Review Article

A Small RNA-Based Immune System Defends Germ Cells against Mobile Genetic Elements

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Transposons are mobile genetic elements that threaten the survival of species by destabilizing the germline genomes. Limiting the spread of these selfish elements is imperative. Germ cells employ specialized small regulatory RNA pathways to restrain transposon activity. PIWI proteins and Piwi-interacting RNAs (piRNAs) silence transposons at the transcriptional and posttranscriptional level with loss-of-function mutant animals universally exhibiting sterility often associated with germ cell defects. This short review aims to illustrate basic strategies of piRNA-guided defense against transposons. Mechanisms of piRNA silencing are most readily studied in *Drosophila melanogaster*, which serves as a model to delineate molecular concepts and as a reference for mammalian piRNA systems. PiRNA pathways utilize two major strategies to handle the challenges of transposon control: (1) the hard-wired molecular memory of prior transpositions enables recognition of mobile genetic elements and discriminates transposons from host genes; (2) a feed-forward adaptation mechanism shapes piRNA populations to selectively combat the immediate threat of transposon transcripts. In flies, maternally contributed PIWI-piRNA complexes bolster both of these lines of defense and ensure transgenerational immunity. While recent studies have provided a conceptual framework of what could be viewed as an ancient immune system, we are just beginning to appreciate its many molecular innovations.

1. Mobile Genetic Elements Threaten Genomic Integrity

Transposons are mobile genetic elements that can move into novel locations within the genome. These genetic parasites have long colonized large portions of all eukaryotic genomes [1]. Transposons are classified based on their movement strategies: retrotransposons move through a "copy and paste" mechanism, involving reverse transcription of initial RNA copies and consecutive insertion into novel genomic regions. This mechanism does not alter the original genomic insertion and results in amplification of the element. In contrast, DNA transposons employ a "cut and paste" mechanism. Transposition to a new genomic location leaves a gap at the donor site that upon repair either results in restoration or in loss of the original insertion [2–7]. While the journey of transposons is often neutral to the host, novel insertions can cause severe damage, or, in rare cases, beneficial changes.

During evolution, host genomes accumulated scars while eliminating deleterious insertions and selecting for advantageous mutations. Above all, genomes devised mechanisms to repress transposon activity. Germline genomes constitute a crucial battleground for the arms race between transposons and their hosts. To ensure vertical transfer and amplification of a mobile element, transposition has to take place in germ cells. Transposons continuously adapt to thrive in this particular environment and germ cells in turn have devised specialized strategies to guard their genomic integrity and thus the continuation of a species [6, 8]. With defense mechanisms in place, host genomes seem to have come to equilibrium with their parasites. Most current transposon insertions are defunct, representing defeated fragments rather than powerful insurgents [1, 9].

Tamed transposons become part of the host's evolutionary toolkit and serve as a rich source of coding and noncoding sequences that allow for genetic innovation [3, 4].

A prominent example of such domestication is Telomere reverse transcriptase, which probably evolved form an ancient retrotransposon [10]. In *Drosophila melanogaster*, retroelements themselves have colonized telomeric regions and directly maintain chromosomal ends without the need for an active telomerase [11, 12]. In addition to domestication of coding sequences, transposon fragments have shaped gene regulatory networks by providing an arsenal of noncoding building blocks [2, 13, 14]. Despite these important positive contributions that have been selected for during evolution, transposons are intrinsically selfish and their activity must be tamed or it will threaten the integrity of host genomes.

Controlling the activity of transposable elements presents two major challenges: (1) to recognize transposons as "nonself" and to (2) mount an efficient defense selectively against active elements. Recognizing transposons as "nonself", thereby discriminating them from host genes is not trivial. Transposons have become integral parts of our genomes and their mastery of camouflage enables them to hijack host machineries for transcription and translation. Additionally, their sequence diversity prevents recognition of specific motifs and their many mechanisms to transpose do not share vulnerable cofactors. In addition to direct genomic damage though transposition, transcription of certain elements can be toxic to the cell through the immense amount of produced RNA transcripts [15]. Adapting to the immediate threat of active transposons requires a prompt and specific defense. Small noncoding RNAs rise to the challenges posed by mobile genomic parasites [16–19]. Specialized small RNA pathways recognize transposons through the molecular memory of individual mobile sequences and have devised an elegant adaptive response against active elements.

2. Small RNA Pathways Are Prevalent in Eukaryotes

RNA interference (RNAi) was first observed as transgene cosuppression in plants [20]. Subsequent studies in *Caenorhabditis elegans* identified double-stranded RNA as the trigger of homology dependent gene silencing with corresponding small RNA products (~20–30 nt in length) serving as executive guides [21, 22]. Conserved small RNA pathways play crucial roles in development and disease [23–27]. These pathways can silence expression of target genes at transcriptional (i.e., recruiting histone and DNA methylation) or posttranscriptional level (i.e., promoting RNA degradation, inhibiting translation) [28–32]. At the heart of all RNA silencing pathways resides an RNA induced silencing complex (RISC), which in essence consists of a small noncoding RNA and its associated Argonaute protein partner [33, 34]. Within RISC, the small RNA determines target specificity by complementary base pairing, while its Argonaute partner governs effector mechanisms. Argonaute proteins are defined by a PAZ (Piwi-Argonaute-Zwille), a MID (middle), and a PIWI domain. The PAZ and MID domains specifically interact with the small RNA partner, anchoring its 3′ and 5′ termini, respectively [35]. The PIWI domain structurally resembles an RNase H fold and harbors

RNA-guided endoribonuclease activity [36]. Phylogenetically Argonaute proteins segregate into two conserved subfamilies: the Ago-clade, similar to *Arabidopsis thaliana* AGO1, and the PIWI-clade, named after *Drosophila piwi* (P-element induced wimpy testis [37]) [38]. Members of the Ago-clade are ubiquitously expressed and associate with microRNAs (miRNAs) and small interfering RNAs (siRNAs). In contrast, the PIWI-clade is mostly restricted to germ cells in animals. PIWI proteins associate with a less well-understood class of small RNAs, piRNAs. PIWI-piRNA complexes silence transposons at the transcriptional and posttranscriptional level to guard the integrity of germline genomes [39–41].

3. Strategies of piRNA Pathways to Guard Genomic Integrity in *Drosophila melanogaster*

PiRNAs greatly differ from miRNAs and siRNAs in their biogenesis, Argonaute protein partners, and expression patterns [42, 43]. In contrast to miRNAs and siRNAs, biogenesis of piRNAs does not depend on the RNase III enzyme Dicer [44]. PiRNAs are thought to be processed from long single stranded precursors that get parsed into an army of small RNAs with the potential to cooperatively target individual transposons. Owing to their sequence diversity and the lack of unique molecular characteristics, piRNAs are best defined by their physical and functional association with PIWI proteins [44–48]. Mechanisms of piRNA biogenesis and effector functions are best understood in the female germline of *Drosophila melanogaster*. It should be noted that piRNA pathways also operate in the male germline of flies, but their mechanisms are less well characterized [49, 50]. Three PIWI proteins, Piwi, Aubergine (Aub), and Ago3, are expressed during oogenesis in *Drosophila* and associate with piRNAs to form piRNA-silencing complexes (piRISCs) (Figure 1). Aub- and Ago3-piRISCs reside in the cytoplasm to degrade transposon transcripts [45, 51, 52]. In contrast, Piwi-piRISC localizes to the nucleus and induces transcriptional silencing at transposon loci [53–55]. Loss of either *PIWI* gene results in sterility of the animals, presumably as a consequence of uncontrolled transposon activity in the germline [37, 56–60].

PiRNAs can be grouped into two classes that represent molecular answers to the two major challenges of transposon control: "nonself" discrimination and selective adaptation to an immediate threat. "Primary piRNAs" and their generative loci, so-called piRNA clusters, achieve "nonself" recognition through extensive genetic memory of individual transpositions. "Secondary piRNAs" defend against transposon activity through selective amplification of sequences that target transposon transcripts. Primary and secondary piRNAs require distinct processing enzymes and cofactors, but they function collaboratively to protect the integrity of germline genomes [32, 61–63].

3.1. Memory of Previous Transpositions Enables "Nonself" Recognition Guided by Primary piRNAs. Most primary piRNAs originate from a limited number of discrete genomic regions termed piRNA clusters that are defined as genomic
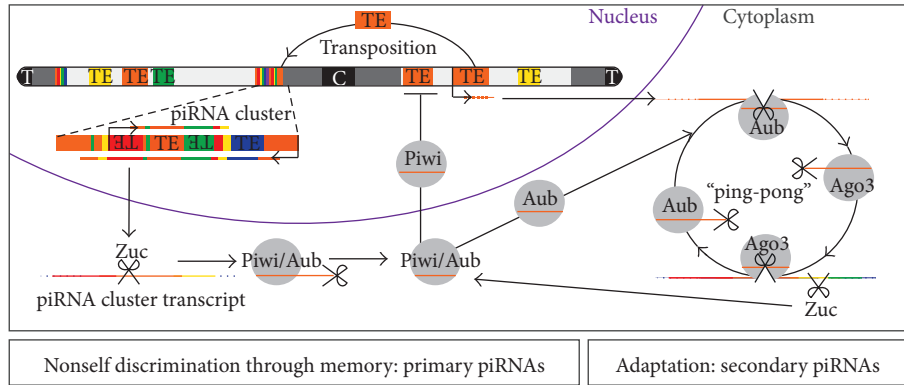
FIGURE 1: Mechanisms of "nonself" discrimination and adaptation in piRNA-guided defense against transposons in *Drosophila*. PiRNA clusters are genomic intervals that accumulate defunct fragments of transposable elements (TE) as a record of prior mobile activity. (1) Cluster regions are unidirectionally or bidirectionally transcribed and give rise to long presumably single stranded transcripts. PiRNA cluster transcripts are specifically processed into primary piRNAs by the consecutive action of at least two nucleases. The endonuclease Zucchini (Zuc) generates the 5′ terminus of a primary piRNA that is loaded into Piwi or Aubergine (Aub) and then trimmed by a 3′-5′ exonuclease. Piwi-piRNA complexes enter the nucleus and recruit chromatin modifying enzymes to silence transposon loci. In contrast, Aub-piRNA complexes initiate an adaptive response against transposon transcripts in cytoplasmic germ granules. (2) Aub and Ago3 engage in a feed-forward adaptation mechanism—the "ping-pong" cycle—that degrades transposon transcripts and concomitantly produces secondary piRNAs to selectively enhance the response against active elements. Through a feedback mechanism secondary piRNAs can initiate further Zuc-dependent piRNAs that are loaded into Piwi and Aub (Centromere (C), Telomere (T)).

intervals with a high density of uniquely mapping piRNAs [45] (Figure 1). Some of these ~140 genomic regions were previously linked to transposon control [64–67]. PiRNA clusters act as transposon traps, accumulating numerous transposon insertions over time and retaining a collection of densely packed defunct fragments. Insertion of novel sequences into piRNA clusters adds information to the repository, marks corresponding elements as "nonself" and confers resistance in trans [68, 69]. piRNA clusters are a fossil record of transposition activity, reflecting the mobile heritage of genomes and providing a molecular database for "nonself" recognition. How piRNA clusters are formed and how their transcripts are specifically marked for processing into piRNAs are major outstanding questions in the field.

Most piRNA clusters contain transposon insertions in mixed orientation and generate transcripts from both genomic strands. Transcriptional regulation of these dual-strand piRNA clusters differs from genic transcription in respect to interpretation of chromatin marks and cotranscriptional processing of nascent transcripts. In contrast to active genes, piRNA clusters preferentially reside in pericentric or subtelomeric regions that mark the boundaries of constitutive heterochromatin and euchromatin [70]. Cluster transcription requires recruitment of Rhino, a fast evolving heterochromatin protein 1 (HP1) family member, to trimethylated lysine 9 of histone H3 (H3K9me3), which otherwise typically marks silent genes [71–74]. Rhino associates with the adaptor protein Deadlock to recruit Cutoff, a homolog of the Rai1/Dom3Z decapping enzyme, which has lost its enzymatic activity. The Rhino-Deadlock-Cutoff complex is suggested to protect the 5′ end of nascent cluster transcripts and to suppress both canonical splicing and transcriptional termination [74–78]. Moreover, Rhino recruits the RNA helicase UAP56 to specify cluster transcripts for transport to their processing site [78]. Using a positive feed-back mechanism, Piwi-piRNA complexes themselves maintain transcriptional activity at cluster loci through recruitment of H3K9me3 [79–81].

PiRNA clusters give rise to long single-stranded transcripts without known structural or sequence determinants or significant formation of double-stranded RNA. These cluster transcripts are processed into a large body of diverse small RNAs by the consecutive action of at least two nucleases. An endoribonuclease generates the 5′ monophosphorylated end of a new piRNA that is consecutively loaded into PIWI's MID pocket. Consecutively, the 3′ end of the PIWI-bound pre-piRNA is trimmed by a 3′ to 5′ exonuclease. The endonucleolytic activity that likely generates the 5′ terminus of a primary piRNA can be assigned to the conserved nuclease Zucchini (Zuc) [82–84]. In contrast, the identity of the 3′ trimming exonuclease remains elusive [85]. The final length of a mature piRNA is marked by 2′-O-methylation and likely represents a footprint of its associated PIWI protein [85–87]. Little is known about the initial processing of piRNA cluster transcripts. While primary processing intermediates or degradation fragments have been observed, the precise mechanisms remain obscure [88]. Further characterization of piRNA cluster transcripts and identification of the factors that specify transcripts for processing into piRNAs are required to understand how hardwired memory of past transpositions is parsed into small RNA guides for "nonself" recognition.

3.2. An Adaptive Mechanism That Produces Secondary piRNAs Defends Selectively against Mobile Elements That Have Evaded Silencing at the Site of Transcription.

PiRNAs engage in a feed-forward adaptive response to specifically eliminate cytoplasmic transposon transcripts and reinforce piRNA production. This robust strategy relies on removal of transposon transcripts through piRNA-guided cleavage

and simultaneous production of select secondary piRNAs [45, 51, 52]. In contrast to primary piRNAs, which represent all indexed transposons irrespective of their transcript abundance, secondary piRNAs shape the overall piRNA pool toward recognition of active elements. Biogenesis of secondary piRNAs relies on the nuclease activity of two PIWI proteins, Aub and Ago3, that collaborate in the so-called "ping-pong" cycle [45, 52]. Processing of secondary piRNAs is believed to be triggered by either primary or maternally contributed Aub-piRNAs. These piRNAs guide Aub to cleave complementary transposon transcripts. Target RNA cleavage by Aub generates the $5'$ end of a new piRNA that is loaded into Ago3. Because Ago3-piRNAs originate from transposon transcripts themselves, they carry selective information about these potentially harmful active elements. Ago3-piRNAs in turn guide cleavage of complementary stretches within cluster transcripts to generate additional Aub-piRNAs and complete the "ping-pong" cycle [57] (Figure 1). Multiple rounds of selection alternate between transposon and cluster transcripts as substrates for secondary piRNA production and mold piRNA populations towards preferentially target active elements. During "ping-pong," transposon-triggered processing of cluster transcripts selects for sequences that are genetically determined as "nonself." This mechanism could act as a protective measure to prevent amplification of piRNAs that accidentally target host genes and thus could cause autoaggression, analogous to the phenomena of autoimmunity.

Coordination of this elegant adaptation mechanism is achieved in specialized perinuclear germ granules called Nuage [89]. Members of the Tudor protein family are key-components of these germ granules and play crucial roles in piRNA biology. Tudor proteins are defined by the presence of one or multiple Tudor domains that recognize methylated Arginine residues and facilitate protein interactions [90]. PIWI proteins are methylated at N-terminal Arginines and various Tudor proteins regulate interactions between PIWI proteins and cofactors to ensure efficient heterotypic processing of secondary piRNAs [91]. Dynamic orchestration of these interactions requires periodic remodeling of PIWI-RNA complexes by the DEAD box helicase Vasa, which chaperones the transfer of piRNA intermediates between PIWI "ping-pong" partners [92, 93].

Additionally, secondary piRNAs have the potential to trigger Zucchini-dependent processing of adjacent piRNA cluster regions into piRNAs that are loaded into Aub and Piwi. This feedback mechanism not only results in diversification of piRNA sequences, but also enables transmission of information from the adaptive defense to the transcriptional silencing machinery [94–96]. Additional genes involved in piRNA pathways emerged from genome-wide screens and further characterization of these factors will add to our mechanistic understanding of this elaborate small RNA based immunity [97–99].

3.3. Maternally Inherited PIWI-piRNA Complexes Bolster Trans-Generational Immunity. Genetic observations have long suggested the role for a maternally contributed factor in transposon control in *Drosophila*. Crosses of males carrying a specific transposon to females naïve to this element result in a loss of germ cells and sterility in the offspring. Interestingly, despite genetic identity, the reciprocal crosses do not exhibit defects in germ cell development. Germ cell defects in the dysgenic progeny are associated with mutations and chromosomal rearrangements attributed to unleashed transposon activity [100–105]. This phenomenon of hybrid dysgenesis suggests the requirement of nongenetic, maternally contributed factors in transposon defense [106, 107]. The nature of these factors remained mysterious until PIWI-piRNA complexes were identified as molecular determinants of maternal immunity [81]. Maternally contributed PIWI-piRNA complexes transmit immunity in two ways: (1) they reinforce memory through recruitment of H3K9me3 to piRNA clusters, thus bolstering noncanonical transcription at these loci, and (2) they directly initiate adaptive processing of secondary piRNAs through the "ping-pong" cycle [55, 80, 81, 108, 109].

4. Small RNA Pathways Guard Genomic Stability in Mammalian Germ Cells

Transposon sequences and active transposon families vary significantly between different species [110]. Considering coevolution between transposons and the host's defense system, adaptive variation in molecular mechanisms is expected. Yet, principles of RNA interference have seemingly proven efficient for defense. Like flies, mouse piRNA pathways employ three PIWI-like (PWIL) proteins: Piwil1/Miwi, Piwil2/Mili, and Piwil4/Miwi2 [46, 47, 111]. Removal of either PIWI gene results in germ cell defects accompanied by a burst in transposon activity in the male germline [112–115]. PiRNA pathways operate at two distinct stages during mouse spermatogenesis. In embryonic testes, Piwil2/Mili collaborates with Piwil4/Miwi2 to protect primordial germ cells. Similar to *Drosophila* piRNAs, mouse embryonic piRNAs mainly correspond to transposon sequences and engage in selective generation of secondary piRNAs in response to transposon transcripts. In contrast to flies, the "ping-pong" cycle in mouse involves the catalytic activity of only one PIWI partner, Piwil2/Mili [116]. Intra-Piwil2/Mili "ping-pong" generates secondary piRNAs that are loaded into Piwil2/Mili and Piwil4/Miwi2. Interaction with piRNAs licenses Piwil4/Miwi2 to transfer to the nucleus and silence transposon loci [113, 115, 116]. Recruitment of Piwil4/Miwi2-piRNA complexes results in histone and DNA methylation to full-length, potentially active transposon-loci [117, 118]. Interestingly, full-length transposons seem to evade a prior piRNA-independent wave of heterochromatinization in the embryonic gonad and require piRNA-guided control [117]. A second phase of piRNA silencing takes place in adult testis during entry into meiosis. Two cytoplasmic PIWI proteins, Piwil2/Mili and Piwil1/Miwi, are involved in this pathway. The functions and precise mechanisms of adult piRNAs remain enigmatic. Adult piRNAs mainly originate from relatively few intergenic clusters with little potential to target cellular transcripts but their own [111, 119]. Although

this class of piRNAs is not enriched in transposon sequences and does not exhibit "ping-pong" activity, loss of Piwil1/Miwi, Piwil2/Mili, or either nuclease activity results in derepression of transposons [120, 121]. How adult piRNAs guide transposon silencing without obvious enrichment in complementary sequences remains elusive. Recent studies suggest a function for these piRNAs in posttranscriptional regulation beyond transposon silencing [122–124].

Curiously, while piRNAs are present in mouse oocytes, they are not required for fertility. In the female germline of mice transposon defense relies on endogenous siRNAs [125, 126]. These siRNAs originate from dsRNA precursors that are generated by the repetitive nature of transposon sequences or antisense transcription at corresponding loci. Long dsRNA substrates are processed into ~21-nt long siRNAs by the RNase III enzyme Dicer and guide Ago2 to slice transposon transcripts. Transposon loci produce piRNAs and siRNAs in a seemingly redundant fashion in the developing oocyte. However, siRNAs dominate piRNAs in abundance and function. It is puzzling how and why mouse oocytes chose siRNAs over piRNAs in transposons control. An unexpected recent finding shed some light on this paradox: mice express an oocyte-specific isoform of Dicer—Dicer^O— that harbors enhanced activity on processing dsRNA into siRNAs [127]. Interestingly, transcription of Dicer^O is driven by an intronic transposon insertion. Deletion of this insertion causes loss of Dicer^O and female sterility and resembles a maternal Dicer null phenotype [128]. This observation emphasizes the tight relationship between transposons and their host even in establishing a transposon defense system. Transposon driven Dicer^O expression is specific to mice and rats. Thus, siRNA-mediated transposon silencing seems to be an exception rather than a rule in the mammalian germline, which prompts a reevaluation of piRNA function in the female germline of other mammals [129, 130].

5. Conclusion

Germ cells have devised small RNA mechanisms to master the challenges of transposon control. PiRNA clusters establish and maintain memory of "nonself" and generate mobile guides to induce an adaptive defense against active transposons. Maternally transmitted PIWI-piRNA complexes add an additional layer to transgenerational immunity against mobile genetic elements in flies. Over the past several years RNA-based immunity against genomic invaders has been discovered in all branches of life ranging from the CRISPR-Cas systems in bacteria to piRNAs in animals [18]. RNA-based strategies for "nonself" discrimination and adaptation have seemingly proven efficient to protect the integrity of genomes. Insights into the elegant mechanisms of these RNA-based immune systems will further our appreciation of the complex relationship between parasitic nucleic acids and their host genomes.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

References

[1] E. S. Lander, L. M. Linton, B. Birren et al., "Initial sequencing and analysis of the human genome," *Nature*, vol. 409, pp. 860–921, 2001.

[2] H. L. Levin and J. V. Moran, "Dynamic interactions between transposable elements and their hosts," *Nature Reviews Genetics*, vol. 12, no. 9, pp. 615–627, 2011.

[3] J. L. Goodier and H. H. Kazazian Jr., "Retrotransposons revisited: the restraint and rehabilitation of parasites," *Cell*, vol. 135, no. 1, pp. 23–35, 2008.

[4] K. H. Burns and J. D. Boeke, "Human transposon tectonics," *Cell*, vol. 149, no. 4, pp. 740–752, 2012.

[5] N. L. Craig, "Unity in transposition reactions," *Science*, vol. 270, no. 5234, pp. 253–254, 1995.

[6] R. K. Slotkin and R. Martienssen, "Transposable elements and the epigenetic regulation of the genome," *Nature Reviews Genetics*, vol. 8, no. 4, pp. 272–285, 2007.

[7] P. K. Mandal and H. H. Kazazian Jr., "SnapShot: vertebrate transposons," *Cell*, vol. 135, no. 1, pp. 192–192.e1, 2008.

[8] K. A. O'Donnell and J. D. Boeke, "Mighty Piwis defend the germline against genome intruders," *Cell*, vol. 129, no. 1, pp. 37–44, 2007.

[9] H. H. Kazazian Jr., "Mobile elements: drivers of genome evolution," *Science*, vol. 303, no. 5664, pp. 1626–1632, 2004.

[10] T. M. Nakamura and T. R. Cech, "Reversing time: origin of telomerase," *Cell*, vol. 92, no. 5, pp. 587–590, 1998.

[11] M.-L. Pardue, S. Rashkova, E. Casacuberta, P. G. DeBaryshe, J. A. George, and K. L. Traverse, "Two retrotransposons maintain telomeres in *Drosophila*," *Chromosome Research*, vol. 13, no. 5, pp. 443–453, 2005.

[12] E. A. Gladyshev and I. R. Arkhipova, "Telomere-associated endonuclease-deficient *Penelope*-like retroelements in diverse eukaryotes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 22, pp. 9352–9357, 2007.

[13] C. Feschotte, "Transposable elements and the evolution of regulatory networks," *Nature Reviews Genetics*, vol. 9, no. 5, pp. 397–405, 2008.

[14] B. McClintock, "Controlling elements and the gene," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 21, pp. 197–216, 1956.

[15] H. Kaneko, S. Dridi, V. Tarallo et al., "DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration," *Nature*, vol. 471, no. 7338, pp. 325–332, 2011.

[16] R. H. A. Plasterk, "RNA silencing: the genome's immune system," *Science*, vol. 296, no. 5571, pp. 1263–1265, 2002.

[17] P. A. Dumesic and H. D. Madhani, "Recognizing the enemy within: licensing RNA-guided genome defense," *Trends in Biochemical Sciences*, vol. 39, no. 1, pp. 25–34, 2014.

[18] F. Jiang and J. A. Doudna, "The structural biology of CRISPR-Cas systems," *Current Opinion in Structural Biology*, vol. 30, pp. 100–111, 2015.

[19] S.-W. Ding, "RNA-based antiviral immunity," *Nature Reviews Immunology*, vol. 10, no. 9, pp. 632–644, 2010.

[20] C. Napoli, C. Lemieux, and R. Jorgensen, "Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans," *Plant Cell*, vol. 2, no. 4, pp. 279–289, 1990.

[21] A. Fire, S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello, "Potent and specific genetic interference by double-stranded RNA in caenorhabditis elegans," *Nature*, vol. 391, no. 6669, pp. 806–811, 1998.

[22] R. C. Lee, R. L. Feinbaum, and V. Ambros, "The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*," *Cell*, vol. 75, no. 5, pp. 843–854, 1993.

[23] V. Ambros, "MicroRNAs and developmental timing," *Current Opinion in Genetics & Development*, vol. 21, no. 4, pp. 511–517, 2011.

[24] J. T. Mendell and E. N. Olson, "MicroRNAs in stress signaling and human disease," *Cell*, vol. 148, no. 6, pp. 1172–1187, 2012.

[25] J. A. Vidigal and A. Ventura, "The biological functions of miRNAs: lessons from in vivo studies," *Trends in Cell Biology*, vol. 25, pp. 137–147, 2015.

[26] L. He, X. He, S. W. Lowe, and G. J. Hannon, "MicroRNAs join the p53 network—another piece in the tumour-suppression puzzle," *Nature Reviews Cancer*, vol. 7, no. 11, pp. 819–822, 2007.

[27] D. P. Bartel and C.-Z. Chen, "Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs," *Nature Reviews Genetics*, vol. 5, no. 5, pp. 396–400, 2004.

[28] M. R. Fabian, N. Sonenberg, and W. Filipowicz, "Regulation of mRNA translation and stability by microRNAs," *Annual Review of Biochemistry*, vol. 79, pp. 351–379, 2010.

[29] H. Guo, N. T. Ingolia, J. S. Weissman, and D. P. Bartel, "Mammalian microRNAs predominantly act to decrease target mRNA levels," *Nature*, vol. 466, no. 7308, pp. 835–840, 2010.

[30] A. A. Bazzini, M. T. Lee, and A. J. Giraldez, "Ribosome profiling shows that miR-430 reduces translation before causing mRNA decay in Zebrafish," *Science*, vol. 336, no. 6078, pp. 233–237, 2012.

[31] F. E. Reyes-Turcu and S. I. Grewal, "Different means, same end—heterochromatin formation by RNAi and RNAi-independent RNA processing factors in fission yeast," *Current Opinion in Genetics & Development*, vol. 22, no. 2, pp. 156–163, 2012.

[32] Y. W. Iwasaki, M. C. Siomi, and H. Siomi, "PIWI-interacting RNA: its biogenesis and functions," *Annual Review of Biochemistry*, vol. 84, no. 1, pp. 405–433, 2015.

[33] L. Joshua-Tor and G. J. Hannon, "Ancestral roles of small RNAs: an Ago-centric perspective," *Cold Spring Harbor Perspectives in Biology*, vol. 3, no. 10, Article ID a003772, 2011.

[34] D. C. Swarts, K. Makarova, Y. Wang et al., "The evolutionary journey of Argonaute proteins," *Nature Structural & Molecular Biology*, vol. 21, no. 9, pp. 743–753, 2014.

[35] C.-D. Kuhn and L. Joshua-Tor, "Eukaryotic Argonautes come into focus," *Trends in Biochemical Sciences*, vol. 38, no. 5, pp. 263–271, 2013.

[36] J.-J. Song, S. K. Smith, G. J. Hannon, and L. Joshua-Tor, "Crystal structure of argonaute and its implications for RISC slicer activity," *Science*, vol. 305, no. 5689, pp. 1434–1437, 2004.

[37] H. Lin and A. C. Spradling, "A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the *Drosophila* ovary," *Development*, vol. 124, no. 12, pp. 2463–2476, 1997.

[38] A. Dueck and G. Meister, "Assembly and function of small RNA—Argonaute protein complexes," *Biological Chemistry*, vol. 395, no. 6, pp. 611–629, 2014.

[39] C. D. Malone and G. J. Hannon, "Small RNAs as guardians of the genome," *Cell*, vol. 136, no. 4, pp. 656–668, 2009.

[40] M. C. Siomi, K. Sato, D. Pezic, and A. A. Aravin, "PIWI-interacting small RNAs: the vanguard of genome defence," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 4, pp. 246–258, 2011.

[41] M. J. Luteijn and R. F. Ketting, "PIWI-interacting RNAs: from generation to transgenerational epigenetics," *Nature Reviews Genetics*, vol. 14, no. 8, pp. 523–534, 2013.

[42] R. C. Wilson and J. A. Doudna, "Molecular mechanisms of RNA interference," *Annual Review of Biophysics*, vol. 42, no. 1, pp. 217–239, 2013.

[43] T. Hirose, Y. Mishima, and Y. Tomari, "Elements and machinery of non-coding RNAs: toward their taxonomy," *EMBO Reports*, vol. 15, no. 5, pp. 489–507, 2014.

[44] V. V. Vagin, A. Sigova, C. Li, H. Seitz, V. Gvozdev, and P. D. Zamore, "A distinct small RNA pathway silences selfish genetic elements in the germline," *Science*, vol. 313, no. 5785, pp. 320–324, 2006.

[45] J. Brennecke, A. A. Aravin, A. Stark et al., "Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*," *Cell*, vol. 128, no. 6, pp. 1089–1103, 2007.

[46] A. Aravin, D. Gaidatzis, S. Pfeffer et al., "A novel class of small RNAs bind to MILI protein in mouse testes," *Nature*, vol. 442, no. 7099, pp. 203–207, 2006.

[47] A. Girard, R. Sachidanandam, G. J. Hannon, and M. A. Carmell, "A germline-specific class of small RNAs binds mammalian Piwi proteins," *Nature*, vol. 442, no. 7099, pp. 199–202, 2006.

[48] N. C. Lau, A. G. Seto, J. Kim et al., "Characterization of the piRNA complex from rat testes," *Science*, vol. 313, no. 5785, pp. 363–367, 2006.

[49] A. A. Aravin, M. S. Klenov, V. V. Vagin, F. Bantignies, G. Cavalli, and V. A. Gvozdev, "Dissection of a natural RNA silencing process in the *Drosophila melanogaster* germ line," *Molecular and Cellular Biology*, vol. 24, no. 15, pp. 6742–6750, 2004.

[50] A. I. Kalmykova, M. S. Klenov, and V. A. Gvozdev, "Argonaute protein PIWI controls mobilization of retrotransposons in the *Drosophila* male germline," *Nucleic Acids Research*, vol. 33, no. 6, pp. 2052–2059, 2005.

[51] A. A. Aravin, G. J. Hannon, and J. Brennecke, "The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race," *Science*, vol. 318, no. 5851, pp. 761–764, 2007.

[52] L. S. Gunawardane, K. Saito, K. M. Nishida et al., "A slicer-mediated mechanism for repeat-associated siRNA $5'$ end formation in *Drosophila*," *Science*, vol. 315, no. 5818, pp. 1587–1590, 2007.

[53] G. Sienski, D. Dönertas, and J. Brennecke, "Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression," *Cell*, vol. 151, no. 5, pp. 964–980, 2012.

[54] N. V. Rozhkov, M. Hammell, and G. J. Hannon, "Multiple roles for Piwi in silencing *Drosophila* transposons," *Genes & Development*, vol. 27, no. 4, pp. 400–412, 2013.

[55] A. Le Thomas, A. K. Rogers, A. Webster et al., "Piwi induces piRNA-guided transcriptional silencing and establishment of a repressive chromatin state," *Genes & Development*, vol. 27, no. 4, pp. 390–399, 2013.

[56] T. Schüpbach and E. Wieschaus, "Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology," *Genetics*, vol. 129, no. 4, pp. 1119–1136, 1991.

[57] C. Li, V. V. Vagin, S. Lee et al., "Collapse of germline piRNAs in the absence of Argonaute3 reveals somatic piRNAs in flies," *Cell*, vol. 137, no. 3, pp. 509–521, 2009.

[58] C. Klattenhoff, D. P. Bratu, N. McGinnis-Schultz, B. S. Koppetsch, H. A. Cook, and W. E. Theurkauf, "*Drosophila* rasiRNA pathway mutations disrupt embryonic axis specification through activation of an ATR/Chk2 DNA damage response," *Developmental Cell*, vol. 12, no. 1, pp. 45–55, 2007.

[59] Y. Chen, A. Pane, and T. Schüpbach, "Cutoff and aubergine mutations result in retrotransposon upregulation and checkpoint activation in *Drosophila*," *Current Biology*, vol. 17, no. 7, pp. 637–642, 2007.

[60] J. S. Khurana and W. E. Theurkauf, "piRNA function in germline development," in *StemBook*, 2008.

[61] K.-A. Senti and J. Brennecke, "The piRNA pathway: a fly's perspective on the guardian of the genome," *Trends in Genetics*, vol. 26, no. 12, pp. 499–509, 2010.

[62] P. M. Guzzardo, F. Muerdter, and G. J. Hannon, "The piRNA pathway in flies: highlights and future directions," *Current Opinion in Genetics & Development*, vol. 23, no. 1, pp. 44–52, 2013.

[63] E. Stuwe, K. F. Tóth, and A. A. Aravin, "Small but sturdy: small RNAs in cellular memory and epigenetics," *Genes and Development*, vol. 28, no. 5, pp. 423–431, 2014.

[64] A. Pélisson, U. S. Song, N. Prud'homme, P. A. Smith, A. Bucheton, and V. G. Corces, "Gypsy transposition correlates with the production of a retroviral envelope-like protein under the tissue-specific control of the *Drosophila* flamenco gene," *The EMBO Journal*, vol. 13, no. 18, pp. 4401–4411, 1994.

[65] S. Ronsseray, M. Lehmann, and D. Anxolabéhère, "The maternally inherited regulation of P elements in *Drosophila melanogaster* can be elicited by two P copies at cytological site 1A on the X chromosome," *Genetics*, vol. 129, no. 2, pp. 501–512, 1991.

[66] N. Prud'homme, M. Gans, M. Masson, C. Terzian, and A. Bucheton, "Flamenco, a gene controlling the gypsy retrovirus of *Drosophila melanogaster*," *Genetics*, vol. 139, no. 2, pp. 697–711, 1995.

[67] C. Biémont, "A brief history of the status of transposable elements: from junk DNA to major players in evolution," *Genetics*, vol. 186, no. 4, pp. 1085–1093, 2010.

[68] J. S. Khurana, J. Wang, J. Xu et al., "Adaptation to P element transposon invasion in *Drosophila* melanogaster," *Cell*, vol. 147, no. 7, pp. 1551–1563, 2011.

[69] F. Muerdter, I. Olovnikov, A. Molaro et al., "Production of artificial piRNAs in flies and mice," *RNA*, vol. 18, no. 1, pp. 42–52, 2012.

[70] S. Yamanaka, M. C. Siomi, and H. Siomi, "piRNA clusters and open chromatin structure," *Mobile DNA*, vol. 5, article 22, 2014.

[71] D. Vermaak and H. S. Malik, "Multiple roles for heterochromatin protein 1 genes in *Drosophila*," *Annual Review of Genetics*, vol. 43, pp. 467–492, 2009.

[72] C. Klattenhoff, H. Xi, C. Li et al., "The *Drosophila* HP1 homolog Rhino is required for transposon silencing and piRNA production by dual-strand clusters," *Cell*, vol. 138, no. 6, pp. 1137–1149, 2009.

[73] C. Beisel and R. Paro, "Silencing chromatin: comparing modes and mechanisms," *Nature Reviews Genetics*, vol. 12, no. 2, pp. 123–135, 2011.

[74] Z. Zhang, J. Wang, N. Schultz et al., "The HP1 homolog rhino anchors a nuclear complex that suppresses piRNA precursor splicing," *Cell*, vol. 157, no. 6, pp. 1353–1363, 2014.

[75] A. Sapetschnig and E. Miska, "Getting a grip on piRNA cluster transcription," *Cell*, vol. 157, no. 6, pp. 1253–1254, 2014.

[76] A. Pane, P. Jiang, D. Y. Zhao, M. Singh, and T. Schüpbach, "The Cutoff protein regulates piRNA cluster expression and piRNA production in the *Drosophila* germline," *The EMBO Journal*, vol. 30, no. 22, pp. 4601–4615, 2011.

[77] F. Mohn, G. Sienski, D. Handler, and J. Brennecke, "The rhino-deadlock-cutoff complex licenses noncanonical transcription of dual-strand piRNA clusters in *Drosophila*," *Cell*, vol. 157, no. 6, pp. 1364–1379, 2014.

[78] F. Zhang, J. Wang, J. Xu et al., "UAP56 couples piRNA clusters to the perinuclear transposon silencing machinery," *Cell*, vol. 151, no. 4, pp. 871–884, 2012.

[79] A. Le Thomas, E. Stuwe, S. Li et al., "Transgenerationally inherited piRNAs trigger piRNA biogenesis by changing the chromatin of piRNA clusters and inducing precursor processing," *Genes & Development*, vol. 28, no. 15, pp. 1667–1680, 2014.

[80] A. de Vanssay, A.-L. Bougé, A. Boivin et al., "Paramutation in *Drosophila* linked to emergence of a piRNA-producing locus," *Nature*, vol. 490, no. 7418, pp. 112–115, 2012.

[81] J. Brennecke, C. D. Malone, A. A. Aravin, R. Sachidanandam, A. Stark, and G. J. Hannon, "An epigenetic role for maternally inherited piRNAs in transposon silencing," *Science*, vol. 322, no. 5906, pp. 1387–1392, 2008.

[82] H. Nishimasu, H. Ishizu, K. Saito et al., "Structure and function of Zucchini endoribonuclease in piRNA biogenesis," *Nature*, vol. 491, no. 7423, pp. 284–287, 2012.

[83] J. J. Ipsaro, A. D. Haase, S. R. Knott, L. Joshua-Tor, and G. J. Hannon, "The structural biochemistry of *Zucchini* implicates it as a nuclease in piRNA biogenesis," *Nature*, vol. 491, no. 7423, pp. 279–282, 2012.

[84] T. Watanabe, S. Chuma, Y. Yamamoto et al., "MITOPLD is a mitochondrial protein essential for nuage formation and piRNA biogenesis in the mouse germline," *Developmental Cell*, vol. 20, no. 3, pp. 364–375, 2011.

[85] S. Kawaoka, N. Izumi, S. Katsuma, and Y. Tomari, "3′ end formation of PIWI-interacting RNAs in vitro," *Molecular Cell*, vol. 43, no. 6, pp. 1015–1022, 2011.

[86] M. D. Horwich, C. Li, C. Matranga et al., "The *Drosophila* RNA methyltransferase, DmHen1, modifies germline piRNAs and single-stranded siRNAs in RISC," *Current Biology*, vol. 17, no. 14, pp. 1265–1272, 2007.

[87] K. Saito, Y. Sakaguchi, T. Suzuki, T. Suzuki, H. Siomi, and M. C. Siomi, "Pimet, the *Drosophila* homolog of HEN1, mediates 2′-O-methylation of Piwi-interacting RNAs at their 3′ ends," *Genes & Development*, vol. 21, no. 13, pp. 1603–1608, 2007.

[88] Y. Murota, H. Ishizu, S. Nakagawa et al., "Yb integrates piRNA intermediates and processing factors into perinuclear bodies to enhance piRISC assembly," *Cell Reports*, vol. 8, no. 1, pp. 103–113, 2014.

[89] E. Voronina, G. Seydoux, P. Sassone-Corsi, and I. Nagamori, "RNA granules in germ cells," *Cold Spring Harbor Perspectives in Biology*, vol. 3, no. 12, 2011.

[90] C. Chen, T. J. Nott, J. Jin, and T. Pawson, "Deciphering arginine methylation: tudor tells the tale," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 10, pp. 629–642, 2011.

[91] H. Ishizu, H. Siomi, and M. C. Siomi, "Biology of Piwi-interacting RNAs: new insights into biogenesis and function inside and outside of germlines," *Genes and Development*, vol. 26, no. 21, pp. 2361–2373, 2012.

[92] J. Xiol, P. Spinelli, M. A. Laussmann et al., "RNA clamping by Vasa assembles a piRNA amplifier complex on transposon transcripts," *Cell*, vol. 157, no. 7, pp. 1698–1711, 2014.

[93] K. M. Nishida, Y. Iwasaki, Y. Murota et al., "Respective functions of two distinct Siwi complexes assembled during PIWI-interacting RNA biogenesis in Bombyx germ cells," *Cell Reports*, vol. 10, no. 2, pp. 193–203, 2015.

[94] F. Mohn, D. Handler, and J. Brennecke, "Noncoding RNA. piRNA-guided slicing specifies transcripts for Zucchini-dependent, phased piRNA biogenesis," *Science*, vol. 348, Article ID 812817, pp. 812–817, 2015.

[95] B. W. Han, W. Wang, C. Li, Z. Weng, and P. D. Zamore, "Noncoding RNA. piRNA-guided transposon cleavage initiates Zucchini-dependent, phased piRNA production," *Science*, vol. 348, Article ID 817821, pp. 817–821, 2015.

[96] H. Siomi and M. C. Siomi, "RNA. Phased piRNAs tackle transposons," *Science*, vol. 348, no. 6236, pp. 756–757, 2015.

[97] D. Handler, K. Meixner, M. Pizka et al., "The genetic makeup of the *Drosophila* piRNA pathway," *Molecular Cell*, vol. 50, no. 5, pp. 762–777, 2013.

[98] F. Muerdter, P. M. Guzzardo, J. Gillis et al., "A genome-wide RNAi screen draws a genetic framework for transposon control and primary piRNA biogenesis in *Drosophila*," *Molecular Cell*, vol. 50, no. 5, pp. 736–748, 2013.

[99] B. Czech, J. B. Preall, J. McGinn, and G. J. Hannon, "A transcriptome-wide RNAi screen in the *Drosophila* ovary reveals factors of the germline piRNA pathway," *Molecular Cell*, vol. 50, no. 5, pp. 749–761, 2013.

[100] G. Picard, "Non mendelian female sterility in *Drosophila melanogaster*: hereditary transmission of I factor," *Genetics*, vol. 83, no. 1, pp. 107–123, 1976.

[101] M. G. Kidwell, J. F. Kidwell, and J. A. Sved, "Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination," *Genetics*, vol. 86, no. 4, pp. 813–833, 1977.

[102] G. M. Rubin, M. G. Kidwell, and P. M. Bingham, "The molecular basis of P-M hybrid dysgenesis: the nature of induced mutations," *Cell*, vol. 29, no. 3, pp. 987–994, 1982.

[103] A. Bucheton, R. Paro, H. M. Sang, A. Pélisson, and D. J. Finnegan, "The molecular basis of I-R hybrid dysgenesis in *Drosophila melanogaster*: identification, cloning, and properties of the I factor," *Cell*, vol. 38, no. 1, pp. 153–163, 1984.

[104] A. Pélisson, "The I–R system of hybrid dysgenesis in *Drosophila Melanogaster*: are I factor insertions responsible for the mutator effect of the I–R interaction?" *Molecular & General Genetics*, vol. 183, no. 1, pp. 123–129, 1981.

[105] D. J. Finnegan, "Hybrid dysgenesis: from darkness into light: a commentary on 'Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility' by William R. Engels," *Genetics Research*, vol. 89, no. 5-6, pp. 405–406, 2007.

[106] J. P. Castro and C. M. A. Carareto, "*Drosophila melanogaster* P transposable elements: mechanisms of transposition and regulation," *Genetica*, vol. 121, no. 2, pp. 107–118, 2004.

[107] S. Chambeyron and A. Bucheton, "I elements in *Drosophila*: in vivo retrotransposition and regulation," *Cytogenetic and Genome Research*, vol. 110, no. 1–4, pp. 215–222, 2005.

[108] A. Le Thomas, G. K. Marinov, and A. A. Aravin, "A transgenerational process defines piRNA biogenesis in *Drosophila virilis*," *Cell Reports*, vol. 8, no. 6, pp. 1617–1623, 2014.

[109] T. Grentzinger, C. Armenise, C. Brun et al., "PiRNA-mediated transgenerational inheritance of an acquired trait," *Genome Research*, vol. 22, no. 10, pp. 1877–1888, 2012.

[110] C. R. L. Huang, K. H. Burns, and J. D. Boeke, "Active transposition in genomes," *Annual Review of Genetics*, vol. 46, pp. 651–675, 2012.

[111] A. A. Aravin, R. Sachidanandam, A. Girard, K. Fejes-Toth, and G. J. Hannon, "Developmentally regulated piRNA clusters implicate MILI in transposon control," *Science*, vol. 316, no. 5825, pp. 744–747, 2007.

[112] S. Kuramochi-Miyagawa, T. Kimura, T. W. Ijiri et al., "*Mili*, a mammalian member of *piwi* family gene, is essential for spermatogenesis," *Development*, vol. 131, no. 4, pp. 839–849, 2004.

[113] S. Kuramochi-Miyagawa, T. Watanabe, K. Gotoh et al., "DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes," *Genes & Development*, vol. 22, no. 7, pp. 908–917, 2008.

[114] M. A. Carmell, A. Girard, H. J. G. van de Kant et al., "MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline," *Developmental Cell*, vol. 12, no. 4, pp. 503–514, 2007.

[115] A. A. Aravin, R. Sachidanandam, D. Bourc'his et al., "A piRNA pathway primed by individual transposons is linked to De Novo DNA methylation in mice," *Molecular Cell*, vol. 31, no. 6, pp. 785–799, 2008.

[116] S. de Fazio, N. Bartonicek, M. Di Giacomo et al., "The endonuclease activity of Mili fuels piRNA amplification that silences LINE1 elements," *Nature*, vol. 480, no. 7376, pp. 259–263, 2011.

[117] A. Molaro, I. Falciatori, E. Hodges et al., "Two waves of de novo methylation during mouse germ cell development," *Genes & Development*, vol. 28, pp. 1544–1549, 2014.

[118] D. Pezic, S. A. Manakov, R. Sachidanandam, and A. A. Aravin, "piRNA pathway targets active LINE1 elements to establish the repressive H3K9me3 mark in germ cells," *Genes & Development*, vol. 28, no. 13, pp. 1410–1428, 2014.

[119] X. Z. Li, C. K. Roy, X. Dong et al., "An ancient transcription factor initiates the burst of piRNA production during early meiosis in mouse testes," *Molecular Cell*, vol. 50, no. 1, pp. 67–81, 2013.

[120] M. Di Giacomo, S. Comazzetto, H. Saini et al., "Multiple epigenetic mechanisms and the piRNA pathway enforce LINE1 silencing during adult spermatogenesis," *Molecular Cell*, vol. 50, no. 4, pp. 601–608, 2013.

[121] M. Reuter, P. Berninger, S. Chuma et al., "Miwi catalysis is required for piRNA amplification-independent LINE1 transposon silencing," *Nature*, vol. 480, no. 7376, pp. 264–267, 2011.

[122] T. Watanabe, E.-C. Cheng, M. Zhong, and H. Lin, "Retrotransposons and pseudogenes regulate mRNAs and lncRNAs via the piRNA pathway in the germline," *Genome Research*, vol. 25, no. 3, pp. 368–380, 2014.

[123] P. Zhang, J. Y. Kang, L. T. Gou et al., "MIWI and piRNA-mediated cleavage of messenger RNAs in mouse testes," *Cell Research*, vol. 25, no. 2, pp. 193–207, 2015.

[124] W. S. S. Goh, I. Falciatori, O. Tam et al., "piRNA-directed cleavage of meiotic transcripts regulates spermatogenesis," *Genes & Development*, vol. 29, pp. 1032–1044, 2015.

[125] O. H. Tam, A. A. Aravin, P. Stein et al., "Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes," *Nature*, vol. 453, no. 7194, pp. 534–538, 2008.

[126] T. Watanabe, Y. Totoki, A. Toyoda et al., "Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes," *Nature*, vol. 453, no. 7194, pp. 539–543, 2008.

[127] M. Flemr, R. Malik, V. Franke et al., "A retrotransposon-driven dicer isoform directs endogenous small interfering rna production in mouse oocytes," *Cell*, vol. 155, no. 4, pp. 807–816, 2013.

[128] E. P. Murchison, P. Stein, Z. Xuan et al., "Critical roles for Dicer in the female germline," *Genes & Development*, vol. 21, no. 6, pp. 682–693, 2007.

[129] E. F. Roovers, D. Rosenkranz, M. Mahdipour et al., "Piwi proteins and piRNAs in mammalian oocytes and early embryos," *Cell Reports*, vol. 10, no. 12, pp. 2069–2082, 2015.

[130] P. Svoboda, "Renaissance of mammalian endogenous RNAi," *FEBS Letters*, vol. 588, no. 15, pp. 2550–2556, 2014.