

Cyp19a1 基因在鱼类性别分化和发育中的研究进展

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摘要:鱼类是人类获得优质动物蛋白的重要途径,蛋白的高效产出是水产研究领域的热点.许多鱼类存在性别异形的特点,因此,培育生长快、个体大的单性群体是增加产量的方式.单性群体的培育工作已在部分鱼类中完成,但鱼类的性别化机制复杂,受遗传、环境等因素影响.鱼类芳香化酶(*Cyp19a*)可将雄激素转化为雌激素,是鱼类卵巢分化和发育的关键因素.*Cyp19a1* 基因在发挥功能时,不仅受到相关转录调控因子的影响,也与外界环境因素(如温度、pH 值、盐度、湿度、光照等)有关.随着分子生物学技术的发展,水产动物性别分化机制日益深入,*Cyp19a1* 在性腺发育过程中的调控机制也愈发明确.通过综述水产动物中的 *Cyp19a1* 基因的研究进展,为鱼类性别分化与性别发育机制的探索提供参考.

关键词:鱼类;性别分化;*Cyp19a1*;环境;转录因子

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鱼类是脊椎动物中分布最广的种类,其物种数量占动物的一半以上.鱼类性别决定与分化机制复杂,受遗传、内分泌与环境因素影响,其性别决定与分化可塑性较强,多种鱼类中可通过温度、性激素诱导性腺分化的方向.芳香化酶由 *Cyp19a1* 基因编码,在动物体内可以将雄激素转化为雌激素.在大多数哺乳动物、鸟类、爬行动物和两栖动物中,仅获得单拷贝芳香化酶基因,但硬骨鱼辐鳍亚纲鱼类存在两种芳香化酶,由两个基因编码(*Cyp19a1a* 和 *Cyp19a1b*).*Cyp19a1a* 与 *Cyp19a1b* 在结构和功能上有差异,具有不同的表达调控机制,可能是进化过程中鱼类特异的基因组复制后,在选择压力下产生亚功能化所导致^[1-2].

1 *Cyp19a1* 基因分布与功能

Cyp19a1a 和 *Cyp19a1b* 结构和功能上的差异与表达位置有关,*Cyp19a1a* 主要在性腺中表达,*Cyp19a1b* 主要在鱼类的脑中表达.类固醇性激素可诱导鱼类性腺发育,而 *Cyp19a1a* 初始表达位于类固醇激素合成细胞的前体细胞,在多种鱼类中的表达早于性腺分化^[3],卵巢中主要在滤泡细胞中表达^[4].采用表达谱、基因敲除、类固醇性激素诱导性腺发育等技术,均证实 *Cyp19a1a* 和 E2 参与鱼类卵巢分化和发育的调控^[5-6],这在鱼类性逆转过程中尤为明显^[7-8].例如,在黄鳝(*Monopterus albus*)中,*Cyp19a1a* 表达随卵巢的退化逐渐降低^[8].

Cyp19a1b 基因对性腺发育的影响研究较少,该基因在性腺分化阶段参与神经内分泌代谢过程,也参与维持精巢的过程^[2,9].在鲶(*Heteropneustes fossilis*)性腺中,主要表达的芳香化酶基因是 *Cyp19a1a*,但也发现有 *Cyp19a1b* 具有较高的表达水平^[10].*Cyp19a1b* 在银汉鱼(*Atherina bleekeri Günther*)精巢中大量表达,表明该基因可能对部分物种的精巢发育有重要作用^[2],这与多数物种的研究结果不同.

Cyp19a1 在哺乳动物精巢成熟过程有重要的调控作用,主要在睾丸间质细胞、生精上皮等细胞表达,参

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与精子发生等过程的调控,基因缺失会导致雄性不育^[11-12].Cyp19a1a也在多种鱼类的精巢中表达^[13-14],但在精巢中的表达远低于卵巢中表达,由于Cyp19a1位于常染色体,该基因的雌雄差异表达可能源于启动子甲基化^[15].在已报道的研究中,仅在斑马鱼(*Danio rerio*)中研究了Cyp19a1对精巢发育的影响.Cyp19a1a缺失会产生全雄鱼,并导致精巢发生延迟^[16],但与哺乳动物不同的是,Cyp19a1a并不决定雄性斑马鱼的繁殖^[17].

鱼类中腺型芳香化酶基因存在性别差异表达,转录起始位点附近的CpG岛在雌雄个体中的甲基化水平差异可能是差异表达的原因之一^[15,18-19].Cyp19a1a在卵巢发育的不同阶段也存在表达差异,在牙鲆(*Paralichthys olivaceus*)中与启动子甲基化水平有关^[5].黄鳝中,可以通过甲基化抑制剂(5-氮杂-2-脱氧胞苷)抑制性逆转的发生^[8].在卵巢成熟过程中,Cyp19a1a表达量在牙鲆^[20]、鮰(*Miichthys miiuy*)^[21]等鱼类产卵前达到峰值,在不同时期的表达量差异与基因的甲基化水平有关^[22],说明该基因也参与卵巢成熟的调控,但在不同发育阶段甲基化水平产生的原因尚待解析.

哺乳动物中,Cyp19a1的组织特异性表达主要是通过组织特异性启动子驱动完成.鱼类中,也存在组织特异启动子,并且功能存在不同.黄鳝脑和垂体Cyp19a1b的启动子存在差异,脑中Cyp19a1b启动子含雌激素反应元件(ERE),但对E2无反应;垂体Cyp19a1b启动子含有雄激素反应元件(ARE),DHT和雌激素仅会诱导垂体Cyp19a1b基因表达,且该反应是一种剂量依赖反应^[23].鱼类中,启动子的差异可能是由于Cyp19a1位于不同染色体上导致^[24],这是硬骨鱼类与哺乳动物的不同之处.

2 环境因子及其对Cyp19a1的影响

鱼类的生活环境多样,性别决定与分化过程复杂.环境对鱼类性腺发育的调控是低等脊椎动物性别发育的重要研究内容,Cyp19a1的表达与环境因素的有很大关联,包括到温度、光照、环境激素等多个方面.

2.1 温度

环境因素对鱼类性别分化有重要影响,温度对性别分化的影响最普遍,也是研究最多的环境因素.DNA甲基化作为表观遗传学调控途径,是温度调控性腺发育的重要途径.许多鱼类Cyp19a1a基因启动子序列包括多个CpG位点^[5,8,19],该区域在不同物种中存在相似或不同的转录因子结合位点.例如,牙鲆启动子CpG富集区域存在CRE、Ad4BP等转录因子结合位点^[5].虽然温度调控性别分化的上游途径尚未明确,但许多鱼类Cyp19a1a启动子甲基化水平与温度高低紧密相关.Cyp19a1a启动子甲基化和高活性区域内转录因子的结合活性对性别分化的影响较大,且该调控过程在鱼类中较为保守.欧洲黑鲈(*European seabass*)^[7]、乌龟(*Mauremys reevesii*)^[25]等动物胚胎发育阶段,在产雄温度孵化条件下,Cyp19a1a启动子甲基化水平升高,基因表达量降低,雌激素合成水平降低并促进精巢发育,产雌温度下则反之.此外,Cyp19a1b与Cyp19a1a在不同温度下的表现不同,在欧洲黑鲈性腺分化的温度敏感阶段,仅在性腺发现Cyp19a1启动子甲基化程度与温度呈现较大关联,在脑中甲基化程度与温度关联度较小^[7].

Cyp19a1启动子甲基化是可逆的,牙鲆(*Paralichthys olivaceus*)中发现启动子在卵巢分化的早期呈去甲基化现象,但是温度升高却能抑制去甲基化过程,提高甲基化水平,造成启动子处于高度甲基化状态^[5].对于温度依赖型性别决定(TSD)类型动物,红耳滑龟(*Trachemys scripta elegans*)胚胎发育阶段,芳香化酶基因启动子区的甲基化水平在不同温度下有差异,在温度敏感时期开始时其CpG位点的甲基化程度较高,而在雌性产生温度下其胚胎内CpG位点呈现去甲基化现象,此时芳香化酶表达水平下降^[26].温度和甲基化程度的高低有着较大的关联,可以通过温度的改变来影响生物体内DNA甲基化程度,从而调节芳香化酶基因的表达,达到性别控制的目的.

2.2 激素

鱼类繁殖及性腺发育过程中,易受到性激素与环境激素的影响.性激素和环境激素可结合雌激素受体(ERs、GPER)和雄激素受体(AR)调节个体内分泌^[27-29],也可以通过结合Lhb启动子调节个体内分泌等^[30],类似的调控方式在其他的温度依赖型性别决定类型动物中也有发现^[31].不同激素对Cyp19a1基因表达调控的作用方式不同,石斑鱼(*Epinephelus coioides*)中,E2可增强Cyp19a1b而非Cyp19a1a启动子活

性^[32].促性腺激素可通过 cAMP 信号通路上调卵巢 *Cyp19a1a* 基因表达^[8].性腺不同发育阶段,激素对鱼类性逆转的影响不同.在石斑鱼^[33]中,MT 诱导性腺发育,早期过程取消 MT 饲喂不会影响性腺的正常发育,晚期阶段会导致性逆转,说明性激素诱导的性逆转可能存在剂量效应.可的松诱导牙鲆由雌性到雄性的性逆转过程中,通过 cAMP 途径抑制 *Cyp19a1* 表达^[34].此外,环境激素(双酚 A)也可以通过非受体途径影响个体的激素代谢,但其具体途径尚待解析^[35].

3 转录调节因子对 *Cyp19a1* 基因表达的影响

Cyp19a1 基因表达除了受环境影响外,还受到诸多转录及转录后调控因子的影响.在鱼类中的研究表明,*Cyp19a1* 基因启动子序列中存在 *Sox*, *GATA* 基因家族、*CREB*, *AP1*, *Foxl1*, *ARE*, *Nr5a1/Sf-1* 等^[36-39] 多个转录调控因子的结合位点,以上转录调控因子与 *Cyp19a1* 基因启动子结合后均会对其表达产生影响.

3.1 核受体家族

核受体家族含 NR0~NR6 共 7 个亚家族,核受体家族成员在动物的胚胎发育及分化、类固醇激素合成和生殖等方面有重要作用,受内分泌激素调节,并参与调控 *Cyp19a1a* 转录^[40].类固醇生成因子 1(*Sf-1/Nr5a1*)是孤儿核受体家族的一员,是调控动物体内类固醇性激素合成的重要基因之一^[41,42].*Nr5a1a* 可以结合 *Cyp19a1a* 启动子,从而调控 *Cyp19a1a* 的表达.在对青鳉(*Oryzias latipes*)、银汉鱼和罗非鱼性别分化中发现 *Sf-1* 作为信号调节因子与 *Foxl2* 共同作用 *Cyp19a1* 基因从而来调控体内雌激素水平以达到性别控制的结果^[43,44].通过 CRISPR/Cas9 敲除罗非鱼 *Sf-1* 基因,在 *Sf-1* 缺失的雌性罗非鱼中发现卵巢组织中 *Cyp19a1* 的表达水平下降,同时血液中雌激素水平也随之下降,但是对 *Sf-1* 缺失的雌性罗非鱼进行雌激素处理进行回救,可以使卵巢产生卵母细胞,但是这些卵母细胞不能发育成为成熟卵细胞,说明 *Sf-1* 与性腺发育和卵子细胞的成熟机制有关^[44].

Nr5a2 和 *Nr0b1* 作为芳香化酶的转录调节因子对芳香化酶的表达有重要调控作用,在鱼类卵巢滤泡和精巢支持细胞中表达^[45].*Nr5a2* 可上调 *Cyp19a1* 基因表达,*Nr0b1* 表达增加会下调芳香化酶在卵巢中的表达^[38,40].在对牙鲆(*Paralichthys olivaceus*)的研究表明,*Nr5a2* 可能作为一个上游调节基因去促进牙鲆性腺分化,而 *Nr0b1*(性类固醇合成拮抗因子)可以抑制 *Nr5a2* 的表达,抑制芳香化酶的表达^[45].

3.2 叉头框(Fox)基因家族

Fox 基因家族是转录因子家族之一,其特征是分子结构中有一个叉头 DNA 结合域.哺乳动物中,*Fox* 基因家族命名从 *Foxa* 到 *Foxs*,在生物个体发育过程中参与调控细胞分化、胚胎发育等^[46].*Foxl2* 在脊椎动物进化过程的中较为保守,在青鳉、罗非鱼、石斑鱼等鱼类中发现 *Fox* 基因家族多个成员参与调控鱼类卵巢分化及发育过程^[37,47-49],是性别发育调控研究中的关键基因之一.目前,*Foxl2* 可作为生理雌性的标志基因,并且在动物精巢发育过程,需要雄性性别决定基因对其参与的卵巢分化途径进行抑制^[50].用雌激素处理太平洋丽龟(*Lepidochelys olivacea*)发现,*Foxl2* 表达早于 *Cyp19a1*,说明 *Foxl2* 在 TSD 类型动物中对性腺发育有重要作用^[51].在罗非鱼进行研究发现,*Foxl2* 可直接作用于 *Cyp19a1a* 调节体内雌激素的表达量,从而达到间接诱导卵巢分化的目的^[52].*Cyp19a* 也可以通过启动子甲基化,阻遏 *Foxl2* 的结合,达到负调控 *Foxl2* 表达^[53].此外,*Foxo1*,*Foxo3b* 也可以通过 *Foxo* 结构域结合 *Cyp19a1a* 启动子从而调节该基因的表达^[54].

3.3 Dmrt1

Dmrt 基因家族在性别决定和性别分化中非常重要,*Dmrt1* 基因被认为是半滑舌鳎(*Cynoglossus semilaevis* Gunther)^[55]、斑马鱼^[56]、中华鳖(*Pelodiscus sinensis*)^[57] 等多种动物的雄性性别决定基因.*Dmrt1* 是目前已知的与性别相关基因中较保守的基因,对于爬行动物、鸟类等 ZW/ZZ 型动物精巢的形成和发育具有重要的作用.罗非鱼中,*Dmrt1* 可结合 *Cyp19a1a* 基因启动子中 ACATATGT 回文序列,通过下调 *Cyp19a1a* 基因表达,调控精巢分化及发育^[58].但在其他鱼类中,*Dmrt1* 是否也通过下调 *Cyp19a1* 的表达调控精巢发育尚待深入研究.此外,鸟类的性别决定与 ZZ 个体或 ZW 个体中 *Dmrt1* 的拷贝量有关(剂量补

偿效应)。在对牙鲆性腺发育时期 *Dmrt1* 基因与 *Cyp19a1* 基因的表达进行分析,发现 *Dmrt1* 在精巢中的表达水平要高于卵巢,而 *Cyp19a1* 在卵巢中表达高于精巢^[15],这种差异虽然与启动子甲基化有关,但是否存在剂量补偿效应,也有待研究。

3.4 CREBP(CRE 结合蛋白)

促性腺激素可以促进卵泡刺激素(FSH)和黄体生成素(LH)的生产,这两种类固醇激素都需要通过胞内信使 cAMP 的变化来表达从而间接调节芳香化酶表达的活性^[38]。在卵巢中, *Cyp19a1a* 启动子区域具有 CRE(cAMP 反应元件),该位点可与 CREBP 或其类似物结合,从而上调基因表达^[59]。在黄鳝中, *Cyp19a1a* 启动子 CREBP 结合位点,该位点的甲基化程度影响了 *Cyp19a1a* 基因表达;同时,CREBP 的磷酸化也会影响与 CREB 的结合,从而调控促性腺激素对 *Cyp19a1a* 基因的调控^[8]。

4 非编码 RNA 在性别分化中的调控

MicroRNAs (miRNAs) 作为一类非编码 RNA,是表观遗传学的研究内容之一,其对动物的性腺发育具有重要的调控功能。在哺乳动物、鸟类中,通过转录水平和翻译水平的检测,表明 miRNA 可以通过转录后调节的方式参与调控性腺分化及发育^[60-61]。在斑马鱼、青鳉、罗非鱼(*O. niloticus*)及黄鳝等多种鱼类中,多位学者筛选到了与性别分化和性腺发育相关的 miRNAs。MiR-9 与黄鳝精巢发育有关,它可能与 *Foxl3* 共同作用抑制精子生成^[49]。Tao 等^[62]在罗非鱼中筛选并预测了对类固醇激素合成关键基因有影响的 miRNAs,但其功能尚待深入研究。随着研究的深入,发现 miRNA 与靶基因的关系复杂,一个 miRNA 可以调控多个靶基因,也可能多个 miRNA 共同作用一个靶基因^[63]。非编码 RNA 中,lncRNA 也参与调控 *Cyp19a1* 等基因,从而调控动物性腺分化与发育^[64]。非编码 RNA 的表达调控研究是一种较新的技术,虽然在鱼类性别分化和发育的研究较少,但这为基因功能研究中的转录后调控研究提供了重要的方法,对于鱼类性别发育与调控具有重要意义。

5 小 结

性类固醇激素影响鱼类性腺分化方向,芳香化酶是雄激素转化为雌激素的关键因子。鱼类在性别决定和发育过程中易受环境因素的影响, *Cyp19a1* 是环境因素参与 TSD 类型动物性别发育的重要途径。虽然已证实了多个 *Cyp19a1* 基因的转录因子,但转录因子在不同物种中是否具有启动子结合活性依然需要继续研究,这对于转录水平的调控有重要意义。随着表观遗传学的发展,研究证实 miRNA 和 lncRNA 也参与了 *Cyp19a1* 基因的转录后调控,这对于研究性别发育也很重要。鉴于 *Cyp19a1* 对与性别分化和发育的重要性,进一步开展相关研究不仅对深入认识鱼类性别决定机制有重要意义,也对培育单性品系具有重要的借鉴。

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Spring birds biodiversity and community composition of Ningxia Yuehai national wetland park

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Abstract: From February 24 to May 5 2018, a survey has been conducted on the bird species and quantity of the Ningxia Yuehai National Wetland Park and its surrounding environment. The local and surrounding habitats mainly include water areas, herbaceous swamps, green belts, and farmland. We selected belt and sample point in each of the four habitats for observation. The species and number of birds are mainly observed and analyzed in various aspects from species composition, residence type, distribution, protection level, ecological type, habitat and community diversity and bird dynamics of birds. A total of 11 investigations were conducted and 96 bird species were recorded, belonging to 15 orders and 34 families respectively. Shannon-wiener index and Pielou index were used to analyze the species diversity and evenness of local spring birds. The study found that the spring bird species diversity and homogeneity in the Ningxia Yuehai National Wetland Park were high, but the stability was poor, and the natural and human disturbance factors had a great impact on the bird's habitat. Finally, suggestions are made for bird protection.

Keywords: wetland park; spring bird species; diversity

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A review on *Cyp19a1* function in sex differentiation and development of fish

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Abstract: Aquaculture plays an important role in providing a healthy animal protein, therefore the efficient supply of the protein is crucial in fisheries. Many species of growth showed dimorphic between male and female fish, and monosex breeding is an efficient way to increase the production of aquaculture. However, mechanisms of sex determination and differentiation in fish are diversity with largely unclear. The cytochrome P450 aromatase (aromatase) could convert testosterone into estradiol, encoded by *cyp19a1*, which plays an important role in ovary differentiation. Function of *cyp19a1* was influenced by transcription factors and environmental factors. To provide a reference for further study of sex differentiation development in aquaculture animals, the article reviewed the advances of *cyp19a1*.

Keywords: fish; sex determining; *cyp19a1*; environmental factor; transcriptional factor

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