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鱼类 DNA 甲基化研究进展*

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摘要 DNA 甲基化作为 DNA 化学修饰的一种形式，能够在不改变 DNA 序列的前提下改变生物的遗传表现，对于理解基因调控机制和生物学过程具有重要作用。近年来，DNA 甲基化的研究已成为热点并取得了长足进展，但其在鱼类生长发育及环境响应过程中的功能及调控机制尚不够清晰。本文对鱼类 DNA 甲基化的模式、生物学功能以及环境因子对 DNA 甲基化的影响等方面进行综述，并对未来的研究方向做出展望，以期为鱼类 DNA 甲基化的深入研究与应用提供参考。

关键词 DNA 甲基化；鱼类；生物学功能；环境因子

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DNA 甲基化是生物体内重要的表观遗传学调控机制，可以在不改变基因序列的情况下，通过改变染色体和蛋白质结构来调节基因组稳定性，从而调节基因组功能(Niederhuth *et al*, 2016; 池小娜等, 2023)。近年来，DNA 甲基化已在医学以及农林牧业等领域得到广泛的研究和应用(Onabote *et al*, 2022; Leiva *et al*, 2021; Wang N *et al*, 2020; Zhao *et al*, 2021; Zhu *et al*, 2021)。研究表明，DNA 甲基化在鱼类的基因表达调控、胚胎发育、生殖发育、肌肉生长、体色、疾病及进化等方面发挥重要作用(Angeloni *et al*, 2024; Xiong *et al*, 2018; Hu *et al*, 2020; Zhang Y Q *et al*, 2017; Wang X *et al*, 2020; Liu *et al*, 2020; 曹文怡, 2021)；同时，温度、重金属、饥饿胁迫、营养饲料和激素等环境因子也会影响 DNA 甲基化的调控作用(Hu F *et al*, 2021; Gao *et al*, 2020; Zhang *et al*, 2014; Kho *et al*, 2024; Wood *et al*, 2016; Liu *et al*, 2014; Saito *et al*, 2022; Lin *et al*, 2023)。本文概述了鱼类 DNA 甲基化的模式、主要生物学功能的基本信息以及环境因子对 DNA 甲基化的调控等多方面的研究进展，以期为鱼类 DNA 甲基化的研究及其应用提供参考。

1 鱼类 DNA 甲基化模式

1.1 甲基化与去甲基化

DNA 甲基化是指在 DNA 分子中添加甲基基团的过程，通常甲基通过特定 DNA 甲基转移酶(DNA methyltransferase, DNMT)转移到 DNA 胞嘧啶(C)残基上，可以调控基因表达而不会改变基因序列(Jones *et al*, 2012; Blondeau-Bidet *et al*, 2023)。在鱼类中，DNA 甲基化存在于 3 种 C 环境中：CG、CHG 和 CHH (H 是除鸟嘌呤 G 以外的任何碱基)，其中，DNA 甲基化主要发生在 CG 位点上(Zhou H *et al*, 2019; Yang *et al*, 2022)。与 CG 位点甲基化(mCG)相比，CHG 和 CHH 位点的甲基化率要低得多。例如，在草鱼(*Ctenopharyngodon idella*)中，CG 位点的甲基化胞嘧啶为 98.21%~98.22%，而 CHG 和 CHH 位点分别为 1.40%~1.41%和 0.37%~0.39% (He *et al*, 2022)。在其他鱼类中，CG 位点与 CHG 和 CHH 位点甲基化也

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存在类似情况(Li L *et al*, 2022; Yao *et al*, 2022)。

去甲基化则是指由去甲基化酶介导从 DNA 中去除甲基基团的过程。DNA 去甲基化模式分为被动和主动 DNA 去甲基化。在被动去甲基化中，甲基化的 DNA 在连续的复制周期中由于维持甲基化模式的 DNA 甲基化转移酶或含有辅因子泛素样的 PHD 和环指结构域 1 (ubiquitin-like protein containing PHD and RING finger domains 1, uhrf1)的失活或核排斥而去甲基化(Onabote *et al*, 2022) (图 1A)。而主动 DNA 去甲基化过程则是在双加氧酶(ten-eleven-translocation, TET1/2/3)作用下，先将 DNA 5-甲基胞嘧啶(5mC)氧化成 5-羟甲基胞嘧啶(5hmC)，再由胸腺嘧啶 DNA 糖基化酶(thymine DNA glycosylase, TDG)作用下切除，并被未经修饰的胞嘧啶替换，从而实现去甲基化(Zeng *et al*, 2019; Xu *et al*, 2023; Banks *et al*, 2021) (图 1B)。斑马鱼(*Danio rerio*)胚胎中，TET2 和 TET3 是主要的 5mC 双加氧酶，可以将 5mC 氧化成 5hmC (Li *et al*, 2015)。

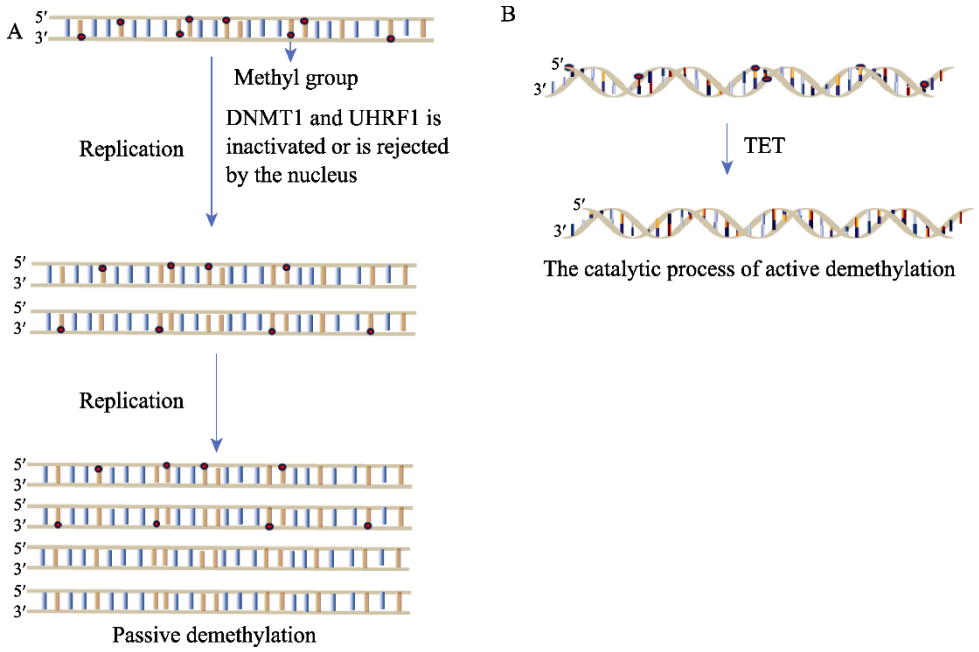

图 1 去甲基化的催化过程

Fig.1 The catalytic process of demethylation

1.2 甲基化转移酶

在生物体内，DNA 甲基化转移酶能够催化甲基从 S-腺苷甲硫氨酸(S-adenosylmethionine, SAM)转移到生物分子上(DNA、RNA、蛋白质和小分子) (Fontecave *et al*, 2004; Gade *et al*, 2023)。在鱼类中，DNA 甲基化转移酶广泛存在于大脑、脾脏、肌肉等许多细胞和组织中(Zhou *et al*, 2021; Xiong *et al*, 2018; 曹文怡, 2021; Pan *et al*, 2021; Liu *et al*, 2014)。鱼类 DNA 甲基化转移酶种类繁多，包括 2 个同源 DNMT1 (DNMT1a 和 DNMT1b)、1 个 DNMT2 和 8 种同源 DNMT3。DNMT3 同源基因较为复杂，但均为 DNMT3a 和 DNMT3b 的旁系同源(Rai *et al*, 2007; Campos *et al*, 2012; Lai *et al*, 2020; Tang *et al*, 2022; Lallias *et al*, 2021; Wang *et al*, 2018)。DNMT1 可以维持细胞现有 DNA 甲基化模式(Magnani *et al*, 2021)。DNMT2 可以催化甲基从辅助因子 SAM 转移到细胞质 tRNA 胞嘧啶残基的 5 号碳原子上，SAM 转变为 S-腺苷高半胱氨酸(SAH) (Alata Jimenez *et al*, 2023; Jeltsch *et al*, 2017)。DNMT3 是从头合成 DNA 甲基转移酶，是一种在没有甲基化 DNA 模板的情况下将 DNA 甲基化精确标记到新位置的酶，涉及到调节多种重要的生物过程(Zhou R *et al*, 2019; Chen *et al*, 2011) (图 2)。在青鳉(*Oryzias latipes*)中，DNA 甲基通过 DNMT3bb1 转移到胞嘧啶残基上，并通过 DNMT1 来维持卵母细胞、胚胎卵裂期至囊胚期的现有 DNA 甲基化模式，最后通过 TET 的氧化作用去除甲基(Wang X G *et al*, 2020)。在斑马鱼中，DNMT2 通过 tRNA 甲基化来调控相关基因的表达，从而促进斑马鱼的肝脏、大脑和视网膜发育(Rai *et al*, 2007) (图 3)。在鳜鱼(*Siniperca chuatsi*)中，也发现了 *dnmt2* 基因在胚胎发育早期高表达(Zhou *et al*, 2021)。这些研究结果表明 DNA 甲基化转移酶在鱼类发育中发挥重要作用。

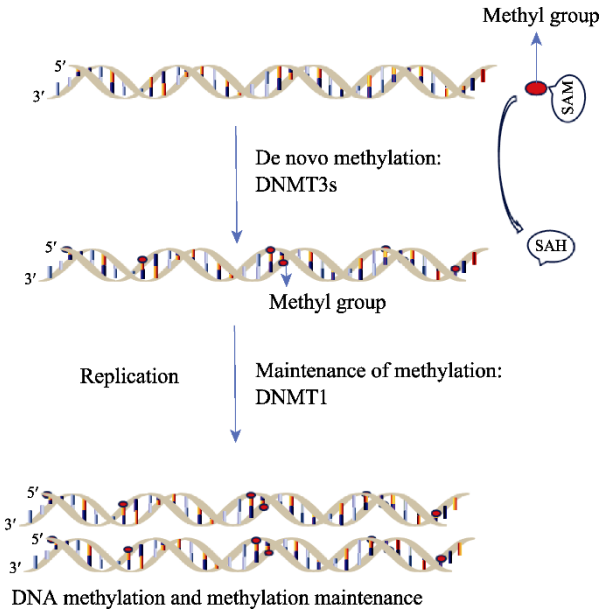

图 2　DNMT1 和 DNMT3 的催化过程

Fig.2　The catalytic process of DNMT1 and DNMT3

鱼类 DNA 甲基转移酶因物种差异，其种类和数量也有所不同(表 1)。在虹鳟(*Oncorhynchus mykiss*)中，除 DNMT2 外，DNMT1、DNMT3 共鉴定出 10 种 DNMTs，是目前鉴定数量最多的鱼类。其次是斑马鱼，共鉴定出 DNMT1、DNMT2、DNMT3bb1、DNMT3bb2、DNMT3bb3、DNMT3ab、DNMT3ba 和 DNMT3aa 8 种 DNMTs。而大西洋鲱鱼(*Clupea harengus*)仅鉴定到一种 DNMT3a DNA 甲基化转移酶，是目前鉴定最少的鱼类。虽然在不同种鱼类中，DNA 甲基转移酶的种类和数量有所差异，但对于同一种类的 DNA 甲基转移酶，其保守性非常高(图 4 和图 5)。

2　鱼类 DNA 甲基化的生物学功能

2.1　DNA 甲基化与基因表达调控

DNA 甲基化是一种重要的表观遗传调控机制，它有助于定义细胞的特性和功能(Simó-Mirabet *et al*, 2020)。DNA 甲基化使鱼类能够更精确地调节基因表达，以适应不同环境因素(Wang *et al*, 2008)。差异甲基化(DM) CpG 位点[胞嘧啶(C)–磷酸(p)–鸟嘌呤(G)]通过调控鱼类基因表达，来参与细胞凋亡、表观遗传调控、细胞自噬、胶原代谢、细胞膜功能和同源盒蛋白的发生(Salem *et al*, 2022)。DNA 甲基化会导致 DNA 构象、DNA 稳定性以及 DNA 与 RNA(或蛋白质)相互作用的方式发生变化，从而调控基因表达(Jones *et al*, 2012)。在斑马鱼中，DNA 甲基化通过募集几种甲基和结合蛋白相互作用，阻止 RNA 聚合酶与 DNA 结合，进而抑制基因表达(Faillace *et al*, 2022) (图 6A)。通过甲基化启动子中 CpG 岛，鱼类基因可以在特定组织中沉默(Wang *et al*, 2008)。DNA 甲基化通过对转录的激活或抑制，在转录水平上影响基因组表达调节(Liu *et al*, 2022)。在转录起始位点(TSS)附近的甲基化会阻断起始，但在基因体中甲基化不会阻断起始甚至可能会刺激转录的延伸(Jones *et al*, 2012) (图 6B、C)。例如在虹鳟中，在 TSS 两侧±1 kb 内的 CpG 位点高甲基化与基因表达量的降低呈现轻/中度相关，相反，在基因体下游+2 kb 至+10 kb 区域，CpG 位点的高甲基化水平与基因表达量的增加呈现微弱相关(Salem *et al*, 2022)。

2.2　DNA 甲基化与胚胎发育

鱼类的胚胎发育始于受精卵，是生命早期最具活力的过程之一。DNA 甲基化作为一种表观遗传修饰，能够调控基因的表达(池小娜等, 2023)，这对生物体的胚胎发育、细胞分化至关重要(Andersen *et al*, 2012; 王湘秀等, 2017; Murphy *et al*, 2018)。与哺乳动物不同，斑马鱼早期胚胎从 16 细胞时期开始到囊胚中期转换(mid-blastula transition, MBT)，受精卵中来自卵子的基因甲基化模式通过细胞分裂逐渐去甲基化，并逐渐转变为与精子甲基化模式类似，而且此时胚胎中的甲基化水平与精子中的甲基化水平相当(王湘秀等, 2017)。Ross 等(2023)比较研究青鳉和青鳉–斑马鱼杂交胚胎全基因组甲基化测序(whole genome bisulfite sequencing, WGBS)图谱也发现，受精后早期胚胎整体甲基化水平与父本甲基化水平类似。在青鳉中，受精卵前 4 次卵裂期间基因组总体上甲基化程度低，而从

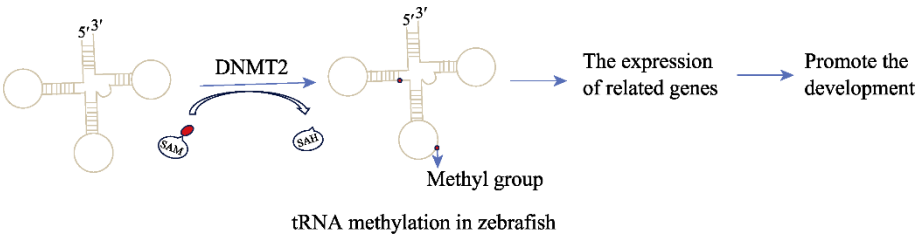

图 3　斑马鱼 tRNA 甲基化

Fig.3　tRNA methylation in zebrafish

表 1 不同鱼类 DNMT 的种类及数量

Tab.1 Different sets of DNMTs in fishes

鱼类 Fish	DNMT1	DNMT2	DNMT3	参考文献 Reference
鲤形目 Cypriniformes				
斑马鱼 *Danio rerio*	1	1	6	Rai *et al*, 2007; Campos *et al*, 2012; Lai *et al*, 2020; Tang *et al*, 2022
草鱼 *Ctenopharyngodon idellus*	1	1	2	Xiong *et al*, 2018
鲫鱼 *Carassius auratus*	1	0	1	Zhang *et al*, 2014
黑头呆鱼 *Pimephales promelas*	1	0	4	Wood *et al*, 2016
鲃鲃 *Gobiocypris rarus*	1	0	3	Liu *et al*, 2014
泥鳅 *Misgurnus anguillicaudatus*	1	0	4	曹文怡, 2021
合鳃鱼目 Synbgranchiformes				
黄鳝 *Monopterus albus*	0	0	4	Zhang Y Z *et al*, 2017
鲈形目 Perciformes				
翘嘴鳜 *Siniperca chuatsi*	1	0	5	Pan *et al*, 2021
尼罗罗非鱼 *Oreochromis niloticus*	1	1	5	Wang *et al*, 2018
斑马天使 *Astatotilapia latifasciata*	1	0	2	Cardoso *et al*, 2019
鳉形目 Cyprinodontiformes				
青鳉 *Oryzias latipes*	1	0	3	Wang *et al*, 2019
鲽形目 Pleuronectiformes				
牙鲆 *Paralichthys olivaceus*	1	1	5	Firmino *et al*, 2017
半滑舌鳎 *Cynoglossus semilaevis*	1	0	2	Wang N *et al*, 2021
鲱形目 Clupeiformes				
大西洋鲱鱼 *Clupea harengus*	0	0	1	Kho *et al*, 2024
刀鲚 *Coilia nasus*	1	0	2	Gao *et al*, 2020
鲑形目 Salmoniformes				
大西洋鲑鱼 *Salmo salar*	1	0	2	Burgerhout *et al*, 2017
虹鳟 *Oncorhynchus mykiss*	2	0	8	Liu *et al*, 2020; Lallias *et al*, 2021
鲇形目 Siluriformes				
黄颡鱼 *Pelteobagrus fulvidraco*	1	0	2	卓梅琴等, 2019
鳕形目 Gadiformes				
大西洋鳕鱼 *Gadus morhua*	1	2	3	Reinardy *et al*, 2019

16 细胞期到原肠胚形成晚期，DNA 甲基化水平和 *dnmt3bb.1* 表达量逐渐提高(Wang *et al*, 2019)。另外，在胚胎发育过程中，DNA 甲基化酶可以促进发育，但缺失会导致胚胎致死现象。例如，在斑马鱼中，敲除 *dnmt1* 会导致严重的发育缺陷和胚胎致死，这可能与合子基因组激活(zygotic gene activation, ZGA)前胚胎中富含 CpG 增强子的异常激活有关(Wu *et al*, 2021; Angeloni *et al*, 2024)。以上都说明 DNA 甲基化在鱼类的胚胎正常发育中扮演着重要角色。

2.3 DNA 甲基化与生殖发育

表观遗传调控在鱼类的性别决定和性别分化中起到重要作用。DNA 甲基化是一种重要的表观遗传修饰，在性腺发育、性别分化中扮演重要角色。配子早期发育过程中随着原始生殖细胞(primordial germ cells, PGC)的产生而开始发育，从 PGC 到配子发生过程中 DNA 甲基化有所改变(Maamar *et al*, 2022)。例如，在斑马鱼中，从受精后 4 h (4 hours post fertilization, 4 hpf)到 24 hpf 早期 PGC 的总体甲基化水平没有实质性变化，PGC 在 36 hpf 时总体甲基化水平开始下降，并在受精后 9 d (9 days post fertilization, 9 dpf)恢复到卵母细胞/卵巢的甲基化水平(Wang X *et al*, 2021)；此外，成年斑马鱼性别转变期间的 DNA 甲基化组也会发生变化(Wang X *et al*, 2021)。黄鳝(*Monopterus albus*)是一种雌雄同体鱼类，在性腺发育中要经历从卵巢到精巢的性逆转(Hu Q *et al*, 2021)。在此过程中，DNA 甲基化通过甲基化和去甲基化影响性别相关转录因子 *dmrt1* 和 *foxl2* 结合 *plastin-2* 基因启动子，从而在性

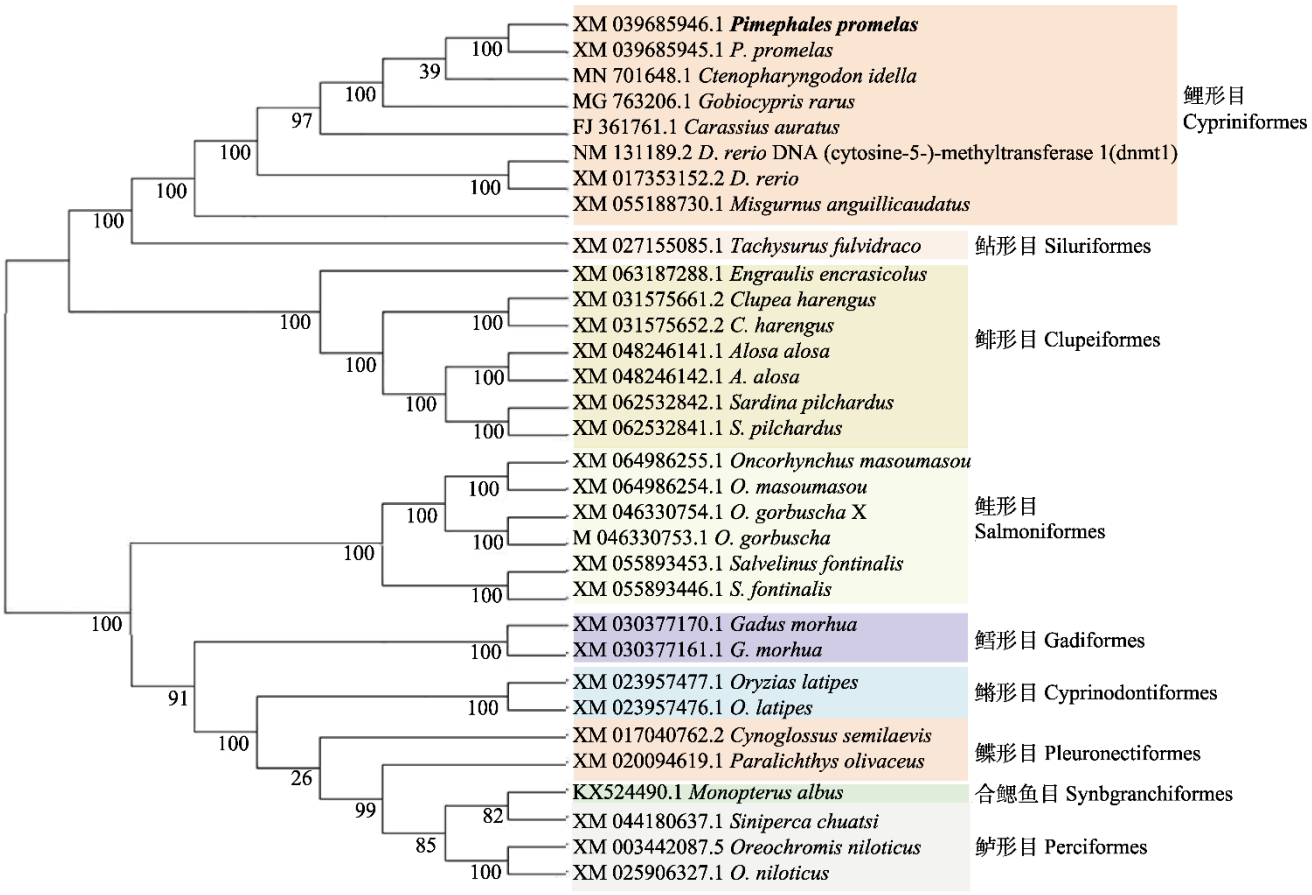

图 4　鱼类的 DNMT1 进化树

Fig.4　DNMT1 phylogenetic trees of fishes

腺发育过程中调节 *plastin-2* 基因表达(Hu *et al*, 2022)。还有研究表明，在黄鳝性转变过程中，精巢和卵精巢的 *cyp19a1a* 启动子 cAMP 反应元件(环腺苷酸 cyclic adenosine 3′-5′ monophosphate, cAMP response element, CRE)内 CpG 甲基化水平要高于卵巢，而且甲基化水平与基因表达为负相关(Zhang *et al*, 2013)。性腺芳香化酶基因 *cyp19a1a* 是调节体内雌激素合成以及调控性别分化的重要分子，而 *foxl2* 能直接调控 *cyp19a1a* 的转录表达。在牙鲆(*Paralichthys olivaceus*)卵巢发育过程中，*foxl2* 和 *cyp19a1a* 的 DNA 甲基化水平与其基因表达呈负相关；CpG 位点的甲基化和去甲基化可以直接影响 *foxl2* 表达，进而影响 *cyp19a1a* 的表达来调控性腺发育方向(Si *et al*, 2016)。

DNA 甲基化酶在不同发育阶段的性腺呈现不同的时空表达模式。在牙鲆卵巢中，DNA 甲基化酶主要存在于Ⅰ期卵母细胞中(Liu *et al*, 2021)；在睾丸中，*dnmt1* 和 *dnmt3b* 存在于精母细胞、精子和支持细胞中，而 *dnmt3a* 主要存在于精母细胞中(Liu *et al*, 2021)。在尼罗罗非鱼(*Oreochromis niloticus*)中，*dnmt3aa* 在卵原细胞、卵巢Ⅰ、Ⅱ期卵母细胞和颗粒细胞以及睾丸精原细胞和精母细胞中高表达，而 *dnmt3ab* 主要在卵巢颗粒细胞和睾丸精母细胞中表达(Wang F *et al*, 2021)。在翘嘴鳜(*Siniperca chuatsi*)精巢的生精小管中，存在 *dnmt2*，精母细胞中存在 *dnmt2* 和 *dnmt3a*，在Ⅱ或Ⅲ期翘嘴鳜卵巢初级卵母细胞中 *dnmt2* 高表达(赵紫维, 2021)。这反映了不同的 DNA 甲基化酶在卵巢和睾丸发育过程中有着不同的生物学功能。

2.4　DNA 甲基化与肌肉生长

性别二态性是指同一物种在雄性和雌性中呈现不同形态(如肌肉生长差异)的现象(Horne *et al*, 2020; Shi *et al*, 2023; He W *et al*, 2021)，这会对鱼类养殖造成重大的经济影响。半滑舌鳎(*Cynoglossus semilaevis*)口感鲜美、营养价值高，其雄性个体比雌性小得多，并且其性别大小二型性(sexual size dimorphism, SSD)与 DNA 甲基化有关(Zhao *et al*, 2015)。Wang N 等(2021)研究雌性(ZW)、雄性(ZZ)和伪雄性(ZW)半滑舌鳎的性别二态性遗传和表观遗传变化，发现了在雄性和伪雄性个体中观察到相对较高的 DNA 甲基化水平，在伪雄性中染色体 W 高甲基化比雌性明显，并且雌

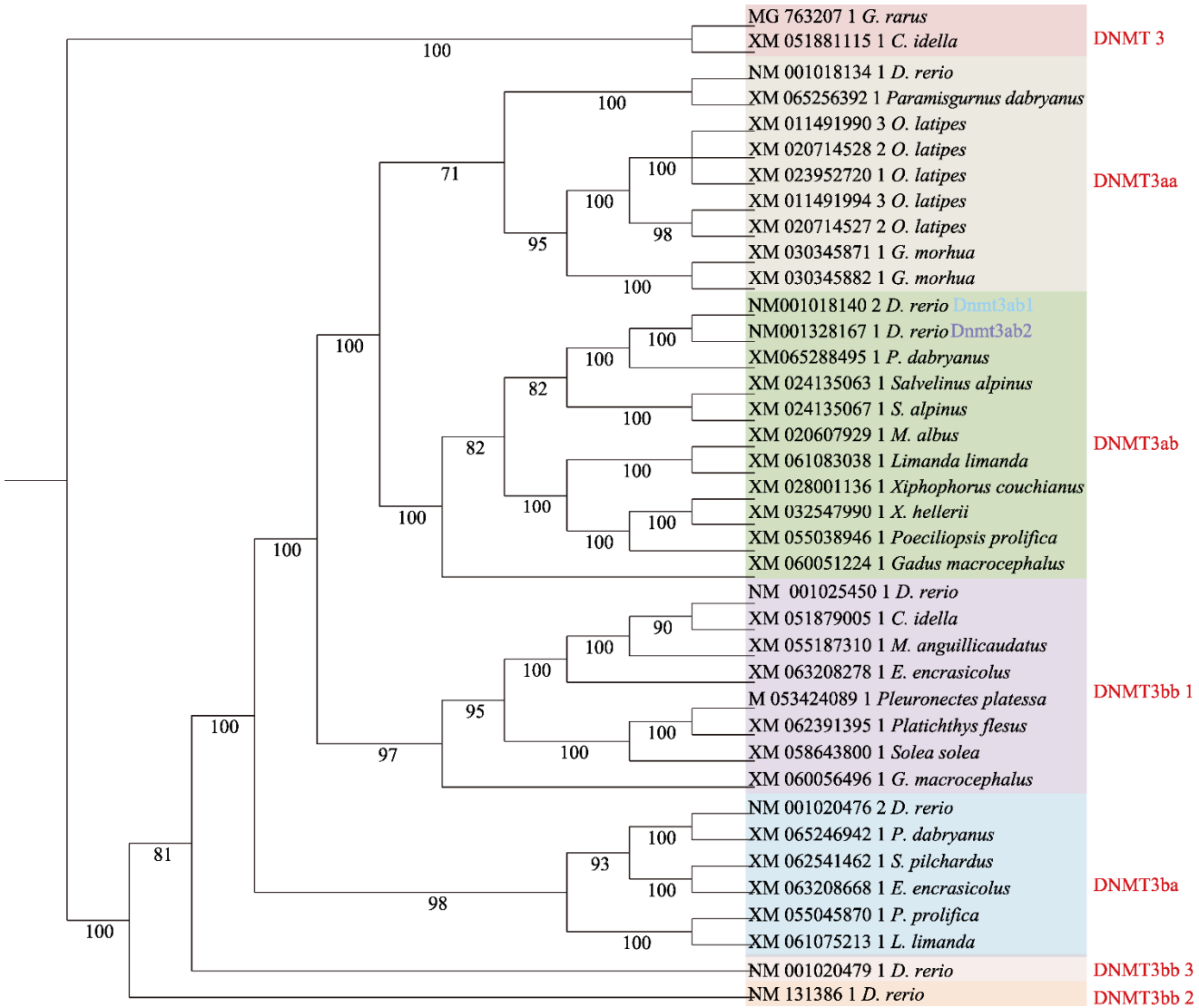

图 5 鱼类 DNMT3 进化树

Fig.5 DNMT3 phylogenetic tree of fishes

性偏向的 mRNA 转录酶和低甲基化水平分别与细胞周期相关基因的上游和下游区域有关。尽管雌性和伪雄性具有相同遗传背景，但它们表现出显著的生长差异，这可以用 DNA 甲基化表观遗传机制来解释。类似的，在尼罗罗非鱼中其雄鱼比雌鱼生长快，雌鱼的生长激素(growth hormone, GH)启动子甲基化水平明显高于雄性的；DNA 甲基化抑制雌性个体 *gh* mRNA 表达，使得雄性个体生长快于雌性(Zhong *et al*, 2014)(图 7A)。DNA 甲基化调控生长相关基因的表达从而导致不同性别鱼类肌肉生长存在差异性。

骨骼肌占鱼类大部分总体重，具有促进生长的作用。骨骼肌由快肌(FM, 又称白肌)和慢肌(SM, 又称红肌)组成，FM 纤维位于身体深处占总肌肉质量大约为 90%，而 SM 纤维位于身体浅表层(Wang H *et al*, 2021)。在红鳍东方鲀(*Takifugu rubripes*)中，SM 的总体甲基化水平高于 FM；共鉴定了 8 479 个差异甲基化基因(differentially methylated, DMG)和 3 407 个在 FM 和 SM 之间的启动子区域中含有差异甲基化区域(differentially methylated regions, DMR)的 DMG，并且 2 个差异基因 *kapca* (是 Hedgehog 信号通路的关键成员，在肌肉发育中发挥重要作用)和 *tsp4b* (细胞外基质介导的局灶粘附途径，是调节细胞生长和分化的众多细胞内通路的信号中心)在 SM 中甲基化水平高于 FM，并且 mRNA 的表达水平较低(Wang H *et al*, 2021)。在翘嘴鳜中也发现了类似结果，翘嘴鳜 SM 中 5mC 水平显著高于白肌 FM (P<0.05)；3 种 DNMT 亚型的 *dnmt1*、*dnmt3ab* 和 *dnmt3bb1* 的表达水平在 SM 中明显高于 FM，而 SM 中较高的 DNA 甲基化水平

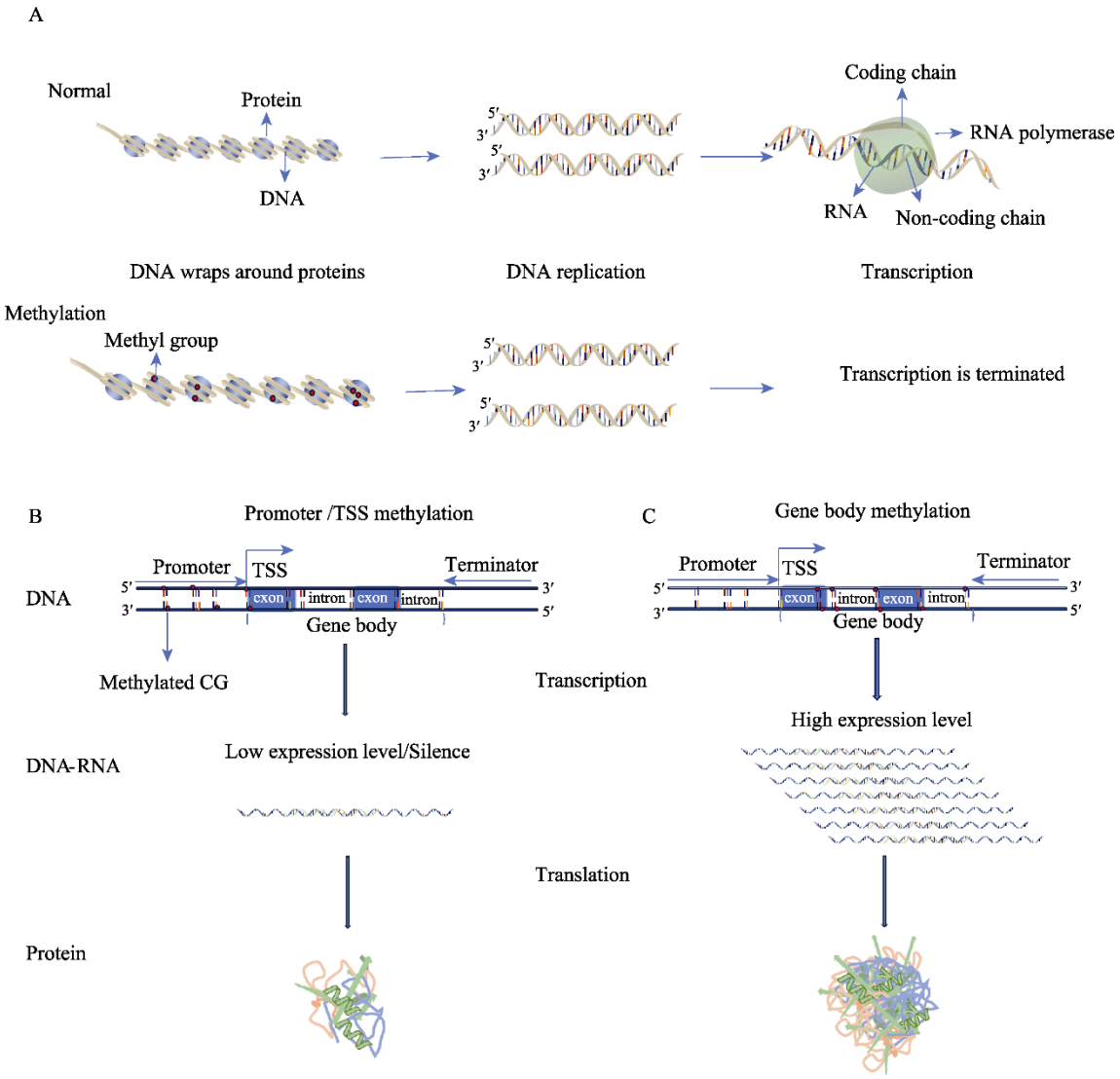

图 6　DNA 甲基化与基因表达调控

Fig.6　DNA methylation and regulation of gene expression

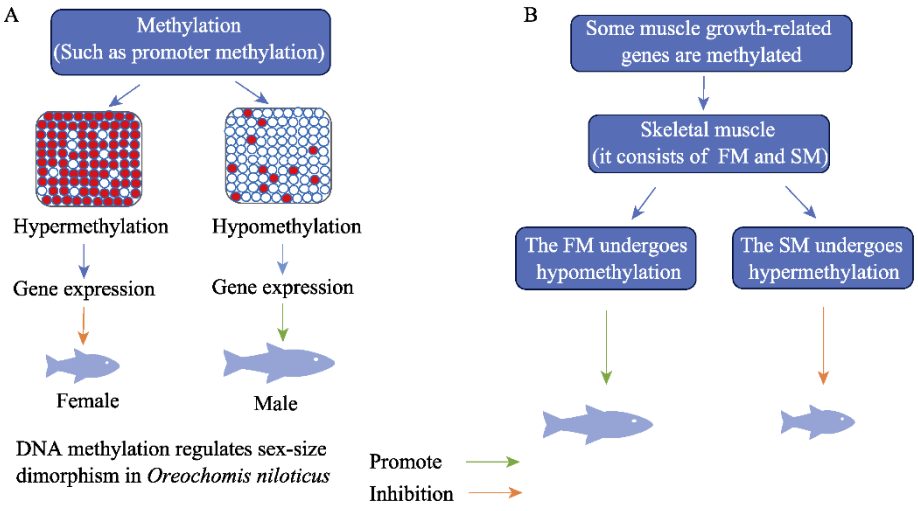

图 7　DNA 甲基化与肌肉生长

Fig.7　DNA methylation and muscle growth

而 SM 中较高的 DNA 甲基化水平可能是由于 SM 中较高的 DNA 甲基化转移酶表达所致(曹文怡, 2021)。这些研究表明，具有低甲基化水平的 FM 生长速度要高于具有高甲基化水平的 SM (图 7B)。

2.5 DNA 甲基化与体色

鱼类体色的形成受遗传因素决定，并受神经和内分泌调节而发生变化。研究表明，DNA 甲基化在鱼类体色的形成中发挥重要作用。在红鲫(*Carassius auratus* red var., RCC)与白鲫鱼(*C. auratus cuvieri Temminck et Schlegel*, WCC)中，RCC DNA 甲基化水平略高于 WCC (Zhang Y Q *et al*, 2017)；DNA 甲基化位点主要分布在基因体、内含子和基因间区域，并且 RCC 中的 DNA 甲基化位点略多于 WCC (Zhang Y Q *et al*, 2017)；在 RCC 和 WCC 差异表达的 *mitfa* 等酪氨酸合成关键基因其 DNA 甲基化位点与色素沉着有关，这些差异表达的 DNA 甲基化位点主要涉及丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)、cAMP、内吞作用和黑素生成等信号通路(Zhang Y Q *et al*, 2017)。总的来说，DNA 甲基化在鱼类体色的形成中起着重要的作用。

2.6 DNA 甲基化与疾病

鱼病是鱼类养殖中常见的问题，对养殖业造成巨大损失(Abinaya *et al*, 2023; Heys *et al*, 2020)。鱼病的产生与 DNA 甲基化调控有关，在调节病原体的反应中发挥相关作用(Leiva *et al*, 2021; 王晨诗等, 2022)。鲑鱼立克次体败血症(SRS)由鲑鱼立克次体引起，是导致智利养殖鲑鱼大量死亡的原因(Happold *et al*, 2020; Moraleda *et al*, 2021)；与健康大西洋鲑鱼(*Salmo salar*)相比，随着受感染的时间增加，大西洋鲑鱼头肾组织 *dnmt3a* 甲基化水平也随之增加，并且与 *dnmt3a* 表达量呈负相关(Mukiibi *et al*, 2022)。Hansen 等(2023)利用 20 条鲑鱼远端肠道组织进行 WGBS，比较未感染个体与患病鱼远端肠道组织全基因组 DNA 甲基化水平，发现在患病鱼中，CpG 位点和区域发生高度甲基化；二者存在超过 19 000 个差异甲基化胞嘧啶位点，这些位点通常位于差异甲基化区域，并聚集在基因周围，其中 68 个差异甲基化基因具有与溃疡病相关的功能。这表明了特定基因可能参与宿主–微生物群相互作用。

草鱼呼肠孤病毒(grass carp reovirus, GCRV)引起的草鱼出血病是水产养殖业的严重病害(Wang N *et al*, 2022)。在易感五月龄(five-month-old, FMO)和抗性三岁(three-year-old, TYO)草鱼中，TYO 鱼整体甲基化水平高于 FMO 鱼(He *et al*, 2022)；WGBS 显示，大多数 DMR 和 DMG 在 TYO 鱼中呈高甲基化模式，TYO 鱼启动子 hypo-DMGs 在免疫反应中显著富集，而 TYO 鱼基因体 hyper-DMGs 在 RNA 转录、生物合成和能量产生相关方面显著富集，DNA 甲基化和基因表达参与免疫反应、生物合成和能量产生，这可能是导致草鱼对 GCRV 年龄依赖易感染的原因(He *et al*, 2022)。核因子-kB (nuclear factor-κB, *nf-κb*)和肿瘤坏死因子(tumor necrosis factor, *tnf*)是免疫系统中最重要的细胞因子之一，鳗弧菌(*Vibrio anguillarum*)感染鱼类可激活其相关通路(Sheng *et al*, 2021; Liu Z *et al*, 2023)；比目鱼(*Paralichthys olivaceus*)感染鳗弧菌可激活转录因子 *nf-κb*，从而促进 *tnfα* 基因的表达，而甲基化修饰可以抑制比目鱼 *tnfα* 基因的表达(Yang *et al*, 2021)。

2.7 DNA 甲基化与进化

进化是指生命形态发生、发展的演变过程。DNA 甲基化在生物进化中具有重要意义。在不同种生物中，同源基因的甲基化模式可能存在差异(Williams *et al*, 2023)。与海鞘(玻璃海鞘 *Ciona intestinalis*、胶州湾萨氏海鞘 *C. savignyi*)同源基因相比，由甲基化 CpG 位点的背景依赖性引起的鱼类同源基因突变，使其 CpG 侧翼位置的 GC 含量增加(Wang *et al*, 2008)。CpG 侧翼位置 GC 含量增加可以减少鱼类基因中 CpG 的丢失并减弱 CpG 密码子的甲基化，这可能是无脊椎动物向脊椎动物进化的原因之一(Wang *et al*, 2008)。

连续全基因组复制(whole-genome duplication, WGD)是指生物体经过不断演化和适应，基因组内所有序列出现加倍，这导致基因数量的增加，从而改变生物的遗传多样性(Zhang *et al*, 2023)。鱼类在演化过程中，DNMT3 会优先固定在基因组中，且增加它们的拷贝数，*dnmt3* 转录模式差异突出表明它们可能在 WGD 后经历亚功能化或新功能化(Liu *et al*, 2020)。如，*dnmt3aa* 参与比目鱼肌肉组织分化，*dnmt3bb1* 或者 *dnmt4* 可能在比目鱼和斑马鱼的造血干细胞和祖细胞谱系分化及维持中发挥作用(Takayama *et al*, 2014; Firmino *et al*, 2017)。

3 环境因子对 DNA 甲基化的影响

3.1 温度

水温在鱼类的早期发育、生长和生理过程中，以及在饲养、新陈代谢和繁殖等方面起着重要作用。在某一特定基因型中，因环境(温度等)而改变生物表型

可塑性是生物对环境条件变化做出反应能力的一个关键组成部分(Pigllucci *et al*, 1996; Huang Z *et al*, 2021)。DNA 甲基化因热适应而发生变化，可能与生命阶段的高度特异性有关(Jiang *et al*, 2024)。

温度会对生长相关基因甲基化造成影响(Burgerhout *et al*, 2017)。虹鳟 5 个品系(A03h、AP2h、G17h、N38h 和 R23h)在 2 个孵育温度(11 ℃和 16 ℃)下，*dnmt3* 基因表达在不同品系呈上调或下调，并且 5 个品系 DNA 甲基化水平也有差异(Lallias *et al*, 2021)。在翘嘴鳜中，与 25 ℃组相比，21 ℃组其生长率和体重增加率显著低于对照组，并且 21 ℃组 GH-3029 和-3032 CpG 位点的 DNA 甲基化水平高于 25 ℃组(Zhang *et al*, 2022)。这说明温度对 DNA 甲基化调控生长发育起到重要作用。

在动物界，性别并不总是由遗传因素决定，有时环境因素，特别是温度，也可以对性别产生影响。鱼类性别决定机制可分为基因型(GSD)、温度(TSD)和附加效应(GSD + TE)(Teng *et al*, 2020)。在关键热敏感期(TSP)，对许多鱼进行人为高温或低温处理也会导致其性别比发生变化，例如雌鱼雄性化或雄鱼雌性化，这些鱼被归类为 GSD + TE (Yao *et al*, 2021)。罗非鱼是典型的 GSD + TE 型鱼类，研究发现，尼罗罗非鱼的热休克转录因子 5 (heat-shock transcription factor 5, *hsf5*)和环指蛋白 43 (ring finger protein 43, *rnf43*)基因存在融合的现象，称为 *hsf5-rnf43*；DNA 甲基化可以调控尼罗罗非鱼早期发育过程中 *hsf5-rnf43* mRNA 的表达；而且 DNA 甲基化水平受到温度的影响，在孵化后 5~17 d 热敏感期，高温(36 ℃水温)诱导罗非鱼雄性化条件下，*hsf5-rnf43* 启动子 CpG 岛 DNA 甲基化水平与 *hsf5-rnf43* 的表达水平呈负相关(Shen *et al*, 2023)。单个基因在高温下会影响 DNA 甲基化，从而影响鱼类性腺发育，在基因组中也是如此。Yao 等(2022)研究发现，在基因启动子中，尼罗罗非鱼性腺分化相关基因 *myb* 的 DNA 甲基化水平在 FT (XX 雌性在 TSP 期间从第 5~17 天接受高温处理)中显著低于 FC (XX female control)中水平，并且 *myb* 在 FT 中表达水平显著高于 FC 中的表达水平；基因体区性腺分化调节因子 *wnt11* 的 DNA 甲基化水平在 MC (XY male control)中显著高于 FC (P<0.05)；亚硫酸氢盐测序 PCR (bisulfite sequencing PCR, BSP)和定量实时 PCR (qRT-PCR)结果显示，*wnt11* 的 DNA 甲基化水平和表达水平在 MC 和 FT 组中显著高于 FC 组。在泥鳅(Huang G *et al*, 2021)(*Misgurnus anguillicaudatus*)、斑马鱼(Valdivieso *et al*, 2023; Han *et al*, 2021)、红鳍东方鲀(Zhou H *et al*, 2019)、半滑舌鳎(Shi *et al*, 2022; Liu *et al*, 2019)、欧洲鲈(*Dicentrarchus labrax*) (Anastasiadi *et al*, 2018)和比目鱼(Fan *et al*, 2017)等鱼类中也发现了温度影响相关基因甲基化水平上调或下调，或者是影响性腺的全基因组甲基化水平，从而影响鱼类的性别分化。这些研究可为鱼类性别决定和性别维持的分子机制提供参考。

3.2　重金属

重金属在鱼类体内不易排出，会在组织器官中累积，尤其是肝脏和肾脏等重要器官(Ahmad Bhat *et al*, 2023)。这种累积效应可能导致鱼类体内的重金属含量超过安全限度，进而影响食用价值和人体健康(Da Silva *et al*, 2024; Turan *et al*, 2020)。将早期发育的鱼暴露于不同金属浓度下，其 DNA 甲基化酶表达量不同。对早期发育的大西洋鳕鱼(*Gadus morhua*)进行从低浓度到高浓度的金属处理，发现 *dnmt1* 和 *dnmt4* 具有显著性上调，并且浓度越高死亡率越大(Reinardy *et al*, 2019)。在鱼类中，DNA 甲基化可能不会在每一代中被清除，因此，重金属的影响可以通过 DNA 甲基化的形式来传递给下一代。*foxl2a* 和 *mad-3* 相关转录因子 1 (mad-3-related transcription factor 1, *dmrt1*)是鱼类性发育相关基因。在斑马鱼中，*foxl2a/dmrt1* 甲基化水平受到镉(Cd)的影响，导致后代雌性化，并且 Cd 对 *foxl2a* 甲基化水平的影响一直观察到第三代，支持潜在的跨代遗传(Pierron *et al*, 2021)。GH-IGF [growth hormone (GH)–insulin-like growth factor (IGF)]系统是鱼类生长的关键促进剂(Niu *et al*, 2024)，重金属可以通过干扰 GH/IGF 生长轴对内分泌系统产生破坏性来影响鱼类生长(Wang L N *et al*, 2020)。鱼暴露于 Cd 环境下会降低其基因组 DNA 甲基化水平，同时增加了 GH 和 IGF-I 启动子的甲基化，并且甲基化水平的下调可能与 Cd 引起 *dnmt3a* 和 *dnmt3b* 转录异常有关(Hu F *et al*, 2021)。

重金属对鱼类 DNA 甲基化的影响可能产生一系列生物效应。首先，DNA 甲基化水平的改变可能导致基因表达模式的紊乱，进而影响鱼类生长和发育等生物学过程(Bian *et al*, 2021)。其次，重金属诱导的 DNA 甲基化异常还可能降低鱼类生存能力(Park *et al*, 2020)。

3.3　饥饿胁迫

由于环境风险，例如野外恶劣的天气和养殖场投喂不及时，从而导致鱼类挨饿。短期或长期饥饿条件下的营养问题会导致鱼类健康问题(Bersin *et al*, 2023)。营养缺乏会抑制斑马鱼卵巢发育，导致某些

特定位点甲基化发生变化，并削弱后代的适应性，表明 DNA 甲基化对配子质量有潜在干扰作用(Fan *et al*, 2019)。饥饿时，黄鳍棘鲷(*Acanthopagrus latus*)肝脏中 DMG 富集于糖代谢和脂代谢等能量代谢相关通路，且与脂质代谢相关基因 *ppargc1a* 的内含子区域有一个差异 CpG 甲基化位点，该 DMS 在实验组的甲基化水平(100%)高于对照组的(20%) (Lin *et al*, 2023)。这表明 DNA 甲基化与能量相关途径有密切关系。在饥饿了 14 d 和 21 d 的大黄鱼(*Larimichthys crocea*)肌肉和饥饿了 7、14、28 d 的大黄鱼肝脏中，实验组和对照组整体 DNA 甲基化水平存在显著差异(P<0.05) (Zhang *et al*, 2019)；并且生长相关基因 *mstn1* 外显子 1 和 IGF2 外显子 2 的甲基化状态随饥饿时间的不同而不同，饥饿 7 d 时，在肝脏和肌肉中开始有显着差异(P<0.05) (Zhang *et al*, 2019)。以上说明，DNA 甲基化在鱼类饥饿压力中起着重要作用。它不仅能够影响基因的表达，还能够影响细胞的功能和整个生物体的生理状态。

3.4 营养饲料

已有研究表明，DNA 甲基化对鱼类饮食变化高度敏感，并且可以在一生中进行改变以应对这种变化，从而产生新的适应性表型(Marandel *et al*, 2022; 刘通等, 2022)。翘嘴鳜是一种典型的肉食性鱼类，终生只进食活鱼活虾(Liang *et al*, 2008)。与不喂人工饲料组相比，饲喂人工饲料的翘嘴鳜中视黄醇代谢、甘油脂代谢和不饱和脂肪酸的生物合成相关基因的表达量增加，且转录因子 IIF (transcription factor IIF, *tfiif*)的表达和 DNA 甲基化水平显著增加(He S *et al*, 2021)。营养差异还会导致鱼类生长发育不同，直接喂养或在鱼类饲料中添加适当营养物质(蛋白质、微量元素等)，可有效促进生长性能、饲料利用效率，调节脂肪和脂肪酸成分，并且可提高鱼类的氧化功能(Andriopoulos *et al*, 2022; Samat *et al*, 2020)。给草鱼分别喂养蚕豆和商品饲料，发现蚕豆组草鱼的肌肉硬度、咀嚼性、胶粘性和弹性含量显著高于商品饲料组，并且与蚕豆组草鱼胶原蛋白合成有关的 *tgff-1*、*cl1a1*、*cl1a2*、*cl1a3*、*cl4a1* 和 *cl18a1* 基因甲基化水平具有差异，这可能导致表达差异，进一步导致草鱼肌肉硬度的增加(Li Y *et al*, 2022)。当给大西洋鲑鱼喂食不同剂量的微量营养素时，与对照饮食相比，中等剂量和高剂量的微量营养素生长性能明显更好，剂量越高影响谷氨酸受体基因及 NMDA3A 样(*grin3a* 样)启动子区域的 DNA 甲基化状态显著，并且高剂量的微量营养素影响雄性性腺中与赖氨酸降解(SASA00310)和嘌呤代谢(SASA00230)相关基因的 DNA 甲基化水平(Saito *et al*, 2022)；高剂量与低剂量的微量营养素相比，高剂量组的乙酰辅酶 a 羧化酶 α (acetyl-CoA carboxylase α, *acacaα*)表达显著下调，而它们之间所有差异甲基化 CpG 位点(DMC)在高剂量组都处于高甲基化水平(Saito *et al*, 2021)。营养饲料差异会影响鱼类代谢水平、生长性能以及性腺发育等相关基因的表达和甲基化水平，从而影响其相关生长发育。

3.5 激素

人为因素影响(如污水排放)会导致激素分布于自然水体中(Rutherford *et al*, 2019; Jenila *et al*, 2023)。鱼类内分泌系统的核心部分是下丘脑和垂体，它们对大脑的神经信号做出反应并将其转化为化学信号(Liu S *et al*, 2023)。这起到干扰内分泌的作用，从而影响鱼类生长和生殖(Zhang *et al*, 2024)。当这些环境激素进入鱼类时，会影响 DNA 甲基化调控过程，从而干扰内分泌系统(Liu S *et al*, 2023; Oliveira *et al*, 2020)。

17α-炔雌醇(17α-ethinylestradiol, EE2)是一种甾体雌激素，将青鳉胚胎(F_0)暴露于 0.05 μg/L 浓度的 EE2 中 7 d 会导致 F_2 代受精率和胚胎存活率降低，且 F_2 代鱼的雄性生育力降低(Bhandari *et al*, 2015)；EE2 处理后在青鳉 F_2 代大脑中 *dnmt3bb* 表达增加并且在 F_0 和 F_2 代鱼的睾丸中处于整体低甲基化模式(Thayil *et al*, 2020)。EE2 诱导的 F_2 代跨代生殖障碍与大脑中生殖基因表达的改变以及睾丸中整体 DNA 甲基化变化有关。

在鱼类实际生产中，激素诱导可以促使性腺发育，并且甲基化参与了调控作用。芳香酶 CYP19A 和 17α-甲基睾酮(17α-methyltestosterone, MT)激素是鱼类性分化的常见试剂，在鱼类中，*cyp19a* 负责将雄激素转化为雌激素(Ramallo *et al*, 2017; Paixão *et al*, 2022)，MT 诱导雌性变化为早熟雄性(Liu S *et al*, 2023)。在 10 ng/L 左炔诺孕酮下，稀有鮈鲫(*Gobiocypris rarus*) *cyp19a1a* 基因 5′侧翼区域诱导 CpG 甲基化模式发生性别特异性变化，在卵巢中低甲基化且 *cyp19a1a* 基因表达显著上调，但在睾丸处于高甲基化水平(Hua *et al*, 2022)。在斜带石斑鱼(*Epinephelus coioides*)卵巢中广泛观察到远端 *cyp19a1a* 启动子存在低甲基化水平，但在睾丸和其他组织中未显示；经 MT 处理的雄性石斑鱼的整体甲基化水平和 *cyp19a1a* 远端启动子甲基化水平要显著高于未成熟鱼雌性(Guo *et al*, 2021)。这些结果表明，*cyp19a1a* 启动子的表观遗传

性在鱼性别变化过程中发挥重要作用。

2,4-二氯苯酚(2,4-dichlorophenol, 2,4-DCP)是一种环境激素，经不同浓度 2,4-DCP 处理 20~50 d 后，斑马鱼的性别比例会偏向于雌性化(Zhang *et al*, 2020)；与空白组相比，2,4-DCP 处理组雄性基因 *sox9a* 表达量显著降低并且 2,4-DCP 处理组(除 160 μg/L 组外)中 *sox9a* 的甲基化水平均显著增加(P<0.01)，而 *dnmt1*、*dnmt3a1*、*dnmt3a2*、*dnmt3b1* 和 *dnmt3b2* 基因表达普遍高于空白组(P<0.05) (Zhang *et al*, 2020)。同时，2,4-DCP 暴露后，斑马鱼 *cyp19a1a* 基因其 61 bp CpG 位点的 DNA 甲基化水平在显著降低，而表达量显著增加(P<0.01) (Hu Y *et al*, 2021)。这些研究结果表明，2,4-DCP 可以通过调控性腺发育相关基因的 DNA 甲基化和表达从而来诱导鱼类雌性化。

4　总结与展望

近年来，DNA 甲基化是一种重要的表观遗传调控，得到越来越多的关注。鱼类 DNA 甲基化的模式、生物学功能，以及一些重要的环境因子与 DNA 甲基化的关系逐渐被人们所认识，但其深度、广度还远远不够。例如：(1)鱼类因为种类繁多，不同种类的鱼类 DNA 甲基化特点还需要进一步了解；(2)还有许多重要的表观遗传与 DNA 甲基化的关系尚未清楚，需要挖掘更多与 DNA 甲基化相关联的基因，便于提高表观遗传学研究在鱼类育种的应用效率；(3)一些重要的变异与 DNA 甲基化水平的具体机制还不清楚；(4)DNA 甲基化水平在不同世代中的遗传机制还不明确；(5)DNA 甲基化核心调控因子的相互作用、调控分化机理尚不清楚。这些科学问题的进一步研究，将能揭示鱼类甲基化调控生长发育以及对环境因子的应答机制，丰富鱼类表观遗传学的理论体系，也能为遗传育种的应用提供理论依据。

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(编辑　冯小花)

Research Progress of DNA Methylation in Fish

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Abstract　DNA methylation is an important epigenetic regulatory mechanism in organisms that regulates genome stability through chromosome and protein structures without altering gene sequences. DNA methylation has been applied in the fields of medicine, agriculture, forestry and animal husbandry, and has attracted great attention in the field of fish genetics and fish breeding.

Methyl groups are transferred to cytosine residues by specific DNA methyltransferases in fish DNA molecules, such as DNMT3, and the existing DNA methylation cell patterns are maintained by the methylation maintenance enzyme, DNMT1. Finally, the methyl group is removed by the oxidation of ten-eleven translocation dioxygenase (Tet1/2/3). DNMT2 catalyzes the transfer of methyl groups from the cofactor S-adenosylmethionine (SAM) to carbon 5 of the cytosine residues of the cytoplasmic tRNA—SAM is also converted to S-adenosine homocysteine. These DNA methylated transferases are widely present in many cells and tissues and play an important role in fish. DNA methyltransferase catalyzes the transfer of methyl groups from SAM to biomolecules (DNA, RNA, proteins, and small molecules) in vivo. There are many species of fish DNA methyltransferases, including two homologous DNMT1 enzymes (DNMT1a and DNMT1b), one DNMT2 enzyme, and eight homologous DNA methyltransferase 3. The naming of DNA methyltransferase 3 homologous genes is complicated; however, they are all parologous genes of DNMT3a and DNMT3b. Demethylation refers to the demethylase-mediated removal of methyl groups from DNA, which plays a key role in gene

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expression regulation, cell differentiation, embryonic development, and disease occurrence and development.

Demethylation refers to the removal of methyl groups from DNA by demethylases. DNA demethylation patterns can be divided into passive and active DNA demethylation patterns. In passive demethylation, methylated DNA undergoes demethylation in successive replication cycles by inactivation or nuclear rejection of DNA methylation transferases that maintain methylation patterns, as well as ubiquitin-like proteins containing PHD and RING finger domains1 (uhrf1). In active DNA demethylation, methylcytosine is first oxidized by TET1/2/3 and then excised by thymine DNA glycosylase. During this process, DNA 5-methylcytosine is oxidized to 5-hydroxymethylcytosine. These oxidation products act as intermediates in DNA demethylation and are replaced by unmodified cytosines to achieve demethylation.

The biological function of DNA methylation in fish is similar to that observed in other organisms, such as mammals, and is involved in gene regulation and cell development. DNA methylation occurs in three C environments: CG, CHG, and CHH (where H is any basic group other than G). DNA methylation occurs primarily at the CG site and allows fish to precisely regulate gene expression and adapt to different environmental factors. Differential methylation—cytosine-phosphate-guanine sites—is involved in apoptosis, epigenetic regulation, autophagy, collagen metabolism, cell membrane function, and homeobox protein generation through gene expression regulation. DNA methylation leads to changes in DNA conformation and stability, and the manner in which DNA interacts with RNA (or proteins) to control gene expression. It can interact with its binding proteins to inhibit gene expression in fish.

DNA methylation affects genome expression regulation by activating or inhibiting transcription at the transcriptional level. Methylation near the transcriptional initiation site blocks initiation, but in the gene body it does not block and may even stimulate transcriptional elongation. It plays an important role in fish biological functions—gene expression regulation, embryonic development, reproductive development, muscle growth, body color, disease, and evolution. It can also provide insights into how genes are regulated during development and how these patterns are passed on to future generations, contributing to the understanding of epigenetics.

Fish are often used as model organisms in endocrine disruption studies because of their high sensitivity to environmental factors. Environmental factors—temperature, heavy metals, starvation stress, nutritional feed, and hormones—affect the regulation of DNA methylation in fish, affecting their growth, development, and overall health.

Recently, DNA methylation has attracted increasing attention as an important epigenetic regulator. The pattern and biological function of DNA methylation in fish, as well as its relationship with important environmental factors, have been gradually recognized; however, knowledge of its depth and breadth are insufficient. For example: (1) because of the wide variety of fish species, their DNA methylation characteristics still need researching; (2) there are still many important epigenetic relationships between DNA methylation, and more genes associated with it need to be explored to improve the application efficiency of fish breeding; (3) the specific mechanisms of some important variations and DNA methylation levels are still unclear; (4) the genetic mechanism of DNA methylation levels in different generations is still unclear; and (5) the interaction of core regulators of DNA methylation and the regulation and differentiation mechanisms are not clear. Further studies of these scientific issues will reveal the mechanism of fish methylation regulation of growth and development and the environmental factor response mechanisms, enrich the theoretical system of fish epigenetics, and provide a theoretical basis for the application of genetic breeding.

Key words DNA methylation; Fish; Biological function; Environmental factor