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精子发生转录调控机制的研究进展

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摘要 精子发生过程中的转录调控是由一系列基因表达和调控事件组成的复杂过程, 影响精子的形成、质量和功能。转录调控过程介导与精子形成密切相关的基因, 包括精子特异性基因、组蛋白基因和其他转录因子的基因表达。这些基因的表达和沉默受到转录因子、表观遗传修饰和非编码RNA等多种机制的调控。此外, 转录调控在精子发生的不同阶段起着不同的作用, 包括精原干细胞的自我更新和分化、精母细胞的减数分裂和精子细胞的变形成熟。深入理解精子发生中的转录调控机制对于研究精子形成的生物学过程、解析生育障碍的病理机制以及开发生育问题相关的治疗方法具有重要的意义。

关键词 精子发生; 转录调控; 减数分裂; 表观修饰

Advances in Transcriptional Regulation of Spermatogenesis

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Abstract Transcriptional regulation during spermatogenesis is a complex process. It consists of a series of gene expression and regulatory events that affect sperm formation, quality and function. Transcriptional regulatory mediates the expression of genes including sperm-specific genes, histone genes, and other regulatory factors. The expression and silencing of these genes are regulated by a variety of mechanisms, which includes transcription fac-

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tors, epigenetic modifications, and non-coding RNA. Furthermore, transcriptional regulation plays different roles in different stages of spermatogenesis, including self-renewal and differentiation of spermatogonia stem cells, meiosis of spermatocytes, and metamorphosis and maturation of sperm cells. Thorough comprehension of transcriptional regulatory mechanisms in spermatogenesis is of great significance for studying the biological processes of spermatogenesis, resolving the pathological mechanisms of fertility disorders, and developing treatments for fertility problems.

Keywords spermatogenesis; transcriptional regulation; meiosis; epigenetic modification

精子发生(spermatogenesis)是一个高度复杂且调控精密的生物学过程,涉及到多种生精细胞和基因表达调控模式。精原干细胞(spermatogonia)经多次有丝分裂产生前细线期(pre-leptotene)精母细胞,随后的两次减数分裂产生圆形精子(round spermatid)。圆形精子通过精子变形(spermiogenesis)过程,即核浓缩、组蛋白-精蛋白转化等一系列形态和功能转变,最终产生长形精子(elongated spermatid)。长形精子进入附睾后,在附睾微环境中形成具有受精能力的成熟精子^[1-2]

精子发生以生精波(spermatogenic wave)形式进行,例如小鼠精子发生全程经历35天^[3]。该过程涉及到多种细胞类型,生精细胞在不同阶段具有不同的基因表达模式,从而启动不同的生物学事件。基因的表达是指一个基因经过转录和翻译,最终形成一个具有特定功能的蛋白质,这个过程涉及到转录调控、转录后调控和翻译调控。转录(transcription)作为基因表达中心法则中的第一步,需要维持高度的准确性,在特定的细胞类型中表达特定的基因集合。转录调控是指DNA在转录成RNA的过程中受到多种转录因子或染色质结构的作用而影响转录准确性和效率。转录调控受到多种因素,包括:特定的转录因子和辅因子、DNA甲基化修饰、组蛋白翻译后修饰等的调控^[4-6]。

1 转录相关因子调控

人类基因组中仅有不到2%的DNA序列是蛋白质编码序列,其中5%~10%的蛋白是专门用来调控转录过程的。这些蛋白主要有三类:(1)转录激活或转录抑制因子;(2)组成以RNA聚合酶为核心的转录复合物;(3)核小体组蛋白,以及组蛋白翻译后修饰的酶。此外,基因组大量非蛋白质编码序列组成了转录过程中的顺式作用元件,如TATA元件(TATA box)、启动子(promoter)、增强子(enhancer)等^[7-9]。因此,众

多的调控序列和调控蛋白提示转录是个高度特异、调控精密的生物学过程。在雄性生殖细胞中,大量基因启动子展现高活性状态^[10],通用的调节因子和生殖细胞特异性因子在这些细胞中存在平衡关系^[11]。减数分裂后,精子形成的开始阶段以大量转录活性为特征,这导致早期单倍体细胞中许多必需的减数分裂后基因的激活。为了确保这种有效和及时的转录,各种通用转录因子在生殖细胞中被差异调节,TATA结合蛋白(TATA-binding protein, TBP)、通用转录因子TFIIB和RNA Pol II(polymerase II)在早期单倍体生殖细胞中的积累量远高于任何体细胞。例如,成年脾脏和肝细胞每个单倍体基因组当量分别含有0.7和2.3分子的TBP mRNA,但成年睾丸每个单倍体基因组当量含有80至200分子的TBP转录物^[12]。生殖细胞与体细胞的不同不仅在于这些通用因子的异常表达水平,而且还因为存在睾丸特异性亚型。如通用转录因子TFIIA,可通过稳定TBP与启动子DNA的结合并促进RNA Pol II依赖的转录起始复合物(preinitiation complex, PIC)中激活蛋白依赖的构象变化来刺激转录,但在睾丸中存在两种TFIIA的特异性亚型,ALF和TFIIA τ ^[13-14],它们能够在转录过程中功能性替代TFIIA,这强调了睾丸中的特异性转录调控过程。因此,理解精子发生中特异的转录调控机制对于理解生精细胞的发育过程具有重要意义。

DNA甲基化(DNA methylation)是基因组DNA上最常见的表观修饰标记,常与基因表达沉默相关^[15]。DNA甲基化由DNA甲基转移酶(DNA methyl-transferase, DNMT)催化S-腺苷甲硫氨酸(S-adenosylmethionine, SAM)作为甲基供体,将DNA的CG两个核苷酸的胞嘧啶选择性地添加甲基,主要形成5-甲基胞嘧啶(5-mC,常见于基因的5'-CG-3'序列)。目前已经鉴定出的DNA甲基转移酶主要有两类:持续性甲基转移酶1(DNA methyl-transferase 1, DNMT1)和从头甲基转移酶(*de novo* DNA methyl-

transferase) DNMT3A及DNMT3B^[16-17]。基因组GC富集的区域被称为CpG岛(CpG island), 脊椎动物基因组中超过一半基因含有CpG岛。位于启动子区域和转录起始位点附近的CpG岛高度甲基化常会导致基因转录沉默。

比较胚胎干细胞(embryonic stem cells, ESCs)和生精干细胞(germ stem cells, GSCs)的全基因组甲基化水平发现, 精子发生相关基因(*Dppa3*、*Stra8*、*Piwi1*、*Mov10*、*Scp-family*、*Tex-family*、*Rnf7*、*Sohlh*、*Tdrd1*、*Catsper-family*等)在ESCs中高度甲基化表达沉默, 但在GSC中呈现去甲基化状态^[18]。精子和卵子受精形成合子再发育成雄性后代产生精子的过程要涉及两次基因组DNA甲基化的重编程(reprogramming)。第一次重编程发生在精子卵子受精后, 来自父源和母源的基因组DNA经历主动和被动的DNA甲基化水平降低过程。原始生殖细胞(primordial germ cell, PGCs)迁移并定植到性腺的过程中发生第二次重编程, 基因组DNA经历完全去甲基化^[19]。随后在E13.5~E16.5天重建基因组DNA甲基化标记, 在雄性生殖细胞中形成特定的DNA甲基化表观标记, 产生精原干细胞(spermatogonia stem cells, SSCs)。E16.5天, PGCs的减数分裂相关基因甲基化水平较高接近其基因组甲基化平均水平, 而在成年SSCs中减数分裂相关基因又呈现高度低甲基化水平。成年精原干细胞中特定基因集的甲基化水平降低可能是维持配子发生的一个关键表观修饰标记。

针对临床不育症男性精子的全基因组甲基化测序发现, 不育症患者在多个精子发生相关基因启动子区域高甲基化^[20]。MARSIT等^[21]学者在精子发生障碍患者piRNA加工通路关键基因*PIWIL2/TDRD1*的启动子区域高度甲基化。HAN等^[22]发现*Sox30*基因启动子区域高度甲基化。NANASSY等^[23]通过比较少精症、精子核蛋白成熟异常患者与正常男性的*CREM*(cAMP-response element modulator)启动子的甲基化状态也发现, 核蛋白成熟异常和少精症患者*CREM*基因甲基化水平更高。虽然在精液质量下降的患者中检测到多个精子发生相关基因高甲基化水平, 但导致基因异常高甲基化的临床病理机制仍然研究较少。

2 精子发生特异性转录因子

2.1 Lin28

哺乳动物的精子发生是由SSCs的自我更新及

分化所维持的。Lin28是一种能够结合RNA的多能干细胞因子, 参与重要的睾丸生理过程, 如细胞更新、成熟、生育和衰老^[24-26]。在哺乳动物体内, Lin28存在两个同源蛋白, Lin28A和Lin28B。这两种蛋白存在各自的表达模式但其功能相似, Lin28A位于未分化和A1型精原细胞中, 而Lin28B则局限于精细胞和间质细胞^[27]。在果蝇中, LIN28表达在早期睾丸的Hub细胞中, 随着果蝇衰老相关蛋白表达水平逐渐下调。Hub细胞在果蝇睾丸内具有维持生殖干细胞生态位(niche)的功能, 随着衰老数目和活性下降, LIN28功能的缺失会导致Hub细胞病理性的数目减少和形态异常, 而LIN28的持续表达会影响Hub细胞的完整性和生殖干细胞的功能^[28]。在秀丽隐杆线虫中缺失LIN28也同样导致生殖干/祖细胞数目的减少^[29]。目前研究发现Lin28A的调控机制是由其抑制let-7 miRNA的生成或调节mRNA的翻译效率所决定的。Lin28A存在两种经典的核酸结合结构域, 一个冷休克结构域(cold-shock domain, CSD)和两个CCHC型的锌指结构域。这表明其功能可能与其能够结合DNA相关。通过对小鼠胚胎干细胞(mESCs)的ChIP-seq结果分析发现, 其保守的结合基序“CAGNACC”-NN-“GGACAG”(N为随机序列)与其RNA结合基序(GGAG/A)高度相似, 此外通过ChIP-seq及RNA-seq联合分析发现, Lin28A的结合位点富集在转录起始位点处, 并且其结合基因与许多相关基因的表达水平呈正相关。研究表明, Lin28A将5-甲基胞嘧啶双加氧酶Tet1招募到基因组结合位点, 以协调5-甲基胞嘧啶和5-羟甲基胞嘧啶的动力学。Lin28A或Tet1的敲减都会导致DNA甲基化和常见靶基因表达失调^[30]。此外, LIN28的表达异常与睾丸癌和睾丸疾病, 如低促性腺功能减退症和Klinefelter综合征有关。除了LIN28在维持生殖干细胞方面的功能外, 异常的LIN28表达还与睾丸精原细胞瘤(testicular seminoma)和卵巢畸胎瘤(ovarian dysgerminoma)相关。LIN28可以负向调控Let-7的合成, 进而促进Let-7靶标中多个致癌基因(*MYCN*、*AURKB*、*CCNF*、*RRM2*、*MKI67*、*CL2orf5*)的表达^[31]。在睾丸精原细胞瘤的切片染色中也观察到了LIN28的高表达^[32]。而在生殖细胞瘤细胞系中抑制LIN28的表达可以回补Let-7的表达, 并抑制Let-7的靶标mRNA水平。最近的一项研究表明, LIN28抑制剂C1632可以在体内外有效回补Let-7

的表达并抑制PD-L1的表达,有效促进抗肿瘤免疫的再激活^[33]。因此,LIN28逐渐成为开发新型治疗药物的一个有希望的靶点。

2.2 STRA8

生精细胞从有丝分裂转变为减数分裂是精子发生中关键的一步,调控有丝分裂结束和减数分裂开始的分子机制一直是配子发生领域的研究重点。2006年,BOWLES等^[34]和KOUBOVA等^[35]先后发现视黄酸(retinoic acid, RA)信号通路在减数分裂起始阶段中具有重要作用。RA可以诱导*Strat8*(stimulated by retinoic acid gene 8)基因的表达,后者参与减数分裂启动。动物研究发现,*Strat8*敲除(knockout, KO)雄鼠睾丸内生精细胞能够发育到前细线期精母细胞,但完全缺失其余的精母细胞类型和后续的单倍体精子细胞^[36]。

STRA8具有DNA结合能力,同时STRA8主要结合在DNA序列的启动子区域(84%)^[37-38]。STRA8对于结合启动子区域的偏好性暗示其具有调控基因转录的巨大潜力。KOJIMA等^[39]通过ChIP-seq发现与STRA8结合的靶标基因中有2 809个在前细线期表达。在前细线期进入减数分裂的过程中,有1 351个基因在减数分裂起始过程上调,而只有165个基因在减数分裂中下调,上调基因主要为细胞周期转化和减数分裂相关基因^[39]。该结果提示,*Strat8*作为关键的减数分裂启动基因,通过调控靶基因转录,使生精细胞转录“重编程”,细胞从有丝分裂状态转化到减数分裂状态。

MEIOSIN是新鉴定出的与STRA8相互作用的因子,*Meiosin* KO小鼠睾丸生精缺陷表型与*Strat8* KO小鼠类似^[40]。同时,MEIOSIN的时空表达模式与STRA8高度相似。比较有意思的一点是,STRA8在*Meiosin* KO睾丸内主要定位在细胞质中,而MEIOSIN在*Strat8* KO睾丸中主要定位于细胞核。说明MEIOSIN可能是维持STRA8细胞核定位的关键因子。*Meiosin* KO小鼠睾丸转录组也和*Strat8* KO小鼠睾丸转录组检测结果具有高度相似性,都表现为与精子发生相关基因表达水平的异常下调。MEIOSIN的表达不受STRA8调控,而是和STRA8一样都受到RA信号调控。RA受体结合*Strat8*和*Meiosin*的启动子区域,调控其表达。ChIP-seq结果显示,MEIOSIN结合的靶标中很大一部分是精子发生相关基因,其中有1 085个基因在*Meiosin* KO睾丸中下调。MEIO-

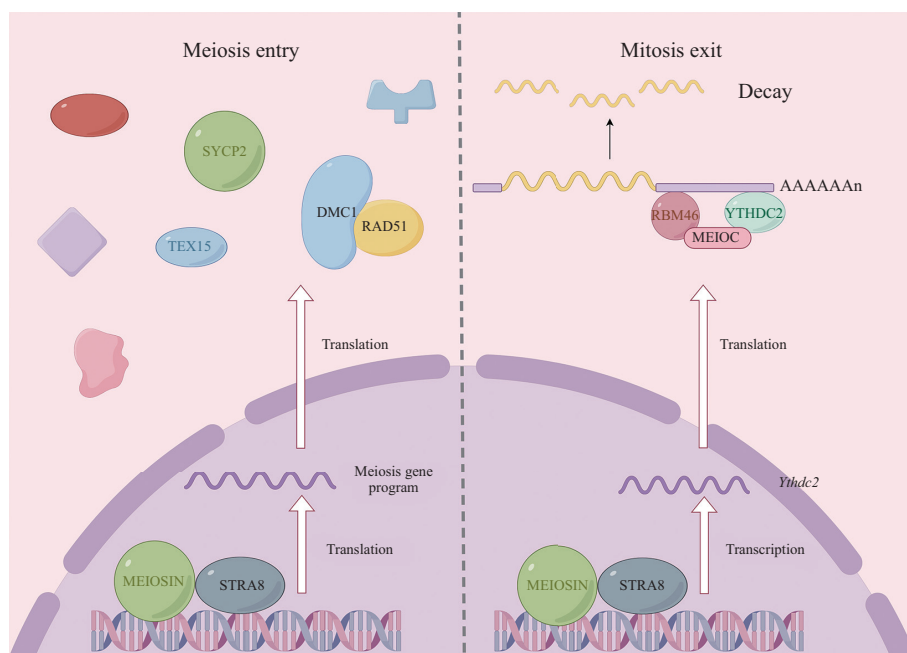
SIN和STRA8结合的靶标中有415个基因是下调的,且这些基因(如:*Sycp2*、*TEX12*、*Rad21l*等)的功能和减数分裂相关。此外,STRA8和MEIOSIN还可以结合*Meioc*、*Ythdc2*的启动子来调控其表达。值得注意的是,*Ythdc2*、*Meioc* KO小鼠都表现出相似表型,即精子发生可以进行到减数分裂起始阶段,但无法继续,大多数生精小管仅能发育到前细线期样细胞类型,随后的研究指出YTHDC2-MEIOC-RBM46复合物能够通过沉默有丝分裂相关的转录本使得生精细胞从有丝分裂向减数分裂发展^[41-44]。

以上研究说明STRA8调控精子发生可能存在两套机制:(1) STRA8直接结合减数分裂相关基因的启动子区域,调控其表达,促进生精细胞进入减数分裂;(2) STRA8结合*Ythdc2*等基因启动子区域促进其转录,最终YTHDC2-MEIOC-RBM46复合物再去降解有丝分裂相关转录本,使生精细胞退出有丝分裂(图1)。

2.3 SOX家族

SOX(Sry-related HMG box)蛋白家族成员是一类含有HMG(high-mobility-group)结构域的转录因子。SOX家族不同成员都含有一个保守的HMG结构域,HMG结构域的氨基酸保守性通常超过50%^[45]。人和小鼠中,已经发现了20种不同的SOX家族成员,根据HMG结构域相似程度分为A至H不同亚组^[46]。SOX蛋白调控转录是通过HMG结构域结合DNA特定序列实现的。已有的研究证明,SOX蛋白在组织器官发育、细胞命运决定、性别决定、配子发生和癌症等多方面都发挥作用^[45,47-48]。虽然SOX蛋白识别相似的DNA基序“(A/T)(A/T)CAA(A/T)G”^[49],但在不同的组织类型和时间点发挥着不同的功能。这可能与SOX蛋白在不同细胞类型中对基序两侧序列具有不同的偏好性^[50]、转录协作因子(co-factor),以及翻译后修饰有关。

近年来的研究发现,一些SOX家族成员在精子发生过程发挥转录调控作用。SOX3蛋白是SOX B1家族的一员,以往的研究证明SOX B1家族成员在神经系统的发育过程中发挥重要调控功能^[51]。在神经系统外,SOX3在睾丸尤其是A型精原细胞中表达水平较高,*Sox3*敲除小鼠睾丸生精小管中只有支持细胞和未分化的精原细胞^[52]。基于RET(ret tyrosine kinase receptor positive,未分化精原细胞marker)和KIT(kit receptor tyrosine kinase,分化精原细胞mark-



精子发生中关键的一步是生殖细胞准确地退出有丝分裂, 并进入减数分裂程序。STRA8蛋白和MEIOSIN主要表达于分化的精原细胞和前细线期细胞中, 并调控两大类基因的表达。第一类是减数分裂相关基因, 如联会复合体相关蛋白(synaptonemal complex protein, *sycp*)和DNA减数分裂重组酶(DNA meiotic recombinase, *dmc*)等。这些减数分裂相关蛋白可以促进生殖细胞进入减数分裂, 完成减数分裂相关事件。第二类是YTHDC2等蛋白。YTHDC2和RBM46、MEIOC蛋白相互作用形成复合物, 该复合物可以结合*Ccna2*等有丝分裂相关基因的转录本并促进其降解, 从而退出有丝分裂。

One crucial step in spermatogenesis is the accurate exit from mitosis and entry into meiosis by germ cells. STRA8 and MEIOSIN are predominantly expressed in differentiating spermatogonia and preleptotene spermatocytes, and they regulate the expression of two major classes of genes. The first class is composed of meiosis-related genes, such as *sycp* (synaptonemal complex protein) and *dmc* (DNA meiotic recombinase). These meiosis-related proteins can facilitate the entry of germ cells into meiosis and participate in meiosis-related events. The second class includes proteins like YTHDC2. YTHDC2 interacts with RBM46 and MEIOC to form a complex that can bind to transcripts of mitosis-related genes, such as *Ccna2*, and promote their degradation. This process leads to a decrease in the transcription levels of mitosis-related genes, allowing the spermatocytes to exit from mitosis.

图1 STRA8调控减数分裂机制(本图由Figdraw绘制)

Fig.1 The mechanism of STRA8 regulating meiosis (picture supported by Figdraw)

er)的流式结果也发现, *Sox3*敲除小鼠睾丸中未分化精原细胞数目不变, 但分化的精原细胞数目大大减少^[53]。已有的ChIP-seq数据库显示SOX3可以结合在*Ngn3*(neurogenin 3)的上游启动子区域, ChIP-PCR进一步证实睾丸中SOX3蛋白能够结合*Ngn3*基因^[54-55]。NGN3是bHLH(basic helix-loop-helix)转录因子家族一名成员, 主要在精原细胞中表达, 参与调控精子发生过程^[56]。以上说明, SOX3可能通过调控NGN3的表达进而调控生精过程, 更具体的调控机制还需要后续的研究完成。

SOX蛋白H亚组成员SOX30是另一个鉴定到调控精子发生的转录因子。SOX30是一个睾丸高度特异性表达的转录因子, 在21天小鼠睾丸中表达达到峰值。特异性的时空表达模式提示SOX在圆形精子变形阶段可能发挥重要作用^[57]。*Sox30*敲除小鼠减数分裂过程不受影响, 但完全缺失长形精子, 生精细

胞最远可以发育到早期精子变形阶段^[57-60]。ChIP-seq结果显示, 小鼠睾丸中SOX30可以结合精子发生减数分裂后调控基因的启动子区域。这些减数分裂后调控基因(顶体形成相关基因、组蛋白替换相关基因和精子细胞发育相关基因)在*Sox30*敲除小鼠睾丸内表达水平也剧烈下降^[57,60]。因此, SOX30可能通过调控精子变形相关基因的转录来调控精子发生。

在以往的研究中发现, SOX家族E组成员SOX9是小鼠睾丸支持细胞分化的关键调控因子, 也是哺乳动物性别决定(sex determination)的关键调控因子。在小鼠10.0天的胚胎中, 生殖器官的前体生殖嵴(genital ridges)出现。此时雄性胚胎和雌性胚胎的生殖嵴没有明显的形态和功能上的差异, 都具有分化为睾丸支持细胞和卵泡颗粒细胞的能力。在胚胎10.0~10.5天时, 雄性胚胎生殖嵴中体细胞开始表达*SRY*基因并启动SOX9的表达。SOX9表达后继

而促进下游多个调控支持细胞分化的基因表达, 雄性胚胎生殖嵴向睾丸方向发育^[61]。SRY和SF1协同结合到*Sox9*基因转录起始位点上样的增强子的核心区域(TESCO)来上调*Sox9*基因的表达。SOX9也可以与SF1协同结合TESCO来促进自身的表达, 可以在SRY表达下调后依靠自身的正反馈机制促进睾丸发育^[62]。雄性性别决定不仅需要促进睾丸发育的基因表达, 还需要抑制卵巢发育的基因表达。SRY和SOX9在转录层面上结合并促进许多共同靶标的表达, 其中富集到一类支持细胞和支持细胞之间紧密连接相关的基因, 这些蛋白连接支持细胞形成血-睾屏障并维持生精微环境的稳定。同时, SRY也可以独自干扰WNT/ β -catenin信号通路活性来抑制多个卵巢发育相关的基因, 如*Fst*、*Lrx3*、*Bmp2*等。在SRY基因表达下调后, SOX9仍可以调控支持细胞分化相关的基因表达^[63]。

2.4 CREM

CREM是一个重要的转录因子, 在多种组织类型中都有表达。CREM基因可以通过可变剪接、可变多聚腺苷化等多种机制产生超过30种不同的蛋白变体, 既有激活型的变体也有抑制型的变体^[64-65]。CREM的不同变体可以识别一个共同的保守回文序列“TGACGTCA”, 这个保守序列也被称为CRE位点(CRE site)^[66-67]。在体细胞中, CREM发挥功能需要自身特定氨基酸残基发生磷酸化修饰以及辅助因子的协助。但是在睾丸中, CREM在ACT(activator of CREM)蛋白的协助下发挥功能^[68]。

在唯支持细胞综合征患者SCOS(sertolli-cell-only syndrome)和圆形精子阻滞患者中发现CREM τ (激活型变体)和它的辅因子ACT表达下调^[69]。在CREM τ 缺陷小鼠中也发现精子发生相关基因(如*Prm1*、*Prm2*、*Tnp1*和*Tnp2*等)表达水平下调^[70-71]。小鼠精子发生过程中, 粗线期精母细胞之前的生精细胞内表达抑制性的CREM变体, 激活型变体CREM τ 开始表达于粗线期精母细胞中, 在圆形精子变形VII~VIII阶段达到峰值, 随后在长形精子中表达水平下降直至不表达^[72-73]。人类睾丸中CREM τ 也呈现和小鼠相似的表达模式, 例如在少弱精不育症患者中更多表达抑制型的CREM变体^[74]。MARTIANOV等^[75]通过ChIP-seq检测到单倍体精子细胞中CREM τ 结合了超过5 000个靶标, 其中80%结合位点位于靶标基因的启动子区域, 这与CREM τ 发挥转录激活作

用相符。CREM τ 缺陷小鼠睾丸转录组差异表达基因中也发现与精子变形相关的基因有很大一部分表达下调^[76]。

2.5 RFX2

转录因子RFX2是调控精子变形过程的一个重要的转录因子^[77]。RFX2主要调控精子变形中鞭毛形成相关的基因表达^[78]。在非哺乳动物脊椎动物中, *Rfx2*对于携带活动纤毛的细胞的分化^[79]以及左/右不对称的发展至关重要^[80]。然而, *Rfx2*在哺乳动物中的功能尚未明确。*Rfx2* mRNA表达量最初在精子发生的减数分裂阶段增加并且RFX2主要结合在靶基因的转录起始位点(transcription start site, TSS)附近^[81]。*Rfx2*基因敲除小鼠可以正常存活, 但表现为生育力的完全丧失。ChIP-seq联合RNA-seq结果也揭示, RFX2所结合调控的基因主要与精子鞭毛形成相关并在基因敲除小鼠中表达下调^[82-83]。同时*Rfx2*基因缺陷小鼠的睾丸中会形成多核的巨大细胞并经历凋亡^[84]。

2.6 BRDT

BRDT包含8个WD重复序列和2个串联溴结构域。溴结构域是高度保守的110个氨基酸基序, 可识别乙酰赖氨酸残基^[85]。BRDT是BET蛋白亚家族成员之一, 含有两个溴结合域(bromodomain)。BRDT蛋白在睾丸中特异性表达, 主要表达在粗线期和双线期精母细胞以及后续的圆形精子阶段^[86-87]。BRDT可能是减数分裂后转录激活因子复合物的一部分, 该复合物与减数分裂后基因周围的乙酰化组蛋白互相作用^[88]。特异性缺失BRDT蛋白第一个溴结构域会导致精子发生阻滞在精子变形的第9步。*BRDT*^{-/-}小鼠睾丸切片组蛋白10号位点丝氨酸磷酸化(H3S10Ph)水平会下降, 但染色质联会等事件不受影响。这说明BRDT蛋白在第一次减数分裂粗线期精母细胞晚期发挥作用。同时, BRDT对于精子发生过程中减数分裂后精子的变形过程也具有重大作用^[89]。ChIP-seq结果显示, BRDT和组蛋白乙酰化在很多基因的TSS位点存在共定位, 这些基因主要分为两类。一类在减数分裂完成后与BRDT的结合能力上升, 并在圆形精子阶段高表达。另一类在减数分裂完成后与BRDT的结合能力减弱, 并且其表达水平在减数分裂过程中达到峰值^[90]。BRDT与组蛋白乙酰化修饰结合位点的共定位主要依赖溴结合域与组蛋白乙酰化修饰的相互作用。而BRDT第一个

溴结构域的缺失(Brd^tΔBD1/ΔBD1)会导致精子延长和组蛋白替换的障碍,但不会影响到减数分裂的完成^[91]。但Brd^tΔBD1/ΔBD1小鼠精子细胞中正常表达PRM和TP等组蛋白替换蛋白。同时在组蛋白替换前,组蛋白一般呈现高度乙酰化的状态。BRDT调控组蛋白转化更主要是通过结合高度乙酰化组蛋白来调控组蛋白转换过程的。

2.7 TAF7L

TAF7L是小鼠和人类中TAF7的X连锁生殖细胞特异性旁系同源物^[92-93],是TFIID的普遍表达成分^[94]。Taf7l编码区被12个内含子中断,Taf7编码区完全缺乏内含子^[95]。这表明Taf7l是祖先基因,Taf7是Taf7l的逆向旁系同源物。当Taf7在进化过程中通过逆转录产生并在体细胞组织中广泛表达时,Taf7l在没有体细胞选择压力的情况下逐渐成为睾丸特化^[92-93]。Taf7^{-Y}睾丸中6个基因转录本(包括Fscn1)的丰度降低至原有三分之一。FSCN1(fascin actin-bundling protein 1)是一种F作用捆绑蛋白,可能是正常精子形态和精子活力所必需的^[96]。与体细胞组织中的TAF7一样,TAF7L与TAF1相互作用,并与睾丸中的TBP相关,这表明TAF7L是真正的TBP相关因子(TBP-associated factor, TAF),TAF7L在雄性生殖细胞中的亚细胞定位是动态的。减数分裂精母细胞中的特定染色质结构域。TAF7L存在于精原细胞和早期精母细胞(细线期、细线期和合子期)的细胞质中,但TAF7L易位到粗线期精母细胞和圆形精子细胞的细胞核中^[92]。Taf7l^{-Y}精子的结构缺陷和运动受损,在Taf7l^{-Y}小鼠中较高比例的精子尾部发生折叠。精子尾部在中片内或中片与主片交界处经常出现180°弯曲。在中片与主片交界处也出现精子尾部急剧成角的情况^[97]。TAF7L突变精子的BCF明显较高,这与尾部折叠的精子跳动更快但不能产生向前运动的观察结果一致^[97]。突变精子获能后运动能力进一步降低。TAF7L的缺失导致生育能力降低,但不会导致不育^[97]。因为精子的半合子状态,男性X染色体上的Taf7l突变可能会导致人类少精症。Taf7l的靶向突变导致精子发生在圆形精子细胞阶段末期停滞。

3 染色质结构

3.1 组蛋白变体

组蛋白是生物体内表达最丰富的一类蛋白,经典的组蛋白H2A、H2B、H3和H4组装形成核小

体核心成分,同时被基因组DNA链缠绕。除了经典的组蛋白,目前还发现许多组蛋白变体,组蛋白变体赋予染色质独特的特性,并且组蛋白变体受特定的产生和去除机制来调控其在基因组区域的分布^[98-99]。H2A.Z是组蛋白H2A的一个变体,ZNHIT1可以调控H2A.Z在基因组上装配。H2A.Z可通过调控MEIOSIN的表达来促进减数分裂的启动^[100]。ChIP-seq结果显示,H2A.Z通常在TSS附近调控下游基因的转录。精子发生过程中,H2A.Z结合的位点常常和STRA8结合位点高度重叠,提示H2A.Z变体可能有和STRA8参与相似的调控模式。Znhit缺陷小鼠也具有与Stra8、Meiosin缺陷小鼠相似的生精缺陷表型和转录组改变。此外,最近十几年研究人员在小鼠睾丸里还鉴定出TSH2B、H1T2和H2B.W等组蛋白变体,在动物层面上证明其对精子发生的必要性,但是少有研究去探究背后的机制^[101-102]。

3.2 组蛋白修饰

核小体(nucleosome)是染色质的基本组成单元^[103]。组蛋白碱性的氨基端(N-term)通常暴露在核小体外部,可以被甲基、乙酰基和泛素等基团修饰,称为组蛋白修饰^[104]。组蛋白翻译后修饰通常是可逆的,通常由修饰酶加上修饰基团,由去修饰酶消除修饰基团。常见的组蛋白修饰类型有甲基化修饰、乙酰化修饰和泛素化修饰等^[103]。不同的修饰类型具有不同的转录调控功能,通常认为组蛋白乙酰化修饰和转录激活相关,而组蛋白甲基化修饰的功能依赖甲基化的位点和数目。

组蛋白甲基化主要指在组蛋白甲基转移酶的作用下,将甲基基团转移到组蛋白精氨酸或者赖氨酸侧链氨基上。不同于组蛋白乙酰化修饰,组蛋白甲基化修饰通常不改变组蛋白带电性。但组蛋白的甲基化更加复杂,组蛋白精氨酸残基的甲基化可以是单甲基化或二甲甲基化,赖氨酸残基的甲基化可以是单甲基化、二甲甲基化、三甲甲基化。甲基化位点的不同和形式的不同都将产生不同的转录调控命运。组蛋白乙酰化是指在组蛋白乙酰基转移酶(histone acetyltransferases, HAT)的催化下,将乙酰辅酶ADAM乙酰基团转移到组蛋白赖氨酸的ε-氨基上^[105]。乙酰化修饰会中和赖氨酸的正电性,将削弱组蛋白与DNA的相互作用。与HAT作用相拮抗的是组蛋白去乙酰化酶(histone deacetylases, HDAC),HDAC可逆性地去除组蛋白赖氨酸上的乙酰化修饰^[106]。相比组蛋白甲

基化复杂的转录调控活性, 组蛋白乙酰化目前普遍认为是促进转录的因素。

SSCs根据分化方向可以分为两类: (1) 自我更新 (THY1⁺); (2) 倾向进入减数分裂型 (KIT⁺)。THY1⁺将会分裂为两个单独未配对的细胞 (As细胞), KIT⁺细胞将要分裂两个通过细胞间桥连接的配对细胞 (Apr)。Apr细胞后续再分裂为Aal细胞和B型精原细胞, B型精原细胞将会进入减数分裂产生精子。THY1⁺和KIT⁺细胞的H3K27me3水平在某些基因上具有差异, 其中有一部分基因 (*Aldh2*、*Stra8*、*Spo11*) 是减数分裂相关基因。H3K27me3作为一种转录的表观修饰, 其在基因组中表达水平下降有利于该区域基因的表达。KIT⁺细胞中 *Stra8* 等基因启动子区域组蛋白H3K27me3水平下降促进转录, 促进生精细胞进入减数分裂^[18]。H3K79me2是一种促进转录的组蛋白甲基化修饰, DOT1L是目前鉴定到的唯一的H3K79甲基转移酶, *Dot1l*缺失导致HOXC的基因组区域的H3K79me2表达水平下降, 引起其mRNA水平降低, 后者在SSCs的命运决定中发挥作用, 因此*Dot1l*缺陷小鼠无法维持SSCs的自我更新, 使得精子发生无法进行下去^[107]。另有研究指出, *Dot1l*条件性敲除小鼠中发现精子发生中的组蛋白-精蛋白转换 (histone-to-protamine exchange) 过程受损。正常情况下, DOT1L调控H3K79me2堆积在参与精子变形阶段的*Brdt*基因上, 而*Dot1l*缺失小鼠睾丸会下调BRDT的表达^[108]。因此, DOT1L调控的H3K79me2修饰可能通过以上两种机制来调控小鼠的精子发生。

4 总结与展望

临床上, 不孕不育是指夫妻同房在不避孕的前提下12个月无法怀孕。不孕不育是目前人们面对的一个主要健康问题, 大约影响到全球15%的人群^[109]。其中, 有一半是来自男性的因素。精液参数异常, 包括少精子症、弱精子症、畸形精子症和无精子症等是男性不育的重要诱因之一。无精子症是指在男性精液未发现成熟精子。按照原因无精子症可以分为梗阻性无精子症 (obstructive azoospermia, OA) 和非梗阻性无精子症 (non-obstructive azoospermia, NOA)。梗阻性无精子症通常由输精管或射精管梗阻导致, 而非梗阻性无精子症则主要是精子发生障碍。常见的非梗阻性无精子症原因有染色体异常、Y染色体微缺失、睾丸发育不良、感染等^[110-111]。随

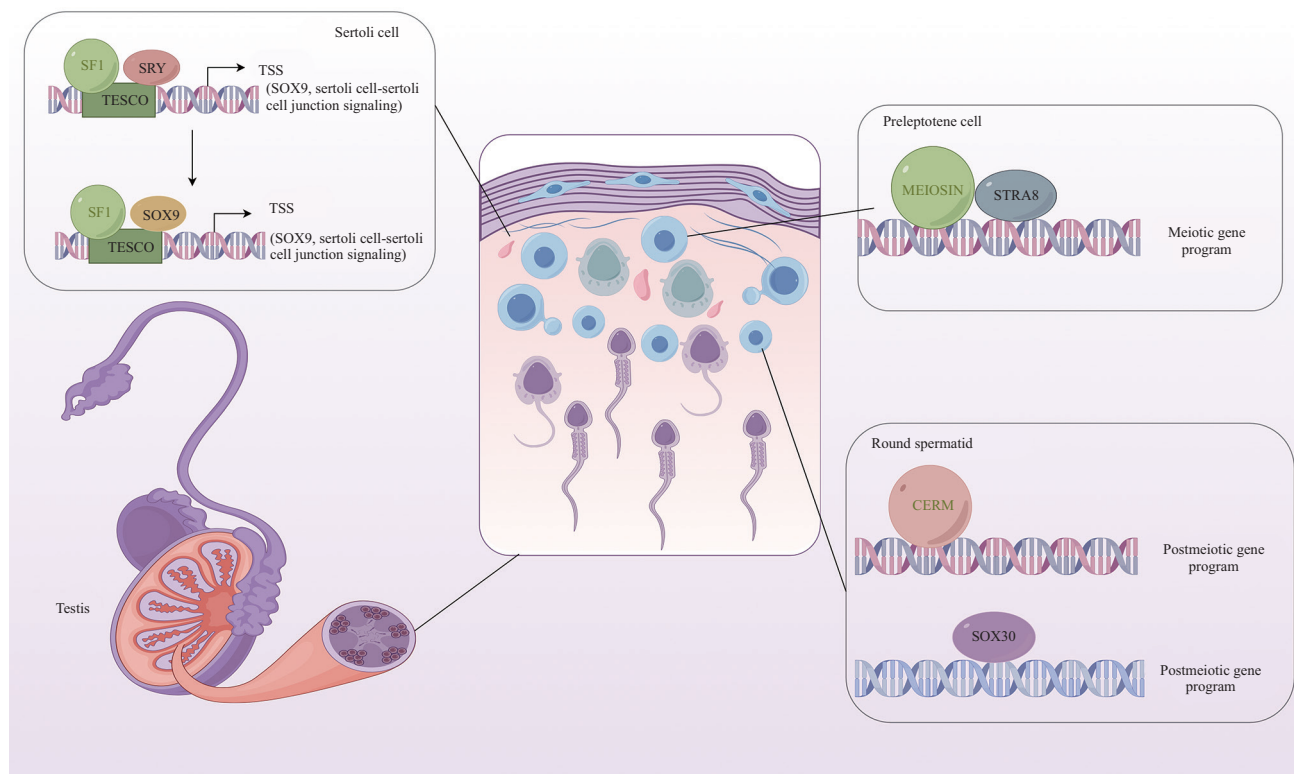
着检测技术的进步, 通过外显子测序发现, 非梗阻无精子症患者基因组上存在很多致病突变, 其中很多与精子发生过程中的转录调控过程相关。因此, 研究精子发生过程中的转录调控机制, 挖掘精子发生的关键基因对于临床诊断非梗阻性无精子症病因具有重要意义。

雄性精子发生最远可以追溯到胚胎时期生殖嵴的形成。之后在性别决定因子SRY的调控下, 支持细胞特异性转录因子SOX9开始表达。SOX9的功能研究证明其对于支持细胞前提的发育分化具有至关重要的作用, 并且SOX9所调控的支持细胞之间的细胞连接蛋白表达可以形成生精小管特异性的血-睾屏障。血-睾屏障对于维持生精微环境的稳定具有重要作用。同时支持细胞作为生精小管内唯一的体细胞, 各级生精细胞与支持细胞之间的相互作用也是维持精子发生稳定的关键因素。精原干细胞是精子发生最初的细胞类型, 其由原始生殖细胞发育而来。精原干细胞通过多次分裂分化形成B型精原干细胞, 其间受到SOX3和LIN28等多个因子的调控来维持自我更新以及定向分化潜能。而STRA8则表达于B型精原细胞和前细线期细胞中, STRA8的表达和调控减数分裂基因表达的能力是进入减数分裂的必要条件。精母细胞经过减数分裂产生圆形精子后, SOX30、CREM和RFX2等转录因子开始调控精子变形相关基因的表达, 最终产生成熟的具有受精能力的精子。在此过程中组蛋白修饰、变体和基因组DNA甲基化水平等因素可以通过改变染色质可及性、维持染色质“开放状态”来促进基因转录 (图2)。

本综述通过总结最近十多年精子发生领域中转录调控相关的知识, 以期为大家展示生理条件下不同的转录因子在不同的时相内准确发挥转录调控功能来维持精子发生稳定进行的必要性, 以及为临床不孕不育患者的诊断和治疗提供一些见解。

参考文献 (References)

- [1] DE ROOIJ D G. The nature and dynamics of spermatogonial stem cells [J]. *Development*, 2017, 144(17): 3022-30.
- [2] HERMO L, PELLETIER R M, CYR D G, et al. Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 1: background to spermatogenesis, spermatogonia, and spermatocytes [J]. *Microsc Res Tech*, 2009, 73(4): 241-78.
- [3] GRISWOLD M D. Spermatogenesis: the commitment to meiosis [J]. *Physiol Rev*, 2016, 96(1): 1-17.



不同类型的生精细胞中表达特定的转录因子来调控特定基因集的表达。在支持细胞(sertoli cell)中, 性别决定因子SRY和转录因子调控支持细胞之间细胞连接基因表达, 从而为此血睾屏障的完整和生精微环境的稳定。在前细线期细胞(preleptotene cell)中, STRA8和MEIOSIN蛋白调控减数分裂相关基因的表达, 促使细胞进入减数分裂。在圆形精子(round spermatid)阶段, SOX30、CREM等蛋白调控减数分裂后基因表达, 促进精子变形过程, 最终产生成熟的精子。

Specific transcription factors are expressed in different types of germ cells to regulate the expression of specific sets of genes. In sertoli cells, the sex-determining factor SRY and transcription factors regulate the expression of cell junction genes between sertoli cells, ensuring the integrity of the blood-testis barrier and the stability of the microenvironment for spermatogenesis. In preleptotene cells, proteins like STRA8 and MEIOSIN regulate the expression of meiosis-related genes, promoting the entry of cells into meiosis. In round spermatids, proteins such as SOX30 and CREM regulate the expression of post-meiotic genes, facilitating the process of sperm morphogenesis and ultimately producing mature sperm.

图2 精子发生中的转录调控(本图由Figdraw绘制)

Fig.2 Transcriptional regulation in spermatogenesis (picture supported by Figdraw)

- [4] BROWNING D F, BUSBY S J W. The regulation of bacterial transcription initiation [J]. *Nat Rev Microbiol*, 2004, 2(1): 57-65.
- [5] CRAMER P. Organization and regulation of gene transcription [J]. *Nature*, 2019, 573(7772): 45-54.
- [6] HABERLE V, STARK A. Eukaryotic core promoters and the functional basis of transcription initiation [J]. *Nat Rev Mol Cell Biol*, 2018, 19(10): 621-37.
- [7] LEVINE M, TJIAN R. Transcription regulation and animal diversity [J]. *Nature*, 2003, 424(6945): 147-51.
- [8] ANDERSSON R, SANDELIN A. Determinants of enhancer and promoter activities of regulatory elements [J]. *Nat Rev Genet*, 2019, 21(2): 71-87.
- [9] SPITZ F, FURLONG E E M. Transcription factors: from enhancer binding to developmental control [J]. *Nat Rev Genet*, 2012, 13(9): 613-26.
- [10] KLEENE K C. A possible meiotic function of the peculiar patterns of gene expression in mammalian spermatogenic cells [J]. *Mech Dev*, 2001, 106(2001): 3-23.
- [11] SASSONE-CORSI P. Transcriptional checkpoints determining the fate of male germ cells [J]. *Cell*, 1997, 88(2): 163-6.
- [12] SCHMIDT E E, SCHIBLER U. High accumulation of components of the RNA polymerase II transcription machinery in rodent spermatids [J]. *Development*, 1995, 121: 2373-83.
- [13] UPADHYAYA A B, LEE S H, DEJONG J. Identification of a general transcription factor TFIIA α/β homolog selectively expressed in testis [J]. *J Biol Chem*, 1999, 274(25): 18040-8.
- [14] OZER J, MOORE P A, LIEBERMAN P M. A testis-specific transcription factor IIA (TFIIA τ) stimulates TATA-binding protein-DNA binding and transcription activation [J]. *J Biol Chem*, 2000, 275(1): 122-8.
- [15] JONES P A. Functions of DNA methylation: islands, start sites, gene bodies and beyond [J]. *Nat Rev Genet*, 2012, 13(7): 484-92.
- [16] OKANO M, BELL D W, HABER D A, et al. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development [J]. *Cell*, 1999, 99(3): 247-57.
- [17] JONES P A, LIANG G. Rethinking how DNA methylation patterns are maintained [J]. *Nat Rev Genet*, 2009, 10(11): 805-11.
- [18] HAMMOUD SAHER S, LOW DIANA H P, YI C, et al. Chro-

- matin and transcription transitions of mammalian adult germline stem cells and spermatogenesis [J]. *Cell Stem Cell*, 2014, 15(2): 239-53.
- [19] HACKETT J A, ZYLICZ J J, SURANI M A. Parallel mechanisms of epigenetic reprogramming in the germline [J]. *Trends Genet*, 2012, 28(4): 164-74.
- [20] CAMPRUBÍ C, SALAS-HUETOS A, AIESE-CIGLIANO R, et al. Spermatozoa from infertile patients exhibit differences of DNA methylation associated with spermatogenesis-related processes: an array-based analysis [J]. *Reprod Biomed Online*, 2016, 33(6): 709-19.
- [21] MARSIT C J, HEYN H, FERREIRA H J, et al. Epigenetic disruption of the PIWI pathway in human spermatogenic disorders [J]. *PLoS One*, 2012, 7(10): e47892.
- [22] HAN F, JIANG X, LI Z M, et al. Epigenetic inactivation of SOX30 is associated with male infertility and offers a therapy target for non-obstructive azoospermia [J]. *Mol Ther Nucleic Acids*, 2020, 19: 72-83.
- [23] NANASSY L, CARRELL D T. Abnormal methylation of the promoter of CREM is broadly associated with male factor infertility and poor sperm quality but is improved in sperm selected by density gradient centrifugation [J]. *Fertil Steril*, 2011, 95(7): 2310-4.
- [24] AECKERLE N, EILDERMANN K, DRUMMER C, et al. The pluripotency factor LIN28 in monkey and human testes: a marker for spermatogonial stem cells [J]? *Mol Hum Reprod*, 2012, 18(10): 477-88.
- [25] GAYTAN F, SANGIAO-ALVARELLOS S, MANFREDI-LOZANO M, et al. Distinct expression patterns predict differential roles of the miRNA-binding proteins, Lin28 and Lin28b, in the mouse testis: studies during postnatal development and in a model of hypogonadotropic hypogonadism [J]. *Endocrinology*, 2013, 154(3): 1321-36.
- [26] ZHENG K, WU X, KAESTNER K H, et al. The pluripotency factor LIN28 marks undifferentiated spermatogonia in mouse [J]. *Bmc Dev Biol*, 2009, doi: 10.1186/1471-213X-9-38.
- [27] KRSNIK D, MARIC T, BULIC-JAKUS F, et al. LIN28 family in testis: control of cell renewal, maturation, fertility and aging [J]. *Int J Mol Sci*, 2022, 23(13): 7245.
- [28] SREEJITH P, JANG W, TO V, et al. Lin28 is a critical factor in the function and aging of *Drosophila* testis stem cell niche [J]. *Aging*, 2019, 11(3): 855-73.
- [29] WANG D, HOU L, NAKAMURA S, et al. LIN-28 balances longevity and germline stem cell number in *Caenorhabditis elegans* through let-7/AKT/DAF-16 axis [J]. *Aging Cell*, 2016, 16(1): 113-24.
- [30] ZENG Y X, YAO B, SHIN J, et al. Lin28A binds active promoters and recruits tet1 to regulate gene expression [J]. *Mol Cell*, 2016, 61(1): 153-60.
- [31] MURRAY M J, SAINI H K, SIEGLER C A, et al. LIN28 expression in malignant germ cell tumors downregulates let-7 and increases oncogene levels [J]. *Cancer Res*, 2013, 73(15): 4872-84.
- [32] CAO D, ALLAN R W, CHENG L, et al. RNA-binding protein LIN28 is a marker for testicular germ cell tumors [J]. *Hum Pathol*, 2011, 42(5): 710-8.
- [33] CHEN Y, XIE C, ZHENG X, et al. LIN28/let-7/PD-L1 pathway as a target for cancer immunotherapy [J]. *Cancer Immunol Res*, 2019, 7(3): 487-97.
- [34] BOWLES J, KNIGHT D, SMITH C, et al. Retinoid signaling determines germ cell fate in mice [J]. *Science*, 2006, 312(5773): 596-600.
- [35] KOUBOVA J, MENKE D B, ZHOU Q, et al. Retinoic acid regulates sex-specific timing of meiotic initiation in mice [J]. *Proc Natl Acad Sci USA*, 2006, 103(8): 2474-9.
- [36] ANDERSON E L, BALTUS A E, ROEPERS-GAJADIEN H L, et al. Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice [J]. *Proc Natl Acad Sci USA*, 2008, 105(39): 14976-80.
- [37] DESIMIO M G, CESARI E, SORRENTI M, et al. Stimulated by retinoic acid gene 8 (STRA8) interacts with the germ cell specific bHLH factor SOHLH1 and represses c-KIT expression *in vitro* [J]. *J Cell Mol Med*, 2020, 25(1): 383-96.
- [38] NIU C, GUO J, SHEN X, et al. Meiotic gatekeeper STRA8 regulates cell cycle by interacting with SETD8 during spermatogenesis [J]. *J Cell Mol Med*, 2020, 24(7): 4194-211.
- [39] KOJIMA M L, DE ROOIJ D G, PAGE D C. Amplification of a broad transcriptional program by a common factor triggers the meiotic cell cycle in mice [J]. *eLife*, 2019, doi: 10.7554/eLife.43738.
- [40] ISHIGURO K I, MATSUURA K, TANI N, et al. MEIOSIN directs the switch from mitosis to meiosis in mammalian germ cells [J]. *Dev Cell*, 2020, 52(4): 429-45, e10.
- [41] LÜ Y, LU G, CAI Y, et al. RBM46 is essential for gametogenesis and functions in post-transcriptional roles affecting meiotic cohesin subunits [J]. *Protein Cell*, 2022, 14(1): 51-63.
- [42] DAI X, CHENG X, HUANG J, et al. Rbm46, a novel germ cell-specific factor, modulates meiotic progression and spermatogenesis [J]. *Biol Reprod*, 2021, 104(5): 1139-53.
- [43] LI L, KRASNYKOV K, HOMOLKA D, et al. The XRN1-regulated RNA helicase activity of YTHDC2 ensures mouse fertility independently of m⁶A recognition [J]. *Mol Cell*, 2022, 82(9): 1678-90, e12.
- [44] QIAN B M, LI Y, YAN R Y, et al. RNA binding protein RBM46 regulates mitotic-to-meiotic transition in spermatogenesis [J]. *Sci Adv*, 2022, 8(34): eabq2945.
- [45] WEGNER M. From head to toes: the multiple facets of Sox proteins [J]. *Nucleic Acids Res*, 1999, 27(6): 1409-20.
- [46] SCHEPERS G E, TEASDALE R D, KOOPMAN P. Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families [J]. *Dev Cell*, 2002, 3(2): 167-70.
- [47] WEGNER M. All purpose sox: the many roles of Sox proteins in gene expression [J]. *Tint J Biochem Cell Biol*, 2010, 42(3): 381-90.
- [48] SARKAR A, HOCHEDLINGER K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate [J]. *Cell Stem Cell*, 2013, 12(1): 15-30.
- [49] ROUANET C, REVERCHON S, RODIONOV D A, et al. Definition of a consensus DNA-binding site for PecS, a global regulator of virulence gene expression in and identification of new members of the PecS regulon [J]. *J Biol Chem*, 2004, 279(29): 30158-67.
- [50] PEIRANO R I, WEGNER M. The glial transcription factor Sox10 binds to DNA both as monomer and dimer with different

- functional consequences [J]. *Nucleic Acids Res*, 2000, 28(16): 3047-55.
- [51] BYLUND M, ANDERSSON E, NOVITCH B G, et al. Vertebrate neurogenesis is counteracted by Sox1-3 activity [J]. *Nat Neurosci*, 2003, 6(11): 1162-8.
- [52] RAVEROT G, WEISS J, PARK S Y, et al. Sox3 expression in undifferentiated spermatogonia is required for the progression of spermatogenesis [J]. *Dev Biol*, 2005, 283(1): 215-25.
- [53] JAMESON J L, LARONDA M M. Sox3 functions in a cell-autonomous manner to regulate spermatogonial differentiation in mice [J]. *Endocrinology*, 2011, 152(4): 1606-15.
- [54] ZHENG D, MCANINCH D, THOMAS P. Identification of highly conserved putative developmental enhancers bound by SOX3 in neural progenitors using ChIP-Seq [J]. *PLoS One*, 2014, 9(11): e113361.
- [55] MCANINCH D, MÄKELÄ J A, LA H M, et al. SOX3 promotes generation of committed spermatogonia in postnatal mouse testes [J]. *Sci Rep*, 2020, 10(1): 6751.
- [56] YOSHIDA S, TAKAKURA A, OHBO K, et al. Neurogenin3 delineates the earliest stages of spermatogenesis in the mouse testis [J]. *Dev Biol*, 2004, 269(2): 447-58.
- [57] BAI S, FU K, YIN H, et al. Sox30 initiates transcription of haploid genes during late meiosis and spermiogenesis in mouse testes [J]. *Development*, 2018, 145(13): dev164855.
- [58] FENG C W A, SPILLER C, MERRINER D J, et al. SOX30 is required for male fertility in mice [J]. *Sci Rep*, 2017, 7(1): 17619.
- [59] CHEN Y, ZHENG Y X, GAO Y, et al. Single-cell RNA-seq uncovers dynamic processes and critical regulators in mouse spermatogenesis [J]. *Cell Res*, 2018, 28(9): 879-96.
- [60] ZHANG D, XIE D, LIN X, et al. The transcription factor SOX30 is a key regulator of mouse spermiogenesis [J]. *Development*, 2018, 145(11): dev164723.
- [61] JAKOB S, LOVELL-BADGE R. Sex determination and the control of Sox9 expression in mammals [J]. *FEBS J*, 2011, 278(7): 1002-9.
- [62] SEKIDO R, LOVELL-BADGE R. Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer [J]. *Nature*, 2008, 453(7197): 930-4.
- [63] LI Y, ZHENG M, LAU Y F. The sex-determining factors sry and SOX9 regulate similar target genes and promote testis cord formation during testicular differentiation [J]. *Cell Rep*, 2014, 8(3): 723-33.
- [64] FOULKES N S, MELLSTRÖM B, BENUSIGLIO E, et al. Developmental switch of CREM function during spermatogenesis: from antagonist to activator [J]. *Nature*, 1992, 355(6355): 80-4.
- [65] DON J, STELZER G. The expanding family of CREB/CREM transcription factors that are involved with spermatogenesis [J]. *Mol Cell Endocrinol*, 2002, 187(1/2): 115-24.
- [66] FOULKES N S, BORRELLI E, SASSONE-CORSI P. CREM gene: use of alternative DNA-binding domains generates multiple antagonists of cAMP-induced transcription [J]. *Cell*, 1991, 64(4): 739-49.
- [67] BEHR R, WEINBAUER G F. cAMP response element modulator (CREM): an essential factor for spermatogenesis in primates [J]? *Int J Androl*, 2001, 24(3): 126-35.
- [68] DE CESARE D, FIMIA G M, SASSONE-CORSI P. Signaling routes to CREM and CREB: plasticity in transcriptional activation [J]. *Trends Biochem Sci*, 1999, 24(7): 281-5.
- [69] JAZIREIAN P, FAVAEDI R, GILANI M A S, et al. Dynamic expression and chromatin incorporation of ACT and CREM transcription factors in testis tissues of infertile men [J]. *Cell J*, 2021, 23(7): 736-41.
- [70] BLENDY J A, KAESTNER K H, WEINBAUER G F, et al. Severe impairment of spermatogenesis in mice lacking the CREM gene [J]. *Nature*, 1996, 380(6570): 162-5.
- [71] NANTEL F, MONACO L, FOULKES N S, et al. Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice [J]. *Nature*, 1996, 380(6570): 159-62.
- [72] RUPPERT S, COLE T J, BOSHART M, et al. Multiple mRNA isoforms of the transcription activator protein CREB: generation by alternative splicing and specific expression in primary spermatocytes [J]. *EMBO J*, 1992, 11(4): 1503-12.
- [73] GREEN C D, MA Q, MANSKE G L, et al. A comprehensive roadmap of murine spermatogenesis defined by single-cell RNA-Seq [J]. *Dev Cell*, 2018, 46(5): 651-67, e10.
- [74] LIU Y, KAMITAKAHARA A, KIM A J, et al. Cyclic adenosine 3',5'-monophosphate responsive element binding protein phosphorylation is required but not sufficient for activation of corticotropin-releasing hormone transcription [J]. *Endocrinology*, 2008, 149(7): 3512-20.
- [75] MARTIANOV I, CHOUKRALLAH M A, KREBS A, et al. Cell-specific occupancy of an extended repertoire of CREM and CREB binding loci in male germ cells [J]. *BMC Genomics*, 2010, doi: 10.1186/1471-2164-11-530.
- [76] TORA L, KOSIR R, JUVAN P, et al. Novel insights into the downstream pathways and targets controlled by transcription factors crem in the testis [J]. *PLoS One*, 2012, 7(2): e31798.
- [77] HORVATH G C, KISTLER W S, KISTLER M K. RFX2 is a potential transcriptional regulatory factor for histone H1t and other genes expressed during the meiotic phase of spermatogenesis [J]. *Biol Reprod*, 2004, 71(5): 1551-9.
- [78] KIM M, LI D, CUI Y X, et al. Regulatory factor interactions and somatic silencing of the germ cell-specific ALF gene [J]. *J Biol Chem*, 2006, 281(45): 34288-98.
- [79] DUBRUILLE R, LAURENÇON A, VANDAELE C, et al. regulatory factor X is necessary for ciliated sensory neuron differentiation [J]. *Development*, 2002, 129(23): 5487-98.
- [80] CHUNG M I, PEYROT S M, LEBOEUF S, et al. RFX2 is broadly required for ciliogenesis during vertebrate development [J]. *Dev Biol*, 2012, 363(1): 155-65.
- [81] KISTLER W S, BAAS D, LEMEILLE S, et al. RFX2 is a major transcriptional regulator of spermiogenesis [J]. *PLoS Genet*, 2015, 11(7): e1005368.
- [82] SCHOLEY J M, ANDERSON K V. Intraflagellar transport and cilium-based signaling [J]. *Cell*, 2006, 125(3): 439-42.
- [83] HORVATH G C, KISTLER M K, KISTLER W S. RFX2 is a candidate downstream amplifier of A-MYB regulation in mouse spermatogenesis [J]. *Bmc Dev Biol*, 2009, doi: 10.1186/1471-213X-9-63.
- [84] FLIEGAUF M, BENZING T, OMRAN H. When cilia go bad: cilia defects and ciliopathies [J]. *Nat Rev Mol Cell Bio*, 2008, 9(1): 88-9.
- [85] HUDSON B P, MARTINEZ-YAMOUT M A, DYSON H J, et al. Solution structure and acetyl-lysine binding activity of the GCN5

- bromodomain [J]. *J Mol Biol*, 2000, 304(3): 355-70.
- [86] SHANG E, NICKERSON H D, WEN D, et al. The first bromodomain of Brdt, a testis-specific member of the BET sub-family of double-bromodomain-containing proteins, is essential for male germ cell differentiation [J]. *Development*, 2007, 134(19): 3507-15.
- [87] PHILIPPS D L, WIGGLESWORTH K, HARTFORD S A, et al. The dual bromodomain and WD repeat-containing mouse protein BRWD1 is required for normal spermiogenesis and the oocyte-embryo transition [J]. *Dev Biol*, 2008, 317(1): 72-82.
- [88] BOTTOMLEY M J. Structures of protein domains that create or recognize histone modifications [J]. *EMBO Rep*, 2004, 5(5): 464-9.
- [89] PATTABIRAMAN S, BAUMANN C, GUIADO D, et al. Mouse BRWD1 is critical for spermatid postmeiotic transcription and female meiotic chromosome stability [J]. *J Cell Biol*, 2015, 208(1): 53-69.
- [90] GAUCHER J, BOUSSOUAR F, MONTELLIER E, et al. Bromodomain-dependent stage-specific male genome programming by Brdt [J]. *EMBO J*, 2012, 31(19): 3809-20.
- [91] BRYANT J M, BERGER S L. Low-hanging fruit: targeting Brdt in the testes [J]. *EMBO J*, 2012, 31(19): 3788-9.
- [92] POINTUD J C, MENGUS G, BRANCORSINI S, et al. The intracellular localisation of TAF7L, a paralogue of transcription factor TRID subunit TAF7, is developmentally regulated during male germ-cell differentiation [J]. *J Cell Sci*, 2003, 116(9): 1847-58.
- [93] WANG P J, MCCARREY J R, YANG F, et al. An abundance of X-linked genes expressed in spermatogonia [J]. *Nature Genet*, 2001, 27(4): 422-6.
- [94] CHIANG C M, ROEDER R G. Cloning of an intrinsic human TFIID subunit that interacts with multiple transcriptional activators [J]. *Science*, 1995, 267: 531-6.
- [95] ZHOU T Y, CHIANG C M. The intronless and TATA-less human gene contains a functional initiator and a downstream promoter element [J]. *J Biol Chem*, 2001, 276(27): 25503-11.
- [96] ADAMS J C. Roles of fascin in cell adhesion and motility [J]. *Curr Opin Cell Biol*, 2004, 16(5): 590-6.
- [97] CHENG Y, BUFFONE M G, KOUADIO M, et al. Abnormal sperm in mice lacking the gene [J]. *Mol Cell Biol*, 2007, 27(7): 2582-9.
- [98] BUSCHBECK M, HAKE S B. Variants of core histones and their roles in cell fate decisions, development and cancer [J]. *Nat Rev Mol Cell Biol*, 2017, 18(5): 299-314.
- [99] MARTIRE S, BANASZYNSKI L A. The roles of histone variants in fine-tuning chromatin organization and function [J]. *Nat Rev Mol Cell Biol*, 2020, 21(9): 522-41.
- [100] SUN S, JIANG Y, ZHANG Q, et al. Znhit1 controls meiotic initiation in male germ cells by coordinating with Stra8 to activate meiotic gene expression [J]. *Dev Cell*, 2022, 57(7): 901-13, e4.
- [101] MARTIANOV I, BRANCORSINI S, CATENA R, et al. Polar nuclear localization of H1T2, a histone H1 variant, required for spermatid elongation and DNA condensation during spermiogenesis [J]. *Proc Natl Acad Sci USA*, 2005, 102(8): 2808-13.
- [102] SINGLETON S, ZALENSKY A, DONCEL G F, et al. Testis/sperm-specific histone 2B in the sperm of donors and subfertile patients: variability and relation to chromatin packaging [J]. *Hum Reprod*, 2007, 22(3): 743-50.
- [103] BANNISTER A J, KOUZARIDES T. Regulation of chromatin by histone modifications [J]. *Cell Res*, 2011, 21(3): 381-95.
- [104] LUGER K, MÄDER A W, RICHMOND R K, et al. Crystal structure of the nucleosome core particle at 2.8 Å resolution [J]. *Nature*, 1997, doi: 10.1038/38444.
- [105] ALLFREY V G, FAULKNER R, MIRSKY A E. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis [J]. *Proc Natl Acad Sci USA*, 1964, 51(5): 786-94.
- [106] YANG X J, SETO E. HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention [J]. *Oncogene*, 2007, 26(37): 5310-8.
- [107] LIN H, CHENG K, KUBOTA H, et al. Histone methyltransferase DOT1L is essential for self-renewal of germline stem cells [J]. *Genes Dev*, 2022, 36(11/12): 752-63.
- [108] LIN H, COSSU I G, LEU N A, et al. The DOT1L-MLLT10 complex regulates male fertility and promotes histone removal during spermiogenesis [J]. *Development*, 2023, 150(9): dev201501.
- [109] EVERS J L H. Female subfertility [J]. *Lancet*, 2002, 360(9327): 151-9.
- [110] PEÑA V N, KOHN T P, HERATI A S. Genetic mutations contributing to non-obstructive azoospermia [J]. *Best Pract Res Clin Endocrinol Metab*, 2020, 34(6): 101479.
- [111] LIU X G, HU H Y, GUO Y H, et al. Correlation between Y chromosome microdeletion and male infertility [J]. *Genet Mol Res*, 2016, doi: 10.4238/gmr.15028426.