KDGKKKK-TVKEK DKPEVEDVGADEGADG-VEKAKKKTIKK EKPVEDVGADEGADK-SACKKKTIKK ED-ADKFKIEDVGEDEDAS-KDK-KKKKTIKK EEDKFKVEDVGEDDEDK-ADKOKKKKTIKK EEDKFKIEDVGEDDEDK-STEKKTIKK	YTEDEELNKTKPLWT 297 YTEDEELNKTKPLWT 285 YSVDEELNKTKPLWT 285 YTEDEELNKTKPLWT 288 YTEDEELNKTKPLWT 281 YTEDEELNKTKPIWT 274	Macrobrachium_nipponense Exopaleamon_carinicauda Daphnia_pulex Tigriopus_japonicus Paracyclopina_nana Eurytemora_affinis	IETIRQARADAWIKKSWILULIFETALLASSFHLEPGYM IERIFYAEDAWIKKSWILULFETALLSSSFSLEPEAYH VENLRQARADAWIKKSVKDLVLLFETALLSSSFSLEPEAYH VETLRQARADAWIKKSVKDLVLLFETALLSSSFSLEDEAYH VETLRQARADAWIKKSVKDLVHLFETSLLSSSFSLEDEWH	AARIYRM ASRIYRM SQRIHRM AQRIHRM AMRIHRM
nonag_intoefcutus inomantes_haematocheir acrobrachium_nipponense copaleamon_carinicauda aphnia putes griopus_japonicus aracyclopina_nana aracyclopina_nana	DEKRKIEUVGEDEDAK-KEGGKKKK-TVER UNKDEKSKIEVGROEDADK-KODSKKK-TVER DKEVEVEDVEDEDADK-KODSKKKETIKK EDAKSKIEUVGEDEDAD-EAKKIKKIIKK EDAKSKIEUVGEDEDAD-EAKKIKKIIKK EERKIEUVGEDEDAD-KOKKIKKIIKK EERKIEUVGEDADA-K-SIEKKIKKIKK	YTEDEELNKTKPLMT 297 YTEDEELNKTKPLMT 285 YSVDEELNKTKPIMT 285 YTEDEELNKTKPIMT 288 YTEDEELNKTKPIMT 288 YTEDEELNKTKPIMT 274	Macrobrachium_nipponense Exopaleamon_carinicauda Daphnia_pulex Tigriopus_japonicus Paracyclopina_nana Eurytemora_affinis	IEIIRQAADAMINISKYNI MULEFTIIJAASHILEPOYU IEAIRVAADMINKANKI MULEFTIIJSSESEEPAN VEILAQXAADMINKANKI MULEFTIIJSSESEEPAN VEILAQXAADMINKSYNI MULEFTIIJSSESEEPAN VEILAVAADMINKSYNI MULEFTIIJSSESEEPAN VEILAVAADMINKSYNI MULEFTIIJSSESEEPAN	AARIYRM. ASRIYRM: SQRIHRM: AQRIHRM: AMRIHRM: . **:**
hana material hinomanies haematocheir acrobrachium nipponense copaleamon_carinicauda aphna pules griopus japonicus iracyclopina_nana macyclopina_nana mytemora_afinis anaeus_monodom nineropenaeus_chinensis	DEKRICEUVSEEDADK-KOSOKOKO-TVEEK NUKEEKRIKUUVSEEDADK-KOSOKOKO-TVEEK DKREVIEVOSEDADG-VEEKKOKOKIIVO ESERVINOSEDEADK-S-LAANKOKOKIIVO EEKRIKUEVOSEDEADK-S-LAANKOKOKOKUIIEK EEKRIKUEVOSEDEADK-S-LAANKOKOKOKUIIEK EEKRIKUEVOSEDEADK-S-LAANKOKOKOKUIIEK EEKRIKUEVOSEDEADK-S-LAANKOKOKOKUIIEK EEKRIKUEVOSEDEADK-S-LAANKOKOKOKUIIEK	YTEDEELNKTKPLWT 297 YTEDEELNKTKPLWT 283 YTEDEELNKTKPLWT 283 YTEDEELNKTKPLWT 283 YTEDEELNKTKPLWT 281 YTEDEELNKTKPLWT 274 *: ***********************************	Macrobrachium_nipponense Exopaleamon_carinicauda Daphnia_pulex Tigriopus_laponicus Paracyclopina_nana Eurytemora_affinis Penaeus_monodom Fenneropenaeus_chinensis	IEILBQKADAMMINSKMIMULIFTELLBSGEIEEDAM IELBUKKAANMINSKMIMULIFTELLBSGEIEEDAM VEILBQKAANKINSKMIMULIFTELLSSGEIEEDAM VEILBQKAANKINSKMIMULIFTELLSSGEIEEDAH VEILBYKAEANKINSKMIMULIFTELLSSGEIEEDAH KLOSIDEED-ARMEEATLEEDMPELGODEDASPHEETY KLOSIDEED-ARMEEATLEEDMPELGODEDASPHEETY	AARIYAMI ASRIYRMI SQRIHRMI AQRIHRMI AMRIHRMI . **:*** 720 726
tacheliz alienensis tromanise, heematocheli tromanise, heematocheli tromanise, heematocheli trobuel, japanicus priopuel, japanicus priopuel, japanicus trojectoping, nana trojectoping, nana trojecto	DEKRKIEUVGEDEDAM-KOGSKKKKO-TVEE DEKRKIEUVGEDEDAM-KOGSKKKO-TVEE DKREVEVUVGEDEDAM-KOGSKKKO-TVEE ED-AMPERIEUVGEDEDAM-KOGSKKKOTIEK ED-AMPERIEUVGEDAMG-KOK-KOKKOTIEK EEKREUVGEDAMG-KOKKOKKOTIEK EEKREUVGEDAMAK-SIEKOKKOKKOTIEK EEKREUVGEDAMAK-SIEKOKKOKKOTIEK BIRDEISKEVYGEVKSLINKOKOLAVK BIRDEISKEVYGEVKSLINKOKOLAVK BIRDEISKEVYGEVKSLINKOKOLAVK BIRDEISKEVYGEVKSLINKOKOLAVK	YTEDEELINKTKFLWT 207 YTEDEELINKTKFLWT 285 YTEDEELINKTKFLWT 285 YTEDEELINKTKFLWT 286 YTEDEELINKTKFLWT 274 YTEDEELINKTKFLWT 274 YTEDEELINKTKFLWT 274 HFSVEQLEFRALLF 330 HFSVEQLEFRALLF 330 HFSVEQLEFRALLF 330	Macrobrachium_nipponense Exopaleamon_carinicauda Daphnia_pulex Tigropus_laponicus Paracyclopina_nana Eurytemora_affinis Penaeus_monodom Fenneropenaeus_taninensis Litopenaeus_vanamei Marsupenaeus_iaponicus	IETLOVADANNIKYKCIVILIPTELLAGONILEPPTU IELALVVADANNIKYKCIVILIPTELLGOSTLEPAVH VEILAQVADANNIKYKCIVILIPTELLGOSTLEPAVH VEILAQVADANNIKYKCIVILIPTELLGOSTLEPATH VEILAQVADANNIKYKCIVILIPTELLGOSTLEPATH VEILAVADANNIKYKCIVILIPTELLGOSTLEPATH VEILAVADANNIKYKCIVILIPTELGOSTLEPATH VEILAUTEEDAPPEARTLEENMPLEODENASHEKYK KLAGITEEDAPPEARTLEENMPLEODENASHEKYK KLAGITEEDAPPEARTLEENMPLEODENASHEKYK	AARIYAMI ASRIYRMI SQRIHRMI AQRIHRMI AMRIHRMI . **:*** 720 726 720 720 723
iachel; ainensis iicontel; ainensis iicomarites, haematocheir scrobrachlum, nipponense opaleamon, carinicauda phnis pulex iifopus, japonicus tracyclopina, nana maeus, monodom nneropenaeus, anameli arsupenaeus, japonicus tapenaeus, anais,	DEKRIEZUYGEDDAK-KOGAKKKO-TVER DWEREKRIEZUYGEDDAK-KOGAKKKO-TVER DKREVEUYGADBADA-VERAKKKIIN/ EENREVUSEDBADA-EDAK-KOKKIIN/ EENREVUSEDBADA-EDAK-KOKKIIN/ EENREVUSEDBADA-KOK-KOKKIIN/ EENREVUSEDBADA-KOKKIIN/ RNEDDISKEPYGEFYKSLINJKEDHA/ RAFUMTKIEDISKEPYGEFYKSLINJKEDHA/ RNEDDISKEPYGEFYKSLINJKEDHA/ RNEDDISKEPYGEFYKSLINJKEDHA/	YTEDEELINTKELMT 297 YTEDEELINTKELMT 295 YSVTBEELINTKELMT 283 YTEDEELINTKELMT 288 YTEDEELINTKELMT 281 HTSVEGLEFAALLF 330 HTSVEGLEFAALLF 330 HTSVEGLEFAALLF 330 HTSVEGLEFAALLF 334 HTSVEGLEFAALLF 334	Macrobrachtum_nipponense Exopaleamo, carinicauda Explana Informa_public Formation Eurytemora_affinis Penaeus_monodom Fenneropenaeus_afionisis Litopenaeus_vennameli Marsupenaeus_aporicus Metaponaeus_ancis	IETLOVADAWINKYKCIVILIPTELLASORILEPPUN IELALVVARANVINKYKCIVILIPTELLSORSIEEPPUN VEILAQVARANVINKYKCIVILIPTELLSORSIEEPPUN VEILAQVARANVINKYKCIVILIPTELLSORSIEEPPUN VEILAVARANVINKYKCIVILIPTELLSORSIEEPPUN VEILAVARANVINKYKCIVILIPTELSORSIESSIEEPPUN VEILAVARANVINKYKCIVILIPTELSORSIESSIEEPPUN KLOLDIEEDAPWEERTLEEIMPPLEODENASMERTU KLOLDIEEDAPWEERTLEEIMPPLEODENASMERTU KLOLDIEEDAPWEERTLEEIMPPLEODENASMERTU KLOLDIEEDAPWEERTLEEIMPPLEODENASMERTU	AARIYAMI ASRIYRMI SQRIHRMI AQRIHRMI AMRIHRMI AMRIHRMI 720 726 720 723 720 723 720 721
Sochel; sinensis Tromariles. Desematocheir sarobrachium_nipponense opplearono_canicauda spinia_putex infotus_laponicus sracyclopia_nana sracyclopia_nana sracyclopia_nana sracyclopia_colorisis openaeus, spanodom meroponaeus, chinensis openaeus, spanodom sraupenaeus, aponicus stepnaeus genis yfula_paramamoselin triunus_fitubercultaus	DEXPRICE/V95EDDAM-K806KGK/-VVER HXEREVERI/V95EDDAM-K906KKK/-VVER DKERVI/V95EDDAM-K906KKK/-VVER EEXPRIVE/V95EDDAM-K90KK/IKK EEXPRIVE/V95EDDAM-K90KK/IKK EEXPRIVE/V95EDDAM-K90KK/IKK FNFDDISKEYVEFYKSI/NOMEDIA/K HXEIN/FNFDDISKEYVEFYKSI/NOMEDIA/K FNFDDISKEYVEFYKSI/NOMEDIA/K FNFDDISKEYVEFYKSI/NOMEDIA/K FNFDDISKEYVEFYKSI/NOMEDIA/K FNFDDISKEYVEFYKSI/NOMEDIA/K FNFDDISKEYVEFYKSI/NOMEDIA/K	VTEDEELINKIKENI 297 VTEDEELINKIKENI 283 VTEDEELINKIKENI 283 VTEDEELINKIKENI 281 VTEDEELINKIKENI 274 VTEDEELINKIKENI 274 VTEDEELINKIKENI 330 NESVEGQLEFAALL 330 NESVEGQLEFAALL 330 NESVEGQLEFAALL 330 NESVEGQLEFAALL 332	Macrobrachtum_nipponense Exopaleamon carnicauda Daphnia putke Tarforous_apontous Paracyclopina_nana Eurytemora_affinis Penaeus_monodom Fenneropenaeus_ainnenis Litopenaeus_wannamei Marsupenaeus_japonicus Metapenaeus_ensis Scylia_paramamosain Portunus_triuberculatus	IETERQYADAWGINYKCI WILLPTELLSOFTLEPOYU IELENYALANNOKAYKCI WILLPTELLSOFTLEPOYU VEILEQUALANNOKAYKCI WILLPTELLSOFTLEPOYU VEILEQUALANNOKAYKCI WILLPTELLSOFTLEPOYU VEILEQUALANNOKAYKCI WILLPTELLSOFTLESOFTLEPOYU VEILEQUALANNOKAYKCI WILLPTELLSOFTLESOFTLEPOYU VEILEGUALANNOKAYKCI WILLPTELLSOFTLESOFTLEPOYU KLEGITEEDAPWEERILEENMPPLEODEDASTWEEY KLEGITEEDAPWEERILEENMPPLEODEDASTWEEY KLEGITEEDAPWEERILEENMPPLEODEDASTWEEY KLEGITEEDAPWEERILEENMPPLEODEDASTWEEY KLEGITEEDAPWEERILEENMPPLEODEDASTWEEY KLEGITEEDAPWEERILEENMPPLEODEDASTWEEY KLEGITEEDAPWEERILEENMPPLEODEDASTWEEY KLEGITEEDAPWEERILEENMPPLEODEDASTWEEY KLEGITEEDAPWEERILEENMPPLEODETSHWEEY	AARIYAM. ASRIYRMI SQRIHRMI SQRIHRMI AQRIHRMI AMRIHRMI 720 726 720 723 720 721 721 721
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图 3 蚤状溞 Hsp90 与其它物种氨基酸序列比对

Fig.3 The predicted amino acid sequence of Hsp90 of *D. pulex* was compared with Hsp90 of other species 黑色部分标出的分别为 GxxGxG 模块、LxxLL 模块、胞质 Hsp90 模块



图 4 Hsp90 基因的进化树分析图 Fig.4 Phylogenetic tree connecting the Hsp90 genes





功能调控、基因转录方面发挥着重要的作用(Michael et al, 2005);其C末端的MEEVD序列说明此Hsp90 存在于细胞质中,为胞质蛋白(Pelham, 1989;Gupta, 1995)。这些功能结构都存在于保守区(图 3),与翟会 芳等(2010)报道的甜菜夜蛾(Spodoptera exigua)、张道 伟等(2012)报道的白背飞虱(Sogatella furcifera)的 Hsp90的功能保守结构一致。结构分析说明蚤状溞 Hsp90与其它物种Hsp90基因具有相似的功能。另外 根据是否含有丰富的谷氨酰胺片段,Hsp90可分为 Hsp90和Hsp90(Hickey et al, 1989)。蚤状溞 HSP90不含有Hsp90所特有的磷酸化位点序列 TQTQDQ (Picard, 2002), 因此可以判定本文克隆的 是 Hsp90 基因。

从系统发育树中,作者发现节肢动物Hsp90 明显 分为两大类群,甲壳纲和蛛形纲聚为一亚群,昆虫纲 聚为另一亚群,说明节肢动物甲壳纲和蛛形纲的亲 缘关系比较近。蚤状溞 Hsp90 与剑水蚤、日本沼虾、 红螯相手蟹等甲壳纲的亲缘关系最近;其次与蛛形 纲的肩突硬蜱、龙骨三色蝎相关联;与印度跳蚁、刺 柚釉小蜂、褐飞虱等昆虫纲动物在分子进化上距离相 对较远。

为了验证 Hsp90 与枝角类的生殖转化是否有相 关性,本论文用 Real Time PCR 的方法检测了 Hsp90 mRNA 在蚤状溞不同生殖状态下的表达水平。结果显 示 Hsp90 在孤雌幼溞和孤雌溞中的表达量并无显著 性差异,这一结果说明 Hsp90 的表达与性成熟不相 关。Hsp90 mRNA 在雄溞中的表达量最高,其次是两 性溞(带冬卵),且在冬卵中的表达量最低。在相同环 境条件下 Hsp90 mRNA 在雄溞和两性溞(带冬卵)中的 表达量明显高于孤雌溞(带夏卵),这说明 Hsp90 可能 与两性溞(带冬卵)和雄溞的形成有关,两性溞(带冬 卵)和雄溞是在高密度等环境恶化的的条件下产生的,

高密度的应激条件诱导了 Hsp90 的表达,保护两性溞 和雄溞在环境胁迫条件下的发育生长,从而也提高 雄溞和两性溞(带冬卵)抵抗环境中由高密度引起的低 溶氧、低 pH 和食物稀少的能力。关于大型 番Hsp90 也有研究报道,在环境压力条件下,Hsp90 在胚胎发 育过程中起到积极作用,Hsp90 在环境压力条件下有 保护胚胎细胞的作用(Sass et al, 1997; Feder et al, 1999; Lewis et al, 1999; Soetaert et al, 2006), Hsp90 在蚤状溞冬卵中的表达量最低,可能是因为冬卵处 于休眠状态中、新陈代谢非常缓慢(几乎停滞)、故 mRNA 的表达水平也低于其它四种生殖状态。本实验 还发现, Hsp90 mRNA 在两性溞和雄溞中的表达也有 显著差异(P<0.05), 在雄溞中的表达量明显高于两性 溞, 这说明 Hsp90 可能参与了精子的形成过程。 王枫 (1997)也报道了 Hsp90 在草鱼的睾丸和脑中表达量最 高。同样 Huang 等(1999)报道,在精子冷冻的过程中, Hsp90 的含量急剧下降,从而导致了精子活力下降; 另外 Huang 等(2000)也发现,把 GA(一种 Hsp90 蛋白 的特异性抑制剂)加入到精子稀释液中, 会导致精子 活力下降。张莹(2011)也发现 Hsp90 与精子活力、顶 体完整率和精子畸形率均有一定相关性。Fatima(2013) 明确提出 Hsp90 能为精子的形成过程提供动力。以上 研究表明 Hsp90 基因有可能在调控生殖转化上发挥 作用。至于 Hsp90 基因在蚤状溞中的具体作用及其作 用机理等问题,作者将会通过 RNA 干扰、相关功能 基因过量表达以及免疫组化技术等,进行更深入的 研究。

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CLONING AND EXPRESSION ANALYSIS OF A Hsp90 GENE IN DAPHNIA PULEX

CHEN Ping¹, QIU Cheng-Gong¹, ZOU Xiu¹, ZHOU Jian-Kai¹, XU Shan-Liang¹,

WANG Chun-Lin¹, WANG Dan-Li¹, ZHAO Yun-Long²

(1. School of Marine Science, Ningbo University, Ningbo 315211, China;

2. School of Life Science, East China Normal University, Shanghai 200062, China)

Abstract The full-length cDNA of a Hsp90 gene (DpHsp90) was cloned from cladoceran *Daphnia pulex* using rapid amplification of complementary DNA ends (RACE) method. The DpHsp90 cDNA is 2568bp in length; and it has a 2155-bp open reading frame that encodes a 718-amino-acid polypeptide containing GxxGxG, LxxLL module (leucine zipper) and C-terminal MEEVD sequence. In addition, DpHsp90 shared homology of up to 85% with *Marsupenaeus japonicus* and *Metapenaeus ensis*, and 79% with other crustacean species. Phylogenetic analysis revealed that DpHsp90 protein has a close genetic relationship with crustacea such as *Tigriopus japonicus*, *Macrobrachium nipponense*, *Chiromantes haematocheir* and so on. Results of qPCR (Real-time Quantitative PCR) show that the DpHsp90 expression was significantly higher (P<0.05) in ephippial female (with winter eggs) than in parthenogenetic female (with summer eggs), and was the lowest in the resting egg. Therefore, Hsp90 was closely related to the reproduction conversion of *Daphnia pulex*. Meanwhile, Hsp90 mRNA expression in male was about 2.4 times higher than the parthenogenetic ones, indicating that Hsp90 may have been involved in the formation of sperm.

Key words Daphnia pulex; Hsp90; Real time-PCR; reproduction switching