

研究论文

奥利亚罗非鱼DMRT1和DMRT4抗体制备及组织表达谱分析

曹谨玲¹, 曹哲民², 吴婷婷²

1. 南京农业大学渔业学院, 无锡 214081;
2. 中国水产科学研究院淡水渔业研究中心农业部水生动物遗传育种和养殖生物学重点开放实验室, 无锡 214081

收稿日期 2006-7-21 修回日期 2006-11-20 网络版发布日期 2007-6-6 接受日期

摘要

DMRT1和DMRT4是DMRT基因家族的成员, 该家族成员与果蝇的性别决定基因和线虫性别决定基因一样, 所编码的蛋白质都包含一个具有DNA结合能力的保守基序, 即DM结构域, 并以锌指结构与特异DNA序列相结合, 在性别决定和分化发育中起调控作用。采用RT-PCR方法分别从奥利亚罗非鱼卵巢和精巢中扩增克隆出DMRT1和DMRT4全长cDNA片段, 构建表达载体, 在大肠杆菌中表达了BMP-DMRT4和BMP-DMRT1蛋白。经Xa切割、Amylose-sepharose柱层析纯化后作为抗原免疫新西兰白兔制备了DMRT1和DMRT4多克隆抗体, 并进行纯化。对纯化多抗进行Western blot分析, 结果表明获得了高特异性的DMRT1和DMRT4抗体。为了观察DMRT1和DMRT4在组织中的表达谱, 首先, 我们通过实时荧光定量RT-PCR检测雌雄奥利亚罗非鱼多种组织mRNA的表达, 仅在卵巢和脑中检测到DMRT4, 在精巢中检测到DMRT1; 其次, 制备了多种组织匀浆蛋白, 使用纯化的抗体进行Western blot分析, 分别在卵巢和精巢中检测到DMRT4和DMRT1蛋白的表达; 制备多种奥利亚罗非鱼组织切片, 使用纯化的DMRT4和DMRT1多抗进行免疫组织化学分析, 发现DMRT4仅在卵巢表达, 而DMRT1仅在精巢表达。这些结果有助于阐明DMRT4和DMRT1的功能及在鱼类性别调控中的作用。

关键词 [DMRT1; DMRT4; 原核表达; 多克隆抗体; 组织表达谱](#)

分类号

Generation of Antibodies Against DMRT1 and DMRT4 of *Oreochromis aurea* and Analysis of Their Expression Profile in *Oreochromis aurea* Tissues

Jinling Cao¹, Zhemin Cao², Tingting Wu^{2,①}

1. Fishery College, Nanjing Agricultural University, Wuxi 214081, China;
2. Key Open Laboratory for Genetic Breeding of Aquatic Animals and Aquaculture Biology, Ministry of Agriculture, Freshwater Fishery Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China

Abstract

<P>Sex determination is composed of somatic and germ-line sex differentiation hierarchies whose interaction is poorly understood. A single gene known to control somatic sex determination, the DM-domain containing (Doublesex/Mab-3 DNA-binding motif) gene, is highly conserved across species. Vertebrate DMRT1 (DM-related transcription factor 1) expression occurs predominantly in the testis. Here, however, isolated two distinct DM-domain cDNA from *Oreochromis aurea* ovary and testis have been named DMRT4 (DM-related transcription factor 4) and DMRT1 by BLAST, respectively. Despite high homology in the DM-domain, there is little similarity outside the

扩展功能

本文信息

- ▶ [Supporting info](#)
- ▶ [PDF\(856KB\)](#)
- ▶ [\[HTML全文\]\(375KB\)](#)
- ▶ [参考文献](#)

服务与反馈

- ▶ [把本文推荐给朋友](#)
- ▶ [加入我的书架](#)
- ▶ [加入引用管理器](#)
- ▶ [复制索引](#)

Email Alert

- ▶ [文章反馈](#)
- ▶ [浏览反馈信息](#)

相关信息

- ▶ [本刊中 包含 “DMRT1; DMRT4; 原核表达; 多克隆抗体; 组织表达谱” 的相关文章](#)
- ▶ [本文作者相关文章](#)

- [曹谨玲](#)
- [曹哲民](#)
- [吴婷婷](#)

DM-domain. To better understand the structure, function, and possible roles of [DMRT4](#) and [DMRT1](#) as potential candidates for sex differentiation and sex determination, the intact regions encoding [DMRT4](#) and [DMRT1](#) obtained by PCR were sub-cloned into the vector pMAL-c2x and introduced into the *Escherichia coli* TB1 cell for efficient fusion expression. After purification and cleavage, [DMRT4](#) and [DMRT1](#) proteins were used to immunize adult rabbits following standard protocols. Consequently, it was found by using Western blot analysis that polyclonal antibodies against [DMRT4](#) and [DMRT1](#) had high specificity. The relative expression levels of [DMRT4](#) and [DMRT1](#) mRNA were determined by fluorescent Real-time RT-PCR in female and male *Oreochromis aurea* with β -actin as the internal standard. [DMRT1](#) was expressed only in testis, whereas [DMRT4](#) was over expressed in the ovary, but in both female and male, a slight expression in the brain was also detected. Statistical analysis showed that in the brain, mean [DMRT4](#) mRNA levels in female were significantly higher than in male. Meanwhile, the expression of [DMRT4](#) and [DMRT1](#) protein was also analyzed using the purified antibodies through Western blot and immunohistochemistry. It was found that [DMRT4](#) was exclusively expressed in the ovary and [DMRT1](#) in the testis. Study on [DMRT4](#) and [DMRT1](#) expression facilitated the elucidation of their roles and the understanding of sex differentiation of fish.

Key words [DMRT1](#) [DMRT4](#) [prokaryotic expression](#) [polyclonal antibody](#)
[expression profile](#)

DOI:

通讯作者 曹谨玲 caojinling7928@163.com