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Review

Review

Advances in PIWI-piRNA function in female reproduction in mammals

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Abstract

PIWI-interacting RNAs (piRNAs), which associate with PIWI clade Argonaute proteins to form piRNA-induced silencing complexes (piRISCs) in germline cells, are responsible for maintaining genomic integrity and reproductive function through transcriptional or post-transcriptional suppression of transposable elements and regulation of protein-coding genes. Recent discoveries of crucial PIWI-piRNA functions in oogenesis and embryogenesis in golden hamsters suggest an indispensable role in female fertility that has been obscured in the predominant mouse model of PIWI-piRNA pathway regulation. In particular, studies of piRNA expression dynamics, functional redundancies, and compositional variations across mammal species have advanced our understanding of piRNA functions in male and, especially, female reproduction. These findings further support the use of hamsters as a more representative model of piRNA biology in mammals. In addition to discussing these new perspectives, the current review also covers emerging directions for piRNA research, its implications for female fertility, and our fundamental understanding of reproductive mechanisms.

Key words piRNA, PIWI, transposon, oocytes, fertility

Introduction

During gametogenesis and early embryogenesis in animals, transposon elements (TEs) are actively transcribed as a result of epigenetic reprogramming, which can cause severe damage to the host genome [1–4]. In most animals, genome integrity is guided by a conserved adaptive immune system known as the PIWI-interacting RNAs (piRNAs) pathway in the germline [5–7]. piRNAs are an animal-specific class of small non-coding RNAs that are distinct from microRNAs (miRNAs) and small interfering RNAs (siRNAs) [8–12]. piRNAs guide PIWI clade Argonautes (PIWI proteins) rather than AGO clade proteins, which are known to mediate miRNA- and siRNA-dependent regulatory pathways [13]. PIWI-piRNA complexes, namely, piRNA-induced silencing complexes (piRISCs), primarily function in silencing TEs at both the transcriptional and post-transcriptional levels in animal germ cells [5,6]. Moreover, increasing evidence suggests that the PIWI-piRNA machinery can also mediate the regulation of protein-coding genes [14,15]. These critical activities of piRNAs in germ cells are indispensable for fertility in most animals [7].

In mice, which have served for decades as a common mammalian model for studying PIWI-piRNAs, the disruption of any *Piwi* genes or other genes critical for piRNA biogenesis leads to the arrest of spermatogenesis but does not impair oogenesis or female fertility [10,16–20]. However, recent evidence indicates that small non-coding RNAs in mouse oocytes may not be representative of other mammals, including humans [21,22], as previously thought. In particular, recent studies in golden hamsters have shown that piRNAs are essential for female fertility and that the absence of any piRNA population can lead to arrested embryonic development [23–26]. It is thus apparent that the piRNA pathway plays essential roles in female reproduction, which has long been underappreciated and warrants careful examination in other mammals. Here, we discuss recent advances in our understanding of the biological functions of the piRNA pathway in mammalian oocytes and early embryos, especially with a focus on golden hamsters and humans.

piRNAs Biogenesis and Their Role(s) in Fertility

PIWI proteins belong to the Argonaute family, which comprises the AGO clade and PIWI clade subfamilies. piRNAs specifically associate with PIWI proteins to form piRISCs, similar to their

miRNA and endogenous siRNA (endo-siRNA) counterparts, which associate with AGO clade to form small RNA-induced silencing complexes (RISCs) [27]. AGO-miRNAs are ubiquitously expressed in animals, whereas AGO-endo-siRNAs and PIWI-piRNAs are specifically expressed in gametes and early embryos [5,28].

piRNAs are processed primarily from long single-stranded RNA precursors transcribed from specific genomic regions enriched with piRNAs, known as the piRNA cluster [8,11,12,29,30]. However, an exception is found in nematodes, where piRNAs (21U-RNAs) are processed from 25–27 nucleotide (nt) single-stranded precursors transcribed from their own mini-genes [31–33]. Nascent single-stranded piRNA transcripts are first processed by Zucchini/MitoPLD [30,34–38]. These antisense "primary piRNAs" then associate with PIWI proteins to recognize and cleave complementary transposon RNAs. The resulting 3′ cleavage product is subsequently converted into a new sense piRNA that associates with the same or another PIWI clade protein. In turn, these newly formed piRISCs can recognize and cleave piRNA cluster transcripts. This reciprocal targeting for cleavage by PIWI-piRNA complexes between TEs and piRNA cluster transcripts, referred to as the Ping-Pong cycle, can thus amplify piRNA populations while silencing TE mRNAs and generate signatures between the piRNAs with 10 nt overlap at their 5′-ends [30,34,39]. The Ping-Pong cycle is conjointly accompanied by the phased production of piRNAs downstream of the cleavage site, which increases piRNA sequence diversity to support the arms race against rapidly evolving TEs [39,40]. The 3′ ends of piRNAs are determined by the activity of various exonucleases, depending on the species (*e.g.*, Nibbler in *Drosophila*, Trimmer in silkworm, and PNLDC1 in mice) [41–43], and are ultimately methylated by HENMT1 to form 2′-O-methylation at the 3′ end [44–46].

In most animal models, piRNAs have been identified as the dominant small non-coding RNAs in germ cells and are essential for fertility [7,47]. Seminal PIWI studies in *Drosophila melanogaster* demonstrated that all three PIWI proteins are necessary for fertility in both males and females through contributions to genomic tandem repeats and transposable elements repression, primordial germ cell specification and development, germline stem cell self-renewal and differentiation, and embryonic axis determination [30,48–57]. In contrast, disruption of *Miwi* (*Piwil1*), *Mili* (*Piwil2*), *Miwi2* (*Piwil4*), or other key piRNA pathway genes results in male infertility without affecting female fertility in mice [16–19,58–61]. Furthermore, in mice, loss of function in any key components of the piRNA pathway leads to severe defects in male germ line development, accompanied by TE derepression, but causes no detectable defects in female germ line development (Figure 1A,B) [6,7].

Golden Hamster as a Model for piRNA and PIWI Function in Female Reproduction in Mammals

Although the vast majority of mammalian PIWI protein studies have focused on mice, only three *Piwi* genes (*Piwil1*, *Piwil2*, and *Piwil4*) are encoded in the mouse or rat genomes [62]. However, most other mammalian genomes that have been searched were found to encode four *Piwi* genes (*Piwil1*, *Piwil2*, *Piwil3*, and *Piwil4*), and the *Piwi* that is absent in mice, *Piwil3*, is specifically expressed in the oocytes of other mammals, including humans (Figure 2A,B) [21,22,63–65]. Furthermore, the composition of small RNA populations in mouse oocytes significantly differs from that in human or monkey oocytes, which do not express endo-siRNAs but instead express oocyte short piRNAs (os-piRNAs) [21]. os-piRNAs, located in more than 200 clusters in the human genome, account for more than 70% of the total small RNAs expressed in primate oocytes. Over 85% of os-piRNAs are derived from intergenic regions enriched with antisense strands of TEs and their flanking regions [21]. Additionally, os-piRNAs are enriched with TE sequences of the L1 subfamily, which newly evolve in primates and display a clear Ping-Pong signature, indicating a potential role in silencing active TEs in oocytes. These unexpected differences suggest that mice might not be a sufficiently representative model to study mammalian piRNA functions.

Recent investigations of piRNAs and PIWI clade expression in the oocytes of 12 representative vertebrate species revealed that, surprisingly, endo-siRNAs and the truncated Dicer protein are specific to mice and absent in the oocytes of other mammals, including rats. A MalE insertion element is detected in the same *Dicer* region in mice, rats, and golden hamsters but only influences the *Dicer* transcription start site in mice, resulting in the expression of an N-terminally truncated isoform of Dicer ($Dicer^{O}$), which could process dsRNAs more efficiently than wild-type Dicer, and consequently, abundant endo-siRNA production in mouse oocytes [22,66]. Instead, PIWIL3-associated os-piRNAs are widespread in oocytes of other mammalian species (Figure 2B). Additionally, piRNA clusters evolve more rapidly than protein-coding genes do; therefore, piRNA sequences expressed in oocytes are poorly conserved among mammals [22], suggesting that hosts generate these highly diverse piRNA sequences in response to invasions by actively evolving TE families.

Analysis of oocyte-specific small RNA populations has identified four general categories among 11 mammalian species, as follows: (1) humans, monkeys, guinea pigs, rabbits, pigs, and golden hamsters express os-piRNAs and oocyte long piRNAs (ol-piRNAs, 25–32 nt), but not endo-siRNAs; (2) goats exclusively express os-piRNAs; (3) rats and Chinese hamsters exclusively express ol-piRNAs; and (4) mice express both ol-piRNAs and endo-siRNAs (Figure 2B) [22]. Among those in group 1, monkey, golden hamster, and guinea pig oocytes have similar small RNA profiles, especially for os-piRNAs, and their associated PIWI clades are similar to those in human oocytes. However, the rarity and extremely long developmental and gestational cycles render monkeys unsuitable as a genetic model (Figure 2C). Similarly, the long gestation period and low litter size of guinea pigs limits their usefulness for reproduction studies (Figure 2C).

The golden (Syrian) hamster (*Mesocricetus auratus*) belongs to the *Cricetidae* family and has been employed as an experimental model to study reproduction, development, cancer, and infectious diseases (including COVID-19) because its physiology and pharmacological responses are similar to those of humans [67–72]. Importantly, the golden hamster has a consistent and robust estrous cycle (4 days), high responsiveness to conventional superovulation regimens, and a short gestation period (16 days) (Figure 2C) [73]. Notably, the first successful *in vitro* fertilization (IVF) studies were conducted in golden hamsters, which greatly contributed to our current understanding of mammalian fertilization mechanisms, such as sperm-egg interactions and pronuclear formation [74]. Additionally, methods for manipulating gene expression, including CRISPR/Cas9-mediated genome editing, have been successfully applied in golden hamsters [75]. In summary, golden hamster has more favorable reproductive characteristics and maintenance requirements, with a similar oocyte PIWI-piRNA profile to that of humans, supporting its further adoption as a representative model

for PIWI-piRNA mechanistic and functional studies of female reproduction.

PIWI Proteins Are Required for Fertility in Both Male and Female Hamsters

In golden hamsters, PIWIL1 and PIWIL3 are highly expressed during oogenesis and early embryogenesis, which is consistent with severe impairment of female fertility in *Piwil1*- or *Piwil3*-deficient hamsters (Figure 1B) [26]. Among them, deficiency of PIWIL1 leads to female sterility due to arrested blastomere development, whereas PIWIL3 deficiency leads to female subfertility, characterized by reduced litter size and decreased pregnancy rate, in

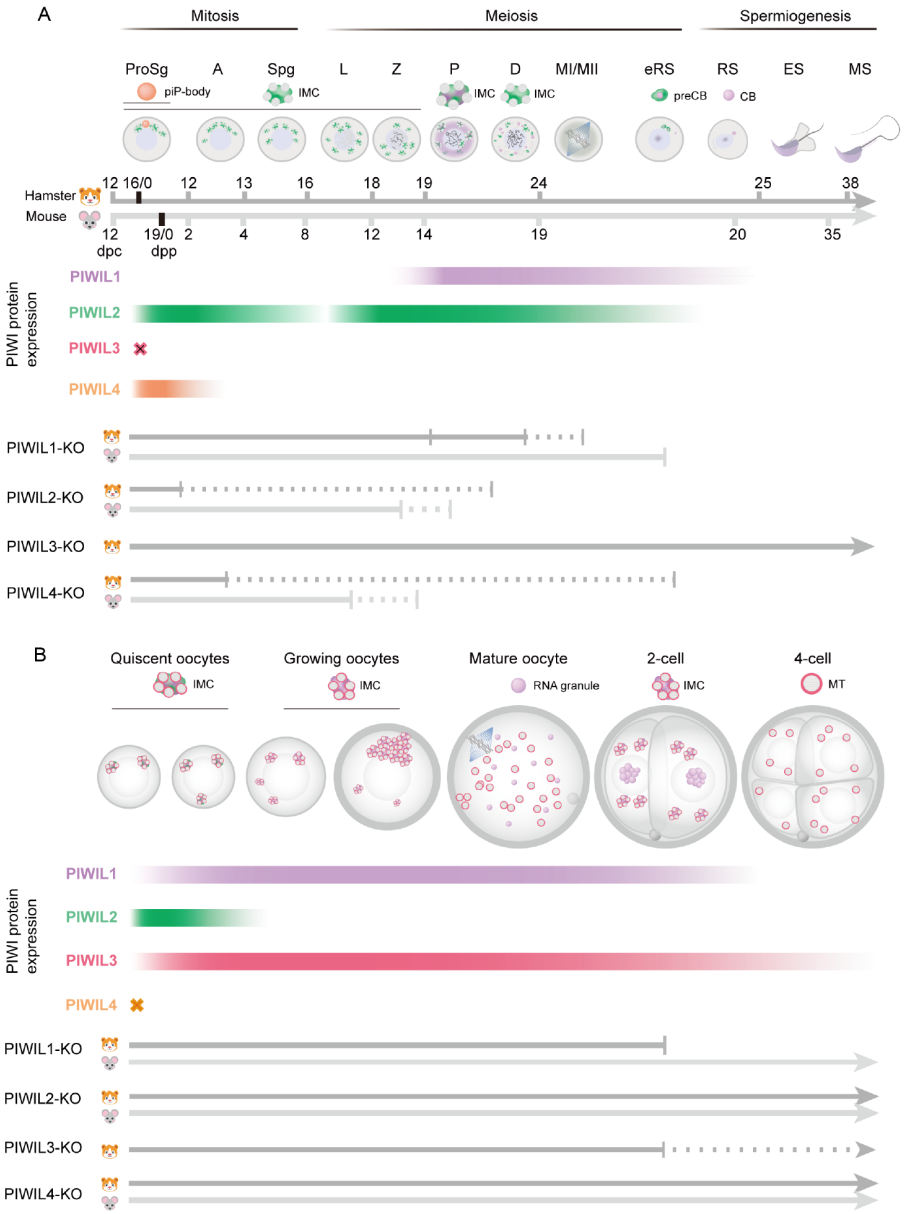
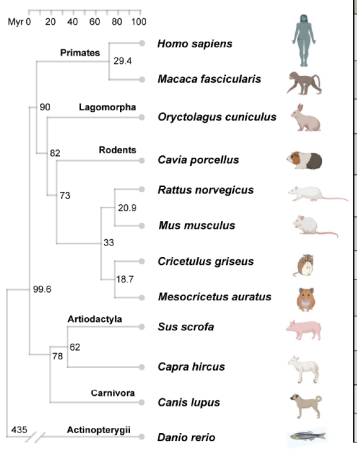


Figure 1. Schematic diagrams showing the expression and the effect of disruption of PIWIs in gametogenesis and early embryogenesis in golden hamsters and mice (A) In spermatogenesis, PIWIL1 (magenta) is specifically expressed in later spermatogenic cells and is located in germ granules. PIWIL2 (green) is present throughout the spermatogenesis and displays exclusively cytoplasmic localization. In contrast, PIWIL4 (orange) is observed in both the nucleus and cytoplasm of prospermatogonia (Prosg). *Piwil1* knockout results in spermatocyte death before the pachytene stage and arrest at the diplotene stage in golden hamsters. The loss of PIWIL2 or PIWIL4 expression leads to the arrest of early spermatogenesis, with only a few germ cells developing to pachytene spermatocytes or round spermatids. PIWIL3 (red) is not expressed in testes, consequently, the loss of PIWIL3 has no detectable impact on spermatogenesis. Spermatogenesis defects in *Piwi*-deficient golden hamsters are much more severe than those in mice. (B) PIWIL1 (magenta) and PIWIL3 (red) are expressed throughout the stages of oogenesis and gradually diminish after zygote cleavage, while PIWIL2 (green) is exclusively expressed in quiescent oocytes. Loss of PIWIL1 or PIWIL3 expression leads to 2-cell arrest in golden hamsters, and only a few *PIWIL3*-deficient blastomeres can develop into live pups. The knockout of *Piwil2* or *Piwil4* does not affect hamster oogenesis and embryogenesis. Solid and dashed lines indicate normal and abnormal development of germ cells or embryos, respectively, while vertical strokes in the grey line signifies the arrested stage. dpc, days postcoitus; dpp, days postpartum; Sg, prospermatogonia; A, type A undifferentiating spermatogonia; Spg, differentiating spermatogonia; PL, preleptotene; L, leptotene; Z, zygotene; P, pachytene; D, diplotene; MI/MII, meiosis I/II; eRS, early round sperm; MS, mature sperm; MT, mitochondria.

A



B

Piwi gene	os-piRNA	ol-piRNA	endo-siRNA
Piwil1-4	17-22nt; PIWIL3-associated	25-32nt; 2′-O-methy 3′-end; PIWIL1-associated	—
Piwil1-4	17-22nt; PIWIL3-associated	25-32nt; 2′-O-methy 3′-end; PIWIL1-associated	—
Piwil1-4	17-21nt	24-29nt; 2′-O-methy 3′-end	—
Piwil1-4	17-22nt; PIWIL3-associated	26-30nt; 2′-O-methy 3′-end; PIWIL1-associated	—
Piwil1-2,Piwil4	—	25-31nt; 2′-O-methy 3′-end; PIWIL1-associated	—
Piwil1-2,Piwil4	—	25-31nt; 2′-O-methy 3′-end; PIWIL1-associated	20-24nt; processed by Dicer^{O}
Piwil1-4	—	25-31nt; 2′-O-methy 3′-end	—
Piwil1-4	17-21nt; PIWIL3-associated	23-31nt; 2′-O-methy 3′-end; PIWIL1-associated	—
Piwil1-4	17-26nt; PIWIL3-associated	22-35nt; 2′-O-methy 3′-end; PIWIL1-associated	—
Piwil1-4	17-27nt; PIWIL3-associated	—	—
Piwil1-4	17-25nt	25-29nt; 2′-O-methy 3′-end	—
Piwil1-2	—	25-30nt; 2′-O-methy 3′-end	—

C

Gestation length (days)	Estrous cycle (days)	Sexual maturity (months)	Body weigh (kg)	Litter size	Litters per year	Genome editing
165	~28	48	4.0	1	1	_
30	~12	4	4.8	6-12	4	CRISPR/CAS9
65	~16	3	0.9	2-8	5	_
16	4	2	0.1	6-15	5	CRISPR/CAS9
115	~21	6	200	11-14	2	_
63	~14	6	6.8	1-10	2	_

Figure 2. Characteristics of *Piwi* genes and piRNAs expressed in GV- or MII-stage oocytes of 12 representative vertebrate species (A) Phylogenetic tree showing relationships between the 12 vertebrate species, including 11 mammals, represent ~81 million years of evolution. The numbers on the tree indicate estimated divergence time in millions of years ago (Myr). (B) *Piwil1*, *Piwil2* and *Piwil4* are encoded in the genome of all 11 mammals. The *Piwil3* gene is absent in the mouse and rat genome, but is encoded in other mammalian genomes. PIWIL3 is associated with oocyte short piRNAs (os-piRNAs), which are shorter than oocyte long piRNAs (ol-piRNAs) and lack the 3′-2′-O-methylation. endo-siRNAs are produced from endogenous, long double-stranded RNAs by Dicer^{O} (oocyte Dicer1) that is specifically expressed in mouse oocytes, which is not expressed in the oocytes of other 11 vertebrate species. (C) Reproductive and developmental characteristics of six mammals that encode four *Piwi* genes and express os-piRNAs and ol-piRNAs in the oocytes.

hamsters [23–26]. PIWIL1 and PIWIL3 protein expression in oocytes is essential for female fertility in hamsters, whereas mice deficient in PIWIs in oocytes exhibit normal female fertility. Additionally, the loss of PIWIL2 has no apparent influence on female fertility in hamsters but is nevertheless highly expressed specifically in primordial or primary stage oocytes (Figure 1B) [26]. This pattern of expression coincides with the highly expressed L1 transcript, which may participate in fetal oocyte attrition by repressing TEs [76]. We speculate that a slight increase in TE insertion frequency arising from PIWIL2 ablation might only cause minor negative effects on fertility in the current generation, whereas the long-term effects of derepressing the TE targets of PIWIL2 in later generations remain unknown.

Notably, PIWIL1, PIWIL2, and PIWIL4 are highly expressed in the same corresponding stage of male gametes, but the loss of any of these PIWIs leads to severe defects in spermatogenesis in both hamsters and mice (Figure 1A) [16–18,23,25,26], suggesting that the function of PIWI in male reproduction is likely conserved across mammals. Notably, individual knockout of *Piwil1*, *Piwil2*, or *Piwil4* led to more severe spermatogenic arrest in golden hamsters than in C57BL/6 mice (Figure 1A). In mice, the "Pachytene piRNAs" associated with PIWIL1 are involved in regulating coding genes essential for meiotic progression in mouse spermatogenesis [15,77–79]. However, expression of the same population of "Pachytene piRNAs" begins earlier in golden hamsters than in mice, which could at least partially explain the greater severity of spermatogenesis defects associated with *Piwil1* deficiency in golden hamsters [26]. Additionally, several recent studies have revealed that loss-of-function mutations in proteins critical for the piRNA pathway also result in impaired spermatogenesis and male infertility in humans [80–84]. These species-specific differences in PIWI functions in male (and female) reproduction highlight the need to interpret mammalian piRNA mechanisms with caution, especially their generalizability to human fertility, regardless of the model used.

PIWI-piRNAs Interplay in Repressing TEs in Mammalian Gametes

PIWI-piRNAs have a conserved function in repressing TEs via complementary base pairing with their target RNAs [27,85]. PIWI proteins are expressed in highly dynamic spatiotemporal patterns throughout the gametogenesis process, which is strictly orchestrated in mice and hamsters [26,58], suggesting that plasticity in the interplay among PIWIs and piRNAs is required for their regulatory activity. For example, in the postnatal testes of mice or hamsters, PIWIL2 produces piRNAs via heterotypic Ping-Pong cycles that bind to PIWIL4, allowing PIWIL4 entry into the nucleus to silence TE transcription (Figure 3). Moreover, the loss of PIWIL4-piRNAs enhances homotypic Ping-Pong signaling by PIWIL2, partially compensating for the loss of transcriptional TE silencing. In contrast, PIWIL4 is blocked from entering the nucleus in PIWIL2-

deficient prospermatogonia, resulting in more severe arrest of spermatogenesis in hamsters [26].

Systematic investigation of PIWI dynamics and characterization of their associated piRNA populations in golden hamster oocytes with individual *Piwi*-knockouts revealed that PIWIL1 binds to 23 nt- and 29 nt-piRNAs, whereas PIWIL3 binds to 19 nt os-piRNAs (Figure 3), resembling PIWI-piRNA profiles observed in primates [21–23,25,26,86]. Both PIWIL1-piRNAs and PIWIL3-os-piRNAs originated from intergenic regions show high overlap (> 90%), and significant correlations were observed in the expression levels of piRNAs from both populations complementary to the same TE [26,86], suggesting that these two PIWI-piRNA systems share some degree of functional redundancy in TE silencing. Indeed, PIWIL1 piRNAs have been shown to suppress active retrotransposons, such as those in the ERVK and ERVL subfamilies, and, to some extent, protein-coding genes, partially compensating for *Piwil3* deficiency in hamster oocytes (Figure 3) [26]. This functional redundancy may explain the negligible effect of *Piwil3* knockout on TE expression and the transcriptomic profile of oocytes, as well as the retention of partial female fertility in golden hamsters.

However, such functional redundancy between PIWIL1-piRNAs and PIWIL3-os-piRNAs observed in hamster oocytes is unlikely to be conserved in humans, as the most abundant piRNAs in hamsters target ERVK class TEs, predominantly comprising rodent-specific intracisternal A particle (IAP) and MYSERV lineage autonomous active retrotransposons that expanded during hamster evolution [24], whereas L1 and Alu lineage retrotransposons are the predominant disease-causing insertion elements in primates [2,87–91]. Additionally, in golden hamsters, most *Piwil3*-deficient females cannot give birth after mating, and few have litters [25,26], suggesting that redundancy in targeting may not complement the full loss of PIWIL3/os-piRNA functions in oocytes, and further investigation is necessary to fully understand the role of PIWIL3 in fertility.

PIWI-piRNAs are Essential for Maternal RNA Regulation and Zygotic Genome Activation

The accumulation of maternal proteins, RNAs and other materials during development is essential for oocyte function and their capacity to initiate totipotency upon fertilization in animals [92–96]. Subsequent clearance of maternal mRNAs is then necessary to eliminate repressive factors and enable zygotic genome activation [97–100]. In golden hamster oocytes, PIWIL1 specifically localizes to RNA-enriched germinal granules in metaphase II (MII)-stage oocytes while translocating into the nucleolus of blastomeres [26], which is consistent with PIWIL1-piRNA deficiency resulting in impaired maternal mRNA degradation and failure of zygotic activation in golden hamsters [23]. It is therefore likely that PIWIL1-piRNAs play a similar role in the maternal-to-zygotic transition in hamster oocytes. In other recent work, maternal RNAs were found to be stored in a mitochondria-associated membraneless compartment (MARDO) in both human and mouse oocytes [93]. This discovery is reminiscent of the preferential co-localization of PIWIL3 with TDRKH on the mitochondrial outer membrane [26], collectively suggesting that PIWIL3-os-piRNAs may be involved in regulating the maternal RNAs required for zygotic reprogramming. Moreover, this possible function is also consistent with the delayed zygotic development and blastomere arrest observed in *Piwil3*-knockout golden hamsters [26]. These findings also illustrate the role of piRNAs in regulating protein-coding gene expression, although their underlying mechanisms remain unknown.

Perspectives

Over the past decade, research in mouse models has uncovered vital roles of PIWI-piRNAs in spermatogenesis, which has led to considerable efforts to identify genetic mutations in piRNA pathway genes that could be linked to male infertility in humans [80,81,101–105]. Consistent with the critical role of the piRNA pathway in spermatogenesis and male fertility, heritable mutations associated with male infertility were indeed found in various piRNA pathway genes [82–84]. However, the dispensable piRNA pathway in mouse female fertility has diminished this research momentum and limited exploration of piRNA biology in mammalian oocytes.

Several studies have recently demonstrated that the piRNA pathway is essential for oogenesis and early embryogenesis in

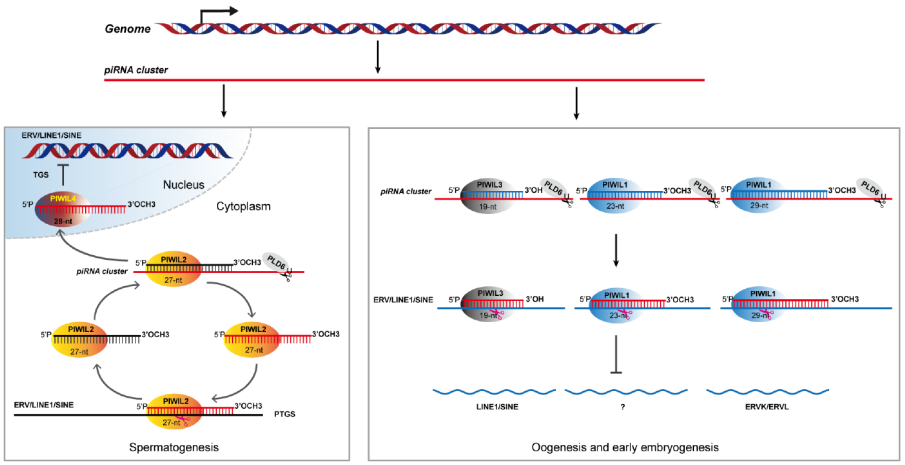


Figure 3. PIWI-piRNA interplay in repressing TEs in mammalian gametes Model for PIWI-piRNA interplay in repressing active TEs in mammalian gametes. In mouse and hamster postnatal prospermatogonia, PIWIL2 produces piRNAs via the Ping-Pong cycle, which disrupts TEs through post-transcriptional gene silencing (PTGS). These piRNAs bind to PIWIL4, allowing PIWIL4 to enter the nucleus and repress TE transcription through transcriptional gene silencing (TGS), as shown in the bottom left. In hamster oocytes and early embryos, the primarily expressed small RNAs are PIWIL1–23 nt piRNA, PIWIL1–29 nt piRNAs, and PIWIL3–19 nt piRNAs (bottom right). PIWIL3–19 nt piRNAs mainly regulate LINE1 and SINE subfamily transposons, while PIWIL1–29 nt piRNAs mainly regulate ERVK and ERVL subfamily transposons. The role of PIWIL1–23 nt piRNAs remains largely unknown.

golden hamsters, challenging the dispensability of PIWI-piRNAs for female fertility in mammals [23–26,106,107]. Furthermore, the conserved expressions and characteristics of PIWI subfamily proteins and piRNAs in the oocytes of several mammal species [22] suggest that the piRNA pathway is potentially essential for female reproduction in most mammals, including humans. This likelihood suggests several new avenues of research that have yet to be explored in the biological functions and regulatory mechanisms of piRNAs in mammalian female reproduction.

First, the molecular mechanism through which PIWI-piRNAs participate in mammalian oogenesis remains elusive. While the common biological functions of PIWI-piRNAs have been identified in both males and females, including transcriptional and post-transcriptional silencing of retrotransposons and regulation of protein-coding genes [6,7], mammalian oogenesis is a complex and unique process involving fetal oocyte attrition, a prolonged quiescent period, and rapid growth and maturation upon hormone stimulation, markedly different from spermatogenesis [108]. Additionally, the oocyte-to-embryo transition involves the restoration and decay of maternal RNAs following the transitions from terminal differentiated to totipotent states and from meiotic to mitotic programs [98]. The role that maternal RNAs play in this process and how PIWI-piRNAs are involved in their regulation remain poorly understood. The dynamic spatiotemporal patterns of PIWI protein expression in oogenesis and early embryogenesis are also noteworthy. For example, PIWIL3 specifically localizes on the mitochondrial surface, which is a common site for piRNA biogenesis [40,109], although little is known about whether and how mitochondrial activity is related to the process of piRNA generation. For example, it is unknown whether the localization of PIWI proteins, such as PIWIL3, to the mitochondrial outer membrane is required for its activity, given that thousands of mitochondria are present in oocytes to provide energy supporting early embryonic development [110–113]. Moreover, PIWIL1 has been detected in dozens of membrane-less RNA-enriched organelles in MII oocytes, which might be involved in RNA processing and regulating stability.

It will also be fascinating to explore the composition and functions of small RNA populations in mammalian oocytes from an evolutionary perspective. Our current limited information suggests that mammals have evolved a variety of small RNA combinations to regulate the expressions of TEs and protein-coding genes, addressing developmental challenges specifically encountered during oogenesis. For example, while most mammals appear to use both os-piRNAs and 30 nt-piRNAs to silence TEs, the requirements for these RNA populations differ among species [22]. In golden hamsters, 30 nt-piRNAs play a central role and can partially compensate for the loss of os-piRNAs in controlling TE bursts [26]. However, in human oocytes, os-piRNAs are primarily responsible for disrupting the large majority of TEs, whereas 30 nt-piRNAs exclusively participate in degrading ERVs (unpublished data). Additionally, some species, such as rats and dogs, utilize only os-piRNAs or 30 nt-piRNAs. In another example, an accidental MalE insertion results in the expression of Dicer^{O} and abundant endo-siRNAs in mouse oocytes. As endo-siRNAs can compensate for the loss of piRNA function in oocytes [66,114], mice are therefore a unique case among mammals. These discoveries underscore the importance of continued exploration of the PIWI-piRNA pathway to ultimately establish a landscape perspective of the evolutionary adaptations and functions of small RNAs in oogenesis.

Finally, the emerging roles of PIWI proteins and piRNAs in human diseases are a topic of considerable interest for the next stage of studies. Future genetic analyses in multiple model organisms will help determine the impacts of PIWI–piRNA functions and evolutionarily-conserved mechanisms underlying piRNA-dependent regulation of TE silencing, RNA degradation, and epigenetic modifications, as well as their roles in female fertility in humans.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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