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Multiple LINEs of retrotransposon silencing mechanisms in the mammalian germline

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Abstract

Retrotransposons play an important role in genome evolution but pose acute challenges to host genome integrity, particularly in early stage germ cells where epigenetic control is relaxed to permit genome-wide reprogramming. In most species, the inability to silence retrotransposons in the germline is usually associated with sterility. LINE1 is the most abundant retrotransposon type in the mammalian genome. Mammalian germ cells employ multiple mechanisms to suppress retrotransposon activity, including small non-coding piRNAs, DNA methylation, and repressive histone modifications. Novel factors contributing to the epigenetic silencing of retrotransposons in the germline continue to be identified. Recent studies have provided insight into how epigenetic changes associated with retrotransposon activation impact on fertility.

Keywords

LINE1; retrotransposon; germline; spermatogenesis; mouse

1. Introduction

Transposable elements (TE) constitute nearly 40% of the mammalian genome. Although often termed “junk DNA” [1], transposable elements play an important role in genome evolution: their integration into new genomic sites can change the level or pattern of expression of neighboring genes, or it can generate new genes, resulting in greater genetic diversity [2]. Transposable elements are highly diverse and include both shared and species-specific elements. Depending on the mode of transposition, mobile DNA segments can be distinguished into retrotransposons that require an RNA intermediate, and DNA transposons that rely on a cut-and-paste mechanism. The vast majority of transposition events produce truncated or inverted inactive copies of transposable elements that accumulate throughout the genome; however, a subset of elements remains intact and is capable of transposition. LINE1 (long interspersed nuclear element-1) retrotransposons are the most abundant class of transposable elements in mammals, accounting for about 20% of the genome in mouse and

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human. Estimates suggest that up to 3000 and 100 copies of LINE1 are intact and active in mouse [3] and in human [4, 5], respectively. The mouse genome also contains the intracisternal A-particle (IAP) element, an active long terminal repeat (LTR)-containing retrotransposon that is not present in human.

LINE1 retrotransposons mobilize in the genome through a “copy and paste” mechanism utilizing reverse transcription. The 6-kb full-length LINE1 element contains two open reading frames (ORF1 and ORF2). The LINE1 ORF1 protein is an RNA-binding protein that forms a trimer and possesses nucleic acid chaperone activity [6]. The ORF2 protein exhibits endonuclease and reverse transcriptase activities [7, 8]. After translation of the bicistronic LINE1 mRNA in the cytoplasm, ORF1 and ORF2 proteins bind the LINE1 mRNA transcript, forming ribonucleoprotein (RNP) particles that are re-imported into the nucleus, followed by reverse transcription and integration at a new genomic location. In addition to the two ORFs, LINE1 contains a 5′ untranslated region (UTR) that functions as an internal promoter. LINE1 is usually methylated at the CpG islands of its promoter and thus silenced.

Genomic integration of retrotransposons can potentially disrupt the function of any gene. More than 60 human genetic diseases are caused by retrotransposon insertion [1]. Retrotransposons, as “genomic parasites”, have enormous capacity to propagate in the host genome. The host genome has evolved mechanisms to suppress the activity of retrotransposons, and the resulting battle between retrotransposons and the host genome is dynamic and continues to evolve [2]. To propagate, retrotransposons must mobilize either in the germline or in early embryonic somatic cells that will give rise to germ cells. However, uncontrolled retrotransposon activity can damage the germline, resulting in sterility. Therefore, given the extreme abundance of LINE1 retrotransposons in the genome and the importance of germline genome integrity, it is critical that the germline ensures silencing of retrotransposons.

The genome is particularly vulnerable to the activity of retrotransposons during the time period of extensive epigenetic reprogramming that occurs in mammalian primordial germ cells (PGCs) at the fetal stage (Fig. 1). Mammalian PGCs originate from epiblast cells that are primed to a somatic cell fate. To acquire germ cell potency, these cells undergo an extensive reprogramming process that leaves PGCs in an epigenetic “ground state” [9], allowing for the subsequent setting of sex-specific epigenetic marks (Fig. 1). In mammals, PGCs remain bipotent till sex determination (embryonic day 12.5 in mice), after which male germ cells enter mitotic arrest till after birth, whereas female germ cells enter meiosis. During mitotic arrest, the germline re-establishes its epigenome including parental-specific imprints through *de novo* DNA methylation by DNA methyltransferases (DNMT3A and DNMT3B) and their non-catalytic homologue DNMT3L [10–12].

The epigenetic reprogramming process in early stage germ cells represents the most extensive erasure of the epigenome during the mammalian life cycle [13] and involves multiple parallel mechanisms, including chromatin remodeling, genome-wide DNA de-methylation, and erasure of parental imprints [14]. Because the associated genome-wide DNA de-methylation results in relaxation of transcriptional repression of retrotransposons,

germ cells have developed molecular mechanisms to control the expression and propagation of retrotransposons at multiple levels. Recent progress in this field has provided new insight into these silencing mechanisms.

2. The piRNA pathway-mediated silencing of transposable elements

The piRNA pathway is a highly conserved RNA-based mechanism that contributes to the silencing of transposable elements in the germline of metazoa. Details of this pathway, which relies on the interaction of Piwi-interacting RNAs (piRNA) with Piwi proteins, have been reviewed elsewhere [15–20]. Here, we will summarize recent advances in the field that provide new insight into its role in retrotransposon silencing. Piwi proteins are a gonad-specific class of Argonaute proteins. In the mouse, three Piwi proteins have been identified: MIWI (PIWIL1), MILI (PIWIL2), and MIWI2 (PIWIL4) (Table 1). Each of these proteins is essential for male fertility and exhibits a distinct temporal and intracellular expression pattern during spermatogenesis [21–23]. The piRNAs are a specific class of small non-coding RNAs of 24–30 nucleotides (nt) that bind to Piwi proteins, together forming the piRNA nucleoprotein complexes (piRNPs). A shared feature of piRNAs from different species is that they are mostly derived from retrotransposons, even though transposable elements are highly divergent among species. Mouse germ cells express two distinct populations of piRNAs: pre-pachytene and pachytene (Fig. 1). The pre-pachytene piRNAs are mainly present in mitotically arrested embryonic male germ cells and originate largely from transposable elements. In contrast, pachytene piRNAs are present in postnatal germ cells, specifically in pachytene spermatocytes and round spermatids, and originate from unique genomic clusters that are depleted of transposable elements. Recent studies have demonstrated that the pachytene piRNAs mediate cleavage and destruction of messenger RNAs in mouse testes [24–26].

In addition to Piwi proteins, the piRNA pathway involves many other proteins, including a group of testis-specific tudor domain containing proteins (TDRD proteins) and RNA helicases such as DDX4 and MOV10L1 (Table 1). TDRD9 and TDRD12 contain both tudor and RNA helicase domains [27, 28]. These proteins interact with Piwi proteins, forming an extensive regulatory network as previously reviewed [20]. For example, the tudor domain of the TDRD proteins binds to dimethylated arginine sites that are present in Piwi proteins [18, 29–32]. Therefore, TDRD proteins are considered scaffold proteins that support the assembly of the piRNA biogenesis machinery [33]. Cytologically, piRNA pathway proteins localize to nuage in germ cells – granules that are located between or adjacent to mitochondria [17, 34].

Unlike the biogenesis of miRNAs, piRNA biogenesis is Dicer-independent and its molecular mechanism remains poorly understood. piRNAs are generated from long single-stranded RNAs that are transcribed by RNA polymerase II [35]. The transcription of pachytene piRNA precursor transcripts is driven by the transcription factor MYBL1, which is essential for male meiosis [35–37]. MOV10L1, a germ cell-specific RNA helicase, interacts with Piwi proteins and is required for the production of both pre-pachytene and pachytene piRNAs [38, 39]. MOV10L1 specifically binds to piRNA precursor transcripts and initiates piRNA biogenesis, which is a stepwise process involving the generation of intermediate fragments

[40]. Biochemical and genomic studies suggest that the processing of precursor RNAs into intermediate fragments relies in part on the formation of G-quadruplex secondary structures [40]. The conserved germ-cell specific RNA-binding protein MAEL is essential for retrotransposon silencing and may be involved at multiple steps, including the primary processing of piRNA precursors. MAEL has endonuclease activity and binds to single-stranded RNA in vitro [41–44]; however, whether it binds to piRNA precursor transcripts in vivo remains an open question. *Drosophila* Zucchini (homologue of mouse PLD6), a mitochondria-associated protein, appears to be the main endonuclease that cleaves precursor RNAs into intermediate fragments [45–47]. Subsequently, Piwi proteins bind to and stabilize the 5′ end of these intermediate fragments, followed by trimming of the 3′ ends, resulting in mature piRNAs [48–50]. Despite these advances, it remains mysterious how precursor transcripts are selected and specifically fed into the primary piRNA biogenesis process.

In addition to binding to piRNAs, Piwi proteins exhibit piRNA-guided endonuclease activity – slicer activity [51]. The slicer activity of Piwi proteins drives the amplification of retrotransposon-associated piRNAs from sense and anti-sense precursor transcripts, a ping-pong mechanism that has been termed secondary piRNA biogenesis. During this cyclic process, retrotransposon transcripts that are base-paired with Piwi-bound piRNAs are cleaved and degraded, resulting in post-transcriptional silencing of retrotransposons and simultaneous generation of more piRNAs. Therefore, retrotransposon-derived piRNAs constitute a small non-coding RNA-based “immune” system to guard the germ cells specifically against retrotransposons. Indeed, the slicer activity of MILI and MIWI is essential for retrotransposon silencing [52, 53].

The de-silencing of transposable elements is a hallmark of piRNA pathway mutants in many species. A large number of mouse piRNA pathway mutants exhibit activation of retrotransposons: *Mili*, *Miwi2*, *Mvh*, *Mov10l1*, *Tdrd1*, *Tdrd5*, *Tdrd9*, *Tdrd12*, *Tdrkh*, *Pld6*, *Asz1*, *Mael*, *Fkbp6*, *Gtsf1*, *Hsp90a*, and *Henmt1* (Table 1). Most mouse piRNA pathway mutants also exhibit meiotic arrest and sterility that is only observed in males but not in females. The de-repression of transposable elements differs between piRNA pathway mutants (summarized in Table 1) but also between transposable elements (for instance, LINE1 vs. IAP) within the same mutant. For example, in *Mov10l1* mutant males, both LINE1 and IAP are activated in mitotically arrested embryonic male germ cells [38]. This observation is consistent with the lack of retrotransposon-derived pre-pachytene piRNAs in *Mov10l1* mutant testis. However, the postnatal *Mov10l1* mutant testis displays an intriguing binary de-repression pattern of LINE1 and IAP: LINE1 is de-repressed in spermatocytes but not in spermatogonia, whereas IAP is activated in spermatogonia but not in spermatocytes [38]. The same binary de-repression pattern has also been observed in *Mili* mutant and *Dnmt3l* mutant testes, suggesting that additional mechanisms may regulate the silencing of LINE1 in spermatogonia and that of IAP in spermatocytes in postnatal testes [54, 55]. The binary de-repression pattern of LINE1 and IAP is likely to be universal in many other piRNA pathway mutants.

3. TE silencing: the role of DNA methylation

DNA methylation leading to transcriptional repression is a major mechanism contributing to retrotransposon silencing in both somatic and germ cells. The majority of DNA methylation in the mammalian genome occurs in repetitive sequences, which encompass the bulk of heterochromatin. The maintenance DNA methyltransferase DNMT1 is required for methylation of IAP retrotransposons in somatic tissues of mouse embryos [56]. Methylation of LINE1 and IAP retrotransposons in male germ cells is carried out by the two de novo DNA methyltransferases DNMT3A and DNMT3B. These methyltransferases exhibit overlapping activity in germ cells, and deletion of either enzyme alone has little effect on DNA methylation of transposable elements [10]. In contrast, inactivation of DNMT3L, a non-catalytic homolog of DNMT3A/3B, causes reactivation of LINE1 and IAP retrotransposons in male germ cells, meiotic arrest, and male sterility [11].

The piRNA pathway is required for de novo methylation of transposable elements in embryonic male germ cells, since male germ cells from many piRNA pathway mutants lack LINE1 promoter methylation [57, 58]. Concomitantly, disruption of the piRNA pathway affects the intracellular distribution of Piwi proteins. In wild type embryonic germ cells, MILI is exclusively cytoplasmic, whereas MIWI2 localizes to the nucleus [58]. In the germ cells of mouse mutants in which piRNA production is impaired or abrogated, MIWI2 fails to enter the nucleus, suggesting that piRNA is a nuclear entry license for MIWI2 [58]. It has been hypothesized that the MIWI2-piRNA complex targets actively transcribing retrotransposons causing methylation at these loci. A recent study has shown that the de novo DNA methylation of retrotransposons in embryonic germ cells occurs in two distinct waves [59]. The first wave reflects nonselective default de novo methylation of the whole genome, during which retrotransposons become methylated in a piRNA-independent manner. During the second wave, actively transcribing retrotransposons are targeted for modification by the piRNA pathway. It is conceivable that the MIWI2-piRNA complex recognizes active retrotransposon loci through base pairing between piRNA and nascent retrotransposon transcripts. However, it remains unknown how piRNAs establish de novo DNA methylation of retrotransposons in mammalian germ cells.

4. Silencing of transposable elements involves histone modifications

Adult mice with defects in the piRNA pathway exhibit an intriguing binary expression pattern of retrotransposons in the testis: LINE1 elements are active in spermatocytes but silenced in spermatogonia [38]. In piRNA pathway mutant spermatogonia, LINE1 elements are de-methylated but remain suppressed, suggesting that an additional silencing mechanism is at work [54]. Histone 3 lysine 9 dimethylation (H3K9me2) marks transcriptional repression and is present in spermatogonia and early spermatocytes (preleptotene to zygotene) but disappears at the pachytene stage [60]. G9a is a major H3K9 mono- and dimethyltransferase with essential functions in embryogenesis and germ cell development [61, 62]. In spermatogonia deficient for *Mili*, H3K9me2 is sufficient to maintain LINE1 silencing in the absence of DNA methylation [62]. Therefore, G9a-mediated H3K9 dimethylation co-suppresses LINE1 elements with DNA methylation in spermatogonia. Moreover, *Dnmt3l*-deficient testes maintain H3K9me2 in spermatogonia but exhibit a precocious loss of

H3K9me2 at the onset of meiosis, apparent from a significant reduction of this mark in preleptotene/leptotene spermatocytes and absence in zygotene spermatocytes [55]. This precocious loss of H3K9me2 combined with absence of DNA methylation may be the cause for the observed activation of LINE1 elements in *Dnmt3l*-deficient spermatocytes prior to the pachytene stage [55]. Thus, LINE1 repression in early spermatocytes appears to rely on both DNA methylation and H3K9me2. These observations may provide a universal explanation for the binary expression pattern of LINE1 in spermatogonia and spermatocytes in piRNA pathway mutants and other mutants with LINE1 DNA de-methylation.

Histone 3 lysine 9 trimethylation (H3K9me3) is a histone modification associated with heterochromatin. In *Drosophila*, the Piwi-piRNA complex is essential for the establishment of H3K9me3 marks on transposable elements and their genomic context [63]. Specifically, the nuclear Piwi-piRNA complex recognizes and binds to nascent transcripts of transposable elements, thereby marking their genomic sites. Silencio, an adaptor protein, interacts with target-engaged Piwi-piRNA complexes and directly or indirectly recruits SetDB1, an H3K9 tri-methyltransferase, leading to the formation of heterochromatin at transposon-rich genomic regions [64, 65]. *Drosophila* lacks DNA methylation, and defects in the fly piRNA pathway cause loss of repressive H3K9me3 at transposable elements resulting in their activation [63, 66]. In mouse germ cells, the piRNA pathway is required to establish repressive H3K9me3 marks on active full-length LINE1 retrotransposons, evident from the specific loss of these marks in *Miwi2* mutant mice [67]. However, whether H3K9me3 is required for retrotransposon silencing in mammals remains to be investigated. It is also unclear how the nuclear MIWI2 protein facilitates the establishment of H3K9me3 marks [67]. Silencio does not have recognizable orthologues outside Drosophilids. In the mouse, SetDB1 is essential for the silencing of H3K9me3-marked IAP retrotransposons in primordial germ cells but dispensable for LINE1 repression in germ cells [68]. Therefore, mechanisms required for the establishment of H3K9me3 marks in mammalian germ cells may involve a yet unknown factor with functional equivalence to Silencio, and the relevant H3K9 tri-methyltransferase remains to be identified.

5. New players in silencing of transposable elements

The conserved MORC1 protein has recently emerged as a novel factor that participates in the silencing of transposable elements in mammalian germ cells. *Morc1*, the founding member of the *Morc* gene family, was originally discovered in a mouse mutant in which insertional inactivation of *Morc1* caused meiotic arrest and male sterility [69, 70]. MORC1 contains a histidine kinase-like ATPase (HATPase) domain and a CW-type zinc finger. In *Arabidopsis*, the *Morc1* and *Morc6* genes are required for heterochromatin condensation and silencing of transposable elements; however, mutations in these two genes do not cause a loss of DNA or histone methylations [71]. It has been hypothesized that MORC proteins may function as chromatin remodeling factors.

More recent studies in the mouse have revealed that MORC1 represses LINE1 and IAP retrotransposons in male germ cells [72]. *Morc1* mutant germ cells fail to establish repressive methylation marks specifically at retrotransposons. In *Morc1* mutant germ cells, hypomethylated genomic loci correspond to retrotransposon transcription initiation sites,

implying that MORC1 modulates DNA methylation locally. In *Morc1* mutant mice, piRNA biogenesis appears to be normal and there is no loss of global DNA methylation, suggesting that MORC1 may function independently or downstream of the piRNA pathway and DNMT3L. The precise molecular mechanism by which MORC gene family factors contribute to the silencing of transposable elements remains to be elucidated.

6. Consequences of LINE1 activation on spermatogenesis

In all mouse mutants studied so far, LINE1 activation in late embryonic male germ cells does not immediately cause developmental arrest. For example, *Miw12*-null male embryonic germ cells continue to develop despite upregulation of LINE1 and enter meiosis at puberty [23, 57, 58]. It appears that embryonic male germ cells are tolerant to a high level of retrotransposon activity.

Meiotic arrest in males is a characteristic phenotype in most mouse mutants with hyper-activation of retrotransposons in the germline, including piRNA pathway mutants, the *Dnmt3l* mutant, and the *Morc1* mutant (Table 1). Meiotic arrest in these mutants presumably results from the activation of the meiotic checkpoint in response to chromatin defects [73]. This notion is further supported by the fact that even though *Miw12* is expressed in embryonic and early postnatal germ cells long before the initiation of meiosis, it is not until meiosis that *Miw12* deficiency manifests with spermatogenic arrest. The correlation between LINE1 activation and meiotic arrest is complex. One hypothesis suggests that LINE1 activation in spermatocytes causes massive DNA damage, which results in activation of the meiotic checkpoint and subsequent apoptosis of spermatocytes. This hypothesis is supported by observations in males that are deficient for the germ-cell specific RNA binding protein MAEL and exhibit LINE1 activation and meiotic arrest. In the spermatocytes of these males, DNA damage accumulates even in the absence of SPO11, which normally induces meiotic double strand breaks (DSBs). The presence of DNA damage in *Mael*^{-/-} *Spo11*^{-/-} double mutant testes therefore indicates that LINE1 is capable of producing DNA damage, which may be initiated through the endonuclease activity of its ORF2 protein [74].

Meiotic arrest in *Dnmt3l* mutant testes has been attributed to a different mechanism involving the formation of recombination sites within retrotransposon sequences [55]. *Dnmt3l*^{-/-} *Spo11*^{-/-} mutant spermatocytes do not exhibit DNA damage, suggesting that the overproduction of LINE1 does not result in the induction of DNA strand breaks. However, the inactivation of *Dnmt3l* and resulting hypomethylation of transposable elements cause major changes in the distribution of epigenetic marks on meiotic chromatin [55, 75]. PRDM9 is a meiosis-specific lysine methyltransferase that specifically catalyzes trimethylation of histone H3 lysine 4 (H3K4me3) [76]. In germ cells, the majority of H3K4me3 marks are associated with active transcription, whereas a small fraction (16%) represent meiotic recombination sites [77]. The formation of DSBs at H3K4me3-marked sites is catalyzed by the SPO11 transesterase leading to recruitment of recombination enzymes such as RAD51 and DMC1 [78–80]. In *Dnmt3l* mutant spermatocytes, de-methylated retrotransposon sequences are enriched for H3K4me3, SPO11, and DMC1, all of which are associated with meiotic recombination. These results suggest that de-methylated and actively transcribing retrotransposon sequences may adopt an open chromatin state,

thereby sequestering the limited meiotic recombination machinery away from normal recombination sites [55, 75]. This relocation of meiotic recombination sites to retrotransposon sequences blocks meiotic progression.

The pachytene piRNA clusters/genes encode long non-coding RNA transcripts, which are processed into piRNAs. *Nct1* and *Nct2* are two of the piRNA genes [81]. Intriguingly, deletion of the *Nct1/2* genes leads to de-repression of LINE1 retrotransposons but does not affect spermatogenesis and fertility (Table 1) [82]. In *Nct1/2*-null testes, LINE1 is selectively de-repressed in early and mid-pachytene spermatocytes. HENMT1 is an RNA methyltransferase responsible for 2'-O-methylation of piRNAs [83, 84]. Deficiency for this factor is associated with de-repression of LINE1 in spermatocytes and round spermatids and leads to spermiogenic arrest at the elongating spermatid stage but does not impair meiotic progression (Table 1) [85]. These studies suggest that LINE1 de-repression in spermatocytes may not necessarily cause meiotic arrest. It is possible that the level of LINE1 de-repression must exceed a threshold to cause meiotic arrest.

The production of high levels of LINE1 RNP particles may not necessarily lead to increased retrotransposition. A large number of proteins are associated with LINE1 RNP particles [86, 87], and some of these associated proteins inhibit LINE1 retrotransposition [87]. In *Dnmt3l*-null germ cells, LINE1 retrotransposition was not detected by qPCR [55], but was detected using the amplified methylation polymorphism (AMP) protocol [75]. The frequency of actual LINE1 retrotransposition events in piRNA pathway mutants (Table 1) remains to be determined. Future studies employing tools for the detection of transposition events, such as a LINE1 retrotransposition reporter transgene may provide more insight into the functional consequences of LINE1 activation [88].

7. A role for LINE1 in fetal oocyte attrition

The LINE1 retrotransposon has been implicated in fetal oocyte attrition in the mouse, which results in elimination of nearly two thirds of all oocytes before birth [89]. LINE1 expression levels are heterogeneous in normal mouse oocytes and correlate with the severity of DNA damage and meiotic defects observed in each oocyte. Oocytes with a high LINE1 level are eliminated through apoptosis. The observation that *Mael* deficiency in mouse leads to de-silencing of LINE1 expression in fetal oocytes and increased oocyte attrition has confirmed a physiological role for LINE1 retrotransposon in fetal oocyte attrition [89, 90].

Female fertility is not impaired in any of the piRNA pathway mutant mouse models studied to date. Intriguingly, several piRNA pathway proteins such as DDX4, MILI, TDRD9 and GASZ are expressed in mouse oocytes [91]. Furthermore, in null mutants for each of these proteins, LINE1 is upregulated in the primordial follicles of the ovary, similar to observations in male germ cells [91]. These results demonstrate that piRNA-dependent retrotransposon de-silencing in oocytes is uncoupled from female fertility, at least in mouse. In the mouse, an oocyte-specific Dicer isoform has been identified that drives the production of endogenous small interfering RNAs – endo-siRNAs, which regulate transcripts in mouse oocytes [92–94]. This Dicer isoform is present only in muridae (mouse and rat) but not in other mammals including human [92]. In the mouse, endo-siRNAs are required for female

meiosis [95], supporting the hypothesis that piRNAs may be dispensable in murine oocytes. Recently, piRNAs have been found in human, macaque, and bovine ovaries [96, 97]. In contrast to the murine (mouse and rat) family, in which only three Piwi genes (*Piwi1*, *Piwi2*, and *Piwi4*) are present, non-murine mammals such as human and cattle have a fourth Piwi gene – *Piwi3*, which is ovary-specific [96]. This raises the possibility that piRNAs may play a more important or essential role in oogenesis in non-murine mammals. The evolution of the oocyte-specific Dicer isoform and oocyte-specific PIWIL3 in muridae and non-muridae mammals respectively provides an explanation for the differential requirement of endo-siRNAs and piRNAs in mouse oocytes.

8. Conclusions

Our understanding of how different species silence transposable elements has rapidly advanced within the past decade. LINE1 is the most abundant retrotransposon type in the mammalian genome. Mammalian germ cells employ multiple and stage-specific mechanisms to silence transposable elements during germ cell development. DNA methylation plays an essential role in transposon silencing in both somatic and germ cells, whereas the piRNA pathway suppresses transposable elements specifically in the germline. This pathway involves base pairing between small non-coding piRNAs and retrotransposon transcripts, and is therefore highly adaptable to any sequence changes within transposable elements. Multiple piRNA pathway protein components have been identified through genetic screens or genetic studies, and most of these are conserved in fly, zebrafish, and mouse. Suppression of LINE1 elements in mouse spermatogonia relies on both DNA methylation and co-suppression by H3K9me2 histone modification. MORC gene family factors appear to regulate the epigenetic silencing of retrotransposon in germ cells by yet unknown mechanisms. Recent studies have identified mechanistic links between LINE1 de-silencing and meiotic failure. Up-regulation of LINE1 may cause DNA damage that is sufficient to induce meiotic arrest in male germ cells and has also been associated with fetal oocyte attrition [74, 89]. Furthermore, DNA de-methylation underlying LINE1 activation has been found to produce profound changes in the epigenetic landscape of meiotic chromatin with redistribution of meiotic recombination sites to regions enriched for retrotransposons, blocking meiotic progression [55, 75]. The piRNA pathway, DNA methylation, and histone modifications play a fundamental role in the silencing of transposable elements in the male germline of mammals, and research into the underlying regulatory mechanisms and interactions between these pathways will remain areas of intense research. Emerging evidence suggests that murine and non-murine species rely on different mechanisms of retrotransposon silencing in oocytes, which relies on endogenous siRNA but not piRNAs in the mouse.

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Fig. 1. Timeline of mouse spermatogenesis illustrating stages and major developmental events. The tan histogram represents the developmental expression pattern of pre-pachytene and pachytene piRNA populations. Pink and grey lines reflect the developmental stage-dependent de-repression of LINE1 and IAP retrotransposons, respectively, in mouse mutants including *Mili*, *Miwi2*, *Mov10l1*, *Dnmt3l*, *Morc1* etc. (Table 1). This figure has been modified from Zheng and Wang [39].

Table 1

Mouse mutants with de-silencing of retrotransposons (LINE1 and/or IAP) in male germ cells

Protein	Mutant phenotype	De-repressed retrotransposons	References
piRNA pathway factors			
PIWIL2 (MILI)	Meiotic arrest	LINE1 and IAP	[57, 98]
PIWIL1 (MIWI)	Spermiogenic arrest	LINE1	[53]
PIWIL4 (MIWI2)	Meiotic arrest	LINE1 and IAP	[23, 57]
MOV10L1	Meiotic arrest	LINE1 and IAP	[38, 99]
DDX4 (MVH)	Meiotic arrest	LINE1 and IAP	[100]
TDRD1	Meiotic arrest	LINE1	[31, 101]
TDRD5	Spermiogenic arrest	LINE1 (incomplete penetrance)	[102]
TDRD9	Meiotic arrest	LINE1	[27]
TDRD12	Meiotic arrest	LINE1 and IAP	[28]
TDRKH	Meiotic arrest	LINE1	[50]
PLD6	Meiotic arrest	LINE1	[103]
ASZ1 (GASZ)	Meiotic arrest	LINE1 and IAP	[104]
MAEL	Meiotic arrest	LINE1 and IAP	[74]
FKBP6	Meiotic arrest	LINE1	[105]
Hsp90α	Meiotic arrest	LINE1	[106, 107]
GTSF1	Meiotic arrest	LINE1 and IAP	[108]
HENMT1	Spermiogenic defect	LINE1 and IAP	[85]
Nct1/Nct2 (non-coding)	fertile	LINE1	[82]
EXD1	fertile	LINE1 (low penetrance)	[109]
Non-piRNA pathway factors			
DNMT3L	Meiotic arrest	LINE1 and IAP	[11, 75]
MORC1	Meiotic arrest	LINE1 and IAP	[72]
EHMT2 (G9a)	Meiotic arrest	Co-suppression of LINE1	[62]