中国科学: 生命科学 2025 年 第 55 卷 第 1 期: 1 ~ 16
SCIENTIA SINICA Vitae lifecn.scichina.com
《中国科学》杂志社 SCIENCE CHINA PRESS

评 述 跨尺度RNA调控与成像专题

母源RNA在卵母细胞向胚胎转变中的转录后调控研究进展

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收稿日期: 2024-12-09; 接受日期: 2025-03-11; 网络版发表日期: 2025-07-03
国家重点研发计划(批准号: 2024YFA1107900)、国家自然科学基金(批准号: 32170606)和中国科学院稳定支持基础研究领域青年团队计划(批准号: YSBR-012)资助

摘要 哺乳动物卵母细胞成熟到受精后胚胎合子基因组激活之间的过程称为卵母细胞向胚胎转变. 在此过程中合子基因组激活之前卵母细胞和胚胎处于转录沉默状态. 因此, 在此期间的生物学事件依赖于卵母细胞中储存的母源RNA与蛋白质. 研究表明母源RNA在卵母细胞和胚胎的早期发育过程中扮演关键的角色, 其转录后调控密切影响卵母细胞向胚胎转变过程, 包括RNA化学修饰(如m^6A, m^5C, ac^4C), 小分子RNA和poly(A)尾重塑等. 这些转录后调控机制通过影响母源RNA的稳定性和翻译效率以及合子基因组的转录, 确保母源RNA支持卵母细胞及早期胚胎的正常发育. 本综述简要总结近年来哺乳动物母源RNA在卵母细胞向胚胎转变中的转录后调控研究进展, 探讨不同转录后调控事件在卵母细胞向胚胎转变过程中的作用机制.

关键词 母源RNA, 转录后调控, 卵母细胞, 胚胎, RNA修饰, poly(A)尾, 翻译, RNA稳定性

哺乳动物的胚胎发育, 起始于精子和卵母细胞结合形成受精卵, 之后经历一系列复杂但有序的过程发育为个体, 是一个受精密调控的过程. 受精后, 受精卵经历一系列细胞分裂和分化, 形成多细胞结构囊胚, 此过程为植入前胚胎发育[1]; 后续囊胚从透明带中孵化出来, 通过与子宫内膜作用侵入子宫中[2], 开始植入后胚胎发育, 开启胚轴建立, 原肠运动, 器官发生等重要的发育事件, 形成生命个体[3].

哺乳动物从卵母细胞成熟到合子基因组激活(zygotic genome activation, ZGA)的发育过程被称为卵母细胞向胚胎转变(oocyte-to-embryo transition, OET), 此过程是确保早期胚胎正常发育的重要基础[4]. 在原始卵泡中的卵母细胞, 被阻滞在第一次减数分裂前期的双线期并保持休眠状态, 等到卵泡被激活后其生长发育才重新启动[5]. 此时卵母细胞中RNA含量成倍增长, 积累大量的蛋白质, 卵母细胞的细胞核呈空泡状的状态, 称为生发泡(germinal vesicle, GV)期[6~8]. GV期的卵母细胞积累大量母源mRNA, 从GV期至受精后早期胚胎发生ZGA之前染色质为转录沉默状态(图1), 因此卵母细胞减数分裂重启和成熟、受精、ZGA等发育

引用格式: 刘静雯, 张晨依, 陆发隆. 母源RNA在卵母细胞向胚胎转变中的转录后调控研究进展. 中国科学: 生命科学, 2025, 55: 1–16
Liu J W, Zhang C Y, Lu F L. Post-transcriptional regulation of maternal RNA during the oocyte-to-embryo transition (in Chinese). Sci Sin Vitae, 2025, 55: 1–16, doi: 10.1360/SSV-2024-0342

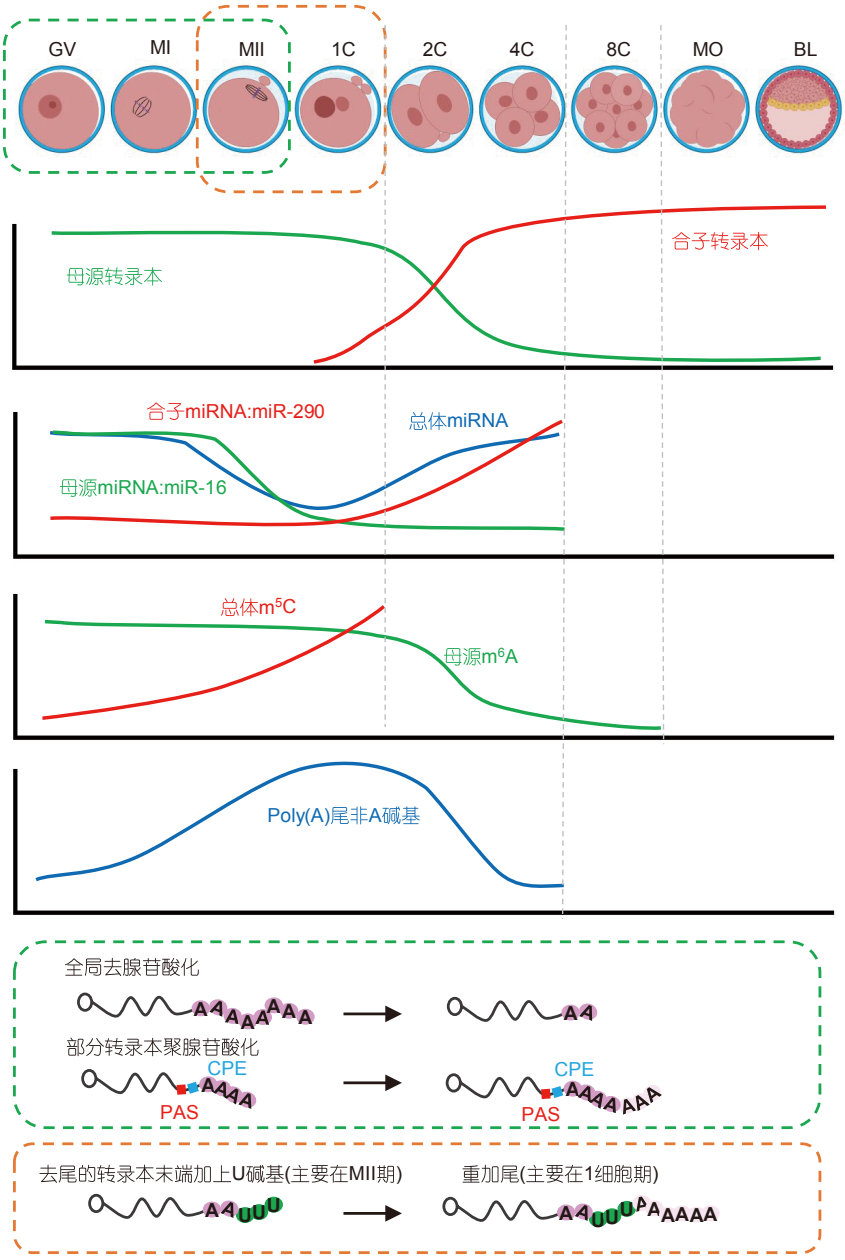

图 1 RNA化学修饰、miRNA和poly(A)尾在小鼠OET过程中的动态变化模式. 小鼠OET过程中母源转录本(绿色线条)逐渐降低, 而合子转录本(红色线条)在晚期1细胞期开始少量激活, 在2细胞期大量激活; miRNA全局变化, 包含整体miRNA水平(蓝色线条), 以及代表性的母源降解的miRNA(绿色线条)和合子激活的miRNA(红色线条); 母源m^6A修饰(绿色线条)全局变化和已知阶段的m^5C修饰(红色线条)变化; Poly(A)尾非A碱基(蓝色线条)呈现高度动态变化. 在卵母细胞成熟过程中, 存在全局的poly(A)尾缩短, 部分转录本发生选择性poly(A)尾延长现象. 在MII期的卵母细胞中去尾的转录本中很大一部分掺入U碱基, 并在1细胞重新加A形成中间含有U碱基的poly(A)尾

Figure 1 Dynamic changes of RNA chemical modifications, miRNAs and poly(A) tails during mouse oocyte-to-embryo transition. During the mouse oocyte-to-embryo transition, maternal transcripts (green lines) gradually decrease, while zygotic transcripts (red lines) are minorly activated in the late 1-cell stage while highly activated in the 2-cell stage; global changes in miRNA, including overall miRNA levels (blue lines), as well as representative maternally degraded miRNAs (green lines) and zygotically activated miRNAs (red lines); global changes in maternal m^6A modification (green lines) and m^5C modification at known stages (red lines); poly(A) tail non-A residues (blue lines) show highly dynamic changes. During oocyte maturation, there is a global shortening of the poly(A) tails, whereas some poly(A) tails are selectively elongated. In MⅡ stage oocytes, a high proportion of the deadenylated transcripts incorporate U residues, which are re-polyadenylated in the 1-cell stage to form poly(A) tails with U residues in their bodies

事件均高度依赖于存储在卵母细胞中的母源RNA的转录后调控[9,10]. 这些母源RNA转录后调控事件通过介导mRNA的翻译和降解来确保胚胎正常发育. 因此, 了解母源RNA的转录后调控机制可以深入认知生命的基本过程, 为发育生物学和生殖医学提供理论依据. 本文将对母源RNA的转录后调控事件, 及其调控胚胎发育的机制和功能进行简要综述, 以期为相关研究提供参考.

1 母源RNA翻译调控

哺乳动物卵母细胞发生过程和OET过程中, 大量的母源mRNA的胞质聚腺苷酸化和翻译激活驱动着减数分裂过程的进行[11]. 聚腺苷酸化是真核生物RNA加工过程中一个关键的转录后修饰步骤, 其主要作用是通过在mRNA分子3′末端添加一段由腺嘌呤核苷酸(A)构成的多聚腺苷酸尾即poly(A)尾, 从而调控mRNA的稳定性、核输出、翻译效率以及降解过程. 聚腺苷酸化对母源mRNA翻译的调控是一个复杂而精细的过程. 储存的mRNA通常带有较短的聚腺苷酸尾, 在翻译前需通过poly(A)聚合酶进行poly(A)尾长度的增加, 这一过程受到胞质聚腺苷酸化结合蛋白1 (cytoplasmic polyadenylation binding protein, CPEB1)等关键因子的调控[12]. 研究表明, CPEB1通过与mRNA的3′非翻译区(3′ UTR)中的蛋白识别元件相互作用来精确控制聚腺苷酸化的程度, 蛋白识别元件主要包括聚腺苷酸化信号(polyadenylation signal, PAS)和细胞质聚腺苷酸化元件(cytoplasmic polyadenylation element, CPE)[13~15]. 有研究表明, 卵母细胞减数分裂成熟过程中不同的PAS和CPE元件的组合, 调控特定发育时期母源mRNA翻译[16]. CPEB1在没有被磷酸化之前, 与Symplekin蛋白和多聚腺苷酸化特异性因子(cleavage and polyadenylation specificity factors, CPSF)组成复合体, 募集其他蛋白组分, 抑制poly(A)尾的延长与翻译; CPEB1被磷酸化后, 抑制因子解离并激活mRNA的翻译[17]. 其中, 细胞外调节蛋白激酶1/2 (extracellular regulated protein kinases, ERK1/2)介导的CPEB1磷酸化和降解是母源mRNA翻译激活的主要机制[18], 也是小鼠(*Mus musculus*)卵母细胞成熟的关键因素. ERK1/2可以直接磷酸化CPEB1, 从而促进母源RNA的聚腺苷酸化并启动蛋白翻译[19]. 在小鼠中敲除ERK1/2会导致卵母细胞纺锤体组装异常, 无法完成减数分裂, 受精后母源mRNA不能适时降解, 无法完成后续胚胎发育[19]. ERK1/2级联信号通路促进一些关键母源mRNA的翻译, 包括*Dazl* (*deleted in azoospermia like*), *Btg4* (*BTG anti-proliferation factor 4*)和*Cnot6l* (*CCR4-NOT transcription complex subunit 6 like*)等. DAZL蛋白的翻译激活进一步促进*Tpx2*和*Btg4*的翻译激活, BTG4负责清除母源mRNA来保障ZGA[19]. 此外, ERK1/2调控的Poly(A) polymerase alpha (PAPα)磷酸化是母源mRNA发生翻译激活的一个关键机制. 激活后的PAPα通过正反馈机制催化编码*Papα* mRNA的poly(A)尾延长和翻译激活, 迅速增加卵母细胞胞质中PAPα的含量, 并催化胞质中mRNA的poly(A)尾延长, 以促进减数分裂的进行[20]. 因此, 在卵母细胞中ERK1/2对母源mRNA翻译的调节在OET中起着举足轻重的作用.

除经典的ERK1/2之外, 实际上还有多种RNA结合蛋白(RNA binding proteins, RBP)已被证明参与母源mRNA的翻译调控. 基因的转录后调控包含多种代谢活动和不同类型RNA的成熟、转运和降解, 这些过程主要由核糖核蛋白复合体(ribonucleoprotein complex, RNP)完成. RNP是由RNA分子与特定结合蛋白质共同组成的功能性复合体, 广泛参与细胞内各种与RNA相关的生物学过程. RNA分子在RNP中起到模板或功能核心的作用, RBP参与RNA加工、修饰、转运、翻译和降解等过程的调控. 在线虫(*Caenorhabditis elegans*)、果蝇(*Drosophila melanogaster*)、小鼠以及人类(*Homo sapiens*)的生殖细胞和早期胚胎发育过程中, RBP能否正确结合母源RNA对于后者能否在卵母细胞成熟过程中维持稳定、正确翻译和定位具有关键作用, 由此决定卵母细胞内RNA和蛋白质的动态变化, 确保后续胚胎发育正常进行[21~29]. 除磷酸化后的CPEB结合到母源mRNA上的CPE激活mRNA外[30], RBP也参与翻译抑制的调控. 在卵母细胞和早期胚胎中, 许多RBP能够通过结合RNA的5′端, 3′端或者一些内源性小RNA来调控翻译. 研究表明, 小鼠卵母细胞中的假基因可以产生内源性的小干扰RNA (endo-siRNA), 这些endo-siRNA通常由来自蛋白质编码基因的剪接转录本与来自同源假基因的反义转录本形成的双链RNA加工而来[31,32]. Argonaute蛋白是一种重要的RBP, 在endo-siRNA介导的基因沉默中起着关键的作用[33], 其中AGO2是Argonaute家族中唯一具有核酸

内切酶活性的蛋白[34]. 有研究在小鼠卵母细胞中鉴定出AGO2, MILI, DDX4, PTBP1等RBP的靶位点, 发现AGO2蛋白可以和endo-siRNA结合形成沉默复合物. 这种复合物不仅能够以完全互补配对的方式切割靶mRNA、转座子元件或逆转座子起始的嵌合体转录本, 还能够以一种类似miRNA的非完全互补配对的方式抑制mRNA的翻译, 从而精细调控卵母细胞蛋白组的变化[35]. MILI的靶RNA与AGO2的靶RNA有99.5%的高度重合, 而AGO2与MILI的结合靶标重合度仅有39.5%, 表明两者在功能上存在冗余, 也很好地解释敲除*Mili*后卵母细胞没有明显表型, 而敲除*Ago2*后卵母细胞存在发育缺陷. 此外, DDX4倾向于结合富含CG的基序来调控翻译, PTBP1在卵母细胞中则倾向于结合富含CU的基序, 从而调控可变剪切[35]. 除稳定性和翻译调控, RBP还在母源RNA的定位中发挥关键作用. 在生殖细胞的细胞质中, 存在一种独特的无膜细胞器: 生殖颗粒(germ granules). 在哺乳动物中, 生殖颗粒只存在于两周龄以下的小鼠原始卵泡中. 当生殖颗粒消失时, 细胞皮层区域会出现新的mRNA储存区域, 其中包括DDX6, CPEB, MSY2等RBP, 其功能还未研究清楚[36]. 近期, 有研究发现哺乳动物卵母细胞的mRNA储存在一个线粒体相关的核糖核酸蛋白域(mitochondria-associated ribonucleoprotein domain, MARDO)中. MARDO由高表达的RBP (ZAR1, YBX2, DDX6, LSM14B和EIF4ENIF1)和mRNA组成, 这一结构在人、小鼠、猪和牛的卵母细胞胞质中保守存在. 在卵母细胞生长期间, 线粒体膜电位升高, 引导线粒体和其他RBP进行组装成MARDO. 在卵母细胞成熟过程中, ZAR1 (zygote arrest-1)的降解驱动MARDO溶解和其中储存mRNA的降解, 因此MARDO通过调节母源mRNA的储存以维持卵母细胞和早期胚胎发育[37]. 研究表明, 组成MARDO的RBP之一ZAR1通过多种机制调节母源mRNA的翻译. ZAR1及其同源蛋白ZAR2 (zygote arrest-2)在卵母细胞减数分裂恢复后, 促进编码重要蛋白质(如*Btg4*, *cyclin B1*和*WEE2*)的母源mRNA poly(A)尾的延伸, 从而激活其翻译. ZAR1/2也与RBP互作, 如与mRNA稳定性相关的MSY2, 以及胞质晶格成分(MATER, PADI6), 共同调节母源转录组的稳定性[38]. 这些研究结果提示卵母细胞到胚胎的转变是由一个复杂的翻译调控网络控制的, 鉴于文章篇幅限制, 此处不再展开讨论.

母源mRNA在OET过程中发生剧烈的翻译重塑. 近年来, 多项研究开发不同方法绘制小鼠和人OET中的mRNA的翻译图谱[39~42]. 有研究通过微量核糖体印迹测序技术LiRibo-seq绘制小鼠OET中mRNA翻译的动态变化, 揭示mRNA翻译的转变在染色质重塑和ZGA过程中的重要作用[40]. 此外, 相关研究通过适用于单个卵母细胞的转录-翻译双组学测序技术(T&T-seq)绘制小鼠和人类卵母细胞成熟过程中的转录组和翻译组图谱, 揭示小鼠和人类卵母细胞成熟过程中的时空顺序调节, 以及小鼠和人类的卵母细胞成熟过程中的翻译组学图谱的异同[39]. 同期研究通过超灵敏翻译组检测方法Ribo-lite绘制OET过程和着床前胚胎的翻译组动态变化, 证明人和小鼠OET中均存在大量的翻译调控事件[41,42]. 小鼠OET过程中, 翻译上调基因在GV卵中维持着较短的poly(A)尾长, 且翻译被抑制. 伴随着减数分裂的恢复, 此部分基因的poly(A)尾被延长, 激活翻译. 翻译下调基因在GV卵中有着较长的poly(A)尾, 并且翻译活跃. 然而, 随着减数分裂的恢复, poly(A)尾被缩短且翻译被抑制[42]. 对人类卵母细胞及早期胚胎翻译组和转录组图谱绘制发现人-鼠同源基因中有一半呈现保守的翻译水平动态变化趋势, 而另一半变化趋势不同, 这种差异一定程度源于人-鼠基因3′端非编码区中调控元件的不同配置.

在非洲爪蟾(*Xenopus laevis*)和小鼠等物种中PAS和CPE元件在3′ UTR上的数量和位置可调控相应mRNA的翻译效率[16,43]. 小鼠GV期卵母细胞中, 含有远离CPE的PAS的转录本可以激活翻译, 含有邻近CPE的PAS的转录本会抑制翻译[16]. 例如, 在*Cpeb1* 3′ UTR中, 当CPE位于PAS下游35~50 bp内时, 能够有效抑制PAS的翻译活性. 而在*Btg*4 3′ UTR中, PASs则被位于上下游66 bp处的CPE显著抑制[16]. 在小鼠OET中, 全转录组分析发现距离PAS 100 nt以内的称为papCPE (PAS proximal CPE)的CPE元件具有明显的上述活性. 在卵子成熟过程中翻译上调的基因通常具有两个或更多papCPE元件, 而翻译下调的基因则往往缺乏papCPE或没有CPE [42]. 在人类中也观察到类似的规律. 在GV期, 3′ UTR上距离PAS较近的CPE元件会抑制翻译, 而在MII期, 这些CPE元件则能激活翻译. 在OET中, 具有papCPE的转录本表现为翻译上调, 而缺乏CPE或非papCPE的转录本则表现为翻译下调[41]. 这一现象的原因可能与小鼠GV期卵母细胞中类似, 即CPE招募

CPEB1, 阻止PAS与CPSF4 (cleavage and polyadenylation specific factor 4)的结合, 从而无法招募CPSF4完成翻译; 在随后卵母细胞成熟的GVBD阶段, CPEB1发生磷酸化和部分降解, 解除CPEB1对mRNA翻译的抑制作用, 使得同时含有CPE和PAS元件的mRNA发生翻译激活[16]. 磷酸化后的CPEB1不仅解除对翻译的抑制, 还增强与CPSF的结合, 并招募末端核苷酸转移酶促进poly(A)尾的延伸, 从而提高mRNA的翻译效率[44]. 通过分析人类翻译组, 研究发现一组Homeobox转录因子(TPRXL, TPRX1和TPRX2)是人类ZGA的关键调控因子[41]. 随后研究发现, OBOX家族是小鼠中与人类TPRX功能同源的家族, 在小鼠中*Obox*$^{♀-/♂-}$的胚胎无法完成ZGA. 机制上OBOX在ZGA时期帮助RNA聚合酶Ⅱ结合到正确靶基因上, 是调控小鼠ZGA的关键转录因子[45]. 该家族转录因子可能在人和小鼠的ZGA中发挥着保守的作用. 需要注意的是, 虽然OET中转录和翻译水平发生剧烈变化, 但是卵母细胞中积累的母源蛋白, 有超过半数能够相对稳定存在至囊胚期(比如CCDC136, SIRT5和MTHFD1L), 因此不能使用翻译组和转录组的变化来简单评估蛋白水平的变化[46]. 对于在此过程中发挥功能的因子需要综合评估其蛋白的初始丰度、翻译水平、半衰期和翻译后修饰等.

2 Poly(A)尾调控母源RNA

mRNA由基因转录产生, 是中心法则的重要组成部分. 几乎所有真核生物的mRNA都会在3′末端发生断裂和多聚腺苷酸化, 生成poly(A)尾[47], 该过程对于mRNA进一步行使功能不可或缺. 细胞核中, pre-mRNA在RNA聚合酶Ⅱ与的转录下生成, 随后经过一系列加工过程, 包括5′端加帽, 3′端的断裂和加尾以及剪切等[47], 产生不同的mRNA变体. 这些变体在卵母细胞成熟过程中受到不同的转录后调控, 共同推动卵母细胞减数分裂的进程[15]. 对个别卵母细胞成熟相关因子研究表明poly(A)尾长度是调控卵母细胞中mRNA翻译效率的关键因素, 长poly(A)尾通常与高翻译效率相关, 而短poly(A)尾则与低翻译效率相关; 在卵母细胞重启减数分裂时, poly(A)尾及其相关调控因子可作为“翻译开关”改变翻译模式的变化[11,48,49], 关于卵母细胞中母源mRNA翻译调控的更详细信息推荐阅读以下近期重要综述文章[48]. 近年来随着测序技术的发展, poly(A)尾介导的RNA转录后调控在卵母细胞和早期胚胎发育过程中的动态变化与功能被更深入地研究, 本文在此简要综述.

2.1 Poly(A)尾长度介导的调控

相关研究开发PAL-seq (poly(A)-tail length profiling by sequencing)技术, 通过在链延伸掺入的标记的UTP信号强度估算相应poly(A)尾长度, 发现在斑马鱼(*Danio rerio*)和非洲爪蟾的早期胚胎中poly(A)尾长度与蛋白翻译效率呈正相关[50]; 另有研究开发TAIL-seq和mTAIL-seq (mRNA TAIL-seq)技术检测poly(A)尾, 其核心是在Illumina测序平台用其开发的Tailseeker算法替代常规碱基读取算法估算poly(A)尾的起止, 可以实现较好的全转录组水平poly(A)尾长度分析. 相关研究通过mTAIL-seq发现在果蝇成熟的卵母细胞和受精卵中存在类似现象: mRNA的poly(A)尾越长, 其翻译的效率越高[51]. 研究者借助PacBio三代测序平台开发PAIso-seq (poly(A) inclusive RNA isoform sequencing)和PAIso-seq2技术, 实现在单细胞水平转录组的全长RNA变体及其poly(A)尾的检测[52~54]. 卵母细胞中poly(A)尾长度分析表明在GV期卵母细胞中mRNA的翻译效率与poly(A)尾长度呈正相关, 并且在卵母细胞GV期至MⅡ期的成熟过程中, mRNA poly(A)尾长度的变化与翻译效率的变化呈高度正相关[42,55]. 如前面母源RNA翻译调控部分所述, 在卵子成熟过程中poly(A)尾长度发生大规模变化从而重塑mRNA翻译景观, 并且在成熟的卵子和合子中部分基因通过较长的mRNA poly(A)尾促进其翻译. 近期研究发现MARTRE (maternal mRNA translation regulator)家族蛋白可以负调控CCR4-NOT核酸去腺苷酸化酶复合体的活性以维持这些mRNA的poly(A)尾长度促进其有效翻译[56]. 这些发现表明在卵母细胞中poly(A)尾长度是mRNA翻译效率的重要调控因素, 受精确调控. 但需要注意的是poly(A)尾长度与mRNA翻译效率的高度正相关性在体细胞中并不存在, 在体细胞中大部分基因只要其mRNA poly(A)尾长度大于20 nt就有相似的翻译效率[57].

在OET中, 母源mRNA的适时降解是激活胚胎自身基因组转录的一个重要前提[58]. 在卵母细胞发育过程中, *Btg4*的mRNA poly(A)尾逐渐延长, 激活BTG4蛋白的翻译, 再将包括CNOT6L在内的CCR4-NOT核酸去腺苷酸化酶复合体招募到母源mRNA上, 降解母源

mRNA, 从而维持早期胚胎发育的正常进行[18,59~61]. 母源mRNA的清除是由两个紧密相连的过程调节, 包括由母源提供的基因产物介导的降解(maternal decay, M-decay)和新表达的合子产物介导的降解(zygotic decay, Z-decay)[9]. 除A碱基, mRNA poly(A)尾中还包含其他碱基, 例如C、G和U碱基, 这些非A碱基在调控mRNA稳定性和降解过程中起着关键作用[62~64]. mRNA末端尿嘧啶化修饰酶TUT4/7 (terminal uridylyl transferase 4/7)介导的3′端尿苷化修饰是Z-decay发生的重要因子[65], 而PABPN1可以招募降解尿苷化修饰的3′-5′核酸外切酶DIS3L2 (DIS3 like 3′-5′ exoribonuclease 2), 至TUT4/7修饰的3′端尿苷化mRNA上, 并促进Z-decay以维持早期胚胎发育正常进行[64]. 在小鼠中对OET过程进行全局poly(A)尾图谱绘制, 发现大部分母源mRNA遵循在卵母细胞成熟过程中poly(A)尾缩短的趋势[55]. 另外, 在人类1细胞中发现, 受精后存在全局的mRNA重新加尾, 该重新加尾在poly(A)末端有或没有3′端U碱基修饰的转录本上都可发生. 如果抑制加尾过程, 将会导致受精卵无法完成第一次卵裂, 暗示着ZGA之前的早期胚胎发育是由重新加尾的母源mRNA驱动的[66]. 此外, 在人和小鼠卵母细胞体外成熟过程中, 去腺苷酸化复合物BTG4和CNOT7的mRNA不能进行充分的poly(A)尾延长, 从而使其翻译不能被有效地激活, 导致其蛋白水平下降, 进而造成卵母细胞中母源mRNA的去腺苷酸化过程受损[67]. 该poly(A)尾重塑缺陷可能是体外成熟卵母细胞来源胚胎发育潜能低的重要原因. 这些研究表明, poly(A)尾重塑影响着母源mRNA的稳定性和翻译, 在发育过程扮演着重要的角色.

2.2 Poly(A)尾中非A碱基介导的调控

Poly(A)尾除了在长度上进行调控外, 近年来的研究还发现poly(A)尾中存在U, G和C这些非A碱基[52,68~74]. Poly(A)尾部添加G碱基可以阻止CCR4-NOT复合物的降解, 从而提高RNA的稳定性[62]; poly(A)末端加上U碱基后, 会在被下游降解因子识别后促进mRNA的降解[75]. 小鼠GV期卵母细胞中有17%的mRNA poly(A)尾区域存在广泛的U, G和C碱基[52]. 有意思的是人类1细胞、2细胞和4细胞胚胎是已知的含有poly(A)尾非A碱基丰度最高的细胞[66], 其内部超过60%的mRNA poly(A)尾内部存在非A碱基, 尤其是U碱基. 在人类MⅡ期卵母细胞中有40%的mRNA 3′末端含有U碱基, 随后在受精后发生重加尾, 形成内部含有U碱基的poly(A)尾[66]. 在小鼠、大鼠和猪中也观察到相似的poly(A)尾介导的母源mRNA重编程模式(图1), 表明这一机制在哺乳动物物种间保守存在[76]. 在斑马鱼和非洲爪蟾早期胚胎的poly(A)尾3′末端[73,77], 以及斑马鱼早期胚胎的poly(A)尾内部[73], 也发现高丰度的非A碱基. 在小鼠卵母细胞的生长过程中, TUT4和TUT7负责介导mRNA的3′端尿苷化. 如果敲除*Tut4*/*7*, 靶向的转录本将无法进行3′端尿苷化, 导致RNA在卵母细胞中积累, 进而造成卵母细胞发育停滞[69]. 此外, 在小鼠敲除*Dis3l2*后, 尿苷化的转录本在卵母细胞中大量积累, 导致卵母细胞发育停滞在减数第一次分裂前期, 最终导致雌性小鼠不孕[78]. 这些研究表明, 非A碱基的掺入对于维持OET以及早期胚胎的正常发育至关重要. 这表明在脊椎动物的卵母细胞和早期胚胎中可能存在保守的poly(A)尾非A碱基动态调控机制.

综上, 在OET过程中非A碱基在poly(A)尾中的动态变化是一种跨物种存在的保守的机制, 其在生命早期发育中的功能和调控机制是非常有意思的有待解决的科学问题. 关于母源RNA poly(A)尾的研究进展, 读者可阅读笔者所撰写的综述文章[79]来获取更多详细信息.

3 MicroRNA调控母源RNA

3.1 MicroRNA的产生和作用机制

2024年, 诺贝尔生理学或医学奖颁发给Victor Ambros教授和Gary Ruvkun教授, 以表彰他们发现microRNA及其在转录后基因调控中的作用. 从1999年Baulcombe团队首先发现一类长度20~25 nt的小分子RNA至今[80], 研究表明, microRNA (miRNA)在胚胎与个体发育、细胞命运及肿瘤发生发展过程中发挥重要的调控作用[81,82]. miRNAs是由约21至24个核苷酸组成的小的非编码RNA分子, 在细胞内通过与靶mRNA结合来调控基因表达[83]. 哺乳动物中miRNAs的生成过程包括多个步骤, 首先是由RNA聚合酶Ⅱ转录生成具有一个或多个发夹结构的初级miRNA (pri-miRNA), pri-miRNA可能由编码基因的内含子或外显子, 或者基因间区转录出来. 随后, 核内的Drosha-DGCR8复合物将pri-miRNA加工成约70个核苷酸的前体miRNA

(pre-miRNA)[84]. 这些pre-miRNA被EXPORTIN-5转运到细胞质中, 在细胞质中由DICER酶进一步加工成成熟的双链miRNA. 最终, miRNA的一条链被加载到RNA诱导沉默复合物(RNA-induced silencing complex, RISC)中, 另一条链则被降解[83,84].

3.2 MicroRNA抑制翻译并促进母源RNA的降解

miRNA可与靶mRNA的3′非翻译区(3′ UTR)结合, 这种结合通常导致mRNA的翻译抑制或降解, 进而影响基因的表达. 在斑马鱼中, 特定的miRNA被证明能够抑制多种母源RNA的翻译, 从而调控细胞分裂和分化[85]. miRNA在母源RNA的选择性降解中也扮演着重要角色. 有研究报道, 在斑马鱼中, miR-430家族是一个重要的miRNA群体, 能够调控数百个mRNA, 其中大多数靶向的mRNA是母源mRNA. 母源mRNA积累的时候miR-430不表达, miR-430表达后促进母源mRNA的去腺苷酸化和降解, 对于母体向胚胎的转变至关重要[86]. miR-430缺失的MZ*dicer*突变体胚胎, 在原肠胚形成及脑形态发生阶段时出现明显的缺陷, 同时其发育进程相较于正常胚胎显著迟缓. 这一现象揭示母源性mRNA的异常累积阻碍胚胎的正常发育[85]. 在果蝇中, miR-309家族以类似的方式清除母源RNA[87]. 在哺乳动物中, miRNA通过结合母源mRNA的3′ UTR来促进poly(A)尾的去尾或者mRNA的降解[88,89]. 在小鼠卵母细胞中去除*Dicer*导致减数分裂停滞, 伴随着严重的纺锤体、染色体分离缺陷和广泛的转录本失调, 其中表达水平升高的mRNA富含miRNA结合的种子序列, 表明其可能是miRNA的潜在靶点[88]. 然而, 在哺乳动物早期胚胎中并没有发现类似于miR-430参与母源mRNA清除的机制. 这些发现表明miRNA对母源RNA的调控有着物种间的保守性与特殊性.

3.3 小RNA调控胚胎发育

在小鼠卵母细胞及早期植入前胚胎的发育过程中, miRNA的功能受到全局性抑制, 且这种抑制状态一直持续到2细胞期之前, 该时间点与大规模母源基因降解的时间一致[90](图1); 然而, 在2细胞期之后, miRNA抑制得到缓解, 参与miRNA调控的蛋白质(如AGO和GW182)在2细胞期之后开始表达, 并组装成RNA诱导的沉默复合体, 激活miRNA的调控功能[91]. miRNA在胚胎发育的不同阶段发挥作用. 在小鼠中, miR-29b通过靶向调控*Dnmt3a*/*b*的表达水平干扰甲基化进程[92]; 在牛中, 抑制miR-10的表达阻碍桑葚胚到囊胚的发育[93]; 在人中, miR-519d, miR-378a-5p, miR-376, miR-155能调控滋养层细胞侵入子宫的能力[94~96]. 这表明miRNA介导的调控在早期胚胎细胞的命运决定和分化过程中发挥重要的作用.

生殖系统中高表达一类特异地与PIWI家族蛋白相互作用的被命名为piRNA (PIWI-interacting RNA)的小分子非编码RNA[97~101], 长度为18~30 nt. piRNA与PIWI家族蛋白结合形成复合物, 在生殖细胞发育中具有重要功能[98]. piRNA调控通路为动物生殖发育必需, 包括斑马鱼[102], 果蝇[103,104]的生殖发育. 而在小鼠中, piRNA调控通路仅为雄性生殖发育必需[105~107], piRNA调控通路异常是男性不育的重要病因之一[108,109]. piRNA在精子细胞中对靶mRNA可发挥双重调控作用. 在早期球形精子细胞中, piRNA通过招募翻译起始因子eIF3F和RNA结合蛋白HuR等激活大量精子形成相关mRNA的翻译; 而在后期精子细胞中, piRNA通过与去腺苷化酶CAF1互作介导精子细胞中mRNA大规模地脱腺苷酸化及降解, 从而保障功能性精子的生成[110]. 其中PIWI蛋白中的PIWI-Ins (PIWI-specific insertion module)元件对于结合更长的piRNA至关重要. PIWI-Ins元件的突变会导致结合的piRNA变短, 从而影响PIWI/piRNA的翻译激活功能, 进而导致雄性不育[111,112]. 2021年, 三个团队发现在黄金仓鼠(*Syrian hamster*)的卵母细胞中敲除piRNA通路重要作用蛋白会导致母源mRNA降解减慢和ZGA失败, 证明piRNA通路对黄金仓鼠雌性生殖发育也是必要的[113~115]. 这些发现表明小鼠卵母细胞中piRNA的种类和功能可能在哺乳动物中不具有广泛的代表性, PIWI-piRNA复合物可能在哺乳动物两性生殖中均发挥广泛的功能, 对理解人类piRNA作用具有重要意义.

综上所述, miRNA和piRNA等小RNA在母源RNA的调控中扮演着多重角色, 通过抑制翻译、促进降解、调节稳定性以及影响时空表达谱等方式, 确保胚胎发育的顺利进行.

4 RNA化学修饰调控母源RNA

RNA化学修饰是指在RNA分子的核苷酸上进行的化学修饰, 常见的修饰类型包括N^6-甲基腺苷(m^6A)、

5-甲基胞嘧啶(5-methylcytosine, m^5C)、乙酰化修饰(ac^4C)和假尿苷(Ψ)等. 这些修饰不仅影响RNA的结构, 还改变其功能, 影响RNA的翻译、降解和定位等, 在早期胚胎发育过程中扮演着重要角色.

4.1 RNA m^6A甲基化修饰

N^6-甲基腺苷(N^6-methyladenosine, m^6A)是高等生物mRNA和长链非编码RNA (long non-coding RNA, lncRNA)上最为普遍的化学修饰[116~118]. N^6-甲基腺苷(m^6A)甲基化是RNA甲基化的主要形式, 即RNA分子腺嘌呤第6位N原子上发生甲基化修饰. 它通过一组特定的酶系统进行修饰, 包括“写入酶”复合物, 如METTL3, METTL14, WTAP, KIAA1429/VIRMA, ZC3H13, RBM15/15B等, 负责在RNA的特定位置添加甲基[119,120]. “擦除酶”主要包括FTO和ALKBH5等去甲基酶, 它们能够识别并去除m^6A修饰, 从而影响RNA的功能和稳定性[121,122]. “甲基化阅读蛋白”, 如YTHDF1, YTHDF2, YTHDF3, YTHDC1, YTHDC2, eIF3, hnRNPC, hnRNPA2B1等, 这些成分共同调控m^6A修饰的动态平衡[119].

在配子发生和胚胎发育过程中, m^6A起着关键的调控作用. 在细胞系中敲降“写入酶”METTL3会显著降低大多数位点的m^6A甲基化水平, 导致胚胎干细胞分化受阻和胚胎植入后的发育停滞[123]; 缺失METTL14会有类似的效果; 缺失METTL16会导致植入后胚胎发育的停滞[124]. 甲基转移酶复合体中的其他蛋白, 如WTAP, ZC3H13和KIAA1429/VIRMA, 对METTL3/METTL14的调控也至关重要[123], 其中KIAA1429在卵母细胞中高表达, 缺失KIAA1429会导致GV期卵母细胞中m^6A修饰显著减少, 进而阻碍卵母细胞的发育[125]. 值得注意的是, m^6A修饰在GV期卵母细胞中倾向于优先降解RNA, 而在MⅡ期卵母细胞中, m^6A修饰则促进转录及翻译过程[126], 表明m^6A修饰影响卵母细胞的成熟和母源RNA的降解. FTO是首个被鉴定的m^6A擦除酶, 研究发现在小鼠胚胎干细胞、卵母细胞和早期胚胎发育过程中, FTO能够调节与染色质相关联的RNA, 尤其是能去除long interspersed nuclear element-1 (LINE-1) RNA上的m^6A修饰, 从而影响LINE-1 RNA的丰度和附近位点染色质状态, 并影响LINE-1下游转录本表达的顺式与反式调控[127]. YTHDC1作为m^6A阅读蛋白参与细胞命运的调控, 在小鼠胚胎干细胞中通过调控H3K9me3和异染色质形成, 沉默逆转录转座子元件, 从而限制该细胞转化为2C-like细胞, 对小鼠胚胎干细胞的维持是必需的[128]; 而METTL3在小鼠胚胎干细胞中也可以通过结合内源性逆转录病毒中的IAPEz (intracisternal A-particles, IAP)转座子, 招募催化H3K9me3的甲基转移酶复合物SETDB1/TRIM28来维持IAPEz转座子上的异染色质状态, 从而抑制IAPEz元件的转录[129]. 这些研究表明m^6A在染色质状态以及早期胚胎细胞命运决定中均发挥重要作用.

在卵母细胞向胚胎转换过程中, m^6A修饰也起着类似的双重调控作用. 小鼠早期胚胎发育过程中转录本上m^6A修饰的动态图谱(图1)表明m^6A修饰分别维持卵母细胞中母源RNA的稳定性, 以及受精后短暂表达的转录本的及时降解, 证明m^6A修饰在卵母细胞和合子基因激活阶段不同的调控功能[130]. 并且m^6A修饰不仅可以从母源继承, 也可以在ZGA时期重写[131]. 一群母源继承且持续存在的m^6A修饰的转录本同时具有明显的翻译信号, 暗示这些转录本上的m^6A修饰有可能通过维持RNA的稳定性来调控母源到合子的转换. 这种双重作用使得m^6A修饰成为母源RNA调控的一个重要机制.

4.2 RNA m^5C甲基化修饰

5-甲基胞嘧啶(m^5C)广泛发生在核糖体RNA (ribosomal RNA, rRNA)、转运RNA (transfer RNA, tRNA)、mRNA、增强子RNA (enhancer RNA, eRNA)和lncRNA上[132]. m^5C修饰发生在胞嘧啶第5位碳原子上, 主要通过“写入酶”DNMT2和NSUN家族写入修饰, “擦除酶”ALBKH1和TET蛋白家族[133,134]去除修饰, “甲基化阅读蛋白”ALYREF[135]和YBX1[136]识别修饰以调控RNA的行为和命运. m^5C甲基化是一种动态、可逆的RNA化学修饰方式, 是RNA分子在转录后水平上进行的一种重要的表观遗传调控方式.

研究发现, m^5C修饰在维持母源RNA的稳定性和翻译中发挥作用. 在小鼠中, 缺失NSUN5会抑制卵泡的发生和发育, 造成胚胎发育迟缓, 胚胎发育至囊胚的比例降低[137]; 在分子水平上, m^5C修饰缺失造成*Mad2l2*和*Gdf9*的翻译效率降低[137], 导致其蛋白减少影响母源mRNA的稳定性. 在线虫中, m^5C甲基转移酶NSUN调控卵母细胞的成熟, 缺失NSUN1导致细胞稳

态失衡[138], 使卵母细胞发育失败.

相关研究绘制斑马鱼早期胚胎发育过程中RNA m^5C修饰图谱, 揭示m^5C修饰调控早期胚胎发育母源mRNA稳定性的机制[139]. 研究发现在OET过程中, 存在m^5C修饰的母源mRNA比未修饰的mRNA更加稳定. m^5C修饰通过其结合蛋白YBX1招募poly(A)结合蛋白PABPC1a来维持母源mRNA的稳定性[139], 保证母源合子转换过程的有序进行. 进一步果蝇、斑马鱼、非洲爪蟾、热带爪蟾(*Xenopus tropicalis*)、小鼠、人类共6个物种不同发育时期的mRNA m^5C图谱揭示母源mRNA普遍存在大规模的m^5C修饰[140](图1). 值得注意的是, 整个发育过程中m^5C只在卵母细胞和胚胎发育早期高度富集, 随着OET, m^5C修饰位点数目和甲基化水平均出现急剧下降, 并保持低水平甲基化状态至个体发育成熟[140]. 在果蝇中敲除*Nsun2*后, 96%的母源mRNA上m^5C修饰消失, 伴随果蝇胚胎发育迟缓的表型. 因此, 在两栖动物和哺乳动物的母源mRNA上均存在大量m^5C修饰, 表明m^5C修饰可能在卵母细胞和早期胚胎中标记和调控母源RNA方面具有保守的功能和机制.

4.3 RNA乙酰化修饰

N^4-乙酰基胞苷(N^4-acetylcytidine, ac^4C)是近期在mRNA上鉴定到的唯一乙酰化修饰[141], 由“书写器蛋白”N-乙酰基转移酶10 (N-acetyltransferase 10, NAT10)[142]催化产生. 早期研究发现在HeLa细胞中存在大量ac^4C修饰, 且修饰主要分布在mRNA的CDS和5′ UTR区域, 通过增强mRNA稳定性来提高翻译效率[143]. NAT10具有乙酰化酶结构域和RNA结合结构域, 是一个在多个物种中保守的组蛋白乙酰化转移酶[142]. 在小鼠中敲除*Nat10*后, 卵母细胞发育阻滞在MI期, 卵泡发育停滞在次级卵泡阶段[144]; 通过*Stra8*和*Zp3*驱动的Cre表达, 在小鼠的原始生殖细胞和次级卵泡中敲除*Nat10*会导致卵巢早衰[144]. 类似地, 通过*Gdf9*驱动Cre的表达, 在小鼠原始卵泡中敲除*Nat10*后, 会在更早期的卵母细胞发生阶段, 即具有两层颗粒细胞的次级卵泡阶段, 出现生长迟缓和阻滞, 并最终导致卵巢早衰[145]. 在分子机制上, NAT10通过建立ac^4C修饰, 调控CCR4-NOT复合体中主要基因(*Cnot6l*, *Cnot7*, *Btg4*)的表达, 从而缩短mRNA poly(A)尾长度来降解母源mRNA[144,145]. 进一步在小鼠卵巢和卵母细胞中绘制ac^4C修饰图谱, 发现两者中均存在广泛的ac^4C修饰, 特别是卵泡发育相关基因的转录本, ac^4C修饰增强这些基因产物的转录后翻译活性来维持卵母细胞发育的正常进行[145]. 这些研究结果表明, ac^4C的形成和动态调控是决定卵泡发育突破次级卵泡阶段的重要分子指标, OET中母源RNA的稳态维持和重塑很大程度上依赖于ac^4C修饰.

5 总结与展望

哺乳动物的卵母细胞成熟及早期胚胎发育是一个极为精细且复杂的过程. 在卵母细胞生长阶段, 卵母细胞中会积累大量的母源mRNA. 生长完成的卵母细胞在GV期转录完全关闭直至受精后ZGA时期被重新激活, 此段过程中染色质均维持转录沉默状态. 因此, 包括卵母细胞减数分裂重启与成熟、受精以及ZGA等在内的关键发育事件, 均依赖于卵母细胞内储存的母源RNA的转录后调控. 这一特性使得母源RNA成为探索RNA转录后调控机制的理想模型.

OET过程受到一系列复杂的转录后机制的精细调控, 包括RNA化学修饰(m^6A, m^5C和ac^4C)和poly(A)尾的调控等, 在RBP以及小RNA等调控因子的共同作用下精细调控母源mRNA的稳定性与翻译. 此前在NIH3T3细胞和小鼠胚胎干细胞(ESC)中的研究发现m^6A修饰的转录本表现出更长的poly(A)尾以及更低丰度的poly(A)尾内部非A碱基修饰[146], 表明这些RNA转录后调控机制之间可能存在着密切的联系. 这些不同机制如何协同调控母源mRNA的稳定性、定位、翻译效率及降解将是未来重要的研究方向.

体外受精(*in vitro* fertilization, IVF)和卵胞质内单精子注射(intracytoplasmic sperm injection, ICSI)等辅助生殖技术已成为治疗不孕症的常用手段. 然而, 文献报道IVF受精失败率仍高达20%至35%[147]. 母源mRNA调控异常在其中的贡献是一个值得关注的重要方面. 例如, *Btg4*纯合突变的女性患者来源的受精卵中母源mRNA大量积累, 无法降解, 最终出现合子分裂失败(zygotic cleavage failure, ZCF)的症状, 导致试管婴儿或ICSI治疗反复失败[148]. 另一方面, 随着RNA修饰检测技术的进步, 母源mRNA上的RNA修饰也可能成为有重大潜力的卵母细胞和早期胚胎发育潜能评估的重要标志物. 对于上述方面进行进一步的深入研究有望为不孕不育的诊疗提供新的理论见解与靶标.

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Post-transcriptional regulation of maternal RNA during the oocyte-to-embryo transition

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The oocyte-to-embryo transition refers to the process from oocyte maturation to zygotic genome activation after fertilization in mammals. During this process, oocytes and embryos are transcriptionally silent before ZGA, relying on maternal RNAs and proteins stored in the oocytes to support their development. Maternal RNA plays a critical roles in oocyte and early embryo development, which is tightly regulated post-transcriptionally to control the oocyte-to-embryo transition. The post-transcriptional regulation mechanisms include RNA chemical modifications (e.g., m^6A, m^5C, ac^4C), small RNAs, and poly(A) tail remodeling, which regulate maternal RNA stability and translation efficiency, as well as zygotic genome transcription. This review briefly summarizes recent advances in the study of post-transcriptional regulation of maternal RNA in mammals during the oocyte-to-embryo transition, highlighting the mechanisms and roles of various post-transcriptional regulatory events in this critical developmental process.

maternal RNA, post-transcriptional regulation, oocyte, embryo, RNA modification, poly(A) tail, translation, RNA stability

doi: 10.1360/SSV-2024-0342