中国科学: 生命科学 2024 年 第 54 卷 第 1 期: 59 ~ 72
SCIENTIA SINICA Vitae lifecn.scichina.com
《中国科学》杂志社 SCIENCE CHINA PRESS
评 述 生殖健康专辑

RNA调控在精子发生及男性不育中的新功能和新机制

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收稿日期: 2023-10-25; 接受日期: 2023-11-02; 网络版发表日期: 2024-01-05
国家重点研发计划重点专项(批准号: 2022YFA1305300)、国家自然科学基金重点项目(批准号: 32330053)和上海市科学技术委员会基础研究项目(批准号: 23JC1403801)资助

摘要 精子发生是哺乳动物体内最复杂且高度协调的细胞发育过程, 尚有许多未解之谜. 男性不育已成为当前一个全球性的人口健康及社会问题, 但一半以上患者病因不明. 已有研究证据表明, 大量RNA结合蛋白在睾丸特异性表达或高表达, 结合蛋白质编码mRNA或/和非编码RNA组成RNA调控网络, 在表观遗传、转录及转录后等多重水平调控雄性生殖细胞的基因表达, 在精子发生中发挥不可或缺的重要作用, 为雄性生育所必需. 近年来的研究发现, RNA调控通路相关基因突变与男性不育密切相关, 显示RNA调控异常是男性不育新病因. 本文总结近期关于RNA调控与精子发生相关的研究进展, 将主要概述RNA修饰调控、可变剪接调控、翻译调控、降解调控等在精子发生中的新功能和新机制, 并探讨其异常调控与男性不育的相关性及其在男性不育临床诊治中的潜在应用.

关键词 男性不育, 精子发生, RNA调控, RNA结合蛋白

据世界卫生组织(World Health Organization, WHO)统计, 全球约有15%~20%的育龄夫妇受不孕不育症的困扰, 其中男性因素导致的不育占比为40%~50%. 导致男性不育的病因较为复杂, 包括泌尿生殖系统异常(主要包括睾丸生精障碍和输精管道梗阻)、内分泌异常、免疫因素、精神因素、环境因素、遗传因素和性功能障碍等, 其中遗传因素约占15%, 但仍有约50%男性不育症患者无法明确病因; 依据《世界卫生组织人类精液检查与处理实验室手册》(第5版, 2010年; 第6版, 2021年)标准化程序的精液分析可将男性不育症分为无精症(azoospermia; 精液中无精子)、少精症(oligozoospermia; 精子总数低于39×10^6/一次射精或精子浓度低于16×10^6/mL)、弱精症(asthenozoospermia; 前向运动精子百分率低于30%)和畸形精子症(teratozoospermia; 正常形态精子百分率低于4%)等[1~3].

1978年首例试管婴儿诞生, 已有多种辅助生殖技术(assisted reproductive technology, ART)在临床广泛应用, 主要包括人工授精(artificial insemination)、体外受精-胚胎移植(*in vitro* fertilization and embryo transfer, IVF-ET)、配子输卵管移植(gamete intrafallopian transfer, GIFT)、卵胞浆内单精子注射(intracytoplasmic

引用格式: 潘舵, 殷子奇, 刘默芳. RNA调控在精子发生及男性不育中的新功能和新机制. 中国科学: 生命科学, 2024, 54: 59–72
Pan D, Yin Z Q, Liu M F. New functions and mechanisms of RNA regulation in spermatogenesis and male infertility (in Chinese). Sci Sin Vitae, 2024, 54: 59–72, doi: 10.1360/SSV-2023-0172

sperm injection, ICSI)和植入前胚胎遗传学诊断(preimplantation genetic diagnosis, PGD)等. 尽管如此, ART也仅能帮助部分不孕不育夫妇成功孕育后代. 为有效提高生育率及保障优生优育, 临床和基础科研人员已投入了大量人力和物力, 进行系统探索生殖障碍的病因及机制. 与之对应的是, 近期研究已发现一系列与男性不育相关的基因突变和调控通路异常, 促进了人们对男性不育病因及致病机制的认识[4,5].

哺乳动物精子发生(spermatogenesis)是十分复杂的细胞发育过程, 该过程起始于精原干细胞(spermatogonial stem cell, SSC)的自我更新和分化. 具体来讲, 精原干细胞经有丝分裂(mitosis)形成精原细胞(spermatogonia); 进一步进行DNA复制产生初级精母细胞(primary spermatocyte), 随即进入减数分裂(meiosis)过程, 精母细胞(spermatocyte, SC)通过两轮连续分裂产生单倍体圆形精子细胞(round spermatid, RS); 圆形精子细胞经历一系列复杂和剧烈的细胞形态和结构变化, 包括顶体组装、鞭毛形成、细胞核压缩、细胞质丢失等, 最终形成高度特化的精细胞即精子(spermatozoa)[6]. 精子发生的有序进行依赖于在转录、转录后和表观遗传等多重水平进行的精准且时空特异性的基因表达调控. 在精子发生过程中, 生精细胞会经历两次转录停止: 第一次是在减数分裂的前期; 第二次则是在精子形成过程, 即圆形精子细胞向延长型精子细胞分化[7]. 在减数分裂前后, 生精细胞也会经历两波活跃转录, 让后期发育所需基因被提前转录, 生成mRNA转录本储存至后期发育阶段翻译[8,9]. 在精子发生过程中, 至少有三个阶段会发生大量转录后调控事件, 即原始生殖细胞(primordial germ cell, PGC)的形成后、减数分裂DNA重组阶段和精子形成过程; 与之对应的是, 超过1500个RNA结合蛋白(RNA-binding protein, RBP)以及包括piRNA(PIWI-interacting RNA)、miRNA(microRNA)和lncRNA(long noncoding RNA)在内的非编码调控RNA在睾丸中特异性高表达[10]. 这些RNA结合蛋白与非编码调控RNA组装形成RNA调控复合物, 通过多重水平调控雄性生殖细胞基因表达命运, 在精子发生过程中发挥不可或缺的作用. 近期的基础及临床研究证据表明, RNA调控异常可能是男性不育的新病因. 本综述将总结RNA修饰调控、可变剪接调控、翻译调控、降解调控等在精子发生及男性不育中的功能机制研究进展, 并探讨这些调控作用在男性不育临床诊治中的潜在应用.

1 RNA修饰调控与雄性生殖

RNA修饰是RNA转录后调控的重要组成部分, 在配子形成和胚胎发育中扮演重要角色. 随着RNA修饰检测方法和技术手段的发展, 越来越多的修饰类型被发现, 且被认为广泛存在于编码及非编码的RNA中, 包括甲基化修饰、羟甲基化修饰、乙酰化修饰和磷酸化修饰等. 大量研究表明, RNA修饰调控异常可干扰精子发生并致雄性不育.

1.1 RNA修饰类型

RNA修饰广泛存在于编码RNA(mRNA)和非编码RNA(包括miRNA, lncRNA, circRNA等调控性RNA和tRNA, rRNA等功能性RNA)中, 可调控RNA的稳定性及mRNA翻译活性[11]. 目前研究较多的RNA修饰类型为甲基化修饰、乙酰化修饰、羟甲基化修饰和磷酸化修饰. 其中, 甲基化修饰包括N^1-甲基腺苷(N^1-methyladenosine, m^1A)、5-甲基胞嘧啶(5-methylcytidine, m^5C)、N^6-甲基腺苷(N^6-methyladenosine, m^6A)、7-甲基鸟苷(N^7-methylguanosine, m^7G)和假尿嘧啶(pseudouridine, ψ)等; 乙酰化修饰包括N^4-乙酰胞苷(N^4-acetylcytidine, ac^4C)、N^4-乙酰-2′-*O*-甲基胞苷(N^4-acetyl-2′-*O*-methylcytidine, ac^4Cm)和N^6-乙酰腺苷(N^6-acetyladenosine, ac^6C)等, 其中ac^4Cm和ac^6C目前被发现仅存在于极端嗜热古菌的RNA中, 而ac^4C则存在于原核和真核生物的RNA中[12]; 羟甲基化修饰包括5-羟甲基胞苷(5-hydroxymethylcytidine, hm^5C)和6-羟甲基腺苷(6-hydroxymethyladenosine, hm^6A); 磷酸化修饰(2′-磷酸尿苷, 2′-uridine phosphate, UP)最近才被鉴定到, 目前被认为其主要功能是赋予tRNA热稳定性, 有助于细胞应对热应激[13].

1.2 RNA修饰调控过程

RNA修饰参与调控RNA的代谢、剪接、翻译、定位、稳定性等, 从而使遗传信息多样化, 介导多种生物过程的精细调控. RNA修饰通常由一组相关蛋白特异性调控, 包括“书写器(writer)”——催化特定修饰的产生、“擦除器(eraser)”——催化特定修饰的去除和“阅读器(reader)”——识别和结合修饰的核苷酸, 从而

影响靶RNA的命运[14].

(1) RNA m^6A修饰调控过程. m^6A修饰于1974年被发现, 是丰度最高的一种RNA修饰, 主要存在于mRNA的5′和3′非翻译区(non-translational region, UTR)和编码区(coding sequence, CDS)近终止密码子区, 可调控mRNA的转录、成熟、定位、翻译和降解等[15,16]. m^6A修饰也存在于非编码RNA中, 如miRNA的m^6A修饰存在于初级miRNA(primary miRNA, pri-miRNA)中, 环状RNA(circular RNA, circRNA)的m^6A修饰常来源于mRNA中未被甲基化的外显子, 而lncRNA的m^6A修饰分布于整个转录本且在经可变剪接的lncRNA中存在较多. m^6A修饰被证明能够促进非编码RNA的加工或者增强其生物学功能[14].

在生物体中存在多种调控m^6A修饰的蛋白, 包括甲基转移酶复合体(由METTL3, METTL14, METTL16, WTAP, RBM15等组分组成)、去甲基化酶FTO和ALKBH5, 阅读蛋白YTH结构域家族蛋白、IGF2BP家族蛋白和HNRNP家族蛋白等. 其中, 甲基转移酶复合体和去甲基化酶共同调节m^6A修饰产生和去除的可逆动态调控, 阅读蛋白可识别和结合不同的RNA而介导特定的生物学过程, 如YTHDC1参与mRNA的剪接、出核转运以及非编码RNA的降解过程, YTHDF1促进mRNA的翻译, IGF2BP1/2/3维持mRNA的稳定等[14,17].

(2) RNA m^5C修饰调控过程. m^5C修饰广泛存在于mRNA中, 主要分布于mRNA的CDS和UTR, 并富集于翻译起始位点, 参与调控mRNA的出核、稳定、剪接、降解和翻译等[18]. 同时, m^5C修饰也存在于miRNA和lncRNA中, 参与miRNA的出核和稳定性调控、增加lncRNA的稳定性等[16].

m^5C甲基转移酶包括DNMT2和NOL1/NOP2/SUN(NSUN)两大蛋白家族. NSUN蛋白家族由7个成员组成, 即NSUN1~7, 其中NSUN2是最主要的m^5C甲基转移酶, 主要作用是调控细胞质中tRNA的修饰[19]; NSUN1和NSUN5主要参与调控28S rRNA的修饰; NSUN3调控线粒体tRNA的修饰; NSUN4则调控线粒体rRNA的修饰; 而NSUN6主要靶向mRNA的3′UTR发夹结构中的CTCCA基序而影响其翻译终止. 此外, TET双加氧酶(ten-eleven translocation protein, TET)可作为m^5C去甲基化酶[15,20]. m^5C修饰阅读蛋白为ALYREF, YBX1和YBX2[21].

(3) RNA ac^4C修饰调控过程. ac^4C修饰存在于mRNA中, 主要分布于CDS和5′UTR区, 促进mRNA的稳定性和翻译. 此外, ac^4C修饰也存在于18S rRNA中, 可促进核糖体的生成[8]. ac^4C修饰乙酰转移酶主要为*N*-乙酰转移酶10(*N*-acetyltransferase 10, NAT10), 可通过促进mRNA稳定性来提高翻译效率[22]. 此外, NAT10在催化tRNA和rRNA乙酰化时分别需要辅因子THUMPD1和核仁小RNA(small nucleolar RNA, snoRNA)的协助[12]. 但目前关于ac^4C去乙酰化修饰酶及其识别蛋白尚不明确.

1.3　RNA修饰调控过程与雄性不育

m^6A, m^5C和ac^4C是精子发生中最受关注的RNA修饰类型. 其中, 对m^6A修饰的研究主要集中于METTL3, METTL14, ALKBH5, YTHDC1, YTHDC2和YTHDF2等在精子发生中的调控机制[23]; m^5C修饰目前主要研究的是NSUN2和NSUN7在精子形成中的调控作用[24,25]; ac^4C修饰的研究主要有NAT10介导精子发生调控作用[26], 见表1. 目前对其他RNA修饰在精子发生中的调控作用的了解还有限.

(1) RNA m^6A修饰与雄性不育. 大量的研究显示, m^6A修饰调控蛋白在精子发生过程中发挥了极其重要的作用. 甲基转移酶*Mettl3*或*Mettl14*经*Vasa*-Cre特异性敲除后, 造成m^6A修饰缺失及精原干细胞无法正常维持自我更新能力; 而*Mettl3*和*Mettl14*经*Stra8*-Cre特异性双敲后, m^6A修饰水平显著下调, 精子发生阻滞在延长型精子细胞时期(第13步)[27]. 此外, 在弱精症患者中, METTL3和METTL14介导的m^6A修饰水平异常增加[28]; 去甲基化酶ALKBH5缺失会造成m^6A修饰累积, 转录本被异常剪接, 导致小鼠精子发生受阻[29]; m^6A阅读蛋白YTHDF2调控m^6A修饰的mRNA进行降解, 进而调控精原细胞的增殖, 其缺失后造成特异性转录本的积累, 导致小鼠精子形态异常和自然受精能力受损[30,31]; m^6A的另一阅读蛋白YTHDC2缺失, 会导致减数分裂相关基因表达下调[32].

(2) RNA m^5C修饰与雄性不育. NSUN家族介导的m^5C修饰参与调控睾丸分化和精子发生. NSUN2缺失会导致小鼠睾丸变小, 精原细胞数量减少且无精子产生[19,25]. NSUN7在睾丸中高表达, 其突变缺失会导致小鼠附睾内成熟精子数量减少、精子尾部中段的线粒体鞘异常致游动能力下降, 表现为雄性亚不育[24]. 在

表 1 雄性生育关键的RNA修饰相关基因

Table 1 RNA modification-related genes critical for male fertility

修饰类型	基因名	小鼠		人类	
		可育性	阻滞/影响时期	患者表型	突变位点
m^6A	*Mettl3*	*Vasa-Cre*敲除, 雄性不育	精原干细胞	N/A	N/A
		与*Mettl14*经*Stra8-Cre*双敲, 雄性不育	延长型精子细胞	N/A	N/A
	Mettl14	*Vasa-Cre*敲除, 雄性不育	精原干细胞	N/A	N/A
		与*Mettl3*经*Stra8-Cre*双敲, 雄性不育	延长型精子细胞	N/A	N/A
	Wtap	敲除, 雄性不育	精原干细胞/延长型精子细胞	N/A	N/A
	Alkbh5	敲除, 雄性不育	精母细胞	N/A	N/A
	Fto	敲除, 年龄依赖性雄性不育	精原细胞	非梗阻性无精症	c.964C>T, p.Arg322* c.1277delT, p.Leu426fs
	Ythdc2	敲除, 雄性不育	精母细胞	N/A	N/A
m^5C	*Nsun2*	敲除, 雄性不育	粗线期精母细胞	N/A	N/A
	Nsun7	敲除, 雄性不育	延长型精子细胞	弱精症	c.11337delA, p.Val157LeufsTer15 c.26248T>G, p.Ser308Ala
ac^4C	*Nat10*	敲除, 雄性不育	精母细胞	N/A	N/A

弱精症患者中, 发现*NSUN7*基因存在男性不育的致病性突变[33]. 另有研究显示, 在父系小鼠高脂饮食后, 其精子tRNA衍生的小RNA(tRNA-derived small RNAs, tsRNA)中的m^5C与m^2G修饰水平升高, 影响精子tsRNA的生成, 从而使子代小鼠获得父代的高脂表型, 但m^5C修饰如何调控父系小鼠精子tsRNA的形成过程还不清楚[34~36].

(3) RNA ac^4C修饰与雄性不育. ac^4C修饰对于维持精子发生正常进行至关重要. ac^4C乙酰化修饰酶NAT10在第一次减数分裂前的精母细胞、精原细胞和支持细胞中高表达, 提示其在染色体联会和同源染色体重组及分离过程中发挥重要作用. NAT10缺失后, 小鼠睾丸中的转录本水平紊乱, 参与减数分裂的关键基因下调, 导致减数分裂异常且无法产生精子, 致使小鼠不育[26].

2 可变剪接调控与雄性生殖

2.1 可变剪接调控

转录后的mRNA前体(pre-mRNA)的可变剪接(alternative splicing, AS)是一种重要的转录后水平基因表达调控方式, pre-mRNA被剪接体(spliceosome)通过不同方式的剪接产生不同的剪接异构体, 对于提高转录组的复杂性和蛋白功能的多样性具有十分重要的生物学意义[37,38]. 剪接体的核心由U1, U2, U4/6和U5等核内小RNA(small nuclear RNA, snRNA)构成, 并通过富含丝氨酸-精氨酸的蛋白保持稳定[39]. 其中, U6 snRNA是经典剪接体中的重要组分, 在进化上最为保守, 与多种snRNA和核内小核糖核蛋白颗粒(small nuclear ribonucleoprotein particles, snRNP)结合, 在可变剪接事件中发挥重要作用[40]. U6虽仅有约为106 nt, 但存在m^6A修饰、ψ修饰以及大量的2′-*O*-甲基化修饰[41,42]. 有证据表明, 人类95%的基因存在可变剪接事件[37]. 可变剪接的方式较多, 最常见的5种为外显子跳跃(exon skipping, ES)、内含子保留(retain intron, RI)、外显子互斥(mutually exclusive exons, MXE)、5′端可变剪接(alternative 5′ splice site, A5SS)以及3′端可变剪接(alternative 3′ splice site, A3SS), 其中ES是哺乳动物中最普遍的可变剪接方式[43]. 通过以上可变剪接方式及不同组合, 使得同一个基因能够编码多个具有不同功能的蛋白, 还可以调控mRNA的稳定性并影响蛋白翻译的效率. 因此, 可变剪接作为RNA调控的重要模式, 在生物体

的生命活动中发挥着关键作用，且发现其调控异常与多种人类疾病密切相关.

2.2　可变剪接调控与雄性不育

睾丸是可变剪接发生水平最高、mRNA异构体数量最为丰富的组织之一[44]. 研究显示，发生可变剪接的基因数量变化模式与小鼠精子发生过程中基因表达模式非常相似，且ES和RI是生殖细胞中最常见的可变剪接方式，显示可变剪接与精子发生密切相关[45]，见表2.

可变剪接关键蛋白SRSF1通过调控精母细胞中减数分裂相关基因表达, 影响同源染色体联会过程, 其缺失致小鼠雄性不育，精子发生阻滞在粗线期精母细胞时期[46]. 支架蛋白SYMPK为精原细胞存活和精母细胞减数分裂所必需，其缺失会造成减数分裂缺陷[47]. RANBP9，MRG15和RBM5被认为在精母细胞和精子细胞的可变剪接过程中发挥重要调控作用. 其中，RANBP9是Ran结合蛋白家族成员，与剪接调控因子SF3B3和HNRNPM结合, 其缺失可致小鼠雄性不育[48]; MRG15可识别并结合染色质上的H3K36me3，并招募剪接调控因子PTBP来调控剪接过程，*Mrg15*敲除小鼠精子发生阻滞在圆形精子细胞[49,50]; RBM5是经典的剪接调控因子，在小鼠精母细胞和圆形精子细胞中高表达，*Rbm5*敲除小鼠雄性不育，圆形精子细胞出现凋

表 2　雄性生育关键的可变剪接调控相关基因和可变剪接异常基因

Table 2　Alternative splicing-related and aberrant alternative splicing genes critical for male fertility

基因名	小鼠		人类	
	可育性	阻滞/影响时期	患者表型	突变位点
Srsf1	*Stra8-Cre*敲除, 雄性不育	粗线期精母细胞	N/A	N/A
Sympk	*Stra8-Cre*敲除, 雄性不育	精母细胞	N/A	N/A
	*Vasa-Cre*敲除, 雄性不育	精母细胞	N/A	N/A
Ranbp9	敲除, 雄性不育	精原细胞/双线期精母细胞	N/A	N/A
Mrg15	*Stra8-Cre*敲除, 雄性不育	圆形精子细胞	N/A	N/A
Rbm5	敲除, 雄性不育	圆形精子细胞	N/A	N/A
Ptbp1	生殖细胞条件性敲除, 雄性不育	精原细胞	N/A	N/A
Sam68	敲除, 雄性不育	延长型精子细胞	N/A	N/A
Larp7	*Stra8-Cre*敲除, 雄性不育	精母细胞/圆形精子细胞	N/A	N/A
Sun5	敲除, 雄性不育	延长型精子细胞	无头精子症	c.475C>T, p.Arg159*
M1ap	敲除, 雄性不育	精母细胞	严重少精症	c.1435-1G>A
			非梗阻性无精症	c.676dup, p.Trp226LeufsTer4
				c.1166C>T, p.Pro389Leu
Dnah8	N/A	N/A	弱精症	c.11771C>T, p.Thr3924Met; c.6689A>G, p.Lys2230Arg
				c.9427C>T, p.Arg3143Cys; c.12721G>A, p.Ala4241Thr
				c.6962_6968del, p.His2321Profs*4
Dnah17	敲除, 雄性不育	延长型精子细胞	弱精症	c.1293_1294del, p.Tyr431∗; c.7994_8012del, p.Gly2665Glufs∗4
				c.5486G>A, p.Cys1829Tyr
				c.10496C>T, p.Pro3499Leu; c.10784T>C, p.Leu3595Pro
				c.10486_10497dup, p.Val3496_Pro3499dup

亡[51,52]. 多种剪接调控因子(如PTBP1, PTBP2, RBMXL2和SAM68等)都已被证明通过调控精子发生中的可变剪接事件, 保证精子生成. 其中, PTBP1和PTBP2分别为调控精原细胞有丝分裂和精母细胞减数分裂的剪接调控因子, PTBP1缺失影响新生儿时期精原细胞的增殖[43]; RBMXL2缺失导致减数分裂不能正常进行, 从而阻碍精子发生正常进行[53]; SAM68主要调控精母细胞和精子细胞中的可变剪接, 防止目标转录本过早终止, 对精子生成至关重要[54]. 本团队[55]此前还发现, RNA结合蛋白LARP7通过调控U6 snRNA的2′-*O*-甲基化修饰而确保雄性生殖细胞mRNA可变剪接的保真性, *Larp7*生殖细胞特异性敲除小鼠雄性不育, 其减数分裂和精子形成发育期均出现异常. 此外, 在男性不育患者中, *SUN5*基因突变(c.475C>T, p.Arg159*)引起可变剪接发生异常而导致翻译提前终止, 临床表现为无头精子症[56]; 减数分裂相关基因*M1AP*的剪接位点发生纯合突变而引起蛋白缺失, 患者表现为严重少精症[57]; 弱精症患者携带的*DNAH8*和*DNAH17*基因错义突变可引起其可变剪接异常, 导致精子尾部结构不稳定而影响精子活力[58,59].

3 翻译调控与雄性生殖

3.1 RNA翻译调控与精子发生

减数分裂后的单倍体精子细胞需历经一系列细胞形态及结构变化, 最终发育为高度特化的精子, 此过程被称为精子形成(spermiogenesis)[60~62]. 在小鼠中, 基于精子细胞的形态和结构变化, 可将精子形成过程划分为16个步骤: 第1~8步圆形精子细胞期, 第9~11步延长型精子细胞期, 第12~14步长型或浓缩型精子细胞期, 第15~16步精子细胞则基本发育完成, 呈现典型的弯钩状[63]. 值得注意的是, 在哺乳动物中, 减数分裂后的精子细胞会发生染色质重塑过程, 细胞核中的组蛋白会逐渐被转换蛋白、鱼精蛋白依次替换, 细胞核逐渐压缩, 转录活动逐渐降低直至完全停止[9,64]. 因此, 精子形成相关的基因提前在精母细胞或早期圆形精子细胞中转录为mRNA, 并与RBP结合以翻译抑制状态储存在信使核糖核蛋白(messenger ribonucleoprotein, mRNP)中, 当精子细胞发育至特定时期, mRNA被激活并进入翻译机器进行相关蛋白的合成, 该过程被称为“转录-翻译解偶联”, 是精子细胞基因表达调控的典型特征[65,66]. 虽然“转录-翻译解偶联”过程为精子形成提供了一个基因表达时空性调控的精巧机制, 但长期以来并不清楚储存于精子细胞中的mRNA是如何被有序激活翻译的.

3.2 RNA翻译调控与雄性不育

以转换蛋白和鱼精蛋白为代表的一群后期精子细胞所需蛋白的翻译如何被调控一直是领域内研究的重点问题. 研究发现, 小鼠的鱼精蛋白存在转录后水平表达调控[67]. 鱼精蛋白*Prm1* mRNA 3′UTR存在保守的TCE元件(translation control element), 可维持其mRNA处于翻译抑制状态[68], 而其翻译激活依赖于蛋白TARBP2[69]. RNA解旋酶GRTH在粗线期精母细胞和圆形精子细胞中表达, 也会参与调控鱼精蛋白和转换蛋白的翻译调控[70]. 在精子发生过程中, poly(A)结合蛋白(poly(A)-binding protein, PABP)对于mRNA的稳定和翻译均发挥重要作用, 且在正常男性及男性不育患者的睾丸组织中存在差异表达[71]. 在后期精子细胞中, 保守的PABP(如PABPC1和PABPC2)非特异性地结合mRNA的3′末端poly(A)尾, 并与EIF4G1, EIF4G3和PAIP1等翻译机器相关蛋白组分, 以满足精子发生后期的翻译需求[72]. 另外, PAIP2a在精子发生后期的表达量上升, 且PAIP2a与PABP结合而很少与eIF4G结合, PAIP2a缺失小鼠中PABP表达水平显著上升、mRNA翻译受到抑制而造成精子发生受阻[73,74].

除翻译机器相关蛋白组分外, RBP是精子发生过程中mRNA翻译调控的重要组成部分. SAM68被认为是精子发生过程中重要的RNA调控蛋白之一, 参与mRNA剪接、核内mRNA转运和mRNA翻译. 在精母细胞中, SAM68存在于多聚核糖体(polysome)组分中并结合特定mRNA的poly(A)尾, 其缺失会影响多聚核糖体组分的分布[75]. 小鼠*Dazl*的3′UTR结合大量mRNA, 并与PABP蛋白结合, 其缺失影响多聚核糖体中靶mRNA的分布而影响蛋白翻译, 导致精子发生受阻[76]. 本团队[77]近期发现, 后期精子细胞中特异性高表达的RNA结合蛋白FXR1通过相分离富集mRNA形成FXR1颗粒, 同时招募EIF4G3和PABPC1等翻译相关因子, 介导了一群后期精子细胞发育必需基因的翻译激活, 从而保障了精子细胞发育和雄性生殖, 且*Fxr1*缺失或相分离缺陷突变小鼠均雄性不育. 此外, 本团队[78]还发现, 在圆形精子细胞中小鼠PIWI(MIWI)及其

相互作用piRNA与翻译起始因子eIF3f相互作用，并与RNA结合蛋白HuR形成MIWI/piRNA/eIF3f/HuR超级翻译复合体，促进mRNA翻译起始阶段“close loop”结构的形成, 进而激活一组中含有ARE元件(AU-rich element)的靶mRNA的翻译，为小鼠精子形成中顶体组装必需. 有趣的是, 本团队[79]还发现, 少弱精子症患者中存在*PIWIL1*基因突变(c.1108C>T, p.R370W; c.1315C>T, p.R439X), 通过突变小鼠模型发现该突变影响MIWI/piRNA翻译激活功能，MIWI/eIF3f/HuR复合体组装受阻、靶基因蛋白表达减少，且突变小鼠雄性生育力低下、精子畸形率剧增，证实该突变为男性不育致病性突变.

4　mRNA降解代谢调控与雄性生殖

4.1　mRNA降解代谢调控

RNA代谢主要通过多种途径的RNA降解来精细调控RNA的质量和数量，不仅参与生物体正常的生理活动，而且RNA代谢紊乱常常与疾病发生发展密切相关. 绝大多数真核细胞中的mRNA降解依赖于脱腺苷酸化(deadenylation), 即对mRNA 3′端poly(A)的特异性水解，在细胞生长、增殖、分化、应激及死亡等重要生命活动中精确维持RNA稳态或快速调控基因表达[80]. 此外，因无义突变、移码突变、转录错误、基因重排或选择性剪接引入的含有提前终止密码子(premature termination codon, PTC)的mRNA常会产生有害的截短蛋白质，该过程可被无义突变介导的mRNA降解(nonsense-mediated mRNA decay, NMD)所调控, NMD参与调控细胞分化、应激、部分遗传性疾病以及肿瘤的发生发展等生理病理过程[81~83].

(1) 脱腺苷酸化与RNA代谢调控. 作为RNA水平的重要调控方式，脱腺苷酸化参与了绝大部分细胞生命活动和多种重要生理和病理过程，而脱腺苷酸酶(deadenylase)在此过程中起到核心作用. 脱腺苷酸酶作为mRNA稳定性的负调控因子，属于3′-5′核酸外切酶，特异性地催化RNA 3′端poly(A)尾的水解、调节RNA 3′端poly(A)尾的长度, 从而调控RNA稳态和降解过程[84]. 目前，已知CCR4(哺乳动物中称为CNOT6/6L)，CAF1(哺乳动物中称为CNOT7/8)，PAN2和ANGEL等脱腺苷酸酶存在于绝大多数真核生物中，而PARN，Nocturnin以及PDE12等主要存在于高等生物中[85~87]. 一般认为，细胞质中mRNA的降解主要依赖于PAN2-PAN3和CCR4-NOT两种复合体的两步降解调控过程：PAN2-PAN3复合体起始脱腺苷酸化过程, 将靶mRNA的poly(A)尾降解约至110 nt左右, 而CCR4-NOT复合体继续降解至约10 nt左右；当mRNA的poly(A)尾小于10 nt时，mRNA将走向进一步的降解过程[88,89].

在真核细胞中，大量RBP被认为是mRNA命运调控的核心组分，根据mRNA的状态或细胞需求识别特定的靶mRNA，并招募特定脱腺苷酸酶，对mRNA的3′端进行降解或修剪, 从而调控mRNA命运. 例如，促进脱腺苷酸化的相互作用蛋白，结合靶mRNA 3′UTR的特定元件和脱腺苷酸酶，提高脱腺苷酸化速率并实现脱腺苷酸酶对靶mRNA的特异性降解；抑制脱腺苷酸化的相互作用蛋白与脱腺苷酸酶结合后，阻止脱腺苷酸酶与靶mRNA结合, 从而保护靶mRNA不被降解; 影响脱腺苷酸酶亚细胞定位的相互作用蛋白，实现对特定时空靶mRNA的精准脱腺苷酸化. 此外，还存在同一种RBP对不同的脱腺苷酸酶具有不同影响的情况, 如PABP会抑制PARN的酶活，而促进PAN2-PAN3和CCR4-NOT复合体的酶活；对于CCR4-NOT复合体中的两种脱腺苷酸酶CCR4和CAF1，PABP促进CCR4但却抑制CAF1的酶活[90].

(2) 无义突变介导的mRNA降解与代谢调控. NMD是真核生物中高度保守的RNA代谢调控途径, 通过降解含有PTC的mRNA控制RNA质量，降解含有3′UTR、上游开放阅读框(upstream open reading frame, uORF)等结构的mRNA来调控基因表达[81,89,90]. 研究显示，NMD可直接或间接地影响哺乳动物细胞中约10%正常mRNA的稳态[91,92]，在神经发生[93]、精子发生[94]、肿瘤发生发展[95~97]等多种生理与病理过程发挥重要作用.

NMD的关键效应蛋白包括UPF蛋白(UPF1，UPF2和UPF3，哺乳动物中UPF3为UPF3A和UPF3B)、SMG蛋白(SMG1, SMG5, SMG6, SMG7, SMG8和SMG9)和外显子拼接复合体(exon-junction complex, EJC)[98]. EJC在RNA剪接过程中结合在外显子-外显子连接处上游的20~24 nt处. 随着翻译的进行，EJC因核糖体的滑动而被取代，当核糖体识别出终止密码子时，eRF1和eRF3使核糖体停滞，PABPC1与eRF3结合可促进多肽链释放及核糖体循环. 而当核糖体识别出PTC而非

正常终止密码子时，导致eRF3与PABPC1不结合而与UPF1结合[99,100]. 若mRNA含有长3′UTR，翻译终止时也会导致eRF3与PABPC1不结合，而使eRF3与UPF1结合，且长3′UTR mRNA会增加其非特异性结合UPF1[101]. UPF1以EJC依赖的方式与UPF2和UPF3B结合，并与其他NMD调控因子共同参与降解诱导复合物(decay-inducing complex, DECID)的形成，诱导mRNA的降解. SMG1可将UPF1磷酸化，其磷酸化活性由SMG8和SMG9调节，UPF2被认为是UPF1与UPF3B的分子桥梁，SURF复合物通过UPF2和UPF3B与EJC共同形成DECID[102,103]. 磷酸化的UPF1招募多种引发mRNA降解的因子，通过SMG5-SMG7异二聚体复合物，招募脱腺苷酶CCR4-NOT复合物和5′-3′核糖核酸外切酶XRN1，分别介导mRNA脱腺苷和5′-3′的核酸外切. SMG5，SMG6和SMG7可招募蛋白磷酸酶PP2A介导UPF1去磷酸化，其与再循环NMD调控因子引发新一轮的NMD[104,105].

4.2 RNA降解代谢调控与雄性不育

(1) 脱腺苷酸化与雄性不育. 通过调节RNA代谢稳态，多种脱腺苷酸酶在精子发生中发挥至关重要的调控作用. 在果蝇和小鼠中的机制研究发现，NANOS, Pumilio，DND1等RBP通过招募CCR4-NOT复合体靶向降解包括Mei-P26在内重要调控因子的mRNA, 从而维持生殖干细胞的自我更新和定向分化[106~108]. CCR4-NOT复合体中RNA结合亚基*Cnot4*经*Stra8*-Cre特异性敲除后，小鼠精子发生阻滞在粗线期/双线期精母细胞时期，其缺失主要影响XY染色体的配对和DNA双链断裂修复等减数分裂中重要的生物学过程[109]; CCR4-NOT复合体中催化亚基*Cnot7*敲除后，小鼠雄性不育，精子质量显著下降，呈现少弱畸形的特征; 进一步研究发现，CAF1a/CNOT7与核视黄醇受体RXRB结合可增强其酶活，从而参与精子发生[110,111]; 然而, CCR4-NOT复合体中另一催化亚基*Cnot6*/*6l*双敲后, 小鼠雄性可育、雌性不育[112], 这说明CNOT6/6L缺失并不影响精子发生，提示CCR4-NOT复合体并非以复合体整体来发挥功能. 本团队[113]发现，MIWI/piRNA与其结合蛋白和脱腺苷酶CAF1组成piRNA诱导的沉默复合物(piRNA-induced silencing complex, pi-RISC), 介导了后期精子细胞中数千种mRNA的脱腺苷酸化降解，提供了精子形成前mRNA大规模清除降解的分子机制.

(2) 无义介导的mRNA降解与雄性不育. NMD途径主要针对含有PTC的mRNA进行降解清除，在精子发生过程中同样重要. 在体细胞中，UPF1识别长3′UTR区域并结合其他降解相关蛋白及PABPC1，从而对长3′UTR mRNA进行降解. 即使睾丸特异性表达基因的3′UTR区普遍较短, 但仍会受UPF1调控. 在生殖细胞中，PTBP1可结合UPF1并干扰NMD正常进行，使mRNA避免被降解. PTBP1同源蛋白PTBP2在雄性生殖细胞中可稳定长3′UTR mRNA，从而保证精子发生的正常进行. 特异性敲除Sertoli细胞中*Upf2*的小鼠雄性不育，睾丸显著变小，生精小管严重退化且仅出现Sertoli样细胞或生殖细胞样细胞，提示UPF2介导的NMD在青春期前Sertoli细胞的发育和雄性生育中发挥了重要作用[94]. UPF3B主要在精原细胞及减数分裂后的生精细胞中存在，并富集在拟染色质小体(chromatoid body, CB)中; 而UPF3A在精母细胞及后期精子细胞中表达, *Upf3a*生殖细胞特异性敲除小鼠精子数量减少，其通过EJC介导的NMD靶mRNA丰度上升，而3′UTR介导的NMD靶mRNA变化不大[114]. 有趣的是，UPF1，UPF2，UPF3B等NMD调控相关蛋白均富集于圆形精子细胞的CB中，提示CB可能是精子形成过程中NMD途径发挥功能的重要场所. 现有研究表明, CB主要组分蛋白TDRD6可帮助UPF1正确定位于CB并帮助其招募UPF2，确保3′UTR介导的NMD途径正常进行, 而*Tdrd6*敲除小鼠长3′UTR mRNA丰度增加[115,116].

此外，已有报道R-loop相关调控因子突变可能导致无精症[117]. R-loop被认为是由DNA-RNA杂合链以及相应单链DNA组成的特殊染色质结构，在DNA复制、转录及维持基因组稳定性等方面发挥重要作用[118]. 近期研究显示, R-loop稳态调控因子RNase H1介导R-loop中RNA的降解清除同样对于精子发生至关重要, 生殖细胞特异性敲除*Rnaseh1*影响重组酶RAD51和DMC1的招募，导致减数分裂同源重组修复异常、雄性不育[119,120].

5 总结与展望

精子发生是复杂且有序的细胞发育过程，其有序进行依赖于在转录、转录后和表观遗传等多重水平进行的精准且时空特异性的基因表达调控. 睾丸组织中

特异性表达或高表达了超过1500个RNA结合蛋白，它们与mRNA或/和非编码调控RNA组装形成RNA调控复合物, 参与雄性生殖细胞基因表达调控, 在精子发生过程中发挥了不可或缺的重要作用. 然而, 领域内对生殖细胞中庞大且复杂的RNA调控网络的认识和了解仍极其有限. 更为重要的是, 近年来临床结合基础生物学研究发现，RNA调控通路相关基因突变与男性不育紧密相关，使得系统性地揭示精子发生中RNA调控的功能机制更为迫切. 因此，深入阐析RNA调控在精子发生及男性不育中的功能机制，不仅有助于人们理解精子发生过程中重要生物学事件，同时也将为男性不育临床诊疗提供理论依据和方法策略.

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New functions and mechanisms of RNA regulation in spermatogenesis and male infertility

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Spermatogenesis is the most complicated and highly orchestrated cell development processes in animals, in which many mysteries remain. Male infertility has become a major global concern for population health. Strikingly, more than half of infertile men are of unknown pathologic causes, representing the major challenge in understanding the disease mechanisms. It is evident that a large number of RNA-binding proteins are specifically or highly expressed in testis, forming RNA regulatory networks through binding to the protein-coding mRNAs or/and regulatory non-coding RNAs. Importantly, many RNA-binding proteins and their associated regulatory non-coding RNAs have been shown to regulate gene expression in male germ cells at epigenetic, transcriptional and post-transcriptional levels, playing an indispensable role in spermatogenesis and essential for male fertility. Moreover, genetic mutations in various RNA regulation-related genes have been demonstrated to play causative roles in infertile patients, suggesting abnormal RNA regulation as a new cause of male infertility. In this Review, we summarize the most recent advances in the field, focusing on the new functions and mechanisms of RNA modifications, alternative splicing, translation, degradation in spermatogenesis and male infertility. We also discuss the potential application of RNA regulation in the clinical diagnosis and treatment of male infertility.

male infertility, spermatogenesis, RNA regulation, RNA-binding proteins

doi: 10.1360/SSV-2023-0172