Molecular Cloning and Evolutionary Implications of Growth Hormone/Prolactin Family Gene cDNAs in Grouper Epinephelus coioides

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Abstract Total RNA was isolated from pituitary of grouper Epinephelus coioides and SMART cDNA was synthesized from 50 ng RNA. Two genes in the growth hormone/prolactin family were cloned from SMART cDNAs plasmid library. The growth hormone cDNA contains 938 bp and encodes 204 amino acids. The prolactin cDNA contains 1429 bp and encodes 212 aa. The deduced aa sequences of grouper GH and PRL were compared with those of other species. Average genetic distances and a dendrogram showing the hierarchical structure of affinity among different nine fishes were implemented in Mega 2 and CLUSTAL W 1.64b. Branch and bound search analysis 1000 replicates were performed and the degree of support for particular nodes were produced. The three phylogenetic trees drawn from the whole aa sequence of GH, PRL and SL were almost identical Epinephelus coioides Pera flaescens Sparus aurata and Paralichthys olivaceus were clustered into one group. Oncorhynchus keta and Oncorhynchus mykiss were clustered into one group. Carassius auratus and Ictalurus punctatus were clustered into one group while Anguilla japonica alone was clustered into another group. Alignment of the deduced aa sequence and the phylogenetic tree based on GH/ PRL/SL showed the same clustering as the present hierarchy of fish and a higher conservation of SL than GH and PRL among various fish species is suggested in this text. Although there is only 24% – 31% identity between the three hormones overall the C-terminal region shows a higher identity. Grouper SL appears to be more closely related to GH
30.8% than to PRI25.6% which is slightly more than the identity of GH to PRI24.1%.

Key words Epinephelus coioides Pituitary SMART cDNA GH/PRL family Growth hormone Prolactin

取出垂体。垂体总质量。

结果与分析

将斜带石斑鱼的生长激素、催乳素及生长催乳素基因及其表达模式做了初步分析，以及家族内成员的氨基酸序列进行系统分析，构建分子克隆及其进化意义。合成所用的

豌豆 Serranidae Epinephelus coioides Boonyaratpalin 1997 Yao et al.

Jia et al. 2004

SMART cDNA

PRL SL

1 RNA

1.1 RNA

500 g RNA SV Total RNA Isolation System Promega

20 30 μL 0.5 μL

1.2 SMART cDNA

SMART switching mechanism at 5’ end of RNA transcript cDNA

Xie et al. 2001 SMART cDNA Library Construction Kit Clontech

CDS 10 μmol/L 5’-AAGCAGTGTAACAACGCAAGACT

Smart II 10 μmol/L 5’-AAGCAGTGTAACAACGCAGAGT-3’ PCR 10 μmol/L 5’-AAGCAG TGGTAACAACGCAGAGT-3’ ID

RNA 50 ng Smart II 1 μL 72 °C 2 min 2 min 10 μL 10 μL 5 250 mmol/L Tris-HCl pH 8.3 375 mmol/L KCl 30 mmol/L MgCl2 2 μL DTT 20 mmol/L 1 μL dNTP 10 mmol/L 1 μL PowerScript 1 μL 1 μL 42 °C 1 h 2 μL cDNA 2 μL PCR 4 μL 2 μL 50 × Advantage 2 cDNA Polymerase Mix 100 μL

PCR 95 °C 1 min 95 °C 5 s 65 °C 5 s 68 °C 6 min 72 °C 10 min 13 72 °C 5 μL

PCR

1.3 SMART cDNA

SMART cDNA pGEM-T Promega DH5α X-gal/IPTG GenBank

SMART cDNA Library Construction Kit Clontech

2 SMART cDNA

2.1 SMART cDNA
Table 1  Accession number of GenBank of GH, PRL, and SL of eight kinds of fish

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2.2 28S rRNA, 18S rRNA, and RNA 1a SMART cDNA: 1.0% ds cDNA 0.1–4 kb SMART cDNA

2.2.1 GH cDNA 5’ 3’ 204 bp 5’ 3’ 59 bp 3’ Signal P 5’ 3’ 267 bp 19 18 N 18

750 bp 18 18 18 18 41%–96% pituitary

Fig. 1 Total RNA isolation a and SMART cDNA synthesis b electrophoresis pattern of grouper Epinephelus coioides pituitary
斜带石斑鱼生长激素基因的序列及其推导的开放阅读框的氨基酸序列

催乳素基因全长序列及由此推断的氨基酸序列见图D。全长为@ '!E F7，编码含有!@!个氨基酸的蛋白。

ABC #L端的!'个氨基酸肽段为信号肽，信号肽酶的可能切割位点位于第!'和!>位氨基酸之间。斜带石斑鱼的ABC基因具有硬骨鱼所特有的'个半胱氨酸，而非四足动物所特有的?个半胱氨酸。

ABC SL端的序列相对较保守，几乎均有?个亮氨酸和!个半胱氨酸。SC有?个保守的半胱氨酸，其中$L端有!个半胱氨酸，SL端有'><个半胱氨酸。

通过对斜带石斑鱼与虹鳟、鳗鲡、鲫鱼、金头鲷、金鲈、大马哈鱼、牙鲆、鲶鱼的ABC及SC的氨基酸序列的进行比较，我们发现ABC的UL端的序列相对较保守，几乎均有?个亮氨酸和!个半胱氨酸。SC有?个保守的半胱氨酸，其中$L端有!个半胱氨酸，UL端有'><个半胱氨酸。

石斑鱼生长激素催乳素基因家族的序列比较表明，石斑鱼的GH、PRL、ABC和SC个成员的氨基酸序列构建的系统树相似性较高，均为石斑鱼与金头鲷、金鲈和牙鲆聚成一类；虹鳟与大马哈鱼聚成一类；鲫鱼与鲶鱼聚成一类；鳗鲡成另外一类。

石斑鱼生长激素R催乳素基因家族#个成员的序列比较

Fig.2  Nucleotide sequence of group© Epinephelus coioides© GH and the predicted open reading frame contains 204 amino acids as shown under the nucleotide sequence
图斜带石斑鱼催乳素基因的序列及其推导的开放阅读框的氨基酸序列在的氨基酸同源性在的保守性高于CD和B：的遗传距离较小，而与89：的遗传距离较大。89：与B：的遗传距离为。这4个基因C端的氨基酸同源性非常高，尤其是J K端的22是严格保守的。

讨论CD、89：和B：都是由垂体分泌的结构相关的激素。CD和89：在脊椎动物中广泛存在，但B：仅在硬骨鱼类中被鉴定出来。CD、89：和B：在结构上的一致性表明这些蛋白可能起源于一个共同的祖先。B：的氨基酸大小范围较窄，为个；CD为个；而89：的大小范围最大，为个。因而B：比CD和89：有较高的保守性。我们的分析也同样表明了这一点。鳗鲡B：与CD的基因序列的同源性高于与89：的同源性；大麻哈鱼B：与CD的同源性也高于与89：的同源性，据此，推测B：可能起源于CD，而非89：。

成熟肽的氨基酸序列的同源性为，由此认为这3种激素是相互独立进化的。但肺鱼的B：与CD的同源性高于动物研究卷

Fig. 3 Nucleotide sequence of group[Epinephelus coioides] PRL and the predicted open reading frame contains 212 amino acids as shown under the nucleotide sequence
根据斜带石斑鱼与“种鱼的生长激素（GH）、催乳素（PRL）和生长催乳素（IGF）的氨基酸序列，利用软件构建的系统树

![系统树图](image)

图4 由Epinephelus coioides和其他八种鱼使用NJ方法推断的系统树

对于斜带石斑鱼和PRL的同源性（21%）与May et al. 1999得出的完全一致，而对于斜带石斑鱼和IGF的同源性（25.6%）与May et al. 1997得出的也完全一致。


