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· 综述 ·

贝类性别决定与分化相关基因研究进展

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摘要：性别决定与分化是生命发育的基本事件，性别决定与分化机制一直是生命科学研究的热点问题。贝类具有雌雄同体、雌雄异体、雄性先熟和性转换等复杂的性别类型，是研究无脊椎动物性别决定与分化机制及其演化进程的理想动物类群。挖掘贝类性别决定与分化调控基因，阐明相关基因的调控作用，对于揭示贝类性别决定与分化的分子机制具有重要意义。本文就贝类性别决定与分化相关基因的研究进展进行了综述，并对该研究领域进行展望，以期为贝类的性别决定和分化机制、生殖操作和遗传改良等研究提供参考。

关键词: 贝类；性别决定；性别分化；基因

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有性生殖是多细胞动物增殖的一种普遍方式，是多细胞动物物种繁衍的基础。性别决定与分化是动物有性生殖的关键过程。性别决定一般指确定性别形成方向的方式，而性别分化则指未分化的性腺发育为精巢或卵巢并出现第二性征的过程[1]。性别决定与分化作为最基本的生命发育事件之一，一直是生命科学研究领域的热点问题。动物种群中的性别比例是反映种群生存能力和稳定性的重要参数，性别决定与分化研究不仅具有重要的理论意义，在指导动物繁育、性别控制育种、提高养殖效益等方面也具有重要应用价值。

软体动物 (Mollusca) 又称贝类，是动物界中的第二大类群，种类繁多、分布广泛，具有重要的生态和经济价值。贝类不仅具有复杂多样的有性繁殖模式，而且拥有多种多样的性别类型，有雌雄同体、雌雄异体、雄性先熟、性转换等多种形式[2]，且有的物种同时具有多种性别类型，性别决定机制非常复杂，迄今除腹足纲 (Gastropoda)

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等少数种类外[3]，贝类中尚未发现异型性染色体的报道。有学者认为，贝类处于性别进化过程中的原始阶段，因此性别类型复杂多样[2]。研究者对贝类性别决定与分化的探索可追溯至 20 世纪 40 年代[4]，但目前有关贝类性别决定与分化的机制尚不明确。动物性别决定机制主要有 3 种：遗传性别决定 (genetic sex determination，GSD)、环境性别决定 (environmental sex determination，ESD) 以及遗传和环境因素共同决定[5]。遗传性别决定机制又可分为染色体决定机制 (chromosomal SD) 和多基因决定机制 (polygenic SD)，如常见的 XY 型、ZW 型和 XO 型等性别决定系统属于 GSD 类型。环境性别决定是指个体的性别由温度、营养条件、光周期等环境因素决定，如红耳龟 (*Trachemys scripta*)胚胎发育过程中的环境温度可决定其性别[6]。已有的贝类性别决定与分化相关研究表明，贝类性别受遗传调控，但也发现环境因素对贝类的性别产生影响。大多数双壳类的性别决定受遗传和环境因素共同作用，其中性别不稳定的种类 [如长牡蛎 (*Crassostrea gigas*)]，其性别受环境影响较大，而性别比较稳定的种类 [如虾夷扇贝 (*Patinopecten yessoensis*)]，主要受遗传调控。腹足类中既有遗传性别决定的类群 [如履螺属 (*Crepidula*)]，也有环境性别决定类群 [如田螺属 (*Viviparu*)] [7-8]。

近年来，随着生物技术的不断发展，贝类性别决定与分化相关研究进展快速，研究人员利用候选基因法、QTL 定位法、转录组等方法在一些重要经济贝类中筛选到一些性别决定与分化相关基因[9-11]。本文就贝类性别决定与分化相关基因的研究进展进行简要综述，重点探讨相关基因在贝类性别决定与分化中的调控功能，并对该研究领域进行展望，以期为贝类的性别决定机制研究、性别控制育种、生殖操作等研究提供参考。

1　组学技术在贝类性别决定与分化相关基因鉴定中的应用

早期研究者主要利用基于同源序列的候选基因法挖掘贝类性别决定与分化相关基因，即根据其他动物中已知的性别决定与分化相关基因信息，进行同源基因克隆及相关功能分析。例如研究者利用该方法分别在牡蛎、扇贝中鉴定出 *Dml* (*DmrtA2-like*)、 *Dmrt2* 和 *Dmrt5* 等性别决定与分化相关基因[9, 12]。然而候选基因法成本较高，且费时费力，进展缓慢。近年来高通量测序技术的发展快速，为生命科学研究创造了前所未有的灵敏度和深度。研究人员从全基因组水平利用转录组测序 (RNA-Sequencing，RNA-Seq) 等技术鉴定出一批与贝类性别决定和分化相关的候选基因 (表 1)，极大地推动了贝类性别决定与分化机制的研究进程。

2　贝类性别决定与分化相关基因研究进展

2.1　***Dmrt* 基因**

Dmrt (doublesex and mab-3 related transcription factor) 基因是一类能编码锌指 DM 结构域的基因。DM 结构域最早发现于黑腹果蝇 (*Drosophila melanogaster*) 的 *Doublesex* (*Dsx*) 基因和秀丽隐杆线虫 (*Caenorhabditis elegans*) 的 *Maleabnormal*-3 (*mab*-3) 基因，二者高度同源，是性别调控的关键基因[34-35]。随后 Raymond 等[36]在人类 (*Homo sapiens*) 中获得一个编码 DM 结构域、睾丸特异性表达的基因并命名为 *Dmrt*1。在此背景下，研究者陆续在青鳉 (*Oryzias latipes*)[37]、大型溞 (*Daphnia magna*)[38] 和红耳龟[6] 等动物中发现参与性别决定与分化的 *Dmrt* 基因，说明 *Dmrt* 基因在脊椎动物和无脊椎动物中比较保守，其中青鳉常染色体上的 *Dmrt*1 基因经复制转座到 Y 染色体上，形成了性别决定基因 *DMY*[39]。*Dmrt* 基因也是贝类中研究最多的性别决定与分化相关基因，研究者利用同源基因序列，从牡蛎、扇贝、珍珠贝和鲍中克隆了 *Dmrt*1、*Dmrt*2、*Dmrt*3、*Dmrt*4、*Dmrt*5 (*DmrtA*2) 以及 *Dmrt*1*L* 等 *Dmrt* 家族基因 (表 2)，其中 *Dmrt*1、*Dmrt*2、*Dmrt*5 和 *Dmrt*1*L* 表现出与性别决定和分化相关。

长牡蛎中已发现 *DmrtA*2、*Dmrt*1 (*Dsx*) 和 *Dml* (*DmrtA2-like*) 3 个含 DM 结构域的基因，其中 *Dmrt*1 (*Dsx*) 与性别分化相关。Zhang 等[14] 研究发现，长牡蛎中 *Dsx* 基因只在性腺中表达，转录组数据和定量 PCR 结果显示，*Dsx* 在精巢中的表达量显著高于卵巢[40]，推测 *Dsx* 基因可能在长牡蛎的性别决定或雄性性腺发育中起关键作用。Sun 等[57] 利用 RNA 干扰技术敲降长牡蛎 *Dmrt*1 基因，发现 54% 的个体性腺无法发育，表明 *Dmrt*1 参与长牡蛎性腺初始分化过程。*Dmrt*1 基因在栉孔扇贝[43]、虾夷扇贝[46] 和海湾扇贝[47] 中也呈现出性别二态性表达，均在精巢发育早期高表达，推测其参与扇贝精巢发育和性别分化调控。在海湾扇贝中利用 RNA 干扰 *Dmrt*1 表达后，卵巢发育相关基因 (*GATA*、

表 1　贝类中运用组学技术筛选的性别决定与分化候选基因

Tab. 1　Candidate genes related to sex determination and differentiation screened by omics in molluscs

物种 species	技术类型 technologies	基因名称 gene names	参考文献 references
长牡蛎 *C. gigas*	microarray	*Bindind*、*py*-30、*foxL*2、*nanos*3、*cd*63、*vitellogenin*	[13]
	RNA-Seq	*SoxH*、*FoxL*2、*Dsx*、*DmrtA*2、*Sh*3*kbp*1、*Malrd*1-*like*、*Trophoblast glycoprotein-like*、*Protein PML-like*、*Protein singed-like*、*PREDICTED: paramyosin*	[14-16]
	Genome-wide screening	*nanos*、*piwi*、*dax*1、5-*HT receptor*	[17]
	Genotyping-by-Sequencing	*proteasome subunit beta type*-3、*G-protein coupled estrogen receptor* 1	[10]
香港牡蛎 *C. hongkongensis*	RNA-Seq	*nanos*、*piwi*、*ATRX*、*FoxL*2、*beta-catenin*	[18]
栉孔扇贝 *Chlamys farreri*	RNA-Seq	*FSHR*、*GABBR*、*MTR*、*CYP*17、*Vtg*4、*Wnt*10*a*、*Htr*4、*PTGR*1、*Zar*1	[11]
虾夷扇贝 *Patinopecten yessoensis*	RNA-Seq	*Foxl*2、*SoxH*、*Dmrt*1	[19-20]
华贵栉孔扇贝 *C. nobilis*	RNA-Seq	*Foxl*2、*β-Catenin*、5-*HT receptor*、*Vitellogenin*、*Dmrt*2、*SRY*、*fem*-1、*Sfp*2、*Sa*6、*AMY*-1、*vasa*、*nanos*、*sox*9	[21-22]
狮爪扇贝 *Nodipecten subnodosus*	RNA-Seq	*dmrta*2、*wnt*4、*sex*-1、*sfrp*2、*sox*9	[23]
	Suppressive Subtraction Hybridization and Pyrosequencing	*dmrt*1、*clk*-2、*fkbp*52	[24]
马氏珠母贝 *Pinctada fucata*	Genome-wide screening	*vasa*、*nanos*、*dmrt*、5-*HT receptors*、*vitellogenin*、*estrogen receptor*	[25]
黑蝶贝 *P. margaritifera*	RNA-Seq	*Dmrt*、*fem*-1、*foxl*2、*vitellogenin*	[26]
三角帆蚌 *Hyriopsis cumingii*	RNA-Seq	*DMRT*1、*SOX*9、*SF*1、*FOXL*	[27]
池蝶蚌 *H. schlegelii*	RNA-Seq	*wnt*4、*rspo*1、*fem*1、*tra*1、*Sry*、*Dmrt*1、*Dmrt*2、*Sox*9、*GATA*4、*WT*1、*Wnt*4、*Rspo*1、*Foxl*2、*β-catenin*	[28-29]
缢蛏 *Sinonovacula constricta*	RNA-Seq	*DmrtA*2、*Sox*9、*Fem*-1*b*、*Fem*-1*c*、*Vg*、*CYP*17*A*1、*SOHLH*2、*TSSK*	[30]
泥蚶 *Tegillarca granosa*	RNA-Seq	*Foxl*2、*Sox*、*β-catenin*、*CBX*、*Sxl*	[31]
中国蛤蜊 *Mactra chinensis*	RNA-Seq	3*β*-*HSD*、17*β*-*HSD*、*LH*、*CYP*17、*CYP*1*A*1、*CYP*1*B*1、*Estrogen*、*vitellogenin*	[32]
红鲍 *Haliotis rufescens*	RNA-Seq	*VTG*、*fusion protein*、*tektin*、*lysin*、*SOX*	[33]

表 2　贝类中已鉴定的 ***Dmrt*** 基因

Tab. 2　*Dmrt* genes identified in molluscs

序号 no.	物种 species	基因名称 gene name	参考文献 references
1	长牡蛎　*C. gigas*	*Dml*、*Dsx*	[9, 40-41]
2	栉孔扇贝　*C. farreri*	*Dmrt*4-*like*、*Dmrt*1	[42-43]
3	华贵栉孔扇贝　*C. nobilis*	*Dmrt*2、*Dmrt*5	[12]
4	虾夷扇贝　*P. yessoensis*	*Dmrt*1*L*、*Dmrt*2、*Dmrt*1	[44-46]
5	海湾扇贝　*Argopecten irradians*	*Dmrt*1、*Dmrt*1*L*	[47-48]
6	马氏珠母贝　*P. martensii*	*Dmrt*2、*Dmrt*3、*Dmrt*4、*Dmrt*5	[49-52]
7	三角帆蚌　*H. cumingii*	*Dmrt*1、*DmrtA*2-1	[53-54]
8	企鹅珍珠贝　*Pteria penguin*	*Dmrt*2	[55]
9	耳鲍　*H. asinina*	*Dmrt*1	[56]

*Wnt*4、*Foxl*2 和 *β-catenin*) 表达量显著升高，表明 *Dmrt*1 基因参与调控精巢分化。Nagasawa 等[45]在虾夷扇贝中发现，*Dmrt*2 基因在精巢中高表达，且在精原细胞增殖期间表达量更高，因此可将

*Dmrt*2 基因作为雄性特异性标记用于性别分化早期的性别鉴定。另外，研究者在虾夷扇贝和海湾扇贝中均鉴定到一个 *Dmrt* 同源基因，并命名为 *Dmrt*1*L*，*Dmrt*1*L* 呈性别二态性表达，利用 lg(*Dmrt*1*L*/*Foxl*2) 值鉴定扇贝发育早期的性别[44, 48]。在马氏珠母贝中已克隆得到 4 个 Dmrt 家族基因 (*Dmrt*2、*Dmrt*3、*Dmrt*4 和 *Dmrt*5)，其中 *Dmrt*2 和 *Dmrt*5 可能参与性别决定和分化，二者均在精巢中特异性高表达，*Dmrt*2 在成熟期精巢中表达量最高，而 *Dmrt*5 在精巢发育早期表达量最高[50-51]。企鹅珍珠贝中 *Dmrt*2 基因的表达模式与马氏珠母贝中类似，推测其参与调控企鹅珍珠贝雄性性别分化[55]。三角帆蚌中，*Dmrt*1 和 *DmrtA*2-1 基因均在性腺分化早期高表达，前者在雄性中高表达，后者反之，前者表达定位于精细胞，后者定位于滤泡壁，推测这两个基因参与三角帆蚌的性别分化调控[53-54]。Klinbunga 等[56] 在耳鲍中也鉴定到精巢特异性表达的 *Dmrt*1 基因，但其在精巢发育和性别分化中的作用仍需进一步研究。

2.2　*Foxl2* 基因

*Foxl*2 (forkhead box transcription factor l2) 基因是 *Fox* 基因家族的一员，参与调控动物性别分化、卵巢功能和维持、基因组完整性以及细胞周期进程、细胞增殖和凋亡等细胞途径[58]。*Foxl*2 最早在患有睑裂狭小综合征及卵巢早衰的人类中克隆获得[59]，随后发现其在山羊 (*Capra hircus*) 卵巢发育中发挥重要作用[60]。后续众多研究表明，*Foxl*2 基因在脊椎动物和无脊椎动物中均比较保守，参与调控卵巢分化[61]，在多种贝类中也发现了 *Foxl*2 基因的存在。研究者通过表达模式分析、RNA 干扰等方法证实了 *Foxl*2 在贝类卵巢发育和性别分化过程中发挥重要作用 (表 3)。

表 3　已报道的贝类 *Foxl2* 基因

Tab. 3　Reported *Foxl2* genes in molluscs

序号 no.	物种 species		基因名称 gene name	参考文献 references
1	长牡蛎	*C. gigas*	*Foxl*2/ *Foxl*2*os*	[57, 62-63]
2	香港牡蛎	*C. hongkongensis*	*Foxl*2	[64]
3	栉孔扇贝	*C. farreri*	*Foxl*2	[65-67]
4	虾夷扇贝	*P. yessoensis*	*Foxl*2	[45, 68-69]
5	海湾扇贝	*A. irradians*	*Foxl*2	[48, 70]
6	三角帆蚌	*H. cumingii*	*Foxl*2	[71-72]

长牡蛎中 *Foxl*2 在卵黄发生期表达上调，敲降 *Foxl*2 后 42% 的个体性腺停止分化，*Esr* (estrogen receptor，雌激素受体) 基因表达受到抑制，表明 *Foxl*2 参与性腺初始分化[57, 62]。Santerre 等[63] 发现，长牡蛎中存在 *Foxl*2 的天然反义转录本 (natural antisense transcript，NAT) *Foxl*2*os*，其可能在性腺分化过程中调控 *Foxl*2 表达。香港牡蛎中 *Foxl*2 基因与长牡蛎中相似，在性腺中高表达，其具体功能仍有待研究[64]。在栉孔扇贝、虾夷扇贝和海湾扇贝中，*Foxl*2 为卵巢特异性高表达，参与调控卵巢发育和性别分化。栉孔扇贝中 *Foxl*2 在增殖期卵巢中表达量最高，约为同期精巢表达量的 62 倍，约为生长期和成熟期卵巢表达量的 2 倍，可作为早期发育性别标记基因[65-67]。虾夷扇贝中 *Foxl*2 的表达量随卵巢发育先上升后下降，原位杂交信号随雄性生殖细胞分化而逐渐减弱[45, 69]。在海湾扇贝中敲降 *Foxl*2 后，精巢发育相关基因 *Dmrt*1、*Sox*7 和 *Sox*9 上调，卵巢发育相关基因 *Vg*、*HSD*14 和 *gatt*-1 下调[70]，且 *Foxl*2 表达与雌二醇/睾酮指数 (E_2/T) 正相关[48]，推测 *Foxl*2 可能是海湾扇贝性别分化关键驱动基因。研究发现，*Foxl*2 在三角帆蚌卵巢中的表达量高于精巢，在性腺开始分化的 5 月龄个体中表达量最高，RNA 干扰 *Foxl*2 后其拮抗基因 *Wnt*4 表达上调[71- 72]。

2.3　*Sox* 基因

Sox (sry-related HMG box) 基因是一类能编码 HMG (high mobility group) box 结构域的基因，其中 *Sry* 基因是脊椎动物中鉴定出的第一个性别决定关键基因，在哺乳动物中，*Sry* 的存在促使睾丸发育，而其缺失导致卵巢发育[5, 73]，但是在贝类中并未鉴定到 *Sry* 基因的存在。研究者在贝类中克隆获得了 *Sox*2、*Sox*8、*Sox*9、*Sox*11 和 *Sox*14 等 *Sox* 基因家族成员，表达模式分析发现，其均表现为性别二态性表达，但具体功能仍有待后续研究。长牡蛎中 *SoxE* (*Sox*8-*like*) 在性腺分化前高表达，分化后表达量下降，表达定位于生殖系细胞，因此推测其参与牡蛎性别决定[74]。在马氏珠母贝、三角帆蚌、企鹅珍珠贝和池蝶蚌中鉴定出了 *Sox*2、*Sox*9、*Sox*11 和 *Sox*14，除 *Sox*2 为雌性性腺高表达之外，其余均在雄性性腺中高表达，推测这些 *Sox* 基因可能参与调控性别决定和分化[71, 75-78]。

2.4　*Wnt*4 基因

研究者最初于患乳腺癌小鼠 (*Mus musculus*) 中发现 *int*-1 基因，后发现其与果蝇 *wingless* 基因为同源基因，故将二者合并命名为 *Wnt* 基因[79]。*Wnt* 基因家族编码的信号分子可激活多种信号通路，在动物早期发育等生命活动中发挥重要作

用。*Wnt*4 是 *Wnt* 基因家族的重要成员，研究表明 *Wnt*4 参与调控哺乳动物雌性性腺分化[80]。近几年研究者在几种贝类中鉴定、克隆了 *Wnt*4 基因并进行了表达分析，*Wnt*4 在贝类各组织中广泛表达，可能在性腺发育和器官形成过程中发挥作用，但是否参与性别分化仍未知。在池蝶蚌、栉江珧 (*Atrina pectinata*) 和三角帆蚌中，*Wnt*4 在卵巢中的表达量高于精巢，在栉孔扇贝和皱纹盘鲍 (*H. discus hannai*) 中反之，推测其参与两性性腺发育。*Wnt*4 在栉江珧、厚壳贻贝 (*Mytilus coruscus*)、长牡蛎的幼虫发育阶段高表达，说明其在早期发育、器官形成等过程中也发挥作用[81-86]。

2.5　*Dax*1、*β-catenin* 基因

*Dax*1 (DSS-AHC critical region, on chromosome X, gene 1) 属于核受体 (nuclear receptor) 超家族。研究表明，*Dax*1 基因在脊椎动物中参与调控性别决定和分化[87-88]。*β-catenin* 最早从非洲爪蟾 (*Xenopus laevis*) 中分离获得，是 *Wnt* 通路的关键转录效应因子[89]，研究发现，*β-catenin* 参与调控哺乳动物性别决定和分化，是一个抗睾丸的卵巢分化关键因子[90]。在贝类中，有关 *Dax*1 和 *β-catenin* 的研究较少，少数研究表明，二者可能参与调控贝类配子发生，其在性别决定或分化中是否发挥作用仍未可知。*Dax*1 在栉孔扇贝精巢中的表达显著高于卵巢，主要在生殖细胞中表达，表明 *Dax*1 可能参与配子发生[91]。研究发现，在长牡蛎和三角帆蚌中，*β-catenin* 均在卵巢中特异性高表达，主要在早期雌性生殖细胞中表达，因此推测 *β-catenin* 参与贝类卵巢分化和卵子发生[74, 92]。Li 等[93] 在栉孔扇贝精巢细胞培养液中加入 querceti (*β-catenin* 的抑制剂) 后，发现 *Dax*1 表达显著下调，表明 *β-catenin* 是 *Dax*1 的上游调控基因。

2.6　*Fem*-1 基因

Fem-1 (ferminazation-1) 基因最早被发现在秀丽隐杆线虫性别决定通路中发挥关键作用，参与调控雄性体组织致雄化和雄性生殖细胞分化。*Fem*-1 基因家族至今共发现 *Fem*-1*a*、*Fem*-1*b* 和 *Fem*-1*c* 三个成员，均编码一种含 ANK (ankyrinrepeat，锚蛋白重复序列) 结构域的细胞内蛋白。研究人员从无脊椎动物和脊椎动物中都发现了 *Fem*-1 同源基因，其中一些表现出与性别相关的表达模式[94-95]。贝类中有关 *Fem*-1 的研究较少，周祖阳等[96] 发现，*Fem*-1*b* 和 *Fem*-1*c* 在长牡蛎精巢中高表达，在胚胎发育早期表达上调，与雄性性别分化相关。相反，三角帆蚌中，*Fem*-1*c* 在卵巢中高表达，在性腺发育早期显著上调，可能调控雌性性别分化[97]。熊文芳等[98] 发现，池蝶蚌 *Fem*-1*c* 基因与其他动物 (包括线虫) 的 *Fem*-1*c* 具较高保守性，暗示其在功能上也相对保守，推测其可能与线虫 *Fem*-1*c* 基因在性别决定方面具有相似功能。

2.7　*Vasa*、*Nanos* 基因

Vasa 和 *Nanos* 基因均为发现于黑腹果蝇的母源性基因，对动物生殖系统细胞分化和发育具有重要意义[99-100]。*Vasa* 编码蛋白属于 DEAD-box (AspGlu-Ala-Asp) 蛋白家族，DEAD-box 参与调节细胞 RNA 转录、剪切、修饰、代谢以及核内 mRNA 运输及降解等过程。*Vasa* 基因高度保守，研究人员已相继在多种无脊椎动物和脊椎动物中鉴定到 *Vasa* 同源基因，大量研究表明，*Vasa* 基因在绝大多数物种中为原始生殖细胞和生殖细胞特异性表达[99, 101-102]。*Nanos* 编码蛋白调控果蝇胚胎后部区域细胞分化，为原始生殖细胞发育所必需。目前已在多种动物中鉴定出 *Nanos*1、*Nanos*2 和 *Nanos*3 三个同源基因，其在不同物种中功能相对保守，参与生殖细胞发育和维持[100, 103]。在贝类中，*Vasa* 基因见诸于长牡蛎、马氏珠母贝、虾夷扇贝和中华蛸 (*Octopus sinensis*) 等的报道，*Vasa* 基因主要在贝类生殖干细胞中表达，在生殖细胞成熟过程中表达量不断降低，在牡蛎中，利用 RNA 干扰技术敲降 *Vasa* 基因会引起生殖细胞增殖不足、减数分裂阻滞[104-108]。在对长牡蛎、虾夷扇贝和耳鲍的研究中发现，*Nanos* 均表现出生殖细胞特异性表达[109-111]，因此 *Vasa* 和 *Nanos* 基因可作为研究贝类生殖细胞分化的标记基因。

2.8　*GnRH* 基因

促性腺激素释放激素 GnRH 最初发现于哺乳动物，是下丘脑合成分泌的、调控“下丘脑-垂体-性腺轴”的多肽类激素，通过控制垂体下叶释放卵泡刺激素 FSH 和黄体生成素 LH 的释放来调节动物性别分化、性腺发育等过程。脊椎动物中存在多样的GnRH，主要有GnRH-Ⅰ、GnRH-Ⅱ和GnRH-Ⅲ三种类型[112-114]。无脊椎动物和脊椎动物的 GnRH 在结构和功能上同源性高，贝类中也广泛存在 GnRH，目前已在真蛸 (*O. vulgaris*)、长牡蛎、海兔 (*Aplysia californica*)、剑尖枪乌贼 (*Loligo edulis*)、菲律宾蛤仔 (*Ruditapes philippinarum*)、曼氏无针乌贼 (*Sepiella japonica*)、耳鲍、海湾扇贝、

长蛸 (*O. minor*) 和拟目乌贼 (*Sepia lycidas*) 等贝类中克隆获得了 *GnRH* 基因。研究发现，*GnRH* 基因在贝类性别分化起始阶段表达上调，推测其参与调控性原细胞增殖、性腺分化启动和性腺发育等过程[115-124]。

3　总结与展望

目前在部分脊椎动物和模式动物 (如小鼠、青鳉、果蝇等) 中已经发现了性别决定与分化的关键基因和调控通路[5, 73, 125-126]。虽然有关贝类的性别决定与分化机制已经开展了大量的研究工作，但贝类的性别决定主开关基因 (master-switch gene) 尚未见报道。目前研究者所鉴定出的贝类性别决定与分化相关基因主要是性别决定和分化通路中的下游基因，因此寻找性别决定关键开关基因是今后应重点关注的方向。对于性别稳定的物种 (如扇贝) 可借助新技术，如雌、雄分别进行基因组测序，以筛选鉴定性别关键基因。对于性别不稳定的贝类 (如牡蛎)，可通过对同一个体性别连续追踪，进行性别决定和分化关键基因挖掘。在斑马鱼 (*Danio rerio*) 中，研究发现驯化的斑马鱼品系其性别可能由多基因共同决定[127]。贝类中的性别究竟是单基因还是多基因决定，仍有待于深入研究。

模式动物中，有关性别决定及分化的信号通路已研究得较为深入，部分基因在性别决定和分化中的作用已得到阐释。目前贝类中关于性别决定和分化相关基因的研究还局限于基因的鉴定、克隆和表达分析，而对于基因调控通路的构建还

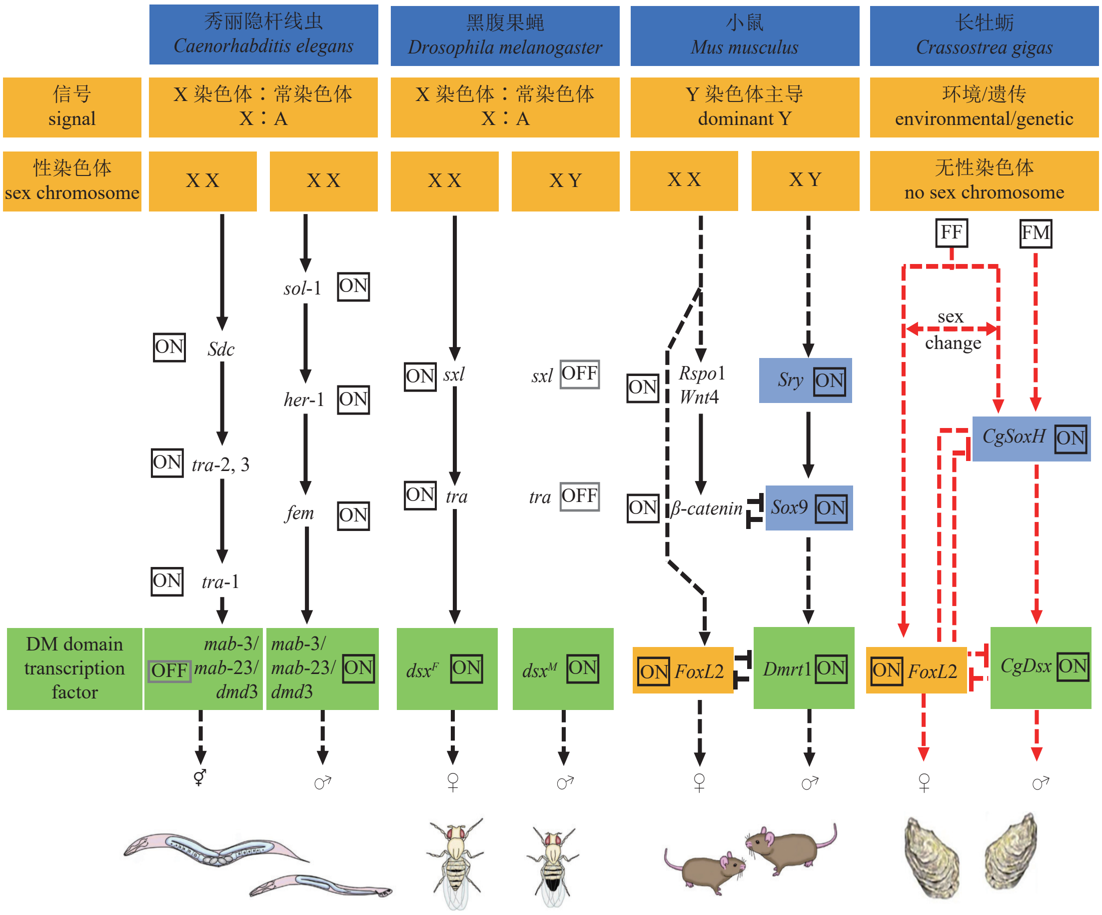

图 1　长牡蛎假定性别决定通路与模式动物性别决定通路比较[14]

为使图简洁易懂，只选择显示关键性别特异性因子。调控互作关系中，黑色实线代表每条通路的调控逻辑而非直接调控作用；黑色虚线表示时序关系，红色虚线表示基于表达数据的假设关系；FF 基因型表示假雄性 (发生性别转换)，FM 基因型表示真雄性 (不发生性别转换)。

Fig. 1　Comparison between putative sex determination pathway in *C. gigas* and model animals[14]

For clarity, only selected key sex-specific regulators are shown. Regulatory interactions (solid lines) are meant to indicate the regulatory logic of each pathway but do not necessarily imply direct regulation. Dashed lines indicate temporal and dashed red lines indicate hypothetical relationships based on expression data only; FF genotype are fake males and permit sex change, FM genotype are true males that do not change sexes.

比较缺乏。有研究人员推测，长牡蛎的性别决定调控通路与脊椎动物性别决定调控通路的相似程度高于线虫和果蝇等无脊椎动物 (图 1)。

表观遗传因其在基因的表达、调控方面发挥重要作用，近年来成为生命科学中一个普遍而又至关重要、不可忽视的研究领域。研究表明，表观遗传在动物性别决定与分化中也发挥重要作用，如哺乳动物和鱼类的性别决定与分化关键基因都受到表观遗传的调控[128-129]，而对于环境性别决定的物种，表观遗传在性别决定与分化中发挥关键作用，如红耳龟中表观遗传因子 *Kdm6b* 在性别调控中至关重要[130]。在贝类中，已有研究探究了组蛋白去甲基化和 DNA 甲基化与性别决定和分化的相关性[131-133]，表明表观遗传在性别决定和分化中可能发挥重要作用。因此，未来针对贝类性别开展研究可关注经典遗传与表观遗传的共同作用机制。

许多双壳贝类如牡蛎、珍珠贝等存在性别逆转现象，但是在相同环境下，并非所有个体都发生性别逆转，对于贝类性别逆转的分子机制仍需深入研究。性逆转个体与非性逆转个体在基因组水平上存在何种差异，贝类发生性逆转时哪些基因表达发生变化，性逆转的分子机制是怎样的，这些都是有待后续研究的重要问题。另外，雌雄同体现象也在贝类中普遍存在，已有的研究主要从组织学和转录组水平进行，但是其性别决定与分化的分子机制尚未解密。

贝类的性别决定与分化研究复杂而困难，目前针对贝类性别决定与分化相关基因的功能验证仍比较局限，仅限于 RNA 干扰技术。近几年，CRISPR/Cas9 基因编辑技术的快速发展为贝类性别决定与分化相关基因的功能研究带来机遇。未来，针对贝类性别特异性标记开发，性别决定与分化相关基因的筛选、功能解析、级联调控通路的构建以及在进化中的地位评估，不仅是进化发育生物学、功能基因学的重要课题，而且有利于探索贝类生殖细胞操作、性别控制、育性控制等遗传改良和濒危物种增殖保护。

（作者声明本文无实际或潜在的利益冲突）

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Research advances in genes related to sex determination and differentiation in molluscs

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Abstract: Sex determination and differentiation are basic events of development and key processes of sexual reproduction. The mechanism of sex determination and differentiation is both baffling and intriguing. Mollusca is the second largest animal phylum, harboring diverse sexual systems including hermaphroditism, gonochorism, protandry and sex reversal. Therefore, molluscs are an ideal animal group to study the mechanisms of sex determination and differentiation. Although efforts towards understanding sex determination and differentiation of molluscs can be traced back to the 1940s, the mechanisms of sex determination and differentiation of molluscs remain enigmatic. Solving the riddle of sex determination and differentiation and identifying key genes would help us better understand the biology of molluscs and provide insights into sexual evolution in the animal kingdom. Here we review the research progress and the prospects of sex determination and sex related genes such as *Dmrt*, *Foxl*2, *Sox*, *Wnt*4, *Dax*1, *β-catenin*, *Fem*-1, *Vasa*, *Nanos* and *GnRH* in molluscs. We also propose several goals about mechanistic underpinning of sex determination in molluscs which require further study.

Key words: molluscs; sex determination; sex differentiation; genes

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