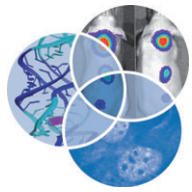
Overview



Small noncoding RNAs and male infertility

Lan-Tao Gou,[1,2,†] Peng Dai[1,2,†] and Mo-Fang Liu[1,2*]

Small noncoding RNAs (ncRNAs) are a novel class of gene regulators that modulate gene expression at transcriptional, post-transcriptional, and epigenetic levels, and they play crucial roles in almost all cellular processes in eukaryotes. Recent studies have indicated that several types of small noncoding RNAs, including microRNAs (miRNAs), endo-small interference RNAs (endo-siRNAs), and Piwi-interacting RNAs (piRNAs), are expressed in the male germline and are required for spermatogenesis in animals. In this review, we summarize the recent knowledge of these small noncoding RNAs in male germ cells and their biological functions and mechanisms of action in animal spermatogenesis. © 2014 John Wiley & Sons, Ltd.

How to cite this article:
WIREs RNA 2014. doi: 10.1002/wrna.1252

INTRODUCTION

Spermatogenesis is one of the most complicated developmental processes, as it requires cellular proliferation, differentiation, and morphologic change, and it produces highly specialized haploid spermatozoa from initially undifferentiated germ stem cells. Mammalian spermatogenesis begins with the self-renewal and differentiation of spermatogonial stem cells (SSCs). In rodents, A_{single} spermatogonia are thought of as authentic SSCs from which A_{paired} (A_{pr}), $A_{aligned}$ (A_{al}), type A1–A4, intermediate, and type B spermatogonia are derived.[1–3] Type B spermatogonia differentiate to preleptotene spermatocytes by mitosis, and spermatocytes then undergo meiosis through the leptotene, zygotene, pachytene, and diplotene stages to produce haploid round spermatids. Round spermatids then undergo a dramatic differentiation process called spermiogenesis that includes acrosome formation, flagellum formation, nuclear condensation, and cytoplasmic exclusion to differentiate into elongated spermatids and eventually spermatozoa.[4] Spermiogenesis in animals is a complex biochemical and morphological process consisting of at least 16 sequential transition steps in mice.[5]

The discovery of microRNAs (miRNAs) by Ambros and colleagues in 1993 revealed noncoding RNAs (ncRNAs) as a novel class of gene regulators in eukaryotes, which has shed new light on gene regulation.[6] Increasing evidence has indicated that ncRNAs, which control gene expression at transcriptional, post-transcriptional, and epigenetic levels, play critical roles in male germ cell development (Figure 1). In this review, we summarize the current understanding of the roles and mechanisms of ncRNAs in spermatogenesis in animals.

miRNAs IN MALE GERM CELLS

miRNAs are derived from long primary transcripts that are synthesized predominantly by RNA polymerase II. These primary transcripts range in length from several hundreds to thousands of nucleotides (nts) and are termed primary miRNAs.[7,8] In animals, primary miRNAs are initially processed into approximately 70 nt precursor miRNAs (pre-miRNAs) by the RNase III member Drosha and its cofactor DiGeorge syndrome critical region 8 gene (DGCR8) in the nucleus[9–13]; the pre-miRNAs are then subsequently

*Correspondence to: mfliu@sibcb.ac.cn

[1]Center for RNA Research, State Key Laboratory of Molecular Biology–University of Chinese Academy of Sciences, Shanghai, China

[2]Shanghai Key Laboratory of Molecular Andrology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

[†]These authors contributed equally to this work

Conflict of interest: The authors have declared no conflicts of interest for this article.

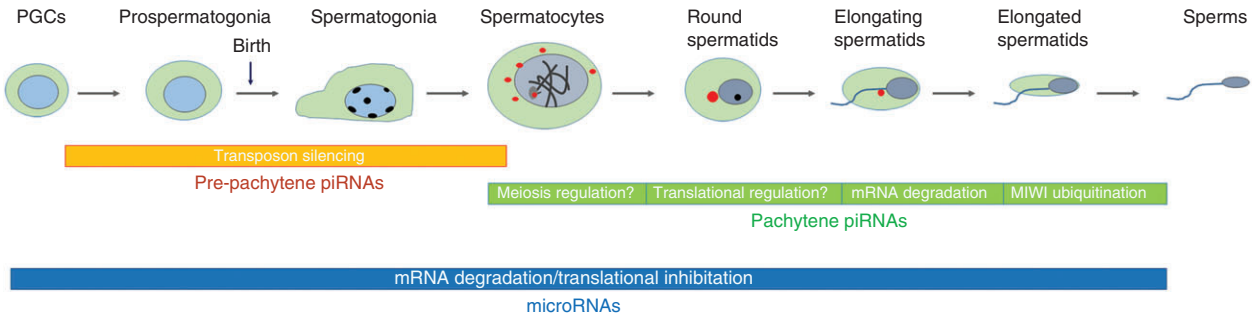


FIGURE 1 | Function of small RNAs in mouse spermatogenesis. A schematic drawing of the expression and function of microRNAs, pre-pachytene piRNAs, and pachytene piRNAs during mouse spermatogenesis.

transported into the cytoplasm via Exportin 5 and Ran GTPase.[14] The pre-miRNAs are then cleaved by Dicer, another member of the RNase III family, into 19–23 bp miRNA/miRNA* duplexes, which consist of a mature miRNA and its complementary miRNA*. These duplexes are loaded onto AGO subfamily members of the Argonaute protein family to assemble an miRNA-induced silencing complex (miRISC), where the mature miRNA strand is retained and the other strand (passenger) is discarded during miRISC assembly.[15–17] The miRISC is guided to target mRNAs by imperfect base pairing between miRNAs and miRNA regulatory elements (MREs) in the 3′ untranslated region (3′UTR) of target mRNAs, which leads to translational repression or/and decay of target mRNAs.[18,19]

Recent findings have indicated a general necessity for miRNAs in spermatogenesis, as genetic or siRNA-mediated ablation of key factors required for the biogenesis of miRNAs, including Dicer and Drosha, blocks male germ cell development. For instance, loss of Dicer in Sertoli cells severely impairs their competence and leads to progressive testicular degeneration, male infertility and a complete absence of spermatozoa,[20,21] thereby suggesting an essential role of the Dicer-dependent miRNA/siRNA pathway in mammalian male germ cell development. Moreover, a recent study reported that selective inactivation of Drosha or Dicer in spermatogenic cells depletes spermatocytes and spermatids in the testes and leads to oligoteratozoospermia or azoospermia, thereby indicating that either the Drosha-dependent canonical miRNA pathway or the Dicer-dependent miRNA/siRNA pathway is critically important for male germ cell development.[22]

Importantly, several miRNAs have been shown to be temporally regulated during male germ cell development. Hayashi et al. showed that the *mir-17-92* and *mir-290-295* clusters were highly expressed in mouse primordial germ cells (PGCs) and spermatogonia.[23] They also found that the levels of miR-141, miR-200a, miR-200c, and miR-323 are reduced with the progression of PGC development, but the *let-7* family miRNAs are upregulated,[23] strongly suggesting that these miRNAs may play roles in spermatogenesis in mammals. Moreover, recent studies have pinpointed the roles of several miRNAs in different steps of spermatogenesis in mammals.

First, a group of miRNAs has been shown to be required for maintenance of the undifferentiated state of SSCs. A recent study showed that miR-21, miR-34c, miR-182, miR-183, and miR-146a were abundant in mouse SSCs.[24] Interestingly, *mir-21* is controlled by ETV5, a critical transcription factor for maintaining the self-renewal of SSCs, and plays an important role in regulating SSC homeostasis.[24] miR-221/222 was also shown to be crucial for maintaining the undifferentiated state of mammalian spermatogonia and SSC capacity through negative regulation of *Kit*.[25] Kit is known to be a critical factor for the differentiation of spermatogonia in mammals.[26]

Second, as the key step in spermatogenesis, spermatogonial differentiation has been demonstrated to be modulated by several miRNAs. Two miRNA clusters, namely *mir-17-92* and *mir-106b-25*, have been found to be downregulated during retinoic acid (RA)-induced spermatogonial differentiation.[27] As a result, their putative targets, namely *Bim*, *Kit*, *Socs3*, and *Stat3*, which are known to be important for spermatogonial development,[28–31] are upregulated by RA in spermatogonia, suggesting that these miRNAs may regulate the differentiation of spermatogonia in mice.[27] *mir-146* is also highly regulated by RA in mouse spermatogonia and modulates RA-induced spermatonial differentiation by targeting *Med1*,[32] which is a co-regulator of retinoid receptors.[33,34] Moreover, a previous study showed that the *mir-17-92* cluster protected meiotic cells from apoptosis through suppression of the E2F1 transcription factor.[35] *mir-18* is highly expressed in spermatocytes and plays a role

in spermatogenesis through regulation of heat shock factor 2 (HSF2).[36] HSF2 is a critical transcription factor controlling a large group of genes in mouse testes and is essential for male germ cell development in mice.[37] In addition, *mir-34c* is highly induced in mouse pachytene spermatocytes, and this enhanced expression persists in spermatids. Overexpression of miR-34c increases male germ cell apoptosis by targeting *Atf1*,[38] a critical factor that sustains cell viability during early embryonic development.[39,40] Interestingly, a recent study reported that miR-34c is expressed in SSCs of dairy goats and promotes apoptosis of SSCs in a p53-dependent manner.[41] Additionally, miR-34c is also present in mouse sperm, and this sperm-borne miRNA has been shown to be important for the first cell division of zygotes via modulation of *Bcl-2* expression.[42] Together, these findings suggest that a number of miRNAs are tightly regulated during spermatogonial differentiation, while these miRNAs play multiple roles in spermatogenic cells by regulating their specific targets.

Third, miRNA regulation is involved in the unique post-transcriptional regulation in spermiogenesis. Because chromatin condensation blocks transcription in the elongating phase, the genes required for spermiogenesis need to be transcribed in pachytene spermatocytes and early round spermatids, and they are then stably stored as messenger ribonucleoproteins (mRNPs) until translation during spermiogenesis.[43–45] Thus, post-transcriptional regulation is important for post-meiotic male germ cell development. Transition nuclear protein 2 (TNP2) and Protamine 2 (Prm2) are chromatin remodelers that are essential for spermatid elongation and completion of spermiogenesis.[46] A recent study showed that testis-specific miR-469 sequestered *Tnp2* and *Prm2* mRNAs from translation in pachytene spermatocytes and round spermatids with a minor effect on their stability.[47] In contrast, miR-122a, which is enriched in late-stage male germ cells and predominantly in polysomes, mediates the degradation of *Tnp2* mRNA.[48] Together, these findings indicate that miRNAs are involved in the post-transcriptional regulation of genes in spermatid development. Moreover, *mir-23b*, *mir-30c*, *mir-30d*, and *mir-690* have been found to be regulated by FSH and androgens in Sertoli cells, and these miRNAs play important roles in cell adhesion and spermiation.[49] In addition, the nucleus of mature spermatozoa contains a complex population of mRNAs and miRNAs, thereby suggesting potential functions in early embryonic development.[50,51]

Collectively, these findings demonstrate the importance of miRNA regulation in each step of spermatogenesis, including the mitotic phase, meiotic phase, spermiogenesis, and final release of spermatozoa into the lumen of seminiferous tubules.

siRNAs IN MALE GERM CELLS

siRNAs are a class of small RNAs that processed from double-stranded RNAs (dsRNAs) by Dicer, which are loaded onto Ago2 and mediate the cleavage of target mRNAs via the perfect or near-perfect base-pairing rule. This biological process has been termed as RNA interference (RNAi) and widely used as a tool to modulate gene expression *in vitro* and *in vivo*.[52–54] Endogenous siRNAs (endo-siRNAs) were first found in yeast and then in plants, worms and flies.[55–59] In addition to their well-characterized roles in post-transcriptional gene silencing in the cytoplasm, endo-siRNAs have been suggested to function in the nucleus as guidance molecules to direct their associated protein factors to specific genomic regions and promote DNA methylation and heterochromatin formation.[56] In yeast and worms, endo-siRNAs are processed by Dicer from dsRNA precursors produced by RNA-dependent RNA polymerase (RdRP).[60,61] Given that RdRP has not been identified in flies or mammals, the naturally occurring dsRNAs in certain cell types, including hairpin-dsRNAs, *trans*-antisense transcript-derived dsRNAs, and *cis*-antisense transcript-derived dsRNAs, are considered to be the endo-siRNA precursors for Dicer processing.[62] In mammals, endo-siRNAs were first reported in murine oocytes and embryonic stem cells.[63–65] Consistent with its evolutionarily conserved roles in antiviral defense in many organisms, RNAi was recently found to function as a mechanism of antiviral immunity in mammals.[66,67] Intriguingly, Song et al. showed that endo-siRNAs were also expressed in mouse spermatogenic cells,[68] but their function in these cells remains largely unclear.

piRNAs IN MALE GERM CELLS

The evolutionarily conserved *piwi* family genes, which encode the germline-specific members of the Argonaute protein family,[69–73] are indispensable to germline development in animals.[74,75] The murine *piwi* homologs, namely *miwi*, *mili*, and *miwi2*, are temporally and spatially regulated during spermatogenesis. *miwi* is expressed from the pachytene stage of meiosis to the haploid spermatid elongating stage.[70] *mili* is expressed from 12.5 dpc in fetal prospermatogonia to the round spermatid stage.[76–78] *miwi2* expression is detected from 15.5 dpc to 3 dpp in the

nonproliferative phase of fetal prospermatogonia.[79] Genetic studies have shown that depletion of any of the *Piwi* genes in mice leads to male sterility, demonstrating that PIWI proteins are essential for spermatogenesis.[70,72,76–81]

Recently, interest in PIWI proteins has been ignited by the finding that they are associated with a novel class of germ cell-specific small ncRNAs. In 2006, four independent groups reported a new class of small ncRNAs in fruit fly, mouse, rat, and human germline cells, which were associated with PIWI proteins and thus termed as PIWI-interacting RNAs (piRNAs).[82–86] The studies later on indicate that PIWI proteins are involved in both the biogenesis and function of piRNAs.[79,87–90] piRNAs are single-stranded RNAs with a size of approximately 24–32 nt and a strong preference for a uridine at the 5′ end.[82,83,86] Another unique feature for this type of small ncRNAs is the Hen1-mediated 2′-O-methylation at their 3′ end, which protects them from the addition of nontemplated uridylation and subsequent destabilization.[91–93]

The biogenesis of piRNAs is distinct from that of other small ncRNAs. Unlike miRNAs and siRNAs produced by Dicer from hairpin/dsRNA precursors, piRNAs are processed from long single-stranded precursors in a Dicer-independent manner.[73,87,94,95] piRNAs originate from a few hundred genomic regions called piRNA clusters, which range in length from several kb to >100 kb in mice. The piRNA clusters are transcribed by RNA polymerase II to generate 5′-capped, polyadenylated, long, and single-stranded transcripts.[96] These primary piRNA transcripts are then exported into the cytoplasm and processed into 24–32 nt piRNAs via the DEAD box proteins UAP56 and Vasa.[97] Two distinct mechanisms have been suggested for piRNA biogenesis, i.e., the primary processing pathway and the 'Ping-Pong amplification loop' pathway.[98] Noticeably, the endonuclease activities of PIWI proteins have been shown to be required for piRNA amplification.[88,89]

The piRNA pathway has been deemed as an innate immune system that prevents mobile genetic elements from destabilizing DNA and that protects genome integrity in animal germ cells. Genetic studies in mice, *Drosophila*, zebrafish, and *C. elegans* have demonstrated a critical function of the piRNA pathway in silencing mobile genetic elements in animal germ cells.[61,74,90] Consistently, the germ cell defects in piRNA pathway mutants are generally accompanied by derepression of transposons and retrotransposons in germ lines. Increasing evidence shows that PIWI/piRNA machinery in animal germline cells silences transposable elements at both epigenetic and post-transcriptional levels.[90,99,100] For example, MILI and MIWI2, which are loaded by transposon-derived piRNAs, mediate the *de novo* DNA methylation of transposon elements in mice.[79] The *mili*-defective mutants show derepression of the long interspersed nuclear element (LINE-1) and intracisternal A particle (IAP), thereby resulting in a loss of DNA methylation of L1 elements.[101] In *mili*-null and *miwi*2-null male fetal testes, DNA methylation of the regulatory regions of the Line-1 and IAP retrotransposons is defective, indicating the failure of *de novo* methylation.[102,103] These findings demonstrate an evolutionarily conserved role for PIWI proteins in transposon suppression.

Intriguingly, in addition to silencing transposable elements, the PIWI/piRNA pathway has also been found to regulate coding genes in *Drosophila*. The traffic jam (tj)-encoding protein controls gonad morphogenesis in *Drosophila*, and the tj transcript also serves as the precursor for piRNAs. Saito et al. showed that these tj-derived piRNAs were loaded on PIWI and silence specific target genes, such as FasIII.[104] Moreover, two Drosophila piRNAs, namely roo and 412, in complex with PIWI, the Smaug RNA-binding protein and the CCR4 deadenylase, degrade the maternal mRNA in early embryos through imperfect base pairing.[105] Similar to previous findings that miRNAs play important roles in transcript clearance during the maternal-to-zygotic transition,[106] piRNAs also appears to function as important components in maternal degradation machinery in animals.

In mice, piRNAs are expressed in two distinct phases during male germ cell development and are respectively termed as pre-pachytene and pachytene piRNAs.[107] Pre-pachytene piRNAs, which are enriched in transposon sequences and co-expressed with MIWI2 and/or MILI in early stages of spermatogenesis, are primarily involved in *de novo* DNA methylation in fetal and perinatal male germ cells.[79,102,108] The function of pachytene piRNAs, which are strongly induced mainly from nontransposon intergenic regions and largely co-expressed with MIWI in pachytene spermatocytes and post-meiotic spermatids, remained elusive. Although MIWI can bind and translationally represses spermiogenic mRNAs in a piRNA-independent manner in mouse testes,[109,110] our recent study indicated that MIWI, in complex with pachytene piRNAs and a deadenylase CAF1, assembles the pi-RISC, which mediates the decay of a large population of mRNAs in elongating spermatids.[111] Interestingly, although we still do not know the molecular mechanisms underlying the assembly of pi-RISC, our data clearly showed that

TABLE 1 | List of Proteins in piRNA Pathway

Gene Symbol	Spermatogenic Arrest Stage in Mutant	Interacting Proteins	References
Miwi	Round spermatid	TDRD8, CAF1, GASZ, MAEL, MOV10L1, TDRD6, TDRD2, MVH	70,111,113–121
Mili	Zygotene	MAEL, MOV10L1, TDRD12, TDRD6, TDRD1, MVH	76,77,115,117,118,120,122,123,124
Miwi2	Leptotene	TDRD9, TDRD2	72,79,114,125
Mvh	Zygotene	MAEL, MIWI, MILI	76,120,121,126
Tdrd1	Spermatocyte, Round spermatid	TDRD12, MILI	122–124,127
Tdrd2	Zygotene	MIWI, MIWI2	113,114
Tdrd5	Round spermatid	Unknown	128
Tdrd6	Round spermatid	MIWI, MILI	115
Tdrd7	Round spermatid	Unknown	129,130
Tdrd8	No obvious defect	MIWI	116,131,132
Tdrd9	Zygotene	MIWI2	125
Tdrd12	Zygotene-Pachytene transition	MILI, TDRD1,	122
MitoPLD	Zygotene	Unknown	133
Mov10l1	Zygotene	MIWI, MILI	117,118
Mael	Pachytene	MIWI, MILI, MVH	120,134
Gasz	Zygotene-Pachytene	MIWI	119
Gtsf1	Leptotene	PIWI	135–137
Caf1	Elongating spermatids	MIWI	111,138

pi-RISC was mainly assembled in elongating spermatids, which may explain the dual role of MIWI in protecting or degrading mRNAs depending on its state of piRNA loading and association with CAF1. Our findings elucidate a key function of pachytene piRNAs in development, and also reveal an important mechanism responsible for the elimination of mRNAs in later stages of spermiogenesis. Intriguingly, we found that at a later stage of spermiogenesis, piRNAs trigger MIWI ubiquitination by a multi-subunit E3 ubiquitin ligase the Anaphase Promoting Complex/Cyclosome (APC/C), thus leading to the degradation of MIWI protein through the Ubiquitin-26S proteasome pathway.[112] Moreover, such piRNA-triggered MIWI destruction leads to piRNA elimination, thereby suggesting a feed-forward mechanism for coordinated removal of the MIWI/piRNA machinery after it fulfills all of its function for spermatogenesis. However, the mechanism of MILI and MIWI2 degradation during spermatogenesis remains unknown.

Remarkably, recent studies have characterized a number of key components in the PIWI/piRNA machinery (Table 1). In mice, several tudor domain-containing proteins, which are preferentially expressed in the germline and essential to germ cell development, were found to be associated with PIWI proteins.[129,139,140,141] Tudor domain-containing proteins most likely serve as scaffolds to recruit PIWI proteins by binding symmetric arginine dimethylation (sDMA) to form germline granules,[113,142] which are essential for piRNA biogenesis and PIWI/piRNA function in spermatogenesis.[143] In addition, germinal granules/nauges are a type of unique electron-dense and amorphous structures in cytoplasm and are ubiquitously present in animal germ cells.[144–146] In mammals, several classes of RNA-containing granules have been observed during spermatogenesis. A typical class of germinal granules is the chromatoid body (CB), which is a large RNP aggregate that is first visible in the cytoplasm of mid-to-late meiotic spermatocytes and persistently develops in round spermatids.[147,148] Because the CB contains many RNA-binding proteins that are involved in different RNA regulation pathways, including RNA helicases, RNA decay machinery and other proteins, the CB has been proposed as a specific site for RNA storage and processing.[149–151] Noticeably, both MIWI and piRNAs are highly enriched in CBs in round spermatids,[149,152] indicating that CBs may be a center for MIWI and pachytene piRNA function.

DYSREGULATION OF SMALL ncRNAs AND MALE INFERTILITY

Growing evidence has shown that aberrant miRNA regulation is associated with human male infertility. By comparing the testicular miRNA profiles between infertile and normal healthy males, recent studies identified a large set of miRNAs dysregulated in asthenozoospermia, Sertoli cell only (SCO), mixed atrophy (MA), and germ cell arrest (GA) patients,[153–155] implicating that aberrant miRNA expression is associated with male infertility. Interestingly, several single-nucleotide polymorphisms (SNPs) have been found in miRNA-binding sites of candidate genes that are critical for male fertility, which may affect the expression of these genes and increase the risk of male infertility.[156] Moreover, a recent study found that SNPs in Dicer and Drosha are associated with semen quality,[157] further suggesting that miRNAs play critical roles in male fertility. Additionally, in line with the findings that extracellular/circulating miRNAs are present in various biological fluids,[158–162] recent studies showed that the miRNA profiles in seminal plasma from the patients with morphologically abnormal/low motility sperm or NOA were significantly different from that in seminal plasma from healthy donors.[163–165] Although the function of the dysregulated miRNAs in sperm movement, structural integrity, and metabolism remains elusive, these miRNA signatures may be used as biomarkers for the diagnosis of male infertility.[163,164]

In addition, Gu et al. analyzed SNPs in *piwi* genes from 490 males with idiopathic azoospermia/oligozoospermia and 468 fertile control males[166] and found that several SNPs in human *piwi* genes might be related to the risk of spermatogenic failure or associated with increased risk of oligozoospermia in the Chinese population.[166] Recent studies found that allele-specific DNA methylation differences in PIWIL1 and PIWIL2 were associated with disturbed spermatogenesis and male infertility.[167] These findings suggest that genetic variations in piRNA pathways may also affect male fertility in humans.

CONCLUSIONS

The existing data demonstrate critical roles of small ncRNAs in spermatogenesis in animals and in human infertility. As small RNAs can be efficiently targeted by stable antisense oligonucleotides, approaches to target specific regulatory ncRNAs should be explored to understand their biological functions and action mechanisms in spermatogenesis and to develop strategies for disease intervention in clinical applications. Additionally, there are many questions that remain in spermatogenesis in animals. (1) What are the determinants for the differentiation or self-renewal of SSCs? (2) What signal(s) triggers meiosis? (3) How is homologous recombination regulated? (4) How is spermiogenesis initiated? Given that ncRNA research has been greatly facilitated by recent advancements in genomic technologies and informatic approaches, we anticipate that more novel species of ncRNAs will be found in male germ cells, which may contribute to answering the remaining questions in the field.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Science and Technology of China (2011CB811303, 2014CB964802, 2014CB943103), National Natural Science Foundation of China (31325008, 91219306, 31270840), and Chinese Academy of Sciences (KJZD-EW-L01-2).

REFERENCES

1. Clermont Y, Bustos-Obregon E. Re-examination of spermatogonial renewal in the rat by means of seminiferous tubules mounted “in toto”. *Am J Anat* 1968, 122:237–247.
2. Huckins C, Oakberg EF. Morphological and quantitative analysis of spermatogonia in mouse testes using whole mounted seminiferous tubules. *Anat Rec* 1978, 192:529–542.
3. Hess RA. Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. *Biol Reprod* 1990, 43:525–542.
4. Dettin L, Ravindranath N, Hofmann MC, Dym M. Morphological characterization of the spermatogonial subtypes in the neonatal mouse testis. *Biol Reprod* 2003, 69:1565–1571.
5. Yan W. Male infertility caused by spermiogenic defects: lessons from gene knockouts. *Mol Cell Endocrinol* 2009, 306:24–32.

6. Lee RC, Feinbaum RL, Ambros VR. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993, 75:843–854.
7. Cai X, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA* 2004, 10:1957–1966.
8. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 2004, 23:4051–4060.
9. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003, 425:415–419.
10. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the microprocessor complex. *Nature* 2004, 432:231–235.
11. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. The microprocessor complex mediates the genesis of microRNAs. *Nature* 2004, 432:235–240.
12. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha–DGCR8 complex in primary microRNA processing. *Genes Dev* 2004, 18:3016–3027.
13. Landthaler M, Yalcin A, Tuschl T. The human DiGeorge syndrome critical region gene 8 and its *D. melanogaster* homolog are required for miRNA biogenesis. *Curr Biol* 2004, 14:2162–2167.
14. Lund E, Güttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of MicroRNA precursors. *Science* 2004, 303:95–98.
15. Hutvágner G, McLachlan J, Pasquinelli AE, Bálint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 2001, 293:834–838.
16. Saito K, Ishizuka A, Siomi H, Siomi MC. Processing of pre-microRNAs by the Dicer-1-Loquacious complex in Drosophila cells. *PLoS Biol* 2005, 3:e235.
17. Lee Y, Hur I, Park SY, Kim YK, Suh MR, Kim VN. The role of PACT in the RNA silencing pathway. *EMBO J* 2006, 25:522–532.
18. Hutvagner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. *Science* 2002, 297:2056–2060.
19. Martinez J, Tuschl T. RISC is a 5′ phosphomonoester-producing RNA endonuclease. *Genes Dev* 2004, 18:975–980.
20. Papaioannou MD, Pitetti JL, Ro S, Park C, Aubry F, Schaad O, Vejnar CE, Kuhne F, Descombes P, Zdobnov EM, et al. Sertoli cell Dicer is essential for spermatogenesis in mice. *Dev Biol* 2009, 326: 250–259.
21. Papaioannou MD, Nef S. microRNAs in the testis: building up male fertility. *J Androl* 2010, 31:26–33.
22. Wu Q, Song R, Ortogero N, Zheng H, Evanoff R, Small CL, Griswold MD, Namekawa SH, Royo H, Turner JM, et al. The RNase III enzyme DROSHA is essential for microRNA production and spermatogenesis. *J Biol Chem* 2012, 287:25173–25190.
23. Hayashi K, Chuva de Sousa Lopes SM, Kaneda M, Tang F, Hajkova P, Lao K, O'Carroll D, Das PP, Tarakhovsky A, Miska EA, et al. MicroRNA biogenesis is required for mouse primordial germ cell development and spermatogenesis. *PLoS One* 2008, 3:e1738.
24. Niu Z, Goodyear SM, Rao S, Wu X, Tobias JW, Avarbock MR, Brinster RL. MicroRNA-21 regulates the self-renewal of mouse spermatogonial stem cells. *Proc Natl Acad Sci USA* 2011, 108:12740–12745.
25. Yang QE, Racicot KE, Kaucher AV, Oatley MJ, Oatley JM. MicroRNAs 221 and 222 regulate the undifferentiated state in mammalian male germ cells. *Development* 2013, 140:280–290.
26. de Rooij DG. Stem cells in the testis. *Int J Exp Pathol* 1998, 79:67–80.
27. Tong MH, Mitchell DA, McGowan SD, Evanoff R, Griswold MD. Two miRNA clusters, Mir-17-92 (Mirc1) and Mir-106b-25 (Mirc3), are involved in the regulation of spermatogonial differentiation in mice. *Biol Reprod* 2012, 86:72.
28. Coultas L, Bouillet P, Loveland KL, Meachem S, Perlman H, Adams JM, Strasser A. Concomitant loss of proapoptotic BH3-only Bcl-2 antagonists Bik and Bim arrests spermatogenesis. *EMBO J* 2005, 24:3963–3973.
29. Rossi P, Sette C, Dolci S, Geremia R. Role of c-kit in mammalian spermatogenesis. *J Endocrinol Invest* 2000, 23:609–615.
30. Winnall WR, Muir JA, Hedger MP. Rat resident testicular macrophages have an alternatively activated phenotype and constitutively produce interleukin-10 in vitro. *J Leukoc Biol* 2011, 90:133–143.
31. Oatley JM, Kaucher AV, Avarbock MR, Brinster RL. Regulation of mouse spermatogonial stem cell differentiation by STAT3 signaling. *Biol Reprod* 2010, 83:427–433.
32. Huszar JM, Payne CJ. MicroRNA 146 (Mir146) modulates spermatogonial differentiation by retinoic acid in mice. *Biol Reprod* 2013, 88:15.
33. Yuan CX, Ito M, Fondell JD, Fu ZY, Roeder RG. The TRAP220 component of a thyroid hormone receptor- associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. *Proc Natl Acad Sci USA* 1998, 95:7939–7944.
34. McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev* 1999, 20:321–344.
35. Novotny GW, Sonne SB, Nielsen JE, Jonstrup SP, Hansen MA, Skakkebaek NE, Rajpert-De Meyts E, Kjems J, Leffers H. Translational repression of E2F1

mRNA in carcinoma in situ and normal testis correlates with expression of the miR-17-92 cluster. *Cell Death Differ* 2007, 14:879–882.

36. Björk JK, Sandqvist A, Elsing AN, Kotaja N, Sistonen L. miR-18, a member of Oncomir-1, targets heat shock transcription factor 2 in spermatogenesis. *Development* 2010, 137:3177–3184.
37. Akerfelt M, Henriksson E, Laiho A, Vihervaara A, Rautoma K, Kotaja N, Sistonen L. Promoter ChIP-chip analysis in mouse testis reveals Y chromosome occupancy by HSF2. *Proc Natl Acad Sci USA* 2008, 105:11224–11229.
38. Liang X, Zhou D, Wei C, Luo H, Liu J, Fu R, Cui S. MicroRNA-34c enhances murine male germ cell apoptosis through targeting ATF1. *PLoS One* 2012, 7:e33861.
39. Bleckmann SC, Blendy JA, Rudolph D, Monaghan AP, Schmid W, Schütz G. Activating transcription factor 1 and CREB are important for cell survival during early mouse development. *Mol Cell Biol* 2002, 22:1919–1925.
40. Jin XL, O'Neill C. The presence and activation of two essential transcription factors (cAMP response element-binding protein and cAMP-dependent transcription factor ATF1) in the two-cell mouse embryo. *Biol Reprod* 2010, 82:459–468.
41. Li M, Yu M, Liu C, Zhu H, He X, Peng S, Hua J. miR-34c works downstream of p53 leading to dairy goat male germline stem-cell (mGSCs) apoptosis. *Cell Prolif* 2013, 46:223–231.
42. Liu WM, Pang RT, Chiu PC, Wong BP, Lao K, Lee KF, Yeung WS. Sperm-borne microRNA-34c is required for the first cleavage division in mouse. *Proc Natl Acad Sci USA* 2012, 109:490–494.
43. Steger K. Haploid spermatids exhibit translationally repressed mRNAs. *Anat Embryol (Berl)* 2001, 203:323–334.
44. Sassone-Corsi P. Unique chromatin remodeling and transcriptional regulation in spermatogenesis. *Science* 2002, 296:2176–2178.
45. Dadoune JP, Siffroi JP, Alfonsi MF. Transcription in haploid male germ cells. *Int Rev Cytol* 2004, 237:1–56.
46. Zhao M, Shirley CR, Hayashi S, Marcon L, Mohapatra B, Suganuma R, Behringer RR, Boissonneault G, Yanagimachi R, Meistrich ML. Transition nuclear proteins are required for normal chromatin condensation and functional sperm development. *Genesis* 2004, 38:200–213.
47. Dai L, Tsai-Morris CH, Sato H, Villar J, Kang JH, Zhang J, Dufau ML. Testis-specific miRNA-469 up-regulated in gonadotropin-regulated testicular RNA helicase (GRTH/DDX25)-null mice silences transition protein 2 and protamine 2 messages at sites within coding region: implications of its role in germ cell development. *J Biol Chem* 2011, 286:44306–44318.
48. Yu Z, Raabe T, Hecht NB. MicroRNA Mirn122a reduces expression of the posttranscriptionally regulated germ cell transition protein 2(Tnp2) messenger RNA (mRNA) by mRNA cleavage. *Biol Reprod* 2005, 73:427–433.
49. Nicholls PK, Harrison CA, Walton KL, McLachlan RI, O'Donnell L, Stanton PG. Hormonal regulation of Sertoli cell micro-RNAs at spermiation. *Endocrinology* 2011, 152:1670–1683.
50. Hamatani T. Human spermatozoal RNAs. *Fertil Steril* 2012, 97:275–281.
51. Sendler E, Johnson GD, Mao S, Goodrich RJ, Diamond MP, Hauser R, Krawetz SA. Stability, delivery and functions of human sperm RNAs at fertilization. *Nucleic Acids Res* 2013, 41:4104–4117.
52. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001, 411:494–498.
53. Paddison PJ, Caudy AA, Hannon GJ. Stable suppression of gene expression by RNAi in mammalian cells. *Proc Natl Acad Sci USA* 2002, 99:1443–1448.
54. Lu PY, Xie F, Woodle MC. In vivo application of RNA interference: from functional genomics to therapeutics. *Adv Genet* 2005, 54:117–142.
55. Martienssen RA, Zaratiegui M, Goto DB. RNA interference and heterochromatin in the fission yeast *Schizosaccharomyces pombe*. *Trends Genet* 2005, 21:450–456.
56. Ruby JG, Jan C, Player C, Axtell MJ, Lee W, Nusbaum C, Ge H, Bartel DP. Large-scale sequencing reveals 21U-RNAs and additional microRNAs and endogenous siRNAs in *C. elegans*. *Cell* 2006, 127:1193–1207.
57. Vaucheret H. Post-transcriptional small RNA pathways in plants: mechanisms and regulations. *Genes Dev* 2006, 20:759–771.
58. Sijen T, Steiner FA, Thijssen KL, Plasterk RH. Secondary siRNAs result from unprimed RNA synthesis and form a distinct class. *Science* 2007, 315:244–247.
59. Michalik KM, Böttcher R, Förstemann K. A small RNA response at DNA ends in Drosophila. *Nucleic Acids Res* 2012, 40:9596–9603.
60. Castel SE, Martienssen RA. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nat Rev Genet* 2013, 14:100–112.
61. Luteijn MJ, Ketting RF. PIWI-interacting RNAs: from generation to transgenerational epigenetics. *Nat Rev Genet* 2013, 14:523–534.
62. Okamura K, Lai EC. Endogenous small interfering RNAs in animals. *Nat Rev Mol Cell Biol* 2008, 9:673–678.

63. Watanabe T, Totoki Y, Toyoda A, Kaneda M, Kuramochi-Miyagawa S, Obata Y, Chiba H, Kohara Y, Kono T, Nakano T, et al. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. *Nature* 2008, 453:539–543.
64. Tam OH, Aravin AA, Stein P, Girard A, Murchison EP, Cheloufi S, Hodges E, Anger M, Sachidanandam R, Schultz RM, et al. Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes. *Nature* 2008, 453:534–538.
65. Babiarz JE, Ruby JG, Wang Y, Bartel DP, Blelloch R. Mouse ES cells express endogenous shRNAs, siRNAs, and other microprocessor-independent, dicer-dependent small RNAs. *Genes Dev* 2008, 22:2773–2785.
66. Li Y, Lu J, Han Y, Fan X, Ding SW. RNA interference functions as an antiviral immunity mechanism in mammals. *Science* 2013, 342:231–234.
67. Maillard PV, Ciaudo C, Marchais A, Li Y, Jay F, Ding SW, Voinnet O. Antiviral RNA interference in mammalian cells. *Science* 2013, 342:235–238.
68. Song R, Hennig GW, Wu Q, Jose C, Zheng H, Yan W. Male germ cells express abundant endogenous siRNAs. *Proc Natl Acad Sci USA* 2011, 108:13159–13164.
69. Cox DN, Chao A, Baker J, Chang L, Qiao D, Lin H. A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes Dev* 1998, 12:3715–3727.
70. Deng W, Lin H. miwi, a murine homolog of piwi, encodes a cytoplasmic protein essential for spermatogenesis. *Dev Cell* 2002, 2:819–830.
71. Klattenhoff C, Bratu DP, McGinnis-Schultz N, Koppetsch BS, Cook HA, Theurkauf WE. Drosophila rasiRNA pathway mutations disrupt embryonic axis specification through activation of an ATR/Chk2 DNA damage response. *Dev Cell* 2007, 12:45–55.
72. Carmell MA, Girard A, van de Kant HJ, Bourc'his D, Bestor TH, de Rooij DG, Hannon GJ. MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline. *Dev Cell* 2007, 12:503–514.
73. Houwing S, Kamminga LM, Berezikov E, Cronembold D, Girard A, van den Elst H, Filippov DV, Blaser H, Raz E, Moens CB, et al. A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. *Cell* 2007, 129:69–82.
74. Thomson T, Lin H. The biogenesis and function of PIWI proteins and piRNAs: progress and prospect. *Annu Rev Cell Dev Biol* 2009, 25:355–376.
75. Siomi MC, Kuramochi-Miyagawa S. RNA silencing in germlines—exquisite collaboration of Argonaute proteins with small RNAs for germline survival. *Curr Opin Cell Biol* 2009, 21:426–434.
76. Kuramochi-Miyagawa S, Kimura T, Ijiri TW, Isobe T, Asada N, Fujita Y, Ikawa M, Iwai N, Okabe M, Deng W, et al. Mili, a mammalian member of piwi family gene, is essential for spermatogenesis. *Development* 2004, 131:839–849.
77. Unhavaithaya Y, Hao Y, Beyret E, Yin H, Kuramochi-Miyagawa S, Nakano T, Lin H. MILI, a PIWI-interacting RNA-binding protein, is required for germ line stem cell self-renewal and appears to positively regulate translation. *J Biol Chem* 2009, 284:6507–6519.
78. Wang J, Saxe JP, Tanaka T, Chuma S, Lin H. Mili interacts with tudor domain-containing protein 1 in regulating spermatogenesis. *Curr Biol* 2009, 19: 640–644.
79. Aravin AA, Sachidanandam R, Bourc'his D, Schaefer C, Pezic D, Toth KF, Bestor T, Hannon GJ. A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. *Mol Cell* 2008, 31:785–799.
80. Cerutti L, Mian N, Bateman A. Domains in gene silencing and cell differentiation proteins: the novel PAZ domain and redefinition of the Piwi domain. *Trends Biochem Sci* 2000, 25:481–482.
81. Kuramochi-Miyagawa S, Kimura T, Yomogida K, Kuroiwa A, Tadokoro Y, Fujita Y, Sato M, Matsuda Y, Nakano T. Two mouse piwi-related genes: miwi and mili. *Mech Dev* 2001, 108:121–133.
82. Aravin AA, Gaidatzis D, Pfeffer S, Lagos-Quintana M, Landgraf P, Iovino N, Morris P, Brownstein MJ, Kuramochi-Miyagawa S, Nakano T, et al. A novel class of small RNAs bind to MILI protein in mouse testes. *Nature* 2006, 442:203–207.
83. Girard A, Sachidanandam R, Hannon GJ, Carmell MA. A germline-specific class of small RNAs binds mammalian Piwi proteins. *Nature* 2006, 442:199–202.
84. Grivna ST, Beyret E, Wang Z, Lin H. A novel class of small RNAs in mouse spermatogenic cells. *Genes Dev* 2006, 20:1709–1714.
85. Lau NC, Seto AG, Kim J, Kuramochi-Miyagawa S, Nakano T, Bartel DP, Kingston RE. Characterization of the piRNA complex from rat testes. *Science* 2006, 313:363–367.
86. Kim VN. Small RNAs just got bigger: Piwi-interacting RNAs (piRNAs) in mammalian testes. *Genes Dev* 2006, 20:1993–1997.
87. Gunawardane LS, Saito K, Nishida KM, Miyoshi K, Kawamura Y, Nagami T, Siomi H, Siomi MC. A slicer-mediated mechanism for repeat-associated siRNA 5′ end formation in Drosophila. *Science* 2007, 315:1587–1590.
88. De Fazio S, Bartonicek N, Di Giacomo M, Abreu-Goodger C, Sankar A, Funaya C, Antony C, Moreira PN, Enright AJ, O'Carroll D. The endonuclease activity of Mili fuels piRNA amplification that silences LINE1 elements. *Nature* 2011, 480:259–263.

89. Reuter M, Berninger P, Chuma S, Shah H, Hosokawa M, Funaya C, Antony C, Sachidanandam R, Pillai RS. Miwi catalysis is required for piRNA amplification-independent LINE1 transposon silencing. *Nature* 2011, 480:264–267.
90. Siomi MC, Sato K, Pezic D, Aravin AA. PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol* 2011, 12:246–258.
91. Kirino Y, Mourelatos Z. 2′-O-methyl modification in mouse piRNAs and its methylase. *Nucleic Acids Symp Ser (Oxf)* 2007, 51:417–418.
92. Ohara T, Sakaguchi Y, Suzuki T, Ueda H, Miyauchi K, Suzuki T. The 3′ termini of mouse Piwi-interacting RNAs are 2′-O-methylated. *Nat Struct Mol Biol* 2007, 14:349–350.
93. Kurth HM, Mochizuki K. 2′-O-methylation stabilizes Piwi-associated small RNAs and ensures DNA elimination in tetrahymena. *RNA* 2009, 15:675–685.
94. Vagin VV, Sigova A, Li C, Seitz H, Gvozdev V, Zamore PD. A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* 2006, 313:320–324.
95. Saito K, Nishida KM, Mori T, Kawamura Y, Miyoshi K, Nagami T, Siomi H, Siomi MC. Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the Drosophila genome. *Genes Dev* 2006, 20:2214–2222.
96. Li XZ, Roy CK, Dong X, Bolcun-Filas E, Wang J, Han BW, Xu J, Moore MJ, Schimenti JC, Weng Z, et al. An ancient transcription factor initiates the burst of piRNA production during early meiosis in mouse testes. *Mol Cell* 2013, 50:67–81.
97. Zhang F, Wang J, Xu J, Zhang Z, Koppetsch BS, Schultz N, Vreven T, Meignin C, Davis I, Zamore PD, et al. UAP56 couples piRNA clusters to the perinuclear transposon silencing machinery. *Cell* 2012, 151:871–884.
98. Guzzardo PM, Muerdter F, Hannon GJ. The piRNA pathway in flies: highlights and future directions. *Curr Opin Genet Dev* 2013, 23:44–52.
99. Saxe JP, Lin H. Small noncoding RNAs in the germline. *Cold Spring Harb Perspect Biol* 2011, 3:a002717.
100. Castañeda J, Genzor P, Bortvin A. piRNAs, transposon silencing, and germline genome integrity. *Mutat Res* 2011, 714:95–104.
101. Aravin AA, Sachidanandam R, Girard A, Fejes-Toth K, Hannon GJ. Developmentally regulated piRNA clusters implicate MILI in transposon control. *Science* 2007, 316:744–747.
102. Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Totoki Y, Toyoda A, Ikawa M, Asada N, Kojima K, Yamaguchi Y, Ijiri TW, et al. DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. *Genes Dev* 2008, 22:908–917.
103. Bao J, Zhang Y, Schuster AS, Ortogero N, Nilsson EE, Skinner MK, Yan W. Conditional inactivation of Miwi2 reveals that MIWI2 is only essential for prospermatogonial development in mice. *Cell Death Differ* 2014, 21:783–796.
104. Saito K, Inagaki S, Mituyama T, Kawamura Y, Ono Y, Sakota E, Kotani H, Asai K, Siomi H, Siomi MC. A regulatory circuit for piwi by the large Maf gene traffic jam in Drosophila. *Nature* 2009, 461: 1296–1299.
105. Rouget C, Papin C, Boureux A, Meunier AC, Franco B, Robine N, Lai EC, Pelisson A, Simonelig M. Maternal mRNA deadenylation and decay by the piRNA pathway in the early Drosophila embryo. *Nature* 2010, 467:1128–1132.
106. Walser CB, Lipshitz HD. Transcript clearance during the maternal-to-zygotic transition. *Curr Opin Genet Dev* 2011, 21:431–443.
107. Meikar O, Da Ros M, Korhonen H, Kotaja N. Chromatoid body and small RNAs in male germ cells. *Reproduction* 2011, 142:195–209.
108. Watanabe T, Chuma S, Yamamoto Y, Kuramochi-Miyagawa S, Totoki Y, Toyoda A, Hoki Y, Fujiyama A, Shibata T, Sado T, et al. MITOPLD is a mitochondrial protein essential for nuage formation and piRNA biogenesis in the mouse germline. *Dev Cell* 2011, 20:364–375.
109. Vourekas A, Zheng Q, Alexiou P, Maragkakis M, Kirino Y, Gregory BD, Mourelatos Z. Mili and Miwi target RNA repertoire reveals piRNA biogenesis and function of Miwi in spermiogenesis. *Nat Struct Mol Biol* 2012, 19:773–781.
110. Nishibu T, Hayashida Y, Tani S, Kurono S, Kojima-Kita K, Ukekawa R, Kurokawa T, Kuramochi-Miyagawa S, Nakano T, Inoue K, et al. Identification of MIWI-associated Poly(A) RNAs by immunoprecipitation with an anti-MIWI monoclonal antibody. *Biosci Trends* 2012, 6:248–261.
111. Gou LT, Dai P, Yang JH, Xue YC, Hu YP, Zhou Y, Kang JY, Wang X, Li H, Hua MM, et al. Pachytene piRNAs instruct massive mRNA elimination during late spermiogenesis. *Cell Res* 2014, 24:680–700.
112. Zhao S, Gou LT, Zhang M, Zu LD, Hua MM, Hua Y, Shi HJ, Li Y, Li J, Li D, et al. piRNA-triggered MIWI ubiquitination and removal by APC/C in late spermatogenesis. *Dev Cell* 2013, 24:13–25.
113. Chen C, Jin J, James DA, Adams-Cioaba MA, Park JG, Guo Y, Tenaglia E, Xu C, Gish G, Min J, et al. Mouse Piwi interactome identifies binding mechanism of Tdrkh Tudor domain to arginine methylated Miwi. *Proc Natl Acad Sci USA* 2009, 106: 20336–20341.
114. Saxe JP, Chen M, Zhao H, Lin H. Tdrkh is essential for spermatogenesis and participates in primary piRNA biogenesis in the germline. *EMBO J* 2013, 32:1869–1885.

115. Vasileva A, Tiedau D, Firooznia A, Müller-Reichert T, Jessberger R. Tdrd6 is required for spermiogenesis, chromatoid body architecture, and regulation of miRNA expression. *Curr Biol* 2009, 19: 630–639.

116. Bao J, Wang L, Lei J, Hu Y, Liu Y, Shen H, Yan W, Xu C. STK31(TDRD8) is dynamically regulated throughout mouse spermatogenesis and interacts with MIWI protein. *Histochem Cell Biol* 2012, 137: 377–389.

117. Frost RJ, Hamra FK, Richardson JA, Qi X, Bassel-Duby R, Olson EN. MOV10L1 is necessary for protection of spermatocytes against retrotransposons by Piwi-interacting RNAs. *Proc Natl Acad Sci USA* 2010, 107:11847–11852.

118. Zheng K, Xiol J, Reuter M, Eckardt S, Leu NA, McLaughlin KJ, Stark A, Sachidanandam R, Pillai RS, Wang PJ. Mouse MOV10L1 associates with Piwi proteins and is an essential component of the Piwi-interacting RNA (piRNA) pathway. *Proc Natl Acad Sci USA* 2010, 107:11841–11846.

119. Ma L, Buchold GM, Greenbaum MP, Roy A, Burns KH, Zhu H, Han DY, Harris RA, Coarfa C, Gunaratne PH, et al. GASZ is essential for male meiosis and suppression of retrotransposon expression in the male germline. *PLoS Genet* 2009, 5:e1000635.

120. Costa Y, Speed RM, Gautier P, Semple CA, Maratou K, Turner JM, Cooke HJ. Mouse MAELSTROM: the link between meiotic silencing of unsynapsed chromatin and microRNA pathway? *Hum Mol Genet* 2006, 15:2324–2334.

121. Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Takamatsu K, Chuma S, Kojima-Kita K, Shiromoto Y, Asada N, Toyoda A, et al. MVH in piRNA processing and gene silencing of retrotransposons. *Genes Dev* 2010, 24:887–892.

122. Pandey RR, Tokuzawa Y, Yang Z, Hayashi E, Ichisaka T, Kajita S, Asano Y, Kunieda T, Sachidanandam R, Chuma S, et al. Tudor domain containing 12 (TDRD12) is essential for secondary PIWI interacting RNA biogenesis in mice. *Proc Natl Acad Sci USA* 2013, 110:16492–16497.

123. Reuter M, Chuma S, Tanaka T, Franz T, Stark A, Pillai RS. Loss of the Mili-interacting Tudor domain-containing protein-1 activates transposons and alters the Mili-associated small RNA profile. *Nat Struct Mol Biol* 2009, 16:639–646.

124. Wang J, Saxe JP, Tanaka T, Chuma S, Lin H. Mili interacts with tudor domain-containing protein 1 in regulating spermatogenesis. *Curr Biol* 2009, 19: 640–644.

125. Shoji M, Tanaka T, Hosokawa M, Reuter M, Stark A, Kato Y, Kondoh G, Okawa K, Chujo T, Suzuki T, et al. The TDRD9-MIWI2 complex is essential for piRNA-mediated retrotransposon silencing in the mouse male germline. *Dev Cell* 2009, 17:775–787.

126. Tanaka SS, Toyooka Y, Akasu R, Katoh-Fukui Y, Nakahara Y, Suzuki R, Yokoyama M, Noce T. The mouse homolog of Drosophila Vasa is required for the development of male germ cells. *Genes Dev* 2000, 14:841–853.

127. Chuma S, Hosokawa M, Kitamura K, Kasai S, Fujioka M, Hiyoshi M, Takamune K, Noce T, Nakatsuji N. Tdrd1/Mtr-1, a tudor-related gene, is essential for male germ-cell differentiation and nuage/germinal granule formation in mice. *Proc Natl Acad Sci USA* 2006, 103:15894–15899.

128. Yabuta Y, Ohta H, Abe T, Kurimoto K, Chuma S, Saitou M. TDRD5 is required for retrotransposon silencing, chromatoid body assembly, and spermiogenesis in mice. *J Cell Biol* 2011, 192:781–795.

129. Tanaka T, Hosokawa M, Vagin VV, Reuter M, Hayashi E, Mochizuki AL, Kitamura K, Yamanaka H, Kondoh G, Okawa K, et al. Tudor domain containing 7 (Tdrd7) is essential for dynamic ribonucleoprotein (RNP) remodeling of chromatoid bodies during spermatogenesis. *Proc Natl Acad Sci USA* 2011, 108:10579–10584.

130. Lachke SA, Alkuraya FS, Kneeland SC, Ohn T, Aboukhalil A, Howell GR, Saadi I, Cavallesco R, Yue Y, Tsai AC, et al. Mutations in the RNA granule component TDRD7 cause cataract and glaucoma. *Science* 2011, 331:1571–1576.

131. Zhou J, Leu NA, Eckardt S, McLaughlin KJ, Wang PJ. STK31/TDRD8, a germ cell-specific factor, is dispensable for reproduction in mice. *PLoS One* 2014, 9:e89471.

132. Bao J, Yuan S, Maestas A, Bhetwal BP, Schuster A, Yan W. Stk31 is dispensable for embryonic development and spermatogenesis in mice. *Mol Reprod Dev* 2013, 80:786.

133. Watanabe T, Chuma S, Yamamoto Y, Kuramochi-Miyagawa S, Totoki Y, Toyoda A, Hoki Y, Fujiyama A, Shibata T, Sado T, et al. MITOPLD is a mitochondrial protein essential for nuage formation and piRNA biogenesis in the mouse germline. *Dev Cell* 2011, 20:364–375.

134. Soper SF, van der Heijden GW, Hardiman TC, Goodheart M, Martin SL, de Boer P, Bortvin A. Mouse maelstrom, a component of nuage, is essential for spermatogenesis and transposon repression in meiosis. *Dev Cell* 2008, 15:285–297.

135. Yoshimura T, Toyoda S, Kuramochi-Miyagawa S, Miyazaki T, Miyazaki S, Tashiro F, Yamato E, Nakano T, Miyazaki J. Gtsf1/Cue110, a gene encoding a protein with two copies of a CHHC Zn-finger motif, is involved in spermatogenesis and retrotransposon suppression in murine testes. *Dev Biol* 2009, 335:216–227.

136. Ohtani H, Iwasaki YW, Shibuya A, Siomi H, Siomi MC, Saito K. DmGTSF1 is necessary for Piwi-piRISC-mediated transcriptional transposon

silencing in the Drosophila ovary. *Genes Dev* 2013, 27:1656–1661.

137. Dönertas D, Sienski G, Brennecke J. Drosophila Gtsf1 is an essential component of the Piwi-mediated transcriptional silencing complex. *Genes Dev* 2013, 27:1693–1705.
138. Berthet C, Morera AM, Asensio MJ, Chauvin MA, Morel AP, Dijoud F, Magaud JP, Durand P, Rouault JP. CCR4-associated factor CAF1 is an essential factor for spermatogenesis. *Mol Cell Biol* 2004, 24:5808–5820.
139. Goulet I, Boisvenue S, Mokas S, Mazroui R, Côté J. TDRD3, a novel Tudor domain-containing protein, localizes to cytoplasmic stress granules. *Hum Mol Genet* 2008, 17:3055–3074.
140. Yabuta Y, Ohta H, Abe T, Kurimoto K, Chuma S, Saitou M. TDRD5 is required for retrotransposon silencing, chromatoid body assembly, and spermiogenesis in mice. *J Cell Biol* 2011, 192:781–795.
141. Yang Y, Lu Y, Espejo A, Wu J, Xu W, Liang S, Bedford MT. TDRD3 is an effector molecule for arginine-methylated histone marks. *Mol Cell* 2010, 40:1016–1023.
142. Kirino Y, Kim N, de Planell-Saguer M, Khandros E, Chiorean S, Klein PS, Rigoutsos I, Jongens TA, Mourelatos Z. Arginine methylation of Piwi proteins catalysed by dPRMT5 is required for Ago3 and Aub stability. *Nat Cell Biol* 2009, 11:652–658.
143. Meikar O, Da Ros M, Korhonen H, Kotaja N. Chromatoid body and small RNAs in male germ cells. *Reproduction* 2011, 142:195–209.
144. Mahowald AP. Polar granules of Drosophila. II. Ultrastructural changes during early embryogenesis. *J Exp Zool* 1968, 167:237–261.
145. al-Mukhtar KA, Webb AC. An ultrastructural study of primordial germ cells, oogonia and early oocytes in *Xenopus laevis*. *J Embryol Exp Morphol* 1971, 26:195–217.
146. Eddy EM. Fine structural observations on the form and distribution of nuage in germ cells of the rat. *Anat Rec* 1974, 178:731–757.
147. Eddy EM. Germ plasm and the differentiation of the germ cell line. *Int Rev Cytol* 1975, 43:229–280.
148. Chuma S, Hosokawa M, Tanaka T, Nakatsuji N. Ultrastructural characterization of spermatogenesis and its evolutionary conservation in the germline: germinal granules in mammals. *Mol Cell Endocrinol* 2009, 306:17–23.
149. Kotaja N, Bhattacharyya SN, Jaskiewicz L, Kimmins S, Parvinen M, Filipowicz W, Sassone-Corsi P. The chromatoid body of male germ cells: similarity with P-bodies and presence of Dicer and microRNA components. *Proc Natl Acad Sci USA* 2006, 103:2647–2652.
150. Kotaja N, Sassone-Corsi P. The chromatoid body: a germ-cell-specific RNA-processing centre. *Nat Rev Mol Cell Biol* 2007, 8:85–90.
151. Nguyen Chi M, Chalmel F, Agius E, Vanzo N, Khabar KS, Jégou B, Morello D. Temporally regulated traffic of HuR and its associated ARE-containing mRNAs from the chromatoid body to polysomes during mouse spermatogenesis. *PLoS One* 2009, 4:e4900.
152. Beyret E, Lin H. Pinpointing the expression of piRNAs and function of the PIWI protein subfamily during spermatogenesis in the mouse. *Dev Biol* 2011, 355:215–226.
153. Lian J, Zhang X, Tian H, Liang N, Wang Y, Liang C, Li X, Sun F. Altered microRNA expression in patients with non-obstructive azoospermia. *Reprod Biol Endocrinol* 2009, 7:13.
154. Abu-Halima M, Hammadeh M, Schmitt J, Leidinger P, Keller A, Meese E, Backes C. Altered microRNA expression profiles of human spermatozoa in patients with different spermatogenic impairments. *Fertil Steril* 2013, 99:1249–1255.
155. Abu-Halima M, Backes C, Leidinger P, Keller A, Lubbad AM, Hammadeh M, Meese E. MicroRNA expression profiles in human testicular tissues of infertile men with different histopathologic patterns. *Fertil Steril* 2014, 101:78–86.
156. Zhang H, Liu Y, Su D, Yang Y, Bai G, Tao D, Ma Y, Zhang S. A single nucleotide polymorphism in a miR-1302 binding site in CGA increases the risk of idiopathic male infertility. *Fertil Steril* 2011, 96: 34–39.
157. Qin Y, Xia Y, Wu W, Han X, Lu C, Ji G, Chen D, Wang H, Song L, Wang S, et al. Genetic variants in microRNA biogenesis pathway genes are associated with semen quality in a Han-Chinese population. *Reprod Biomed Online* 2012, 24:454–461.
158. Zhu H, Fan GC. Extracellular/circulating microRNAs and their potential role in cardiovascular disease. *Am J Cardiovasc Dis* 2011, 1:138–149.
159. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008, 105: 10513–10518.
160. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, et al. Serum microRNAs are promising novel biomarkers. *PLoS One* 2008, 3:e3148.
161. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen TD, et al. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One* 2008, 3:e3694.
162. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The microRNA spectrum in 12 body fluids. *Clin Chem* 2010, 56:1733–1741.

163. Curry E, Safranski TJ, Pratt SL. Differential expression of porcine sperm microRNAs and their association with sperm morphology and motility. *Theriogenology* 2011, 76:1532–1539.

164. Wang C, Yang C, Chen X, Yao B, Yang C, Zhu C, Li L, Wang J, Li X, Shao Y, et al. Altered profile of seminal plasma microRNAs in the molecular diagnosis of male infertility. *Clin Chem* 2011, 57: 1722–1731.

165. Wu W, Qin Y, Li Z, Dong J, Dai J, Lu C, Guo X, Zhao Y, Zhu Y, Zhang W, et al. Genome-wide microRNA expression profiling in idiopathic non-obstructive azoospermia: significant up-regulation of miR-141, miR-429 and miR-7-1-3p. *Hum Reprod* 2013, 28:1827–1836.

166. Gu A, Ji G, Shi X, Long Y, Xia Y, Song L, Wang S, Wang X. Genetic variants in Piwi-interacting RNA pathway genes confer susceptibility to spermatogenic failure in a Chinese population. *Hum Reprod* 2010, 25:2955–2961.

167. Friemel C, Ammerpohl O, Gutwein J, Schmutzler AG, Caliebe A, Kautza M, von Otte S, Siebert R, Bens S. Array-based DNA methylation profiling in male infertility reveals allele-specific DNA methylation in PIWIL1 and PIWIL2. *Fertil Steril* 2014, pii:S0015-0282(13)03490-0.