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鱼类促性腺激素研究进展

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摘 要

鱼类促性腺激素(Gonadotropin Hormone, GtH)是硬骨鱼类关键生殖调控激素之一，在促进鱼类性腺成熟以及排卵过程中起至关重要的作用。本文综述了鱼类促性腺激素的生化结构、生理功能、调控机制以及重组表达研究现状，为进一步研究鱼类促性腺激素以及鱼类的繁育工作提供参考。

关键词

硬骨鱼类，促性腺激素，调控机制，蛋白重组表达

Research Progress on Gonadotropin Hormones in Fish

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Abstract

Gonadotropin hormone (GtH) is one of the key reproductive regulatory hormones in bony fishes, which plays a vital role in promoting gonadal maturation and ovulation in fish. In this paper, the biochemical structure, physiological function, regulatory mechanism and recombinant expression of gonadotropin hormones in fish are reviewed, which provides a reference for further research on gonadotropin hormones and fish breeding.

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Keywords

Bony Fishes, Gonadotropin Hormones, Regulatory Mechanisms, Recombinant Protein Expression

1. 引言

促性腺激素(Gonadotropin Hormone, GtH)是硬骨鱼类关键生殖调控激素之一，在鱼类性腺成熟以及排卵过程中起至关重要的作用。自从上世纪 60 年代鱼类 GtH 的功能被发现，国内外学者对不同鱼类 GtH 的研究便逐步开展。随着鱼类养殖业与分子生物学研究的不断发展，关于促性腺激素的研究也愈发深入。包括鱼类 GtH 的结构与功能，调控机制，以及其与养殖生产结合后的实用性。从鱼类垂体中提取的天然鱼类 GtH 需要耗费大量鱼体材料且产物质量难以保证，进行基因体外重组表达获得鱼类 GtH 的重组蛋白成为趋势。整合梳理国内外研究有助于我们进一步研究鱼类 GtH 的机制，探究 GtH 应用于生产工作的发展性，为鱼类养殖生产及繁育工作提供参考。

2. 使鱼类促性腺激素研究进展

2.1. 硬骨鱼类下丘脑－垂体－性腺(HPG)轴

在鱼类的繁殖过程中，下丘脑－垂体－性腺(Hypothalamic-Pituitary-Gonadal Axis, HPG)轴起着至关重要的作用。下丘脑通过接收光照和温度等外源性环境因子信号，刺激下丘脑分泌促性腺激素释放激素(Gonadotropin-Releasing Hormone, GnRH)。大部分硬骨鱼类缺乏垂体门脉系统，GnRH 通过神经轴突末梢作用于垂体，刺激垂体腺细胞进行促性腺激素(Gonadotropin Hormones, GtH)的合成与分泌[1] [2]，GtH 通过血液循环达到卵巢后，同卵巢表面特异性受体结合，一方面通过直接作用，调控卵母细胞发育、成熟和排出，另一方通过诱导性类固醇激素(雌激素和雌激素)的合成分泌，通过局域性旁分泌和自分泌方式间接影响卵母细胞发育[3]-[5]。

2.2. 鱼类促性腺激素的结构与生理功能

GtH 作为 HPG 轴关键核心激素，在鱼类卵母细胞发育过程中起重要调控作用，直接影响鱼类卵母细胞的成熟和排出。鱼类 GtH 分为两种，促卵泡刺激素(Follicle-Stimulating Hormone, FSH)和促黄体生成素(Luteinizing Hormone, LH)，在结构上与陆生哺乳类的 FSH 和 LH 同源[6]-[8]，由一个共有的 α 亚基(CGα)和特有的 β 亚基(FSHβ、LHβ)以共价键结合而成[9]，二者结合形成异二聚体糖蛋白后，同卵巢表面受体结合后，GtH 与其特异性受体结合后，激活第二信使 cAMP，启动复杂的信号通路，通过信号级联效应，调控卵母细胞的成熟和排出，发挥其生殖调控作用[10]-[12]。随着此方面研究的不断进展，发现在不同物种的 GtH 之间，α 亚基的氨基酸保守性很高，而 β 亚基存在一定的差异。所以 β 亚基决定了两种 GtH，LH 与 FSH 之间的特异性差异[13] [14]。

在硬骨鱼类中，LH 与 FSH 发挥着各自的作用。对于雌鱼，FSH 主要在卵巢成熟前期发挥作用，通过刺激卵泡膜细胞和颗粒细胞合成雌二醇(Exogenous Estradiol-17b, E2)促进卵母细胞的卵黄积累过程。LH 则在卵巢成熟后期通过刺激卵母细胞表面的膜细胞和颗粒细胞分泌成熟诱导激素(Maturation-Inducting Hormone, MIH)促进卵母细胞成熟与排卵[15] [16]。对于雄鱼，在精巢生长与发育早期，FSH 在血液中含

量升高，促进精巢的发育成熟，在精子发生与发育成熟阶段，血液中 LH 水平开始提高，作用于精巢细胞表面的受体，刺激雄激素的分泌，从而促进精子的形成与发育成熟[17] [18]。

2.3. 鱼类促性腺激素的结构与生理功能

GtH 鱼类的下丘脑通过 GnRH、多巴胺、氨基丁酸(GABA)、垂体腺苷环化酶激活肽(PACAP)、去甲肾上腺素、神经肽 Y (NPY)、5-羟色胺和 Kisspeptin 等多种神经激素对 GtH 的释放进行调节。

2.3.1. 促性腺激素释放激素

GnRH 作为 HPG 轴中的关键激素，同时作为 GtH 的上游调节激素，直接调控 GtH 合成分泌。已经在数种硬骨鱼类中发现并证明 GnRH 具有提高 GtH 基因表达水平的功能[19]，其中 GnRH 对于 LH 的 mRNA 水平的调节作用较于 FSH 更为明显，在鲤鱼[20]、罗非鱼[19]、条纹鱼[21]与金鱼[22]的研究中都已经被证实。这表明 GtH 的两种亚型之间存在调控差异，但也有可能是由于垂体中合成分泌 LH 的细胞数量多于 FSH [23]。

GnRH 具有三个亚型，最早发现于罗非鱼与鲑鱼中，命名为 GnRH1 (sbGnRH)、GnRH2 (cGnRH)与 GnRH3 (sGnRH) [24]。在鲷鱼与罗非鱼的研究中发现，GnRH1 在诱导 GtH 分泌时，效力明显低于其他两个亚型[11] [25]。

2.3.2. 多巴胺

在多种硬骨鱼类中，多巴胺可以抑制 GnRH 刺激的 GtH 分泌，从而对鱼体内 GtH 水平进行调控，这一点在金鱼[26] [27]、三文鱼[28]、虹鳟[29] [30]、鳗鱼[31]、鲟鱼[32]以及罗非鱼[11] [33]中都得到了证明。目前在鱼类人工养殖的雌性亲鱼生殖调控过程中，最常用到商品激素为促黄体生成素释放激素类似物(Luteinizing Hormone-Releasing Hormone-A, LHRH-A)，其本质为 GnRH 的类似物[34]，通过诱导 GtH 中 LH 的合成分泌，间接促进卵母细胞发育和成熟。在养殖生产过程中，多潘立酮常用于与 LHRH-A 共同作用[35]，可以抑制多巴胺受体从而控制多巴胺对 GnRH 分泌的反调控[36]，但是该药物有明显的副作用，尤其对海水鱼类会造成生理损伤。

3. 鱼类促性腺激素重组表达

GtH 是硬骨鱼类 HPG 轴调控过程中的关键因子，对于鱼类养殖与生产意义重大。虽然目前技术可以从鱼类垂体提取物中纯化出天然的 GtH，但需要耗费大量的生物资源，且得到的产物效果并不理想[37] [38]。与之相比，重组 GtH 不需要耗费大量生物资源，而且可以连续生产。

3.1. 原核重组表达

目前在研究中最常见的原核重组蛋白表达系统就是大肠杆菌(*Escherichia coli*, *E. coli*)表达系统，大肠杆菌作为蛋白质生产中最常用的宿主生物之一，具有易操作、成本低、效率高等优势。在大肠在大肠杆菌中，蛋白质可在细胞质中胞内产生，直接进入周质，或分泌到胞外环境。细胞质是还原环境，而周质是氧化环境，允许二硫键的形成，并且还具有一定的蛋白水解活性[39]。然而，与真核表达系统相比，大肠杆菌不能进行大多数的翻译后修饰(尤其是糖基化修饰)，并且通常无法进行复杂蛋白的折叠[40]。

在大肠杆菌表达系统的研究中，大肠杆菌 BL21 (DE3)为目前最为常用的菌株为，BL21 适合 T7 启动子驱动的重组蛋白诱导表达系统[41]，该菌株缺失内源性蛋白酶的大肠杆菌 B 菌株，它能有效避免重组表达蛋白的降解，广泛用于重组蛋白的表达。BL21 (DE3)是被 λ 噬菌体 DE3 溶原化所得 BL21 衍生菌株。噬菌体 DE3 中的 T7 RNA 聚合酶受到 lacUV5 启动子控制。外源添加 IPTG 可以诱导 BL21 (DE3)菌株中 T7 RNA 聚合酶的快速表达。T7 RNA 聚合酶进而识别表达质粒载体上的 T7 启动子，从而驱动重组蛋白

的高效表达[42]。但是，受原核生物功能的局限，其生产的重组蛋白不能进行大多数蛋白修饰、无法进行复杂蛋白的折叠并且重组蛋白还可能会出现包涵体导致蛋白无法正常发挥作用[43]。针对这些缺点，目前也存在一些解决方法，例如在蛋白质序列中添加融合标签来增加重组蛋白的产量，或是通过优化密码子来避免包涵体的产生[44]。但是，有些融合标签的存在会影响重组蛋白发挥功能，需要在后续步骤中剪除，密码子的优化也需要准确精密的实验方案设计，同时需要验证重组蛋白中天然结构的回收率[45]-[49]。在鱼类促性腺激素重组表达的研究中，利用原核表达系统进行生产的方法已经趋于成熟，在鲟鱼[50]、鳗鱼[51]、黄颡鱼[52]等许多鱼类中均有研究。

3.2. 真核重组表达

真核重组表达体系的种类很多，包括酵母、哺乳动物细胞、昆虫细胞与植物细胞等。

3.2.1. 毕赤酵母表达系统

酵母是单细胞真核微生物，它们适于高密度培养，并具有进行某些翻译后修饰所必需的细胞机制。其中，甲基营养型酵母巴斯德毕赤酵母在过去近二十年中已经成为最常用的酵母基因表达系统之一[53]。毕赤酵母重组表达体系拥有高水平的重组蛋白产量并且能够正确进行复杂蛋白质的折叠，而且没有脂多糖污染，非常适合药物用重组蛋白的生产。毕赤酵母通常会将外源基因整合到酵母基因组中以产生稳定的高表达菌株，可以进行小规模表达试验来筛选产量较高的克隆。在毕赤酵母表达体系中常用的启动子为甲醇诱导型醇氧化酶(AOX1)强启动子或组成型甘油醛-3-磷酸脱氢酶(GAP)强启动子，此外还有谷胱甘肽依赖性甲醛脱氢酶(FLD1)启动子、过氧化物酶体基质蛋白(PEX8)启动子等，根据重组表达要求不同，选择不同的启动子可以发挥满足不同要求的功能。

目前已经有许多研究成功利用毕赤酵母表达系统进行了不同鱼类促性腺激素的体外重组表达，并且将重组蛋白运用于科学研究与生产应用中[54]-[58]。

3.2.2. 哺乳动物细胞表达系统

哺乳动物细胞表达系统适合于生产分子量大且结构复杂的重组蛋白，因为它可以提供与天然蛋白非常相似的细胞环境。某些重组蛋白需要复杂的翻译后修饰与折叠，而哺乳动物细胞表达系统就具备这些功能[59]。常用与研究与生产的哺乳动物细胞表达系统有人胚肾细胞293 (HEK 293)与中国仓鼠卵巢(CHO)细胞。HEK 293细胞系易于转染，经常用于研究应用，而CHO细胞通常是生产生物药物蛋白的首选表达系统[60] [61]。

HEK293T细胞由HEK293细胞衍生而来，是SV40 largeT抗原稳转得到的细胞株，这意味着当外源质粒载体中含有SV40复制起点时，HEK293T能高水平表达蛋白，显著提高重组蛋白的产量[62]。而且HEK293T保留了原本的高转染效率，使得HEK293T在蛋白的重组表达生产中使用越来越广泛[63]。

3.2.3. 其他表达系统

昆虫细胞表达系统在重组蛋白的生产同样比较常见，在昆虫细胞中，蛋白质可以在细胞内产生，也可以分泌到细胞外环境，这需要N端信号肽的存在。在多数情况下，昆虫细胞肽酶可以识别哺乳动物信号序列[64] [65]，也可以识别天然昆虫细胞信号序列(例如，gp 67、HBM、SP1、SP2) [66]。尽管昆虫细胞能够进行N-和O-糖基化，但它们缺乏复合型N-聚糖，从而影响某些蛋白在昆虫细胞中的重组表达效果[67]。

无细胞表达(Cell-Free Expression, CFE)即在无细胞环境中使用转录和翻译所需的组分产生蛋白质[68]。大多数CFE系统使用细胞的粗提取物来进行表达，这些细胞通常参与高速率的蛋白质合成，如未成熟的红细胞(网织红细胞) [69] [70]。这些粗提取物的内源性DNA和mRNA被去除，随后给细胞裂解产

物补充进行翻译所需的大分子成分，例如核糖体、tRNA、氨酰 tRNA 合成酶和起始、延伸和终止因子。随后，通过添加合适的模板(DNA 或 mRNA)启动翻译程序，并在适当的温度下进行翻译[71]。

4. 总结

GtHs 作为生殖轴的核心关键激素，促进鱼类性腺发育，影响卵子和精子的成熟与排出，在鱼类种苗人工繁育过程中发挥重要作用。目前在养殖生产过程中常用到 LHRH-A 与多潘立酮的共同使用，LHRH-A 本质为 GtHs 上游基因 GnRH 类似物，可以调控 GtHs 合成与分泌。但这两种药物都有明显的副作用，存在对卵子和精子催熟效果不稳定、缩短亲鱼生殖年限等问题，因此通过构建高效鱼类 GtHs 的体外重组表达体系，获得特异性重组蛋白，成为了亟待解决的问题。

国内外的学者在多种鱼类的研究中，利用原核、真核、细胞以及其他表达系统，对鱼类 GtHs 进行了不同程度的体外重组表达以及重组蛋白活性与功能验证，这些研究将丰富鱼类 GtHs 的研究内容，夯实基础理论研究基础，同时为养殖鱼类生殖调控提供重要技术支撑。

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