硬骨鱼类卵黄原蛋白及其在卵子发生中的作用

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摘要 卵黄原蛋白(vitellogenin, Vtg)作为卵黄蛋白(yolk protein, YP)的前体, 参与卵生动物的卵子发生。硬骨鱼类的Vtg为同二聚体, 存在不同的亚型, Vtg单体(monomer)由5个结构域组成。在硬骨鱼类卵子发生过程中Vtg主要有两方面作用: (1)Vtg被卵母细胞吸收后裂解为卵黄蛋白, 贮存在卵母细胞中, 为其生长发育提供必需的营养; (2)随着卵母细胞的发育, 卵黄蛋白裂解为游离氨基酸, 调节卵母细胞的渗透压, 保证水合作用的顺利进行。该文介绍了硬骨鱼类Vtg的种类及结构特性, 并对Vtg的表达、吸收、分解及其参与水合作用的研究进展作简要综述, 以期为硬骨鱼类卵子发生机制的研究提供参考。

关键词 硬骨鱼类; 卵黄原蛋白; 卵黄发生; 水合作用; 渗透压

The Vitellogenin and Its Function during Oogenesis in Teleost Fishs

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Abstract Vitellogenin (Vtg), as the precursor of yolk protein (YP), involved in the oogenesis in oviparous animals. The structure of teleost fishes' Vtg is the homodimer and present different subtypes. The Vtg monomer is consist of five different domains. During the oogenesis in teleost, the Vtg plays two functions. On the one hand, the Vtg is hydrolyzed into yolk protein and store in the oocyte to provide the necessary nutrition for the oocyte development. On the other hand, the yolk protein is hydrolyzed into free amino acid with the development of oocyte. These free amino acids can regulate the osmotic pressure of oocyte to ensure the hydration. In this review, we described the types and structural characteristics of teleost Vtg, summarized the recent advances on the expression, uptake, proteolysis, and the role in hydration of Vtg. We hope this review can provide the basic data for the molecular mechanism of teleost fish oogenesis.

Keywords teleost fishes; vitellogenin; vitellogenesis; hydration; osmotic pressure

卵黄原蛋白(vitellogenin, Vtg)是卵黄蛋白(yolk protein, YP)的前体, 在卵生动物卵子发生及胚胎发育过程中发挥重要作用[1-2]。早在1935年, Roepke等[3]在分析鸡血浆蛋白总磷时发现, 产卵期的母鸡血浆中含有一种与卵黄蛋白相似的磷蛋白, 而在非产卵期的雌性及雄性中未发现。直到1969年, Pan等[4]首次用卵黄原蛋白概念描述雌性昆虫血浆中发现的这一蛋白, 由此卵黄原蛋白概念成专用词被广泛使用。随后, 在鱼类生殖研究中发现, 处在卵黄发生期的或被雌激素处理后的雌鱼, 其血浆中存在一种雌性特异性磷脂蛋白(female-specific serum proteins, FSSP)[5-12]。由Hara等[13]用免疫学方法证明, 虹鳟

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(*Oncorhynchus mykiss*)中的FSSP为卵黄原蛋白, 这是在鱼类中最先确定的Vtg。

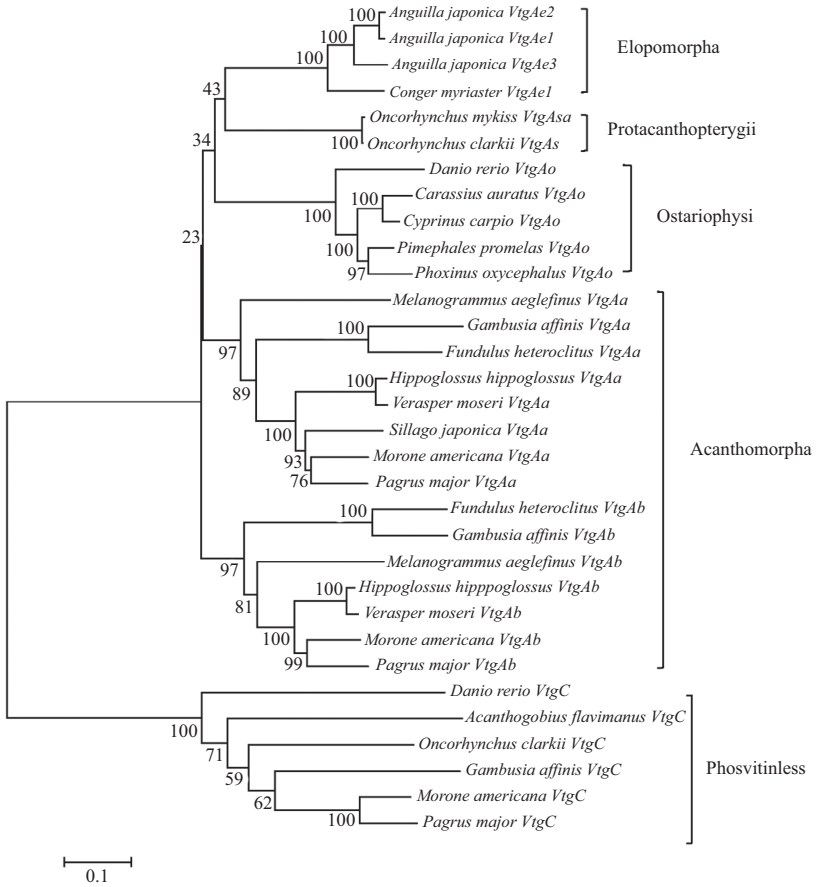
Vtg为大分子磷脂糖蛋白。脊椎动物的Vtg一般是由2个150~200 kDa Vtg单体组成的同二聚体[14-16]。硬骨鱼类的Vtg均为同二聚体, Vtg的单体由5个结构域组成, 不同种鱼类Vtg分子结构不同, 存在不同的亚型[15-19]。在卵子发生过程中, Vtg的表达受激素调控, 该过程中由肝合成的Vtg经血液循环运送至卵母细胞表面, 经细胞膜上受体介导的内吞作用而被吸收入细胞内, 并被组织蛋白酶D水解为YP, 储存在卵黄颗粒或卵质的其他组分中。此时, 卵母细胞内充满卵黄蛋白, 有的种类甚至占整个卵母细胞干重的80%~90%[14]。随后, 卵母细胞进行水合作用, YP在组织蛋白酶B或L的作用下水解为游离氨基酸(free amino acid, FAA)或小分子多肽, 调节细胞渗透压[14]。鱼卵中的YP是胚胎发育和早期仔鱼必要的营养物质[20]。本文主要介绍硬骨鱼类Vtg的种类及结构特性, 并对Vtg的表达、吸收、分解及水合作用研究进展作简要综述。

1 硬骨鱼类卵黄原蛋白的种类及结构特性

Vtg是一种广泛存在于卵生无脊椎和脊椎动物体内的雌性特异性蛋白质, 几乎所有Vtg都起源于一个共同的祖先基因, 经过复杂而漫长的进化过程, Vtg出现了多种亚型[15-19]。早期研究对Vtg亚型的命名并不统一, 例如VtgA、Vtg1和VtgI为同一种亚型, VtgB、Vtg2和VtgII为同一种亚型, VtgC和Vtg3为同一种亚型[17,21-24]。直到2007年, Finn等[19]根据脊椎动物进化过程中全基因组复制(whole genome duplications, WGD)对Vtg进行分类, 此分类方法被普遍接受。据此方法, 在第一轮WGD过程中产生的Vtg为VtgABCD, 这一Vtg亚型仅在七鳃鳗(*Lampetra japonicum*)中发现, 随后产生了VtgAB和VtgCD, VtgAB进一步分化出现了VtgA和VtgB两个分支, 同样地, VtgCD分化成VtgC和VtgD两个分支。但是, VtgB和VtgD这两个分支已经在进化过程中消失[19]。随着硬骨鱼类的不断进化, VtgA又进一步产生不同的亚型。硬骨鱼类中几种A-型Vtg亚型与VtgC之间的进化关系如图1所示, Vtg主要分两支: A-型Vtg和VtgC。A-型Vtg又有多种不同的亚型, 在棘鳍总目(Protancanthopterygii)中为VtgAs, 在海鲢总目(Elopomorpha)中为VtgAe1、VtgAe2及VtgAe3, 在骨鳔总目(Ostariophysi)中为VtgAo, 在棘鳍总目(Acanthomorpha)中为VtgAa和VtgAb[14,19]。VtgC与A-型Vtg的差异较大, 单独形成一个分支(Pv缺失型, Phosvitinless)。

硬骨鱼类Vtg由两个相同单体组成的同二聚体, 每一单体包含5个卵黄蛋白结构域, 自N-端到C-端依次为卵黄脂磷蛋白重链(LvH)、卵黄高磷蛋白(Pv)、卵黄脂磷蛋白轻链(LvL)、β'-区域(β'-c)、C-端多肽(Ct)[14,16]。硬骨鱼类不同亚型的Vtg在结构上存在一定差异, 包含有全部5个卵黄蛋白结构域(LvH、Pv、LvL、β'-c及Ct)的Vtg称为完整型, 即为A-型Vtg。然而, A-型Vtg中, VtgAo1缺少β'-c和Ct两个结构域。VtgC则缺少Pv结构域(图2)[21,25-26]。从不同亚型Vtg的结构可以看出, A-型Vtg之间的差异主要体现在Pv结构域上(VtgAo1除外)。不同物种中, Pv结构域中的多聚丝氨酸的数目不同。例如, 在斑马鱼(*Danio rerio*)中, VtgAo1的Pv结构域中含有26个丝氨酸, VtgAo2的该结构域含有34个丝氨酸[24]; 在美洲狼鲈(*Morone americana*)中, VtgAa的Pv结构域中含有48个丝氨酸, VtgAb中的该结构域含有54个丝氨酸[16]; 在大黄鱼(*Larimichthys crocea*)中, VtgAa的Pv结构域中含有54个丝氨酸, VtgAb中的该结构域含有75个丝氨酸; 在海鲢总目(Elopomorpha)鱼类, Pv结构域中含有更多数量的丝氨酸, 例如, 在日本鳗鲡(*Anguilla japonica*), 该结构域中含87~91个丝氨酸[27], 然而, 在星康吉鳗(*Conger myriaster*), 仅含有38个丝氨酸[28]。有研究指出, Pv结构域中丝氨酸的数量与鱼类的繁殖策略及卵的性质有关, 不论是淡水硬骨鱼类还是海水硬骨鱼类, 所产卵不含大油球鱼类的Pv结构域中丝氨酸的数量较少, 而卵中含有大量中性脂(甘油三酯或蜡脂)鱼类的Pv结构域中丝氨酸的数量较多[29]。

VtgC仅由LvL和LvH两个结构域构成, A型-Vtg分解产生的LvH与VtgC分解产生的LvH存在明显差异, 前者结构域中有一段与受体结合的高度保守的氨基酸序列, 这段序列的第一个赖氨酸($K^{[181]}$, 第181位氨基酸)对其与受体的结合具有至关重要的作用[30], 后者结构域中$K^{[181]}$被谷氨酰胺($Q^{[181]}$)替代, 这一替换普遍存在于硬骨鱼中, 然而, 斑马鱼中该位置的氨基酸则被精氨酸($R^{[181]}$)替代。而且, A型-Vtg中第204位和第207位分别为两个半胱氨酸($C^{[204]}$和$C^{[207]}$)残基, 这两个半胱氨酸残基能够与β'-区域中的半胱氨酸残基

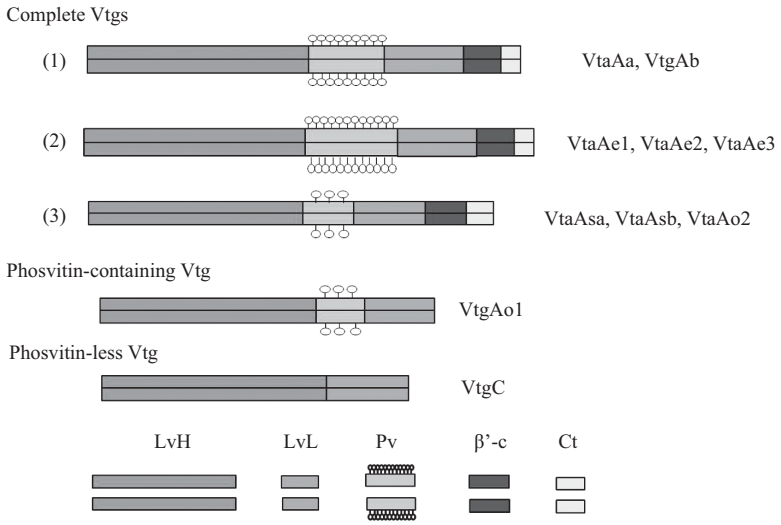


利用MEGA 6.06软件对硬骨鱼类不同亚型的Vtg氨基酸片段构建NJ系统进化树, 不同亚型Vtg的命名参考文献[19]。

A Neighbor-joining (NJ) phylogenetic tree constructed based on the full-length Vtg amino acid sequence deduced polypeptide sequences of teleost Vtg[19].

图1　硬骨鱼类卵黄原蛋白进化关系(根据参考文献[16]修改)

Fig.1　The evolutionary relationship of teleost Vtg (modified from reference [16])



硬骨鱼类完整卵黄原蛋白从氨基末端依次包含5个卵黄蛋白结构域: 卵黄脂磷蛋白重链(LvH)、卵黄高磷蛋白(Pv)、卵黄脂磷蛋白轻链(LvL)、β'-组分(β'-c)、C-端多肽(Ct)。A-型Vtg包含有9个亚型, 分别是: VtgAa、VtgAb、VtgAe1、VtgAe2、VtgAe3、VtgAsa、VtgAsb、VtgAo1和VtgAo2, 但VtgAo1缺少β'-c和Ct; VtgC仅有LvH和LvL两个结构域。

Complete Vtg molecules consist of five linear YP domains from the amino-terminus: lipovitellin heavy chain (LvH), phosvitin (Pv), lipovitellin light chain (LvL), β'-component (β'-c), and C-terminal peptide (Ct). The multiple Vtg forms (VtgAa, VtgAb, VtgAe1, VtgAe2, VtgAe3, VtgAsa, VtgAsb, and VtgAo2) are complete. The incomplete Vtgs are of two types: phosvitin-containing Vtg (VtgAo1) and phosvitin-less Vtg (VtgC).

图2　硬骨鱼类不同亚型卵黄原蛋白结构模式图(根据参考文献[14]修改)

Fig.2　Model of the pentapartite domains organization of native vitellogenin (modified from the reference [14])

形成二硫键, 但在VtgC中分别被天冬氨酸(D[204])和丙氨酸(A[207])替代, 二硫键不能形成, 导致其空间构象(conformation)发生变化。这些半胱氨酸的替换同样也普遍存在于硬骨鱼中[31]。因此, A型-Vtg和VtgC的3D结构及表面电荷的分布具有较大差异。这种结构上的差异导致其与受体结合的特异性。

Vtg的功能与其结构密切相关, Vtg序列结构的同源性比对结果表明, 其与载脂蛋白B-100、微粒体甘油三酯转移蛋白(microsomal triglyceride transfer protein, MTP)及血管假性血友病因子(von Willebrand factor, vWF)的亲缘关系较近[32-34]。并且, 已有研究表明, Vtg中LvH结构域具有疏水性, 三维结构研究表明其可形成腔隙, 两亲性脂质以非共价键的方式连接于LvH所形成的腔隙中, 这说明LvH在卵母细胞脂质积累中具有重要的作用[14], LvL与LvH的功能相似[14,29,33]。但是, 硬骨鱼中, 沉性卵和浮性卵中积累的脂质类型存在差别。产沉性卵(卵中无大量油球)的硬骨鱼类, 发育中的卵母细胞磷脂(极性脂, 约80%为磷脂酰胆碱)的积累主要依靠Vtg; 产浮性卵(卵中无大油球)的硬骨鱼类, 卵母细胞中磷脂的含量超过70%; 而卵中含有大油球的硬骨鱼类, 卵母细胞中的中性脂(例如甘油三酯等)的含量大于50%[14]。目前, 硬骨鱼类卵母细胞脂质代谢机制未见详细报道。硬骨鱼体细胞中脂肪酸是由肝合成的脂质在脂蛋白脂肪酶(lipoprotein lipase, LPL)的作用下分解产生[35], 在卵母细胞中脂质可以通过Vtg转运并贮存在脂滴中。在哺乳动物, 已经证实卵母细胞中的脂肪酸除从细胞外转运吸收外, 还可由自身贮存的脂质代谢产生[36]。脂肪酸进入体细胞后被转运至线粒体进行β-氧化作用, 硬骨鱼中已经证明脂肪酸移位酶(fatty acid translocase, CD36)及脂肪酸转运蛋白1(fatty acid transport protein 1, FATP1)参与脂肪酸在细胞中的转移[37], 但卵母细胞中参与转运脂肪酸的蛋白及酶未见报道。

Vtg的Pv结构域中含有大量丝氨酸, 其对Vtg的磷酸化具有重要作用[14,29]。已有研究表明, 磷酸化的Pv结构域能够帮助Vtg维持其结构的稳定, 提高其亲水性[29]。同时, 磷酸化的Pv结构域带有负电荷, 能够结合Ca^{2+}并将无机磷酸(Pi)转运至卵母细胞, 这表明, 胚胎骨形成所需的Ca^{2+}和Pi是由Pv转运[38-39]。也有实验证明, Pv可作为Fe^{3+}的载体[40-41]。因此, 卵母细胞卵黄发生过程中Vtg在金属离子转运过程中可能起关键作用。

Vtg包含血管假性血友病因子(vWF)D型同源结构域, 硬骨鱼中β'-c与Ct为该区域的裂解产物[18,33,42], 这两个结构域可能参与免疫及凝血过程[14]。已有研究表明, 硬骨鱼类Vtg存在凝集素活性, Shi等[43]证明, 由玫瑰无须鲃(*Puntius conchonius*)中纯化得到的Vtg能够凝集蟾蜍和鸡的红细胞。

2 硬骨鱼类卵黄原蛋白的表达

硬骨鱼类*Vtg*基因的表达受雌激素调控, 下丘脑释放促性腺激素释放激素作用于垂体, 垂体释放促性腺激素作用于卵母细胞外的滤泡层, 在促性腺激素的作用下, 鞘细胞和颗粒细胞释放17β-雌二醇(E_2), 循环的E_2结合到雌激素受体上并进入肝细胞, 这一激素/受体复合物紧密结合在肝细胞核中*Vtg*基因启动子上游的雌激素受体元件(estrogen receptors elements, EREs)上, 激素/受体复合物与EREs结合后启动卵黄原蛋白基因的转录并且能够增强卵黄原蛋白mRNA的稳定性[14,43-44]。在缺乏E_2的条件下, 热休克蛋白90(heat shock protein 90, HSP90)能够和雌激素受体结合, 通过激活激素受体复合物来提高Vtg的转录[45]。肝细胞中表达的Vtg经过大量翻译后修饰, 如酯化、磷酸化和糖基化等, 使蛋白质正确的折叠并二聚化, Vtg进入高尔基体并转变为分泌小泡释放到血液中[14]。性成熟的雌鱼能够自发合成Vtg, 但在雌激素或其类似物的诱导下, 雄鱼或未成熟的雌鱼也能够合成Vtg[44,46-48]。Zhong等[49]研究发现, 在α-乙炔基雌二醇(E_2类似物)存在条件下, 雄性斑马鱼能够大量表达Vtg。类似研究也见在其他鱼类中的报道, 如雄性玉丽体鱼(*Cichlasoma dimerus*)在水体中存在壬基酚时, *Vtg*基因表达上调[50]。Elgaabary等[51]研究高锰酸钾对养殖水体中雌激素类似物的氧化作用时发现, 尼罗罗非鱼(*Oreochromis niloticus*)肝中*Vtg*基因的表达可以作为雌激素类似物的一个精确的生物标志物。Meucci等[52]发现, 水体中存在水溶性壬基酚时, 未成熟的大西洋鲑鱼(*Salmo salar*)也能够大量表达Vtg。由于雌激素能够调控*Vtg*基因的表达, 因此, Vtg对环境中雌激素及其类似物的存在有明显的指示作用。目前, Vtg已被作为雌激素、雌激素类似物以及其他内分泌干扰物污染的生物标志物, 并且已经广泛应用于实验室测试、内分泌干扰物筛选和环境毒理学研究等方面[39,48,53-54]。

3　硬骨鱼类卵母细胞卵黄原蛋白的吸收和分解

3.1　卵黄原蛋白的吸收

卵母细胞吸收Vtg的方式为受体介导的内吞作用[14,55]。Vtg进入血液后随循环系统到卵巢, 通过毛细血管穿过鞘细胞、颗粒细胞到达卵母细胞膜表面, 紧密结合在卵黄原蛋白受体上形成卵黄原蛋白–受体复合物, 并快速内陷形成有被小窝后被网格蛋白(clathrin)包被转化为内生性的有被小泡进入到细胞内部[14,56-57]。Vtg进入卵母细胞后, 与其受体分离。Vtg受体被排出有被小泡并返回卵母细胞表面用于接收和内化后续的Vtg[14]。在卵子发生过程中, 不同发育阶段的卵母细胞Vtg受体的表达量不同, 在卵黄发生的前期, Vtg受体表达量最高。在卵黄发生的后期以及成熟卵中, Vtg受体的表达量极低甚至不表达[14,58]。这一发现证明, 卵黄原蛋白受体在卵子发生过程中可循环利用, 表明生物体建立了一个经济有效的方式确保卵母细胞吸收Vtg的效率[56]。

3.2　卵黄原蛋白的分解

由网格蛋白包被的有被小泡进入到卵母细胞后, 网格蛋白从有被小泡上脱离、重复利用, 有被小泡转变为多泡体[59-60]。H^+通过vATPase(vacuolar H^+-ATPase)进入多泡体, 营造微酸性的环境使卵黄原蛋白–受体复合物分离, 随后Vtg被组织蛋白酶D分解[14,45,59]。Opresko等[60]首次证明, 非洲爪蟾(*Xenopus laevis*)卵母细胞中的Vtg是由组织蛋白酶D裂解。Sire等[59]通过免疫定位的方法证明, 虹鳟卵母细胞的有被小泡中同时存在Vtg和组织蛋白酶D。Brooks等[61]研究了虹鳟中组织蛋白酶D的表达特征, 发现其在卵黄发生时期组织蛋白酶D大量表达。Carnevali等[62]证明, 金头鲷(*Sparus aurata*)中组织蛋白酶D能够将Vtg裂解为卵黄蛋白。随着卵母细胞的发育, 卵黄蛋白经过组织蛋白酶B或L裂解后, 变成FAA或小分子多肽。在这一过程中, H^+通过质子泵进入卵黄泡, 使卵黄泡内维持微酸性的环境, 激活组织蛋白酶原, 有活性的组织蛋白酶作用于卵黄蛋白, 使其分解为FAA或小分子多肽[14]。但不同种硬骨鱼类参与卵黄蛋白裂解的组织蛋白酶不同。例如, 在底鳉(*Fundulus heteroclitus*)中组织蛋白酶B为卵黄蛋白的主要裂解酶[63], 但在金头鲷中发挥主要作用的酶为组织蛋白酶L[64]。

总之, Vtg进入卵母细胞后先经过组织蛋白酶D的作用分解为卵黄蛋白, 随后在组织蛋白酶B或L的作用下分解为FAA或小分子多肽。

4　硬骨鱼类卵黄原蛋白与卵母细胞水合作用的关系

卵母细胞的水合作用过程即为卵母细胞的成熟过程[65-66]。卵母细胞经过水合作用后, 卵质变透明, 体积及浮力增大。该过程中大量的水分子通过卵母细胞膜上的水通道蛋白(aquaporin)进入细胞内[14,65-66]。产浮性卵的硬骨鱼类, 卵母细胞水合作用后含水量从54%~76%上升至76%~93%, 水的质量占整个卵的90%~95%[63-64]。产沉性卵的硬骨鱼类, 卵母细胞水合作用前后含水量仅从53%~79%上升为56%~85%[65-66]。在这一过程中, 水分子依靠渗透压的变化进入卵母细胞。影响卵母细胞渗透压变化的主要因素是细胞内FAA和无机离子的浓度变化[14,65-67]。

卵母细胞中FAA浓度的变化主要受到Vtg的影响, Vtg的裂解产生的FAA与卵母细胞的水合作用相关[68-69]。然而, 沉性卵与浮性卵水合作用的发生与FAA的相关性存在一定差异。产浮性卵的硬骨鱼类, 由VtgAa裂解产生的LvH(LvHAa)经过酶的作用完全裂解为FAA, 而来自VtgAb的二聚化LvH(LvHAb)只被分解为单体, 这一单体蛋白质可能为后期胚胎和幼体的发育提供营养。此外, 由VtgAa和VgAb产生的Pv和β'-c被完全降解, 而由VtgAa和VtgAb产生的两种LvL仅有部分被完全降解为FAA, 未降解的部分与由VtgAb降解产生的LvH(LvHAb)共同作为营养物质储存在卵中[18,26,38,70-74]。这表明, 由Vtg水解产生的FAA参与调节卵母细胞渗透压, 使其能顺利完成水合作用[14,65], 如果卵母细胞中Vtg的水解作用受阻, 则卵母细胞的水合作用受到抑制。Selman等[75]利用洛霉素A1阻断vATPase的功能, 使H^+不能够进入有被小泡激活组织蛋白酶原, 从而阻碍Vtg的水解。Vtg的水解被阻断后, 卵母细胞中FAA含量并没有发生显著变化, 并且卵母细胞的水合作用受到抑制。因此, Vtg的水解后产生的FAA对卵母细胞的水合作用的顺利进行有着重要意义[75]。在产沉性卵的棘鳍鱼类中, 由VtgAa分解产生的LvHAa、LvLAa、β'-c和由VtgAb分解产生的Pv、β'-c仅有一部分被水解为FAA, 其他没有被水解的卵黄蛋白仍保持原来的构象(conformation)或以小分子多肽的形式保存在卵母细胞中[63]。因此, 沉性卵经水合作用后, 其内

FAA浓度的变化较小, 例如, 锯隆头鱼(*Crenilabrus melops*)在水合作用后, 卵中的FAA含量仅增加了2.9~3.8倍[76]。

不同种硬骨鱼类的不同亚型Vtg的裂解方式存在一定差异[76-77], 如底鳉的卵母细胞中有两种不同的LvHAa, 分子量分别为122 kDa和103 kDa, 在卵母细胞成熟过程中仅分子量为122 kDa的LvHAa和Pv完全被水解为FAA[65-76]; 在斑马鱼的卵母细胞成熟过程中, LvHAa、LvHAb、LvLAa和LvLAb等卵黄蛋白仅部分被水解[78]。沉性卵中Vtg的裂解方式表明, FAA在沉性卵水合过程中的作用较小。然而, 另一影响卵母细胞渗透压的因子为无机离子, 特别是K^+和Na^+对其影响较大[14,65,79]。Greeley等[80]和Wallace等[81]均发现, 底鳉卵母细胞水合过程中胞内K^+和Na^+浓度升高, 且K^+为Na^+浓度的2倍。但在香鱼(*Plecoglossus altivelis*)中, 卵母细胞水合后Na^+浓度明显升高[82]。沉性卵中渗透压的调节主要依靠无机离子, FAA的作用较小。浮性卵调节渗透压的方式与沉性卵不同, 水合作用后, 不仅FAA的浓度显著上升, 而且无机离子浓度也产生了明显的变化[65,69,81]。例如, 条纹锯鲈(*Centropristes striata*)卵母细胞水合作用前后, 细胞中Na^+和K^+的绝对含量分别升高2倍和4倍[75]; 大西洋庸鲽(*Hippoglossus hippoglossus*)水合作用后, 卵母细胞中K^+、Cl^-、NH_4^+和Pi的含量均增加[33], 这说明无机离子在卵母细胞的水合过程中也发挥重要作用。可见, 在产浮性卵的硬骨鱼中, FAA与无机离子共同参与调节卵母细胞内的渗透压[38,65]。

综上, 在硬骨鱼卵子发生过程中, 不同亚型Vtg的裂解方式不同。产浮性卵的硬骨鱼类由VtgAa水解产生的LvH(LvHAa)、Pv和β'-c及由VgAb水解产生的Pv和β'-c被组织蛋白酶B或L完全降解为FAA; 由VtgAa和VtgAb产生的两种LvL仅有部分被降解, 这些由Vtg裂解产生的FAA与无机离子共同调节卵母细胞内的渗透压, 保证水合作用的顺利进行, 未降解部分与来自VtgAb的LvH(LvHAb)作为营养物质储存在卵中。产沉性卵的硬骨鱼类由VtgAa分解产生的LvH、LvL、β'-c和由VtgAb分解产生的Pv、β'-c仅有一部分被组织蛋白酶B或L水解为FAA, 其他没有被水解的卵黄蛋白仍保持原来的构象(conformation)或以小分子多肽的形式保存在卵中, 为胚胎发育提供营养物质; VtgAa和VtgAb水解后, 卵母细胞中FAA的含量没有显著升高, 由VtgAa和VtgAb水解产生的FAA对卵母细胞渗透压的影响相对较弱, 因此, 沉性卵中的渗透压主要由无机离子参与调节。然而, 不论在沉性卵还是浮性卵, VtgC几乎没有被酶水解, 以LvH-LvL形式存在于正在发育的卵母细胞中[74,83], VtgC作为大分子磷脂糖蛋白, 为胚胎及幼体发育提供营养而不参与卵母细胞渗透压的调节[14]。

5 小结与展望

Vtg作为硬骨鱼类卵子发生过程中的重要蛋白, 主要在卵黄发生作用及水合作用中发挥功能。Vtg在硬骨鱼卵子发生过程中的作用机制可用模式图(图3)表示。首先, 雌激素诱导肝合成Vtg, 分泌入血液中, 通过血液循环, Vtg到达卵母细胞表面, 通过受体介导的内吞作用被卵母细胞吸收。Vtg进入卵母细胞后, 被组织蛋白酶D水解为卵黄蛋白, 一部分卵黄蛋白贮存在卵母细胞中为卵母细胞的生长及胚胎的发育提供营养, 另一部分卵黄蛋白在组织蛋白酶B或L的作用下完全水解为FAA, 与无机离子共同调节卵母细胞的渗透压, 参与水合作用。

尽管Vtg在硬骨鱼卵子发生中的功能研究报道较多, 但关于卵母细胞吸收VtgC的方式仍不清楚, 已有研究表明, 在鲑科鱼类中已经发现至少两种未知的VtgC受体, 但在鲈形目中并没有发现特异性结合VtgC的受体[55]。因此, 关于卵母细胞吸收VtgC的方式仍是今后的研究重点。除此之外, Vtg具有免疫功能。Vtg能够参与鱼类的自然免疫, Vtg具有多价型的识别模式, 它既能结合革兰氏阴性细菌也能结合革兰氏阳性细菌, 并且对真菌和病毒也具有一定的免疫作用[84-90], 但其参与免疫的分子机制尚不清楚。对Vtg免疫功能的研究有助于揭示母源性免疫的分子机制, 有利于提高子代的免疫力及成活率, 具有重要的应用价值。

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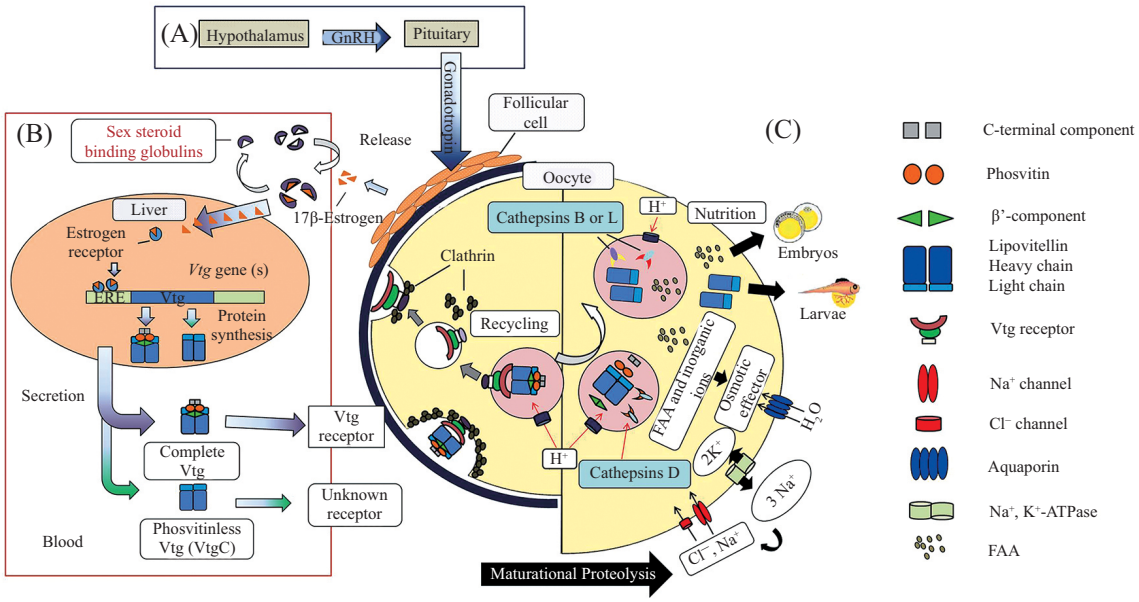

A: 示激素的作用途径; B: 示Vtg的表达及分泌过程; C: 示卵母细胞吸收与分解Vtg。首先, 下丘脑释放GnRH作用于垂体, 垂体释放促性腺激素作用于滤泡细胞层, 滤泡细胞分泌17β-雌二醇并进入血液, 17β-雌二醇与雌激素受体结合, 在肝细胞核中这一激素/受体复合物紧密结合在Vtg基因启动子上游的ERE上并启动*Vtg*基因的表达并将合成的卵黄原蛋白分泌入血液。Vtg随循环系统到达卵母细胞表面经过受体介导的内吞作用被卵母细胞吸收, Vtg受体被重新释放到卵母细胞的表面用于结合新的Vtg。同时, 包含有Vtg的"有被小窝"被网格蛋白包被转变为有被小泡, 随后网格蛋白与有被小泡分离, 循环利用, 有被小泡转变为多泡体。H^+穿过vATPase进入多泡体形成一个弱酸性的环境激活组织蛋白酶D, Vtg在组织蛋白酶D的作用下分解为卵黄蛋白。一部分卵黄蛋白在组织蛋白酶B或L的作用下分解为FAA, 另一部分则储存于卵母细胞中用于胚胎发育; 无机离子穿过离子通道(Na^+和Cl^-通道)和Na^+、K^+-ATPase进入卵母细胞。这时, FAA和无机离子共同参与调节卵母细胞渗透压, 水分子通过水通道蛋白进入卵母细胞完成水合作用。

A: the pathway of hormone; B: the expression and secretion of Vtg; C: absorption and decomposition of Vtg in oocyte. At first, the GnRH released by hypothalamus acts on the pituitary, and in response to GnRH, the pituitary releases gonadotropin, which stimulates follicular cells to secrete 17β-estradiol (E_2) into the blood. E_2 binds to the ER in the liver and the hormone/receptor complexbinds the ERE tightly in the nucleus. Subsequently, the liver starts to synthesize Vtg and secrete it into the blood. A-type Vtgs are transported by the bloodstream to the surface of oocytes and are taken up by receptor-mediated endocytosis. The coated pit containing A-type Vtg is transformed into an endocytic "coated vesicle", a membrane-enclosed organelle that is coated by clathrin. H^+ enter the multivesicular body across the vATPase to provide an acidic environment for the activation of cathepsin D. While Vtgs are subjected to proteolysis into YPs by cathepsin D, the Vtg receptor is released to the surface of the oocyte for recycling. Some YPs are degraded into FAAs by cathenpsin B or L and others are stored in the oocyte to be used for embryonic growth. Inorganic ions enter the oocyte via ion channel (Na^+ and Cl^- channels) and Na^+, K^+-ATPase. The FAAs and inorganic ions serve as osmotic effectors for oocyte hydration. The water enters the oocyte through aquaporin. The oocyte becomes a mature egg after hydration and maturation.

图3　硬骨鱼卵子发生过程中卵黄原蛋白的表达、吸收及分解的机制(根据参考文献[14,55]修改)

Fig.3　The mechanism of Vtg expression, uptake and proteolysis (modified from the references [14,55])

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