

罗氏沼虾3个Dmrt基因的序列分析

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摘要: Dmrt 基因家族是一个与性别决定相关的基因家族。迄今, 已在鱼类、爬行类、鸟类、哺乳类等高等动物中检测到了 Dmrt 基因的存在。为了进一步探讨该家族在系统进化中的保守性, 本研究采用简并 PCR 技术, 扩增了罗氏沼虾 (*Macrobrachium rosenbergii*) Dmrt 基因的 DM 结构域。经序列分析, 获得了 Dmrt 基因家族的 3 个成员, 其编码序列分别与人 DMRT2、DMRT3、DMRT4 基因 DM 结构域编码序列的相似性分别为 93%、80% 和 95%, 根据罗氏沼虾的拉丁名分别命名为 MrDmrt2、MrDmrt3、MrDmrt4。与其他动物相关的 Dmrt 基因进行聚类分析, 结果表明, 不同进化地位动物的 Dmrt 基因 DM 域编码序列存在高度的同源性, 显示 Dmrt 基因在系统进化上高度保守, 序列上的相似性可能暗示着它们在功能上的保守性。

关键词: 罗氏沼虾; Dmrt 基因; DM 结构域; 简并 PCR; SSCP

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Dmrt (Double-sex and Mab-3 related transcription factor) 基因家族的主要特征是其成员与果蝇和线虫的性别决定基因 Dsx 和 Mab-3 相似, 所编码的蛋白质都包含一个具有 DNA 结合能力的保守基序——DM (Double-sex 和 Mab-3) 结构域^[1]。Dmrt 基因是一类转录调控因子, 通过锌指方式与特异 DNA 序列结合, 在性别决定和分化发育中起调控作用^[2]。迄今, 已在哺乳类、鸟类、爬行类、两栖类、鱼类等脊椎动物中检测到了 Dmrt 基因的存在, 并获得了多个成员基因^[1-5], 充分显示该基因家族在进化上具有高度的保守性, 但较低等的甲壳类动物中的相关研究则尚未见报道。

甲壳类是生物系统进化上的一个重要类群。罗氏沼虾 (*Macrobrachium rosenbergii*) 隶属节肢动物门甲壳纲长臂虾科沼虾属, 是我国重要的淡水食用虾, 具有重要的经济价值。在甲壳类中, 性别是一个重要的经济性状, 如中国对虾雌性的个体较大; 罗氏沼虾和日本沼虾雌性的个体较大; 雌性河蟹蟹黄丰富等等。因此, 若能在虾、蟹养殖上获得全雄或全雌的群体, 将会大大提高经济效益。所以, 研究甲壳动物性别决定的机制在理论和应用上都具有重要的意义。罗氏沼虾为 ZW 型性别决定^[6], 其性别决定的分子机制尚未得到阐明。本实验采用简并 PCR 技术首次对

罗氏沼虾 Dmrt 基因的 DM 保守序列进行了研究, 以期进一步研究 Dmrt 基因的功能、阐明甲壳动物性别决定的分子机制, 从而达到人工控制性别的目的。

1 材料与方法

1.1 实验材料

罗氏沼虾 (1♀, 2♂♂) 标本购于安徽芜湖吉和水产市场, 均为活体解剖确定性别。

1.2 基因组 DNA 提取

采用 Sambrook 的方法^[7], 从罗氏沼虾的肌肉组织中提取基因组 DNA。

1.3 PCR 扩增和 DM 的克隆

参考文献^[2]设计以下简并 PCR 引物用于扩增 Dmrt 基因保守区: D1: 5'-TGCG (AGC) (AC) G (AG) TGC (AC) G (AG) AA (CT) CACGG-3'; D2: 5'-C (GT) (GC) AG (GC) GC (GC) ACC TG (GC) GC (AGCT) GCCAT-3', 由上海生工生物工程公司合成, PAGE 纯化。PCR 反应体系为 25 μL, 循环参数为: 97℃ 预变性 7 min; 94℃ 变性 45 s, 64℃ 退火 45 s, 72℃ 延伸 1 min, 35 个循环; 72℃ 再延伸 10 min。PCR 扩增产物经 1.7% 琼脂糖凝胶检测后拍照。

PCR 扩增产物经纯化试剂盒 (上海华舜生物工程有限公司) 纯化, 与 pGEM-T 载体 (TaKaRa 公司)

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连接后,转化大肠杆菌 DH5 α 。

1.4 DMRT 基因片段的筛选和测序

采用菌落PCR(引物同上)和SSCP^[8]方法筛选出不同的阳性克隆。委托上海博亚生物公司进行测序。

1.5 Dmrt 基因的分析

将获得的3条Dmrt基因序列输入www.ncbi.nlm.nih.gov网站,通过BLAST,联机GenBank进行DNA及氨基酸序列相似性检索并命名。通过Clustal X软件对相应的氨基酸序列进行分析,系统进化树通过MEGA V2.1软件UPGMA法构建。

2 结果与分析

2.1 扩增结果

以罗氏沼虾基因组DNA为模板,经PCR扩增获得长约150 bp的产物片段(图1),阳性对照为黑斑蛙Dmrt1基因。此结果初步说明了罗氏沼虾的基因组中存在有Dmrt基因,且雌雄无差异。

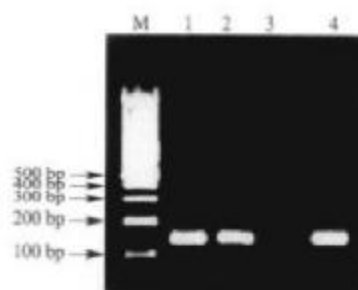


图1 PCR扩增结果

M:100 bp DNA ladder; 1. 雌; 2. 雄; 3. 阴性对照; 4. 阳性对照:黑斑蛙Dmrt1基因

Fig.1 The result of PCR amplification

M: 100 bp DNA ladder; 1. Female; 2. Male; 3. Negative control; 4. Positive control: Dmrt1 gene of *Rana nigromaculata*

2.2 菌落PCR扩增结果

菌落PCR扩增结果表明,克隆后的白色菌落中80%以上都是阳性克隆(图2)。



图2 部分菌落PCR结果

M:100 bp DNA ladder; 1,3,4,5,7,8,9,10,11:阳性克隆;2,6:假阳性克隆;12:基因组PCR扩增结果

Fig.2 PCR amplification result of clones

M: 100 bp DNA ladder; 1,3,4,5,6,7,8,9,10,11: Positive clones; 2,6: Negative clones; 12: PCR Amplification result of genome DNA

2.3 SSCP 分析筛选结果

通过SSCP技术筛选到3种有差异的阳性克隆(图3),且为雌雄个体所共有。

2.4 罗氏沼虾的Dmrt基因

对筛选出的3个有差异的阳性克隆进行测序,结果见图4。其编码的氨基酸序列见图5。经GenBank中同源检索,得到这3个Dmrt基因DM保守区与人相应的DMRT基因保守区编码序列的最高相似性分别为93%、80%和95%,按通用的命名法,根据罗氏沼虾的拉丁名*Macrobrachium rosenbergii*,分别命名为MrDmrt2、MrDmrt3和MrDmrt4。

2.5 不同物种Dmrt基因的聚类结果

不同物种Dmrt基因编码的氨基酸序列的聚类结果见图6。结果表明,罗氏沼虾的MrDmrt2与人

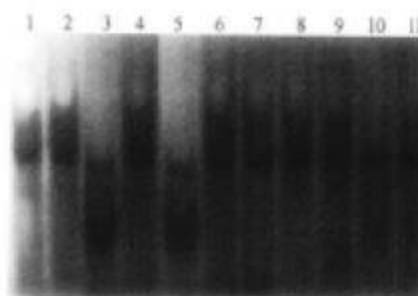


图3 SSCP分析结果

3种有差异的阳性克隆:1♀(2♀,4♂,6♀,7♂,8♂,9♀);3♂(5♀);10♀(11♂)

Fig.3 Result of SSCP analysis

Three different positive clones: 1♀ (2♀, 4♂, 6♀, 7♂, 8♂, 9♀); 3♂ (5♀); 10♀ (11♂)

	20	40	60
MrDmrt2	TGGCCCGGTGCAGAAACCAAGCGTGGTGTCTGCTGAAGGGGCACAAGAAGCTCTGT		
MrDmrt3	TGGCCAGATGCAGGAACCAAGCATGATCAGCTGGCTCAAGGGGCACAAGAGACTGTC		
MrDmrt4	TGGCCCGGTGCGAAACCAAGCGTAGTTTCAGCACTCAAAGGTCATAAACGTTACTGT		
	80	100	120
MrDmrt2	CGGTGGAGGGACTGCGCTGCGCCAACTGCTCTGGTGGTCGAGAGGCAGAGGGTCATG		
MrDmrt3	AAATTCAAGGACTGCACCTGCGCAGGTGTAATCTCATCGCGGGAGGCAGAGGGTGATG		
MrDmrt4	AGATGGAGAGATTGTGTTTGGCTAAATGCACGCTGATTGCTGAGCGGCAGCGGGTGATG		
	140		
MrDmrt2	GCGGCCAGGTGGGCTAAT		
MrDmrt3	GCGGCCAGGTGGGCTCAG		
MrDmrt4	GCGGCCAGGTGGGCTCAG		

图4 罗氏沼虾3条Dmrt基因DM盒区的DNA序列

Fig.4 DNA sequence of 3 Dmrt gene DM domain in *Macrobrachium rosenbergii*

	1	10	20	30	40	46
MrDmrt2	CARCRNHGVVSLKLGHKLCRWRCRCANCLLVVERQVRVMAAQVAL					
MrDmrt3	CARCRNHGMISWLKGRHCKFKDCTCARCNLIAGRQVRVMAAQVAL					
MrDmrt4	CARCRNHGVVSALKGHRKRYCWRDVCVCAKTLIAERQVRVMAAQVAL					

图5 罗氏沼虾3条Dmrt基因DM盒区编码的氨基酸序列

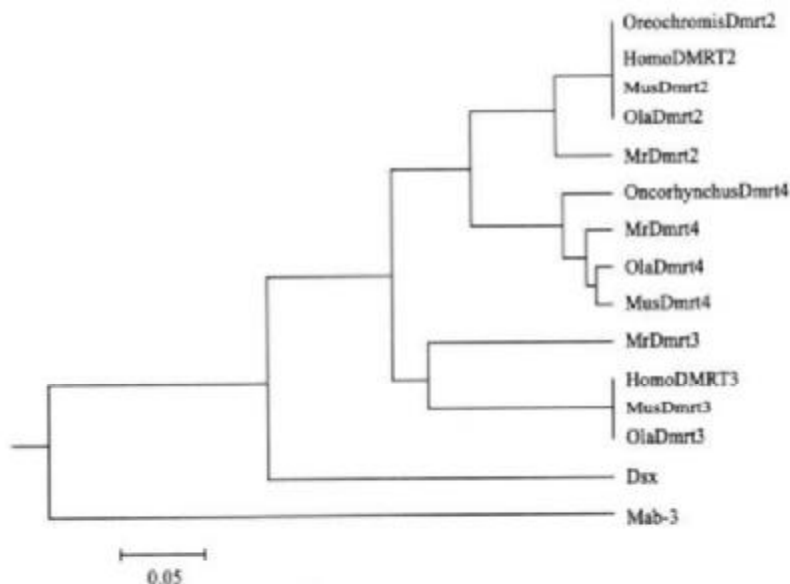
Fig.5 Amino acid sequence of 3 Dmrt gene DM domain in *Macrobrachium rosenbergii*

图6 Dmrt基因家族的聚类分析结果

MrDmrt2、MrDmrt3、MrDmrt4的氨基酸序列为本研究结果;其他序列是从GenBank中检索得到,登录号分别为: HomoDMRT2 (NP_870987.1), MusDmrt2 (AAN77205.1), OreochromisDmrt2 (AAN78446.1), OlaDmrt2 (AAL02163.1); HomoDMRT3 (NP_067063.1), MusDmrt3 (AAH52041.2), OlaDmrt3 (AAL02164.1); MusDmrt4 (AAN77234.1), OlaDmrt4 (BAB63259.1), OncorhynchusDmrt4 (AAG17546.1); *Drosophila* Dsx (P23023); *Caenorhabditis* Mab-3 (018214).

Fig.6 Phylogenetic analysis of Dmrt gene family

The amino acid sequences of MrDmrt2, MrDmrt3, MrDmrt4 were the result of this paper, while the others were searched from GenBank. The accession numbers are HomoDMRT2 (NP_870987.1), MusDmrt2 (AAN77205.1), OreochromisDmrt2 (AAN78446.1), OlaDmrt2 (AAL02163.1); HomoDMRT3 (NP_067063.1), MusDmrt3 (AAH52041.2), OlaDmrt3 (AAL02164.1); MusDmrt4 (AAN77234.1), OlaDmrt4 (BAB63259.1), OncorhynchusDmrt4 (AAG17546.1); *Drosophila* Dsx (P23023); *Caenorhabditis* Mab-3 (018214).

Sequence analysis of three Dmrt genes in *Macrobrachium rosenbergii*

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Abstract: The Dmrt genes constitute a new gene family related to sex-determination. Like the double-sex gene of *Drosophila melanogaster* and the Mab-3 gene of *Caenorhabditis elegans*, they encode transcription factors characterized by a conserved zinc-finger like DNA-binding motif, the DM domain, which is thought to bind DNA in the process of sex differentiation and development. In 1998, Dmrt1 genes were first identified and found containing DM domains in *Homo sapiens* and *Mus musculus*. And Dmrt1 was found to regulate sex determination and differentiation in vertebrates extensively. So far, the Dmrt genes have been discovered in a wide range of animal species, such as fish, amphibian, reptiles, birds, and mammals. These evidently reveal the evolutionary conservation of Dmrt gene family. But the research of Dmrt genes in crustacean, which was a very important group in phylogenic evolution, has not been reported. *Macrobrachium rosenbergii*, which is subjected to *Macrobrachium*, Palaemonidae, Crustacea, is a very important kind of freshwater edible shrimps in China and the economic value is prominent. As to *Macrobrachium rosenbergii*, sex was an important economic character, for the male are bigger than the female. So to clarify the mechanism of *Macrobrachium rosenbergii* sex determination will be of great importance in both theory and application. With the hope of doing further research on the functions of Dmrt genes and clarify the molecular mechanism of *Macrobrachium rosenbergii*, degenerate PCR was used in this research to amplify the conserved DM domains of Dmrt genes in *Macrobrachium rosenbergii*. The PCR products were approximately 150 bp. After DNA cloning SSCP analysis and sequencing, three different DM sequences were identified. The amino acid sequence of one clone is 93% identical with human DMRT2, named MrDmrt2, according to its name *Macrobrachium rosenbergii*. Another sequence encodes an amino acid sequence with 80% identical with human DMRT3, named MrDmrt3. The other sequence encodes an amino acid sequence with 95% identical with human DMRT4, named MrDmrt4. The amino acid sequences encoded by Dmrt genes from different species showed a high degree of sequence homology as revealed in phylogenic tree constructed. In Fig. 7, the comparative analysis of DM domains of Dmrt2, 3, 4 in different species showed that 67% amino acid site of all sequences were the same. These conservative site may form a C2/H2 model zinc-finger structure to bind specific DNA sequences and regulate sex differentiation and development. The results further indicated that Dmrt genes were highly conserved in phylogeny and the strong evolutionary conservation of this gene family suggests that Dmrt genes may be of importance in developmental process.

Key words: *Macrobrachium rosenbergii*; Dmrt gene; DM domain; degenerate PCR; SSCP

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