DOI号: 10.3724/SP.J.0000.2009.61126

鲤鱼发育早期 HPG轴和 GH/IGF轴相关因子的转录起始分析

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摘要: 采用 RT-PCR的方法, 以不同发育时期的鲤鱼胚胎和幼鱼为材料, 研究了与鱼类生殖相关的 HPG轴以及与生长相关的 GH/IGF轴中 *GnRH*、*GH* 以及 *GH*、*GHR* 和 *IGF* 重要信号分子的转录起始特征。结果显示, *sGnRH*、*cGnRH*、*GH-I* β亚基和 *GHR* 于鲤鱼胚胎受精后 20h开始转录, *IGF-1*于受精后 23h开始转录, *GH-II* β亚基于受精后 26h开始转录, *GH* α亚基于受精后 46h开始转录, *GH* 于 1dph(孵出后第 1天)开始转录。其中, *GHR* 和 *IGF-1*均早于 *GH* 开始转录, *GH* α亚基和 β亚基的转录起始时间不同步。研究结果为揭示鲤鱼生殖与生长间的调控机制积累了重要的科学依据。

关键词: *GnRH*/*GH*; *GH*/*IGF*/*GHR*; 鲤鱼胚胎; 转录起始
中图分类号: Q344+.1 文献标识码: A 文章编号: 1000-3207(2009)06-1126-06

脊椎动物的下丘脑分泌促性腺激素释放激素(Gonadotropin-releasing hormone, GnRH)作用于垂体, 促进促性腺激素(Gonadotropin, GtH)的合成[1—3], GtH刺激性腺分泌性激素, 诱导性腺的发育成熟[2,4,5]。GnRH和 GtH作为下丘脑—垂体—性腺(Hypothalamic-pituitary-gonad, HPG)轴的关键信息分子与其他因子共同调节着脊椎动物的生殖发育。生长轴(GH/IGF轴)中, 由垂体分泌的生长激素(Growth Hormone, GH)是调节脊椎动物个体生长的主要因子, 主要通过其受体 Growth Hormone Receptor (GHR)[6,7]及其下游因子 IGF-1发挥功能[8]。在鲤鱼中, sGnRH对 GtH和 GH的分泌也有促进作用[9], GH可提高鲤鱼幼鱼脑部 *IGF* mRNA的水平[10]。同时, 外源 GH也可促进去垂体鲤鱼的生长[11]。可见, 鲤鱼有着与其他脊椎动物相同的 HPG轴和 GH/IGF轴作用机制, 进而对鲤鱼的繁殖和生长进行调控。

有研究表明, HPG轴及 GH/IGF轴之间存在相互作用的关系。雌性鲤鱼中, 一定水平的类固醇激素可刺激垂体细胞分泌 GH[12]。长期的雌激素处理抑制罗非鱼幼鱼垂体中 GH的分泌和组织中 *IGF-1*的转录[13]。而在鲶(*Heteropneustes fossilis*)的研究则发现, GH可以诱导卵巢的成熟[14,15]。

Moles, *et al*. 对孵出后 50d到 300d的欧洲鲈鱼幼鱼中 *GnRH*、*GnRH* 受体和 *GH* 的转录研究发现, 这些重要信号分子的同步激增可能调控着性腺分化的起始[16]。而对一些鱼类的胚胎和幼鱼中 *GH*/*GHR*/*IGF* 的转录分析揭示了 GH/IGF轴对鱼类早期个体的正常生长发育可能发挥着重要作用[17—19]。在犬齿牙鲆(*Paralichthys. adspersus*)的幼鱼[20]和孵出后 1d的鲶鱼(*Epinephelus coioides*)幼鱼[21]中分别发现了 *GHR* 和 *GH* 的转录; 虹鳟鱼[22]和斑马鱼胚胎中[23]则存在 *IGF* 及其受体的转录。这些研究提示, HPG轴和 GH/IGF轴中的重要信号分子对鱼类的早期发育, 各种组织器官的形成与分化起到重要作用, 而且 HPG轴及 GH/IGF轴之间的相互作用可能从胚胎发育的早期即存在。因此, 开展 HPG轴及 GH/IGF轴中重要信号分子的转录起始特征研究, 对于阐明鱼类生殖与生长的相互作用关系具有重要的意义。

本文以我国重要的养殖鱼类黄河鲤(*Cyprinus carpio* L.)为研究对象, 通过分析鲤鱼发育早期 HPG轴和 GH/IGF轴中几个关键信号分子的转录起始特征, 以期为揭示鲤鱼 HPG轴及 GH/IGF轴之间的调控机制提供科学依据。

收稿日期: 2009-03-16; 修订日期: 2009-07-29
基金项目: 国家 973计划(2007CB109206); 国家自然科学基金(30771641)资助
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1　材料与方法

1.1　实验材料　黄河鲤由河南省水产科学研究所提供,本实验室保存。选择发育良好的雌雄黄河鲤为亲本,人工授精获得受精卵。在室温下(23℃)饲养胚胎。分别收集未受精卵、32细胞期、64细胞期、256细胞期、50%外包、受精后 20h、受精后 23h、受精后 26h、受精后 32h、受精后 40h、受精后 46h、受精后 58h、孵出后 1d(1 day post-hatch, dph)、2dph、3dph、5dph、7dph、9dph时期的样品,保存于 1mL TRIzol(invitrogen公司)中备用,用于提取总 RNA。同时,对重要发育时期的胚胎及幼苗进行拍照,观察胚胎及幼苗发育特征。

1.2　总 **RNA** 提取及 **RT-PCR** 分析　取各发育时期的胚胎及幼苗,依 Invitrogen公司试剂盒方法提取总 RNA,取 1μL RNA 样品经 1%的琼脂糖凝胶电泳,同时测 OD 值,分析总 RNA 的含量和质量。

利用 ReverTra Ace酶(TOYOBO公司)反转录 RNA 合成 cDNA。PCR 检测 cDNA 中目标基因的转录,同时以 β-*actin* 作为内参基因。PCR反应体系为 25μL。PCR 反应条件如下:94℃ 5min;94℃ 30s,60℃ 30s,72℃ 30s,共 40个循环;72℃ 延伸 5min。最后,通过 2%琼脂糖凝胶电泳分析 PCR 产物。检测目标基因转录的引物序列(表 1)。

表 1　**PCR** 扩增所用引物及其序列

Tab.1　Sequences of PCR primers used in the study

引物名称 Primers	引物核苷酸序列 Sequence
sGnRH-sen	5′GTCATACGGTTGGCTTCC 3′
sGnRH-ant	5′GCGTCCACCTCATTCACT 3′
cGnRH-sen	5′TTGACTTGAGGGAAACGA 3′
cGnRH-ant	5′CAAACTGGGCACTTAGAAA 3′
GH-Iβ-sen	5′GCTCTTATCTTCCACCCTG 3′
GH-Iβ-ant	5′CTCGTAACGCACATCCC 3′
GH-IIβ-sen	5′GGTCACTGCCTGACAAAG 3′
GH-IIβ-ant	5′ACACCTGGAGGACAATCT 3′
GHα-sen	5′CGCAGGAAGTCAAGAAC 3′
GHα-ant	5′AACAGCATCCCATACAC 3′
GH-sen	5′TGCTATTGTCGGTGGT 3′
GH-ant	5′CTGTCTGCGTTCCTCA 3′
GHR-sen	5′CCACAACACGCAAGTCT 3′
GHR-ant	5′CCAGTCCGTTTCCACA 3′

续表

引物名称 Primers	引物核苷酸序列 Sequence
IGF-sen	5′TCCCAGGACACCAAAG 3′
IGF-ant	5′TCGCCCTACTGTCTTCTA 3′
β-actin-sen	5′GATGATGAAATTGCCGCACTG 3′
β-actin-ant	5′ACCAACCATGACACCCTGATGT 3′

注:IGF-sen、IGF-ant的退火温度为 53℃;其他引物退火温度均为 60℃;sen代表正向引物;ant代表反向引物

Note: The annealing temperature should be chosen at 53℃ for IGF PCR products. Others should be 60℃. Sen stands for sense primers. Ant stands for antisense primers

2　结　果

2.1　总 RNA 的质量鉴定

取各发育时期的胚胎和幼苗总 RNA 1μL,经 1%的琼脂糖凝胶电泳,显示 28S和 18S条带清晰(图 1),紫外分光光度计测定各样品总 RNA 的 A_{260}/A_{280} 比值均在 1.8—2.0之间,RNA 质量符合试验要求。

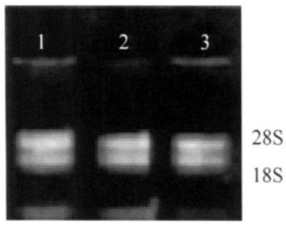

图 1　总 RNA 电泳检测结果

Fig.1　Total RNA extracted from embryos and larvae

各泳道样品代表的时期:1.3dph;2.9dph;3.受精后 20h

Sample run in Lane 1 is RNA extracted from larvae of 3dph, and then larvae of 9dph and embryos of 20hpf

2.2　RT-PCR 分析结果

从图 2可见,在 23℃培养条件下,HPG 轴中的 *sGnRH*、*cGnRH* 和 *GH-I* β亚基于受精后 20h开始转录,*GH-II* β亚基于受精后 26h开始转录,只有 *GH* α亚基的转录起始较晚,于受精后 46h开始转录。而 GH/IGF 轴中的 *GHR* 于受精后 20h开始转录,*IGF-1*于受精后 23h开始转录,*GH* 于 1dph开始转录。可见,HPG 轴和 GH/IGF 轴中的基因转录起始的时间大概一致,只有个别基因如 *GH* α亚基和 *GH* 的转录较滞后,说明其在调控轴中发挥调控作用的可能性。

2.3 胚胎及幼苗发育图谱

为进一步研究 HPG 轴及 GH /IGF 轴重要因子的转录起始与胚胎发育间的相互关系, 本研究观察了几个重要时期的胚胎发育特征 (图 3), 图 3a 受精后 2.5h胚胎, 胚胎分裂到 64细胞期, 可见原生质网正向动物极流动; 图 3b 受精后 11h胚胎, 胚层外包 50%, 胚盾出现; 图 3c受精后 20h胚胎, 肌节 7—10对, 脑区隆起较高; 图 3d受精后 23h胚胎, 肌节 9—14对, 脑区进一步增高; 图 3e 受精后 46h胚胎, 眼球呈透明状, 头部与尾部之间呈 90°角; 图 3f孵出后第 3天鱼苗, 眼球呈乌黑状, 头部与尾部之间呈 180°角, 侧游。

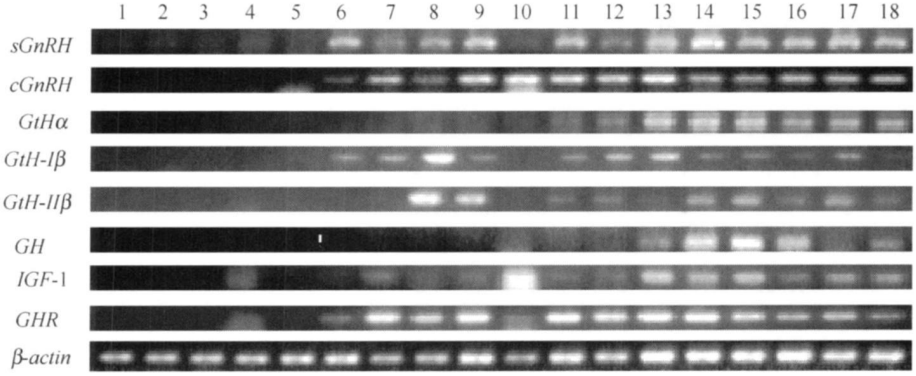

图 2 鲤鱼早期个体目标基因表达分析结果

Fig. 2 Expression pattern of target genes in common carp embryo and early larval stages

各泳道样品代表的时期:1. 64 细胞期; 2. 32 细胞期; 3. 256 细胞期; 4. 未受精卵; 5. 50% 外包; 6. 受精后 20h; 7. 受精后 23h; 8. 受精后 26h; 9. 受精后 32h; 10. 受精后 40h; 11. 受精后 46h; 12. 受精后 58h; 13. 1dph; 14. 2dph; 15. 3dph; 16. 5dph; 17. 7dph; 18. 9dph

Sample runs in Lane 1 is PCR products of 64-cell stage embryos, and then 32-cell stage, 256-cell stage, unfertilized eggs, 50% -epiboly, 20, 23, 26, 32, 40, 46 and 58 hours post fertilization (hpf), and 1, 2, 3, 5, 7 and 9 days post hatching(dph) in turn

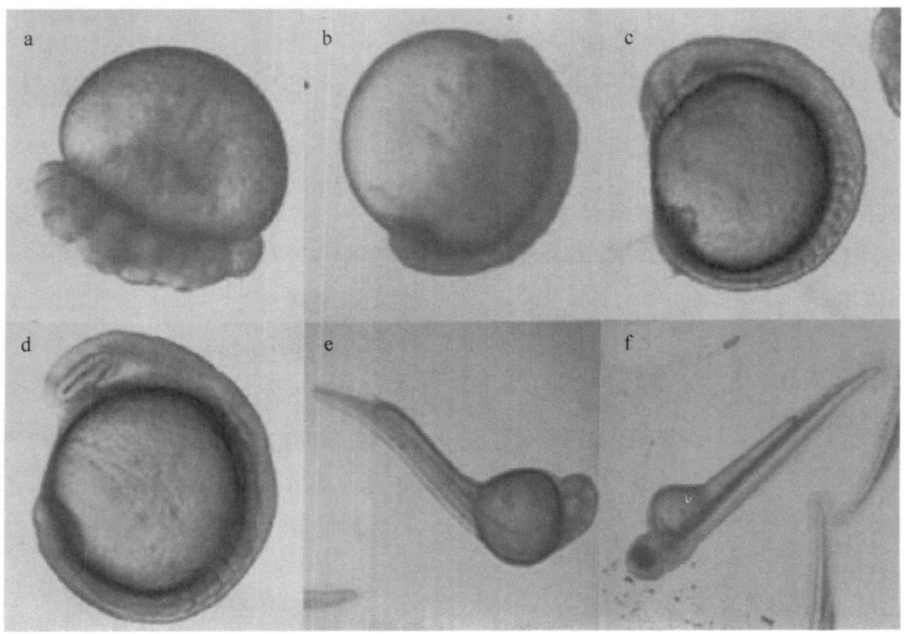

图 3 鲤鱼胚胎及幼苗发育图谱

Fig. 3 Face views of common carp embryos and larvae

a. 64 细胞期; b. 胚层 50% 外包期; c. 胚胎受精后 20h; d. 胚胎受精后 23h; e. 胚胎受精后 46h; f. 孵出后第 3 天幼苗

a. 64-cell stage; b. 50% epiboly; c. 20hpf; d. 23hpf; e. 46hpf; f. 3dph

3 讨　论

3.1 HPG轴关键因子的转录特征

本文研究了在室温(23℃)培养条件下,鲤鱼发育早期胚胎和幼鱼中 HPG 轴关键因子的转录起始特性,结果发现 *sGnRH* 和 *cGnRH* 均于鲤鱼胚胎受精后 20h开始转录。*GH-I* β亚基于受精后 20h表达,*GH-II* β亚基于受精后 26h开始转录,而 *GH* α亚基则于受精后 46h开始转录。*sGnRH* 基因在端脑和下丘脑转录水平较高[24],*cGnRH* 基因主要在端脑和中脑转录[25],而 *GH* 基因主要在垂体中转录,由受精后 20h胚胎图 3c和受精后 23h胚胎图 3d可见,脑区有逐步增高的趋势,可以推测在此时期 *GnRH* 基因与 *GH* 基因的转录起始与鲤鱼胚胎脑区的分化有关,随着脑及垂体等重要器官的发生,相应基因开始转录。

斑马鱼中的研究发现,*sGnRH* 和 *cGnRH* 分别于斑马鱼胚胎受精后 26h和 24h开始检测出转录[26 27];而有报道表明,鲑鱼的 *sGnRH* 的转录起始表达不早于受精后 10d[28]。不同胚胎间 *GnRH* 基因转录起始时间的差异可能与胚胎的发育速率有关。本研究中,*GnRH* 基因开始转录时,鲤鱼胚胎发育至 7—10体节期,斑马鱼受精后 24h和 26h时,发育至 Prim-5期[29];鲤科类的孵化时间与鲑科类有着明显的不同,鲤科类的孵化时间只需 1d左右,而鲑科类虹鳟的孵化时间需要 22d[28],而此时,鲤鱼早已发育成幼鱼。鲤鱼 *GnRH* 的转录起始时间与虹鳟相比大大提前,与鲤鱼胚胎发育速率较虹鳟快的表型相一致。

GtH-I和 GtH-II由相同的 α亚基与不同的 β亚基结合而成[30]。*GH* α亚基于鲤鱼胚胎受精后 46h开始转录,*GH-I* β亚基和 *GH-II* β亚基的转录起始分别在受精后 20h和 26h,可见 α亚基的转录起始靠后,这也许可以认为是一种对 *GH* 转录的调控形式,通过对 *GH* α亚基的转录调控进而控制 GH的合成。

3.2 GH/IGF轴关键因子的转录特征

对鲤鱼发育早期胚胎和幼鱼中 GH/IGF轴重要信号分子转录起始特征的研究发现,*GH* 于 1dph开始转录,非常有趣的是,*IGF-1*于受精后 23h开始转录,比 *GH* 的转录起始早。有研究发现,在虹鳟胚胎中,垂体和肝脏形成之前即 GH/IGF轴形成之前,*IGF* 有转录,说明这时 *IGF* 的转录不受 GH/IGF轴的调控[31 32]。另有观点认为,GH可能主要是对出生后的个体进行生长调控,而胚胎时期的生长发育则主要由 IGF-1来完成,这种作用独立于 GH[33]。斑马鱼中的研究发现,无论是抑制 IGF-1受体的表达,或是对其进行负调控,还是 IGF的过量表达都会对斑马鱼胚胎的正常发育产生影响[34 35];而抑制 GH的表达对受精 10d内的斑马鱼胚胎发育没有明显的影响[36]。本研究关于 *IGF-1*的转录起始早于 *GH* 的研究发现暗示,IGF-1可能独立于 GH在鲤鱼早期胚胎发育中发挥着作用。当然,IGF和 GH在鱼类早期发育中发挥的具体作用还有待进一步研究。

与鲑鱼、罗非鱼、青鳉等鱼类相同[37],鲤鱼胚胎 *GH* 的转录起始于 1dph;*GHR* 于受精后 20h开始转录,比 *GH* 的转录起始早,这种表达次序与日本鳗和虹鳟中的研究结果一致[37,32],究其原因可能与 GH的分泌调节有关[37]。在鱼类的早期发育过程中,GHR可能以游离的 GH结合蛋白的形式存在[38],GH通过与 GHR的结合对鱼类早期个体的生长发育发挥作用,而 GHR在胚胎发育过程中早于 GH表达,对 GH产生调控作用[37]。

HPG轴和 GH/IGF轴的相互作用及其机制是一个非常复杂而又有趣的问题,本研究对 HPG轴和 GH/IGF轴中与生殖和生长相关的重要因子的转录起始特征进行了初步研究,为阐明 HPG轴和 GH/IGF轴的相互作用机制积累了重要的科学依据。

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GENE EXPRESSION PROFILES OF GROWTH AND REPRODUCTION RELATED GENES DURING THE EARLY DEVELOPMENT OF COMMON CARP(*CYPRINUS CARPIO* L.)

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Abstract As many researches revealed some relations between HPG and GH/IGF axis in teleost, we detected gene expression profiles of growth- and reproduction-related genes from HPG or GH/IGF axis in common carp (*Cyprinus carpio*) embryos and early larval stages, in order to find some regulatory relations between HPG and GH/IGF axis in the early development stages of common carp. The genes under detection were *sGnRH*, *cGnRH*, *GH* α subunit and β subunit from HPG axis and *GH*, *IGF*, growth hormone receptor (*GHR*) from GH/IGF axis, with β-*actin* gene as the internal control. Samples were collected from embryos and larvae of unfertilized eggs, 64-cell stage, 32-cell stage, 256-cell stage, 50%-epiboly, 20-, 23-, 26-, 32-, 40-, 46- and 58- hours post fertilization (hpf), and 1-, 2-, 3-, 5-, 7- and 9- days post hatching (dph) reared at 23℃, and then RNA extracted from the samples was used in RT-PCR. *sGnRH*, *cGnRH*, *GH* α subunit, *GH-I* β subunit, *GH-II* β subunit, *GH*, *GHR* and *IGF-1* showed different expression patterns in carp embryos and larvae. Development around 20h post fertilization (hpf) and 23hpf was critical for carp embryos, as *sGnRH*, *cGnRH*, *GH-I* β subunit and *GHR* were first detectable at 20hpf, *IGF-1* at 23hpf and *GH-II* β at 26hpf. Meanwhile, embryos in 20hpf and 23hpf stages showed apparent development in brain region, indicating a regulatory function of GnRH and GH in the development of brain. *GH* α subunit was first detectable at 46hpf while *GH* was first detectable at 1d post hatching (dph). The difference in the timing of the expression of *GH* and *IGF* mRNA may suggest that *IGF* gene expression is not GH-dependent. Additionally, the timing of the expression of *GH* α subunit, *GH-I* β subunit, *GH-II* β subunit were unsynchronized in embryos, suggesting a regulatory function of *GH* α subunit in GtH expression.

Key words: *GnRH*/*GH*; *GH*/*IGF*/*GHR*; Common carp; Embryos; Gene expression