Developmental Cell
Review

Small RNA-Mediated Quiescence of Transposable Elements in Animals

Kuniaki Saito[1,2,*] and Mikiko C. Siomi[1,3,*]
[1]Department of Molecular Biology, Keio University School of Medicine, Tokyo 160-8582, Japan
[2]JST, PRESTO, Saitama 332-0012, Japan
[3]JST, CREST, Saitama 332-0012, Japan
*Correspondence: saito@z6.keio.jp (K.S.), siomim@sc.itc.keio.ac.jp (M.C.S.)
DOI 10.1016/j.devcel.2010.10.011

Transposable elements (TEs) are major components of the intergenic regions of the genome. However, TE transposition has the potential to threaten the reproductive fitness of the organism; therefore, organisms have evolved specialized molecular systems to sense and repress the expression of TEs to stop them from jumping to other genomic loci. Emerging evidence suggests that Argonaute proteins play a critical role in this process, in collaboration with two types of cellular small RNAs: PIWI-interacting RNAs (piRNAs) of the germline and endogenous small interfering RNAs (endo-siRNAs) of the soma, both of which are transcribed from TEs themselves.

Introduction

Transposable elements (TEs) were discovered in maize by Barbara McClintock in the 1940s. Since then, various TEs and TE-like sequences have been identified in many species. Worldwide genome projects have revealed that TEs are actually major genomic components. For example, TEs comprise 45% of the human genome (Lander et al., 2001) and 15%–22% of the genome of *Drosophila melanogaster* (Kapitonov and Jurka, 2003; Biémont and Vieira, 2006). TEs can be separated into Class 1 and Class 2, based on their structures and modes of integration (Gogvadze and Buzdin, 2009). Class 1 comprises retrotransposons and retrotransposon-like elements, such as LINEs and SINEs, which replicate via transcribed mRNA intermediates that are converted back into DNA through multiple rounds of reverse transcription. The retrotransposon DNAs thus transpose into the genome by a mechanism analogous to "copy and paste." Class 2 TEs consist of DNA transposons only. Unlike retrotransposons, DNA transposons move directly from place to place through a "cut and paste" process.

The significance and biological implications of TEs and TE-like sequences have been thoroughly reviewed (Goodier and Kazazian 2008; Gogvadze and Buzdin, 2009). TEs can occasionally play beneficial roles in organisms. Transposition of LINE-1 elements contributes to genetic diversity in neuronal progenitor cells during the development of the nervous system (Coufal et al., 2009). LINE-1 expression may contribute to the establishment of X inactivation in ES cells (Chow et al., 2010). The human endogenous retrovirus (HERV-W) encodes an envelope protein named syncytin, which plays important roles in trophoblast cell fusion and placental morphogenesis (Mi et al., 2000). The telomeric ends of linear chromosomes are protected by TEs in *Drosophila* (Pardue et al., 2005). And in many organisms, heterochromatic centromeres, which are crucial for the segregation of chromosomes during cell division, consist of repeats and TEs (e.g., Volpe et al., 2002). However, the mobilization of TEs is often considered deleterious because it leads to genomic structural rearrangements, such as deletions, duplications, and inversions. Organisms have thus developed effective strategies that constrain TE transposition.

One such mechanism is mediated by small RNAs, in a process that is highly analogous to RNA interference (RNAi) or RNA silencing (Ghildiyal and Zamore, 2009; Kim et al., 2009; Malone and Hannon, 2009; Siomi et al., 2008; Siomi and Siomi, 2009; Thomson and Lin, 2009). RNA silencing is triggered by small RNAs 20–30 nucleotides (nt) in length, which are often, if not universally, processed from double-stranded RNAs (dsRNAs) by the enzyme Dicer. The small RNAs are then loaded onto members of the Argonaute family of proteins. Argonaute proteins bind target transcripts by means of base pairing between the small RNA and the target; this interaction confers target specificity on Argonaute regulation of transcription, transcript stability, and/or translation. Accumulating evidence indicates that TEs are silenced by similar (transcriptional or posttranscriptional) mechanisms, in which small RNAs and Argonaute proteins play key roles.

The numbers and types of Argonaute family members differ between species (Cerutti et al., 2000). In the paradigmatic example of *Drosophila*, five Argonaute proteins are expressed. Among these, the AGO proteins (AGO1 and AGO2) are ubiquitous, whereas the others (AGO3, Aubergine [Aub], and Piwi) are gonad specific (Williams and Rubin, 2002) and are generically referred to as PIWI proteins (Carmell et al., 2002). PIWI-interacting small RNAs (piRNAs) are mainly derived from intergenic regions containing TEs, TE remnants, and other repetitive elements (O'Donnell and Boeke, 2007; Klattenhoff and Theurkauf, 2008; Siomi and Kuramochi-Miyagawa, 2009), and mutations in *PIWI* genes cause derepression of TEs in gonadal cells (Aravin et al., 2001; Kalmykova et al., 2005; Savitsky et al., 2006; Sarot et al., 2004; Vagin et al., 2006; Li et al., 2009). Thus, PIWI/piRNA complexes protect the integrity of the germline by suppressing TE expression and mobilization there. Conversely, AGO2-associated small RNAs have a similar role in nongonadal somatic cells (Golden et al., 2008; Okamura and Lai, 2008; Ghildiyal and Zamore, 2009). The endogenous small RNAs associated with AGO2 also arise from intergenic elements

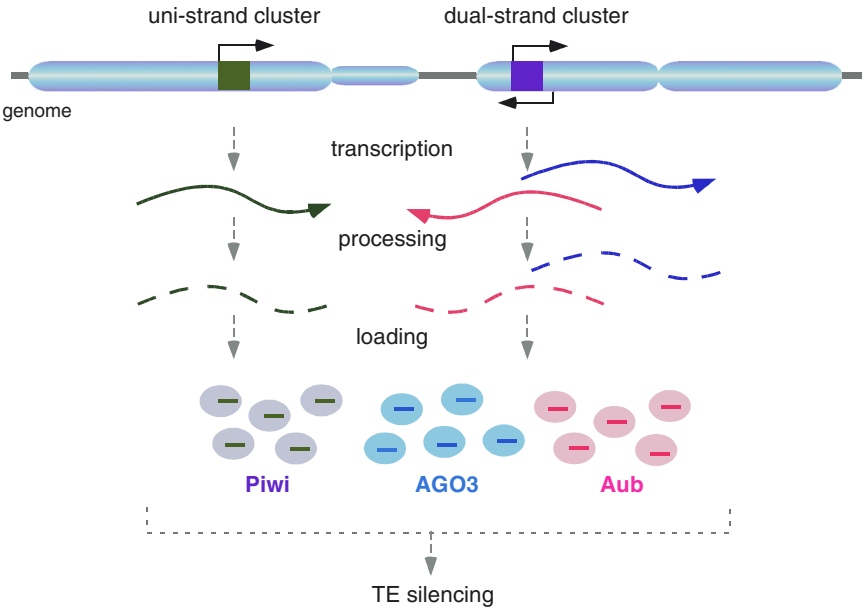

Figure 1. The piRNA Clusters Form Two Groups: Uni-Strand and Dual-Strand Clusters

The uni-strand clusters are transcribed in only one direction whereas the dual-strand clusters produce piRNAs from both strands. The sense (blue) and antisense (red) transcripts are complementary to each other. However, piRNAs are produced in a manner independent of Dicer activity; thus, it is implied that they are produced from single-stranded TE precursors (green, red, and blue). After processing, mature piRNAs are loaded onto PIWI proteins (Aub, Piwi, and AGO3) and silence TEs by mechanisms whose details remain elusive.

that contain TE fragments and repeats, and are now referred to as endogenous small interfering RNAs (endo-siRNAs or esiRNAs). AGO/endo-siRNA components and PIWI/piRNA components are highly conserved in vertebrates and invertebrates, and they all function in TE silencing.

In this review, we discuss how piRNAs and endo-siRNAs are produced and how these small RNAs silence TEs, focusing on studies in *Drosophila melanogaster* and then comparing and contrasting with other organisms such as mice, frogs, and zebrafish.

PIWI Proteins in *Drosophila*

The *piwi* gene was originally discovered as an essential factor for germline stem cell self-renewal in *Drosophila* (Lin and Spradling, 1997; Cox et al., 1998). Further investigation showed that mutations in *piwi* cause hyperactivation of retrotransposons (Vagin et al., 2006; Klenov et al., 2007). Likewise, the paralogous *aub* gene is necessary for pole cell formation (Harris and Macdonald, 2001) and transposon silencing (Vagin et al., 2006), and *PIWI* genes are commonly required for female fertility (Cox et al., 1998; Schmidt et al., 1999; Li et al., 2009).

The PIWI proteins show different subcellular localization and expression patterns, suggesting distinct and discrete roles. Among the PIWI proteins, only Piwi is localized in the nucleus (Cox et al., 2000), and its primary function in TE silencing appears to be in the nucleus (Saito et al., 2010). In ovaries, Piwi can be detected in both somatic and germ cells (Cox et al., 2000; Saito et al., 2006; Brennecke et al., 2007; Nishida et al., 2007). In the testes, however, Piwi is barely detectable in germ cells but is strongly expressed in somatic niche cells, which associate with and maintain the germline stem cells. Aub and AGO3 are expressed in the germ cells of both males and females, but are absent in gonadal somatic cells. Unlike Piwi, Aub and AGO3 are exclusively detected in the cytoplasm at steady-state levels and accumulate in the nuage (Brennecke et al., 2007; Gunawardane et al., 2007; Nishida et al., 2007; Li et al., 2009), an electron-dense perinuclear structure characteristic of germline cells in diverse animals (al-Mukhtar and Webb, 1971; Eddy, 1975). These observations indicate that the PIWI proteins possess distinct functions and utilize different systems for expression and subcellular localization.

piRNAs in *Drosophila*

The first direct link between PIWI proteins and TEs was the discovery of piRNAs, defined as small RNAs associated with PIWI proteins in any organism. Typically, piRNAs are 23–30 nt long. Sequencing of piRNAs in *Drosophila* has revealed that they mainly originate from TEs and TE-related genomic elements (Saito et al., 2006; Brennecke et al., 2007; Gunawardane et al., 2007; Yin and Lin, 2007). Whereas miRNAs are processed by Dicer from double-stranded primary precursors (Lee et al., 2004), piRNAs are produced in a Dicer-independent manner from single-stranded precursors (Vagin et al., 2006). piRNAs are considerably more heterogeneous than miRNAs. More than 70% of piRNAs have been cloned only once, indicating that the piRNA population is very complex, in clear contrast to the miRNA population, which consists of approximately 170 species in *Drosophila* (Brennecke et al., 2007; Yin and Lin, 2007). Mapping of piRNAs on the *Drosophila* genome revealed that several hundred genomic regions could be referred to as piRNA clusters. These clusters contribute to the generation of piRNAs, most of which encode TEs and TE remnants and are mainly localized in the pericentromeric and subtelomeric heterochromatin regions.

The piRNA clusters fall into two groups: dual-strand and uni-strand clusters (Figure 1). The dual-strand clusters produce piRNAs from both genomic strands, with the representative being *42AB* on chromosome 2. The uni-strand clusters are transcribed in only one direction and are represented by the *flamenco (flam)* locus on chromosome X. The *flam* locus was first discovered as a regulator of the activity of the retroviral *gypsy*, *idefix*, and *ZAM* elements (Pélisson et al., 1994; Desset et al., 2008). Antisense-oriented copies of these retro-elements in the *flam* locus result in the production of mainly antisense

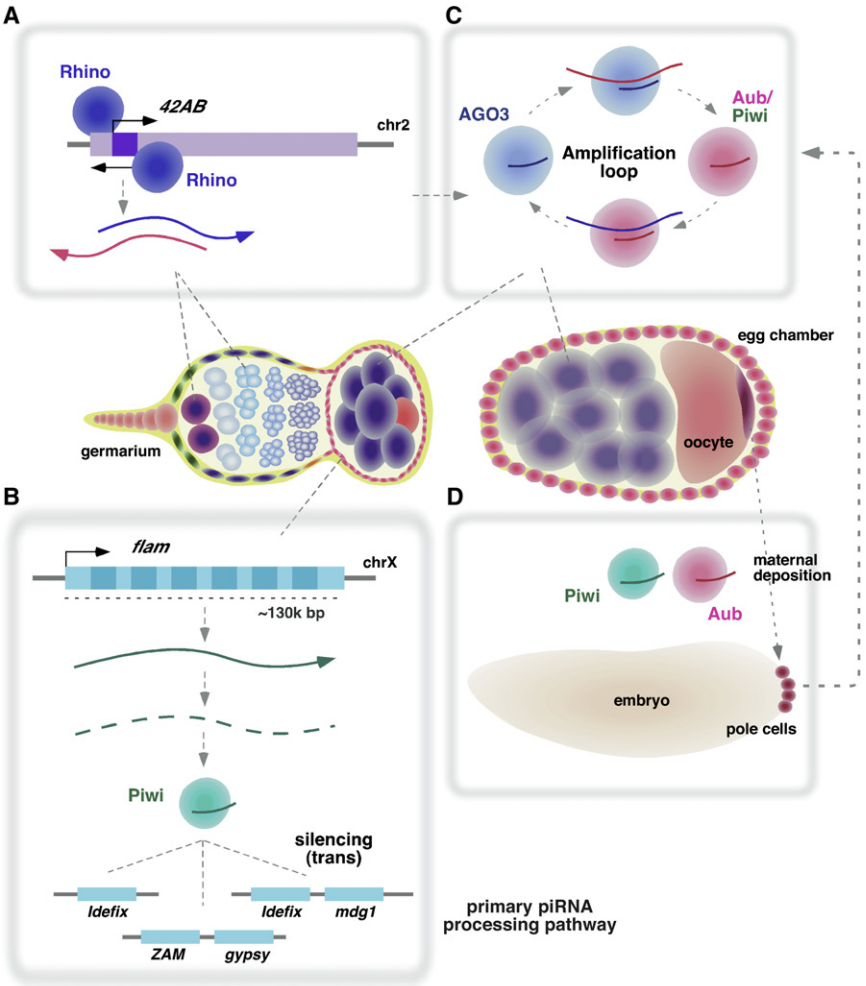

Figure 2. Models for piRNA Biogenesis in *Drosophila* Ovaries

(A) The piRNA *42AB* locus on chromosome 2 is transcribed in both directions and the sense and antisense transcripts serve as piRNA precursors. Rhino associates with DNA from this locus and is required for its transcription. The production of *42AB*-derived piRNAs occurs in germline cells in ovaries. These piRNAs are later amplified by the ping-pong cycle.

(B) The *flam* piRNA locus on chromosome X gives rise to transcripts in one direction. The *flam*-derived piRNAs are loaded onto Piwi and are not amplified. These processes occur only in the ovarian somatic cells.

(C) Amplification of piRNAs derived from the primary processing pathway. The piRNAs deposited from the mother to the offspring are also subject to the ping-pong cycle, specifically in the germline cells of the ovary.

(D) Aub and Piwi associated with piRNAs accumulate at the posterior pole of the oocytes in egg chambers. In developing embryos, the Aub- and Piwi-piRNA complexes are incorporated into the primordial germ cells. AGO3 does not seem to be passed from the mother to the offspring.

piRNAs (Figure 2; Brennecke et al., 2007). Although the mechanisms of transcription from the uni-strand piRNA clusters remain unclear, recent chromatin immunoprecipitation analysis revealed that the heterochromatin protein 1 (HP1) homolog Rhino associates with DNA of the dual-strand *42AB* cluster and is required for its transcription. In *rhino* mutant ovaries, the level of piRNAs was decreased by around 80% compared with wild-type ovaries, and in parallel, a significant reduction in putative precursor RNA was observed, suggesting that Rhino possibly promotes the transcription of all the dual-strand clusters, initiating piRNA biogenesis (Klattenhoff et al., 2009).

piRNA Biogenesis in *Drosophila*: Ping-Pong Amplification

Examination of the nucleotide preferences of piRNAs associated with Piwi and Aub in *Drosophila* ovaries has shown that they have a strong bias for uracil (U) at their 5′ end (1-U) and are mainly antisense to active TE transcripts (Saito et al., 2006; Brennecke et al., 2007; Gunawardane et al., 2007; Yin and Lin, 2007). In contrast, piRNAs bound to AGO3 are mostly derived from the sense transcripts of TEs and show a strong bias for adenosine (A) at the tenth nucleotide from their 5′ end (10-A). In fact, the first 10 nucleotides of antisense piRNAs frequently overlap with the sense piRNAs. These findings (including the 1-U/10-A relationship) are referred to as the ping-pong signature; they imply that AGO3-associated piRNAs may pair with Aub- (and Piwi-) associated piRNAs through their first 10 nucleotides (Brennecke et al., 2007; Gunawardane et al., 2007). Moreover, recombinant Piwi, Aub, and AGO3 cleave complementary target RNAs between nucleotides 10 and 11 of small guiding RNAs (Gunawardane et al., 2007), suggesting an intriguing model in which PIWI proteins generate 5′ ends of piRNAs and amplify piRNAs from longer (e.g., conventional) transcripts in a Slicer-dependent manner (Figure 2). This model is called the ping-pong cycle or amplification loop and explains why piRNAs are generated in a Dicer-independent manner, in contrast to siRNAs and miRNAs (Vagin et al., 2006).

In the ping-pong cycle, the Aub complex associated with an antisense piRNA specifically cleaves TE transcripts in the sense orientation. Therefore, the ping-pong cycle not only produces piRNAs but also degrades TE mRNAs at the same time. This model was confirmed by a recent study on *ago3* mutant flies (Li et al., 2009). In *ago3* mutants, the number of Aub-bound piRNAs was markedly decreased and their antisense strand bias was weakened from 71% to 41%. Some TEs were derepressed in *ago3* mutants, as in *aub* mutants. These observations support the notion that TE silencing and piRNA biogenesis are coupled (Li et al., 2009). Thus, the seeds to initiate the ping-pong cycle are supplied by the primary processed or maternally deposited piRNAs (see below).

piRNA Biogenesis in *Drosophila*: The Primary Processing Pathway

Although Aub- and AGO3-associated piRNAs are mainly produced by the ping-pong cycle, Piwi-associated piRNAs show few ping-pong signatures, suggesting a ping-pong-independent piRNA biogenesis pathway (Brennecke et al., 2007). Piwi is required for the silencing of a subset of TEs in gonadal somatic cells. In fact, the majority of Piwi-associated piRNAs in ovaries are derived from the *flam* uni-strand cluster, which is expressed only in the soma (Brennecke et al., 2007). Further studies suggested the idea that *flam* piRNAs are loaded onto Piwi by a mechanism distinct from the amplification cycle (Malone et al., 2009; Li et al., 2009). In the ovarian somatic cell (OSC) line, which contains only somatic cells of fly ovaries, Piwi is expressed, but Aub and AGO3 are not, suggesting that the piRNAs in OSCs are produced by the primary processing pathway but are not amplified further by the amplification cycle (Saito et al., 2009). In OSCs, Piwi is associated with abundant piRNAs, including *flam*-derived piRNAs, confirming that *flam*-piRNAs are produced only by the primary pathway (Figure 2).

Mechanisms of PIWI-piRNA-Mediated Silencing

Aub and AGO3 show Slicer activity (the RNaseH-like endonuclease activity of their PIWI domains) in vitro (Gunawardane et al., 2007) and localize in the cytoplasm in vivo (Nishida et al., 2007; Li et al., 2009). Therefore, Aub-piRNA and AGO3-piRNA complexes most probably silence TEs posttranscriptionally by cleaving their transcripts. The Aub-piRNA complex may also induce mRNA decay to silence target genes such as *nanos*, the embryonic posterior morphogen, by recruiting deadenylation enzyme CCR4 and the RNA-binding protein Smaug to the target mRNA (Rouget et al., 2010). How then do Piwi and the somatic primary piRNAs act to silence TEs? One model is Slicer-mediated RNA cleavage, because recombinant Piwi produced from *E. coli* possesses Slicer activity (Saito et al., 2006). Another feasible model is that Piwi is involved in heterochromatic gene silencing. This model was suggested from the observations that Piwi influences position-effect variegation (PEV) (Pal-Bhadra et al., 2004). PEV is a probabilistic variegation in gene silencing that depends on the genomic locus; for example, if a euchromatic gene were relocated in close proximity to heterochromatin through a genetic rearrangement such as inversion or translocation, there is a high chance that this gene will be silenced by the heterochromatin. It has also been shown that HP1a and Piwi associate specifically with heterochromatin-containing TEs, such as *1360* and *F element*. Piwi may direct HP1a on heterochromatin to silence TEs in early-stage embryos (Pal-Bhadra et al., 2004). However, *piwi* mutations resulted in an increase of HP1 association with the *3R-TAS* subtelomeric region in ovaries and subsequently increased transcriptional silencing, suggesting that Piwi might act as an activator of TEs at *3R-TAS* (Yin and Lin, 2007). In addition, a recent study showed that HP1 recruitment on heterochromatin does not depend on Piwi (Moshkovich and Lei, 2010). The mechanism by which the piRNA pathway contributes to heterochromatin regulation thus remains controversial.

piRNA-Mediated TE Repression in Vertebrates

PIWI proteins are conserved in vertebrates (Figure 3) and have conserved functions in TE silencing and germ cell development.

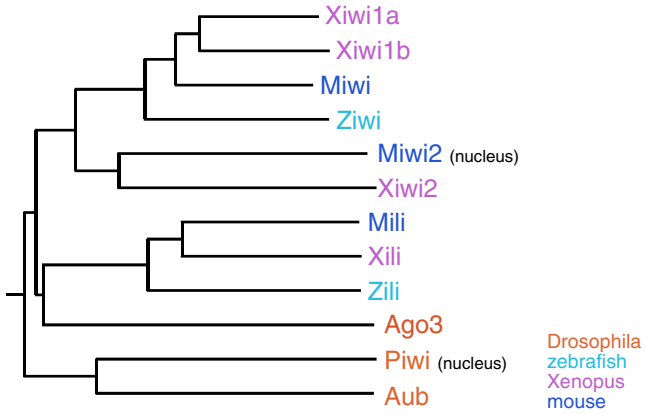

Figure 3. Phylogenetic Tree of Piwi Proteins in Fleis, Mice, Frogs, and Zebrafish

Colors: flies, orange; mice, dark blue; frogs, pink; and zebrafish, sky blue. The tree was based on ClustalW alignment of the predicted peptide sequences of PIWI proteins. Piwi: *D. melanogaster* NP_476875; Aub: *D. melanogaster* CAA64320; Ago3: *D. melanogaster* ABO27430; Ziwi: *D. rerio* NP_899181; Zili: *D. rerio* ABM46842; Miwi: *M. musculus* NM_021311; Miwi2: *M. musculus* NM_177905; Mili: *M. musculus* NM_021308. The peptide sequences of Xiwi1a, Xiwi1b, Xiwi2, and Xili were retrieved from Wilczynska et al. (2009).

In mice, three PIWI proteins, mouse Piwi (Miwi), Miwi-like (Mili), and Miwi2 (Kuramochi-Miyagawa et al., 2001, 2004; Deng and Lin, 2002; Carmell et al., 2007), have been characterized, and loss of each leads to derepression of TEs and spermatogenic arrest (Kuramochi-Miyagawa et al., 2001, 2004; Aravin et al., 2007a; Carmell et al., 2007). Mili expression begins in primordial germ cells at embryonic day (E) 12.5 and continues throughout adult spermatogenesis, up to the round spermatid stage (Kuramochi-Miyagawa et al., 2004). Miwi2 is transiently expressed in embryonic gonocytes from E15.5, with its expression decreasing after birth, prior to meiosis (Aravin et al., 2008, Kuramochi-Miyagawa et al., 2008). Miwi is specific to meiotic and postmeiotic spermatogenesis of the adult; it is expressed from pachytene to round spermatid stages (Kuramochi-Miyagawa et al., 2001; Deng and Lin, 2002). piRNAs isolated from PIWI proteins at each developmental stage can be divided into three groups: prenatal piRNAs, prepachytene piRNAs, and pachytene piRNAs. Mili and Miwi2 associate with prenatal piRNAs, largely corresponding to TEs in fetal gonads (Aravin et al., 2007a, 2008; Kuramochi-Miyagawa et al., 2008). At the prepachytene stage, Mili, the only Piwi protein expressed at this stage, associates with prepachytene piRNAs containing both TE and non-TE sequences (Aravin et al., 2007a, 2008; Kuramochi-Miyagawa et al., 2008). Miwi associates with pachytene piRNAs derived from non-TE elements, with only 12%–17% of pachytene piRNAs corresponding to TEs (Aravin et al., 2006; Girard et al., 2006; Grivna et al., 2006; Watanabe et al., 2006). Consistent with the distinct temporal profiles of each paralog and its associated piRNAs, the loss of zebrafish Mili (Zili) leads to the expression of TEs in the developing gonad and early failure of germline development, while the loss of zebrafish Miwi (Ziwi) causes apoptosis later in the germline, at the premeiotic stage. In zebrafish and *Xenopus*, the majority of piRNAs correspond to TEs (Houwing et al., 2008; Kirino et al., 2009; Lau et al., 2009), similar to piRNAs in *Drosophila*. Unlike *Drosophila* and mice, the

Xenopus genome contains numerous DNA transposons (Jurka et al., 2005), and thus the proportion of piRNAs targeting DNA transposons is high (Lau et al., 2009). These studies indicate that piRNA content differs with development and the genomic context.

Accumulating evidence has demonstrated that piRNA biogenesis occurs through the primary piRNA processing and the amplification loop in mice, frogs, and zebrafish (Aravin et al., 2007b; Ghildiyal and Zamore, 2009; Houwing et al., 2007; Kim et al., 2009; Malone and Hannon, 2009). In gonocytes of fetal mice, where both Mili and Miwi2 are expressed, the ping-pong signature is obvious between Mili-associating sense 10-A piRNAs and Miwi2-associating antisense 1-U piRNAs. Moreover, the ping-pong signature is found in zebrafish and *Xenopus* (Kirino et al., 2009; Lau et al., 2009; Houwing et al., 2008), which strongly supports the existence of conserved mechanisms of biogenesis in vertebrates. Interestingly, piRNA amplification is not limited to cycles between distinct PIWI proteins. At the pre-pachytene stage, Mili shows a tendency to associate with both 1-U and 10-A piRNAs during a certain period (Aravin et al., 2007a). Similar to Mili, Ziwi may be able to amplify piRNAs without Zili expression (Houwing et al., 2008), suggesting that PIWI proteins self-amplify piRNAs via the amplification loop. Taken together, these studies indicate that, as in *Drosophila*, TE mRNAs in vertebrates are degraded by PIWI proteins via a ping-pong cycle.

Intriguingly, *Mili* and *Miwi2* mutant mice exhibit loss of DNA methylation in TEs. Further studies have clarified that the Mili- and Miwi2-piRNA complexes are involved in de novo DNA methylation of TEs (Aravin et al., 2008; Kuramochi-Miyagawa et al., 2008). In general, CpG DNA methylation causes stable repression of transcription (Bourc'his and Bestor, 2004). However, it remains unclear how piRNAs are involved in DNA methylation.

Scaffolds and Modifiers of piRNA Pathways

Tudor (Tud) was identified as a nuage component required for gametogenesis in classical genetic analyses of the *Drosophila* germline (Boswell and Mahowald, 1985; Thomson and Lasko, 2004, 2005; Arkov et al., 2006). More recently, biochemical and structural studies have revealed that the Tudor domains of Tud bind directly to symmetrical dimethyl arginines (sDMAs) in PIWI proteins (Kirino et al., 2009; Nishida et al., 2009; Liu et al., 2010). This arginine methylation is mediated by the methyltransferase PRMT5 (also known as Dart5/Capsuleen) (Kirino et al., 2009), which is also required for germline development. Complexes consisting of Tud, Aub, and AGO3 are heteromeric and contain RNAs resembling the precursors for both sense and antisense piRNAs (Nishida et al., 2009). This suggests the involvement of Tud in piRNA biogenesis, e.g., as a platform for piRNA amplification. Loss of Tud function in ovaries causes Aub to be associated with a greater abundance of piRNAs compared with Aub in wild-type ovaries (Nishida et al., 2009). The population of TE-derived piRNAs was significantly altered by loss of Tud function, although an obvious change in the strand bias was not seen in the mutants (Nishida et al., 2009). Loss of *dprmt5* diminishes piRNA association with Aub (Nishida et al., 2009), suggesting that sDMA modification itself influences the RNA-binding capacity of PIWI proteins, independently of Tudor domain proteins.

In mice too, Tudor-domain-containing proteins (or Tdrds) associate with PIWI proteins specifically through sDMA modification (Chen et al., 2009; Kojima et al., 2009; Reuter et al., 2009; Shoji et al., 2009; Vagin et al., 2009; Wang et al., 2009). Tdrd1 is required for efficient operation of the ping-pong cycle (Vagin et al., 2009). In *Tdrd1* and *Tdrd9* mutants, the sequence profile of piRNAs is altered (Reuter et al., 2009; Shoji et al., 2009). Thus, Tudor domain proteins have conserved roles in the quality control of transposon-derived piRNAs (Table 1; Siomi et al., 2010).

Tejas, another Tud-domain protein expressed in *Drosophila* ovaries, also associates with Aub (Patil and Kai, 2010). However, unlike Tudor, Tejas does not seem to require sDMA modification to associate with Aub. In *Tejas* mutants, steady-state levels of piRNAs were low and TEs reactivated; thus, Tejas modulates piRNA biogenesis. *krimper* (Lim and Kai, 2007), *zucchini* (*zuc*) (Pane et al., 2007), *spn-E* (Vagin et al., 2006), and *maelstrom* (*mael*) (Findley et al., 2003; Lim and Kai, 2007) also affect piRNA accumulation, and TEs are derepressed in the ovaries of mutants for all these genes (Table 1; Vagin et al., 2006; Lim and Kai, 2007; Pane et al., 2007). Deep-sequencing of piRNAs in these mutants has shown distinct requirements: for example, *spn-E* mutations reduce not only piRNA amplification but also the production of primary piRNAs originating from cluster 2, one of ping-pong-independent uni-strand clusters (Malone et al., 2009). However, *spn-E* is apparently unnecessary for producing primary piRNAs from *flam* in ovary (Malone et al., 2009). On the other hand, *zuc*, which encodes a putative nuclease, is necessary for somatic primary piRNA processing but not for the ping-pong cycle (Haase et al., 2010; Malone et al., 2009; Olivieri et al., 2010; Saito et al., 2010). These observations suggest that piRNA pathway components perform different and specific roles in piRNA biogenesis in *Drosophila*. Krimper and Spn-E contain Tud domains; however, whether they interact with PIWI proteins through sDMAs remain unknown.

Krimper, Zuc, and Mael localize to the nuage, suggesting that nuage might be an important site of piRNA processing in germ cells. Recent studies indicate that Yb bodies consisting of Yb and Armitage (Armi) are the sites for somatic primary piRNA processing (Olivieri et al., 2010; Saito et al., 2010). Both Armi and Yb contain an RNA helicase domain and therefore are implicated in RNA metabolism. In fact, mutations in Armi cause a severe decrease in piRNA accumulation in ovaries (Vagin et al., 2006). In Armi- or Yb-depleted cells of the ovarian soma, Piwi is not associated with piRNAs and mislocalizes to the cytoplasm, resulting in a general loss of piRNAs, and suggesting that Armi and Yb are piRNA biogenesis modulators. Yb contains a Tud domain; however, its function remains undetermined. In mice, piRNA pathway components exhibit differential localization. Miwi2/Tdrd9/Mael colocalizes with the components of Processing bodies referred to as piP-bodies, whereas Mili/Tdrd1/MVH/GASZ associates with the intermitochondrial cement or pi-bodies (Aravin et al., 2009; Ma et al., 2009; Shoji et al., 2009; Kuramochi-Miyagawa et al., 2010). piP-body formation depends on pi-bodies, and Miwi2 fails to associate with piRNAs when pi-bodies are not formed. On the other hand, pi-body formation is not affected by piP-body disruption, suggesting a possible progression of piRNA processing from pi-body to piP-body. Thus, both piP-bodies and pi-bodies are implicated as the sites for piRNA biogenesis in mice. It will be interesting

Table 1. Features of TE-Derived Small RNAs in Animals

	Mouse	Frog	Zebrafish	Fly	Function/Motif	References
Small RNA	piRNA	piRNA	piRNA	piRNA		reviewed in Klattenhoff and Theurkauf, 2008; Siomi and Kuramochi-Miyagawa, 2009; Ghildiyal and Zamore, 2009; Kim et al., 2009; Malone and Hannon, 2009; Thomson and Lin, 2009; Lau, 2010; Senti and Brennecke, 2010
Size (nt)	25–30	26–31	27–30	24–30		see entry above
Nucleotide preference	5'U; 10^{th} A	5'U; 10^{th} A	5'U; 10^{th} A	5'U; 10^{th} A		see entry above
Associated Argonaute protein	Miwi; Mili; Miwi2	Xiwi1a,1b; Xili; Xiwi2	Ziwi; Zili	Piwi; Aub; AGO3		see entry above
Amplification of small RNA (5'U-10^{th}A)	Mili-Miwi2; Mili-Mili	not determined	Ziwi-Zili	Piwi-AGO3; Aub-AGO3		see entry above
Other factors required for small RNA-mediated TE silencing	Tdrd-1	Tdrd-1*	Tdrd-1*		Tudor, MYND	Vagin et al., 2009; Reuter et al., 2009
	Tdrd-9	Tdrd-9*	Tdrd-9*	Spindle E	Tudor, DEXDc	Shoji et al., 2009; Vagin et al., 2006
	Maelstrom	Maelstrom*	Maelstrom*	Maelstrom	HMG	Soper et al., 2008; Lim and Kai, 2007
	Tdrd-6*	Tdrd-6*	Tdrd-6*	Krimper	Tudor	Lim and Kai, 2007
	Mov10l1	Mov10l1*	Mov10l1*	Armitage	Helicase?	Zheng et al., 2010; Frost et al., 2010; Vagin et al., 2006
	Tdrd-7*/Tdrd-5*	Tdrd-7*/Tdrd-5*	Tdrd-7*/Tdrd-5*	Tejas	Tudor	Patil and Kai, 2010
	MVH	DDX4*	Vasa*	Vasa	DEAD	Kuramochi-Miyagawa et al., 2010; Lim and Kai, 2007
	PLD6*	PLD6*	PLD6*	Zucchini	Nuclease?	Pane et al., 2007
				Squash	Nuclease?	Pane et al., 2007
	PRMT5*	PRMT5*	PRMT5*	dPRMT5	Methyltransferase	Kirino et al., 2009
				Fs(1)Yb	Tudor	Olivieri et al., 2010; Saito et al., 2010
	GASZ	GASZ*	GASZ*	CG2183*	ANK repeat	Ma et al., 2009

Genes marked with an asterisk show that the involvement on piRNA-mediated TE silencing has not been confirmed experimentally.

to determine what the specificity of these structures might be, for example, whether primary and ping-pong processes are differentially localized between them.

Germline Transmission and Hybrid Dysgenesis

The complex relationship between nuage architecture, germline development, and piRNA pathways suggests that the broader implications of piRNA-mediated germline integrity control are worth considering. During ovary development, Aub and Piwi accumulate in specialized cytoplasm at the posterior pole of the oocyte, where germ cell determinants are localized (Rongo and Lehmann, 1996). Maternally deposited Aub and Piwi are thus incorporated into the primordial germ cells that inherit this "posterior pole plasm" and are transmitted to the next generation directly. In contrast, AGO3 does not seem to be passed on from mother to offspring, suggesting that antisense piRNAs may be selectively inherited, whereas sense piRNAs are not. Brennecke et al. revealed that piRNAs are truly transmitted from mother to offspring (Brennecke et al., 2008). This maternal inheritance of piRNAs was shown to explain a long-standing but poorly understood phenomenon of "hybrid dysgenesis," in which the introduction of new genetic material via the male germline induces genetic instability in female progeny (Kidwell and Kidwell, 1976). The relevant genetic material was known to include TEs for some time (e.g., Rubin et al., 1982), and a maternally transmitted epigenetic suppressor, called a cytotype, had

been proposed (Engels, 1979), but it took several decades before piRNA characterization progressed to a point where the realization could be made that the cytotype corresponds to maternally inherited PIWI/piRNA complexes (Figure 2; Brennecke et al., 2008). These results extended the paradigm of piRNA-dependent germline integrity, to provide molecular insight into the epigenetics of incompatibility between diverging animal subpopulations.

Interestingly, fertility is enhanced by two environmental factors, age and high temperature (Bucheton, 1978). Heat shock protein 90 (Hsp90), a chaperone involved in several cellular and developmental pathways, has been implicated in TE control mediated by piRNAs (Specchia et al., 2010). Mutations affecting Hsp90, or treatment with the specific Hsp90 inhibitor geldanamycin, decreased piRNA biogenesis and increased the expression levels and the mobilization rates for all types of TEs. These observations suggest that Hsp90 may act as a piRNA factor and couple the control of TEs to environmental inputs.

Endogenous Small Interfering RNAs: Biogenesis

Recent studies have indicated that TE silencing by small RNAs is not a gonad-specific event. Rather, TE transcripts are converted into a subset of small RNAs in nongonadal somatic cells, which display different characteristics from those of piRNAs. These small RNAs are designated endo-siRNAs or esiRNAs. In *Drosophila*, endo-siRNAs specifically bind to AGO2, the effector of RNAi (Figure 4). AGO2 was originally demonstrated to associate with exogenous siRNAs (exo-siRNAs) derived from external dsRNAs in RNAi. The exo-siRNAs produced from viral dsRNAs have been shown to serve as a defense mechanism against viral infection (Galiana-Arnoux et al., 2006; van Rij et al., 2006; Wang et al., 2006). However, the recent discovery of endo-siRNAs clarified that AGO2 does not always wait for "external" siRNAs, but rather plays particular roles in cellular RNAi with endo-siRNAs.

Like piRNAs, endo-siRNAs are mainly derived from TEs, heterochromatic regions, and intergenic elements of the genome. The endo-siRNAs occasionally arise from protein-coding genes transcribed from overlapping 3′ untranslated regions (UTRs) or long hairpin RNA genes (Czech et al., 2008; Ghildiyal et al., 2008; Kawamura et al., 2008; Okamura et al., 2008). Sequencing of small RNAs in *Drosophila* embryos, ovaries, and the S2 cell line has revealed that endo-siRNAs are 21 nt long and broadly map across the locations of most TEs in both the sense and antisense orientations (Figure 4). However, they do not show an obvious nucleotide preference at their 5′ end. These characteristics, together with the observation that endo-siRNAs show a typical phasing pattern when mapped on the genome, suggest Dicer2 involvement in the processing pathway. In fact, loss of *dicer2* function abolished the production of endo-siRNAs in vivo (Czech et al., 2008; Kawamura et al., 2008; Okamura et al., 2008). It had previously been shown that Dicer1 partners with the dsRNA-binding partner Loquacious (Loqs) to generate miRNAs (Förstemann et al., 2005; Jiang et al., 2005; Saito et al., 2005), whereas Dicer2 uses the related protein R2D2 during exo-siRNA production (Liu et al., 2003). It therefore came as a surprise to find that endo-siRNA biogenesis occurs by an unusual combination of Dicer2 and Loqs (Czech et al., 2008; Okamura et al., 2008). This confusion was resolved

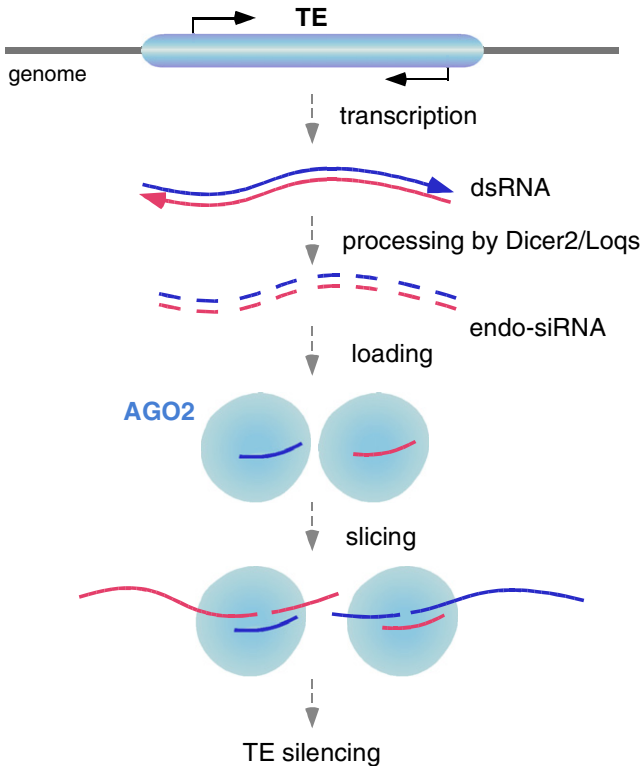

Figure 4. Endo-siRNA Biogenesis
Like piRNAs, endo-siRNAs are mainly derived from TE transcripts. In this pathway, Dicer2 associates with Loqs-PD, both of which are required for endo-siRNA processing from their dsRNA precursors. Mature endo-siRNAs are loaded onto AGO2.

when it was discovered that the *loqs* gene gives rise to four isoforms, *loqs-PA-loqs-PD* (Förstemann et al., 2005; Hartig et al., 2009; Zhou et al., 2009; Miyoshi et al., 2010), such that Loqs-PB and Loqs-PD are involved in the miRNA and endo-siRNA processing pathways, respectively (Hartig et al., 2009; Miyoshi et al., 2010; Zhou et al., 2009). Indeed, Loqs-PB associates with Dicer1, whereas Loqs-PD associates with Dicer2. These specific associations of Loqs isoforms with Dicer proteins most probably confer the ability of Dicer proteins to discriminate small RNA precursor substrates. The involvement of Loqs-PD in TE silencing might be modest, because endo-siRNA production depends less on Loqs than Dicer2 and AGO2 (Chung et al., 2008; Czech et al., 2008).

Endo-siRNA-Mediated TE Silencing

In mouse oocytes, the endo-siRNA pathway plays important roles in TE silencing (Tam et al., 2008; Watanabe et al., 2008; Murchison et al., 2007). However, the effects of endo-siRNAs are subtler in *Drosophila,* where TEs are derepressed (1.5- to 9-fold) when *dicer2* or *ago2* gene functions are lost, but only a small subset of specific TEs are affected, and the mutant flies are viable and fertile (Chung et al., 2008; Czech et al., 2008; Ghildiyal et al., 2008; Kawamura et al., 2008). It is plausible that the absence of strong phenotypes may be due in part to redundancy with piRNAs: Endo-siRNA factors are expressed in germline cells where piRNA-mediated silencing occurs, and sequence analysis has shown that AGO2-associated endo-siRNAs often map to

piRNA clusters. Thus, these regions of the genome are able to produce both endo-siRNAs and piRNAs. This raises important questions about how sense and antisense strands from these piRNA clusters form dsRNAs to produce endo-siRNAs and how they stay single stranded as piRNA precursors.

Conclusions

Despite their sequence diversity, piRNAs and endo-siRNAs do not disturb the expression of protein-coding genes. Thus, there must be strict cellular systems that discriminate TEs from protein-coding genes. How does this operate in vivo? One possibility is that TEs are identified at the transcription stage. Rhino contributes specifically to piRNA production from dual-strand piRNA clusters but not from uni-strand clusters, suggesting that Rhino can discriminate between the two clusters. Because Rhino is an HP1 homolog, it is most likely that Rhino functions at the transcriptional level, although there is no direct evidence to support this. The second possibility is that TEs are identified at the posttranscriptional level. TEs are transcribed by RNA polymerase II and are poly(A)-tailed, suggesting that piRNA precursors and mRNAs may contain similar structural characteristics. This would make it difficult to discriminate between TE transcripts and protein-coding transcripts. However, there should be cellular mechanisms that can separate the two species. It would be of interest to identify these mechanisms in *Drosophila* and in other species, such as mice.

ACKNOWLEDGMENTS

We thank H. Siomi and other members of the Siomi laboratory for discussions and comments on the manuscript. This work was supported by Ministry of Education, Culture, Sports, Science, and Technology of Japan grants to K.S. and M.C.S. K.S. is supported by Precursory Research for Embryonic Science and Technology from Japan Science and Technology Agency. M.C.S. is supported by Core Research for Evolutional Science and Technology from the Japan Science and Technology Agency.

REFERENCES

al-Mukhtar, K.A., and Webb, A.C. (1971). An ultrastructural study of primordial germ cells, oogonia and early oocytes in *Xenopus laevis*. J. Embryol. Exp. Morphol. *26*, 195–217.

Aravin, A.A., Naumova, N.M., Tulin, A.V., Vagin, V.V., Rozovsky, Y.M., and Gvozdev, V.A. (2001). Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the *D. melanogaster* germline. Curr. Biol. *11*, 1017–1027.

Aravin, A.A., Gaidatzis, D., Pfeffer, S., Lagos-Quintana, M., Landgraf, P., Iovino, N., Morris, P., Brownstein, M.J., Kuramochi-Miyagawa, S., Nakano, T., et al. (2006). A novel class of small RNAs bind to MILI protein in mouse testes. Nature *442*, 203–207.

Aravin, A.A., Sachidanandam, R., Girard, A., Fejes-Toth, K., and Hannon, G.J. (2007a). Developmentally regulated piRNA clusters implicate MILI in transposon control. Science *316*, 744–747.

Aravin, A.A., Hannon, G.J., and Brennecke, J. (2007b). The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. Science *318*, 761–764.

Aravin, A.A., Sachidanandam, R., Bourc'his, D., Schaefer, C., Pezic, D., Toth, K.F., Bestor, T., and Hannon, G.J. (2008). A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. Mol. Cell *31*, 785–799.

Aravin, A.A., van der Heijden, G.W., Castañeda, J., Vagin, V.V., Hannon, G.J., and Bortvin, A. (2009). Cytoplasmic compartmentalization of the fetal piRNA pathway in mice. PLoS Genet. *5*, e1000764.

Arkov, A.L., Wang, J.Y.S., Ramos, A., and Lehmann, R. (2006). The role of Tudor domains in germline development and polar granule architecture. Development *133*, 4053–4062.

Biémont, C., and Vieira, C. (2006). Genetics: Junk DNA as an evolutionary force. Nature *443*, 521–524.

Boswell, R.E., and Mahowald, A.P. (1985). *tudor*, a gene required for assembly of the germ plasm in *Drosophila melanogaster*. Cell *43*, 97–104.

Bourc'his, D., and Bestor, T.H. (2004). Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking *Dnmt3L*. Nature *431*, 96–99.

Brennecke, J., Aravin, A.A., Stark, A., Dus, M., Kellis, M., Sachidanandam, R., and Hannon, G.J. (2007). Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. Cell *128*, 1089–1103.

Brennecke, J., Malone, C.D., Aravin, A.A., Sachidanandam, R., Stark, A., and Hannon, G.J. (2008). An epigenetic role for maternally inherited piRNAs in transposon silencing. Science *322*, 1387–1392.

Bucheton, A. (1978). Non-Mendelian female sterility in *Drosophila melanogaster*: influence of ageing and thermic treatments. I. Evidence for a partly inheritable effect of these two factors. Heredity *41*, 357–369.

Carmell, M.A., Xuan, Z., Zhang, M.Q., and Hannon, G.J. (2002). The Argonaute family: Tentacles that reach into RNAi, developmental control, stem cell maintenance, and tumorigenesis. Genes Dev. *16*, 2733–2742.

Carmell, M.A., Girard, A., van de Kant, H.J., Bourc'his, D., Bestor, T.H., de Rooij, D.G., and Hannon, G.J. (2007). MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline. Dev. Cell *12*, 503–514.

Cerutti, L., Mian, N., and Bateman, A. (2000). Domains in gene silencing and cell differentiation proteins: The novel PAZ domain and redefinition of the Piwi domain. Trends Biochem. Sci. *25*, 481–482.

Chen, C., Jin, J., James, D.A., Adams-Cioaba, M.A., Park, J.G., Guo, Y., Tenaglia, E., Xu, C., Gish, G., Min, J., and Pawson, T. (2009). Mouse Piwi interactome identifies binding mechanism of Tdrkh Tudor domain to arginine methylated Miwi. Proc. Natl. Acad. Sci. USA *106*, 20336–20341.

Chow, J.C., Ciaudo, C., Fazzari, M.J., Mise, N., Servant, N., Glass, J.L., Attreed, M., Avner, P., Wutz, A., Barillot, E., et al. (2010). LINE-1 activity in facultative heterochromatin formation during X chromosome inactivation. Cell *141*, 956–969.

Chung, W.J., Okamura, K., Martin, R., and Lai, E.C. (2008). Endogenous RNA interference provides a somatic defense against *Drosophila* transposons. Curr. Biol. *18*, 795–802.

Coufal, N.G., Garcia-Perez, J.L., Peng, G.E., Yeo, G.W., Mu, Y., Lovci, M.T., Morell, M., O'Shea, K.S., Moran, J.V., and Gage, F.H. (2009). L1 retrotransposition in human neural progenitor cells. Nature *460*, 1127–1131.

Cox, D.N., Chao, A., Baker, J., Chang, L., Qiao, D., and Lin, H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. Genes Dev. *12*, 3715–3727.

Cox, D.N., Chao, A., and Lin, H. (2000). *piwi* encodes a nucleoplasmic factor whose activity modulates the number and division rate of germline stem cells. Development *127*, 503–514.

Czech, B., Malone, C.D., Zhou, R., Stark, A., Schlingeheyde, C., Dus, M., Perrimon, N., Kellis, M., Wohlschlegel, J.A., Sachidanandam, R., et al. (2008). An endogenous small interfering RNA pathway in *Drosophila*. Nature *453*, 798–802.

Deng, W., and Lin, H. (2002). *miwi*, a murine homolog of *piwi*, encodes a cytoplasmic protein essential for spermatogenesis. Dev. Cell *2*, 819–830.

Desset, S., Buchon, N., Meignin, C., Coiffet, M., and Vaury, C. (2008). In *Drosophila melanogaster* the COM locus directs the somatic silencing of two retrotransposons through both Piwi-dependent and -independent pathways. PLoS ONE *3*, e1526.

Eddy, E.M. (1975). Germ plasm and the differentiation of the germ cell line. Int. Rev. Cytol. *43*, 229–280.

Engels, W.R. (1979). Extrachromosomal control of mutability in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *76*, 4011–4015.

Findley, S.D., Tamanaha, M., Clegg, N.J., and Ruohola-Baker, H. (2003). *Maelstrom*, a *Drosophila* spindle-class gene, encodes a protein that colocalizes with Vasa and RDE1/AGO1 homolog, Aubergine, in nuage. Development *130*, 859–871.

Förstemann, K., Tomari, Y., Du, T., Vagin, V.V., Denli, A.M., Bratu, D.P., Klattenhoff, C., Theurkauf, W.E., and Zamore, P.D. (2005). Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. PLoS Biol. *3*, e236.

Frost, R.J., Hamra, F.K., Richardson, J.A., Qi, X., Bassel-Duby, R., and Olson, E.N. (2010). MOV10L1 is necessary for protection of spermatocytes against retrotransposons by Piwi-interacting RNAs. Proc. Natl. Acad. Sci. USA *107*, 11847–11852.

Galiana-Arnoux, D., Dostert, C., Schneemann, A., Hoffmann, J.A., and Imler, J.L. (2006). Essential function *in vivo* for Dicer-2 in host defense against RNA viruses in *drosophila*. Nat. Immunol. *7*, 590–597.

Ghildiyal, M., and Zamore, P.D. (2009). Small silencing RNAs: An expanding universe. Nat. Rev. Genet. *10*, 94–108.

Ghildiyal, M., Seitz, H., Horwich, M.D., Li, C., Du, T., Lee, S., Xu, J., Kittler, E.L., Zapp, M.L., Weng, Z., and Zamore, P.D. (2008). Endogenous siRNAs derived from transposons and mRNAs in *Drosophila* somatic cells. Science *320*, 1077–1081.

Girard, A., Sachidanandam, R., Hannon, G.J., and Carmell, M.A. (2006). A germline-specific class of small RNAs binds mammalian Piwi proteins. Nature *442*, 199–202.

Gogvadze, E., and Buzdin, A. (2009). Retroelements and their impact on genome evolution and functioning. Cell. Mol. Life Sci. *66*, 3727–3742.

Golden, D.E., Gerbasi, V.R., and Sontheimer, E.J. (2008). An inside job for siRNAs. Mol. Cell *31*, 309–312.

Goodier, J.L., and Kazazian, H.H., Jr. (2008). Retrotransposons revisited: The restraint and rehabilitation of parasites. Cell *135*, 23–35.

Grivna, S.T., Beyret, E., Wang, Z., and Lin, H. (2006). A novel class of small RNAs in mouse spermatogenic cells. Genes Dev. *20*, 1709–1714.

Gunawardane, L.S., Saito, K., Nishida, K.M., Miyoshi, K., Kawamura, Y., Nagami, T., Siomi, H., and Siomi, M.C. (2007). A slicer-mediated mechanism for repeat-associated siRNA 5′ end formation in *Drosophila*. Science *315*, 1587–1590.

Haase, A.D., Fenoglio, S., Muerdter, F., Guzzardo, P.M., Czech, B., Pappin, D.J., Chen, C., Gordon, A., and Hannon, G.J. (2010). Probing the initiation and effector phases of the somatic piRNA pathway in *Drosophila*. Genes Dev., in press. Published online October 21, 2010. 10.1101/gad.1968110.

Harris, A.N., and Macdonald, P.M. (2001). Aubergine encodes a *Drosophila* polar granule component required for pole cell formation and related to eIF2C. Development *128*, 2823–2832.

Hartig, J.V., Esslinger, S., Böttcher, R., Saito, K., and Förstemann, K. (2009). Endo-siRNAs depend on a new isoform of loquacious and target artificially introduced, high-copy sequences. EMBO J. *28*, 2932–2944.

Houwing, S., Kamminga, L.M., Berezikov, E., Cronembold, D., Girard, A., van den Elst, H., Filippov, D.V., Blaser, H., Raz, E., Moens, C.B., et al. (2007). A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. Cell *129*, 69–82.

Houwing, S., Berezikov, E., and Ketting, R.F. (2008). Zili is required for germ cell differentiation and meiosis in zebrafish. EMBO J. *27*, 2702–2711.

Jiang, F., Ye, X., Liu, X., Fincher, L., McKearin, D., and Liu, Q. (2005). Dicer-1 and R3D1-L catalyze microRNA maturation in *Drosophila*. Genes Dev. *19*, 1674–1679.

Jurka, J., Kapitonov, V.V., Pavlicek, A., Klonowski, P., Kohany, O., and Walichiewicz, J. (2005). Repbase Update, a database of eukaryotic repetitive elements. Cytogenet. Genome Res. *110*, 462–467.

Kalmykova, A.I., Klenov, M.S., and Gvozdev, V.A. (2005). Argonaute protein PIWI controls mobilization of retrotransposons in the *Drosophila* male germline. Nucleic Acids Res. *33*, 2052–2059.

Kapitonov, V.V., and Jurka, J. (2003). Molecular paleontology of transposable elements in the *Drosophila melanogaster* genome. Proc. Natl. Acad. Sci. USA *100*, 6569–6574.

Kawamura, Y., Saito, K., Kin, T., Ono, Y., Asai, K., Sunohara, T., Okada, T.N., Siomi, M.C., and Siomi, H. (2008). *Drosophila* endogenous small RNAs bind to Argonaute 2 in somatic cells. Nature *453*, 793–797.

Kidwell, M.G., and Kidwell, J.F. (1976). Selection for male recombination in *Drosophila melanogaster*. Genetics *84*, 333–351.

Kim, V.N., Han, J., and Siomi, M.C. (2009). Biogenesis of small RNAs in animals. Nat. Rev. Mol. Cell Biol. *10*, 126–139.

Kirino, Y., Kim, N., de Planell-Saguer, M., Khandros, E., Chiorean, S., Klein, P.S., Rigoutsos, I., Jongens, T.A., and Mourelatos, Z. (2009). Arginine methylation of Piwi proteins catalysed by dPRMT5 is required for Ago3 and Aub stability. Nat. Cell Biol. *11*, 652–658.

Klattenhoff, C., and Theurkauf, W. (2008). Biogenesis and germline functions of piRNAs. Development *135*, 3–9.

Klattenhoff, C., Xi, H., Li, C., Lee, S., Xu, J., Khurana, J.S., Zhang, F., Schultz, N., Koppetsch, B.S., Nowosielska, A., et al. (2009). The *Drosophila* HP1 homolog Rhino is required for transposon silencing and piRNA production by dual-strand clusters. Cell *138*, 1137–1149.

Klenov, M.S., Lavrov, S.A., Stolyarenko, A.D., Ryazansky, S.S., Aravin, A.A., Tuschl, T., and Gvozdev, V.A. (2007). Repeat-associated siRNAs cause chromatin silencing of retrotransposons in the *Drosophila melanogaster* germline. Nucleic Acids Res. *35*, 5430–5438.

Kojima, K., Kuramochi-Miyagawa, S., Chuma, S., Tanaka, T., Nakatsuji, N., Kimura, T., and Nakano, T. (2009). Associations between PIWI proteins and TDRD1/MTR-1 are critical for integrated subcellular localization in murine male germ cells. Genes Cells *14*, 1155–1165.

Kuramochi-Miyagawa, S., Kimura, T., Yomogida, K., Kuroiwa, A., Tadokoro, Y., Fujita, Y., Sato, M., Matsuda, Y., and Nakano, T. (2001). Two mouse *piwi*-related genes: *miwi* and *mili*. Mech. Dev. *108*, 121–133.

Kuramochi-Miyagawa, S., Kimura, T., Ijiri, T.W., Isobe, T., Asada, N., Fujita, Y., Ikawa, M., Iwai, N., Okabe, M., Deng, W., et al. (2004). *Mili*, a mammalian member of *piwi* family gene, is essential for spermatogenesis. Development *131*, 839–849.

Kuramochi-Miyagawa, S., Watanabe, T., Gotoh, K., Totoki, Y., Toyoda, A., Ikawa, M., Asada, N., Kojima, K., Yamaguchi, Y., Ijiri, T.W., et al. (2008). DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. Genes Dev. *22*, 908–917.

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

Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., et al; International Human Genome Sequencing Consortium. (2001). Initial sequencing and analysis of the human genome. Nature *409*, 860–921.

Lau, N.C. (2010). Small RNAs in the animal gonad: Guarding genomes and guiding development. Int. J. Biochem. Cell Biol. *42*, 1334–1347.

Lau, N.C., Ohsumi, T., Borowsky, M., Kingston, R.E., and Blower, M.D. (2009). Systematic and single cell analysis of *Xenopus* Piwi-interacting RNAs and Xiwi. EMBO J. *28*, 2945–2958.

Lee, Y.S., Nakahara, K., Pham, J.W., Kim, K., He, Z., Sontheimer, E.J., and Carthew, R.W. (2004). Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. Cell *117*, 69–81.

Li, C., Vagin, V.V., Lee, S., Xu, J., Ma, S., Xi, H., Seitz, H., Horwich, M.D., Syrzycka, M., Honda, B.M., et al. (2009). Collapse of germline piRNAs in the absence of Argonaute3 reveals somatic piRNAs in flies. Cell *137*, 509–521.

Lim, A.K., and Kai, T. (2007). Unique germ-line organelle, nuage, functions to repress selfish genetic elements in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *104*, 6714–6719.

Lin, H., and Spradling, A.C. (1997). A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the *Drosophila* ovary. Development *124*, 2463–2476.

Liu, Q., Rand, T.A., Kalidas, S., Du, F., Kim, H.E., Smith, D.P., and Wang, X. (2003). R2D2, a bridge between the initiation and effector steps of the *Drosophila* RNAi pathway. Science *301*, 1921–1925.

Liu, H., Wang, J.Y., Huang, Y., Li, Z., Gong, W., Lehmann, R., and Xu, R.M. (2010). Structural basis for methylarginine-dependent recognition of Aubergine by Tudor. Genes Dev. *24*, 1876–1881.

Ma, L., Buchold, G.M., Greenbaum, M.P., Roy, A., Burns, K.H., Zhu, H., Han, D.Y., Harris, R.A., Coarfa, C., Gunaratne, P.H., et al. (2009). GASZ is essential for male meiosis and suppression of retrotransposon expression in the male germline. PLoS Genet. *5*, e1000635.

Malone, C.D., and Hannon, G.J. (2009). Small RNAs as guardians of the genome. Cell *136*, 656–668.

Malone, C.D., Brennecke, J., Dus, M., Stark, A., McCombie, W.R., Sachidanandam, R., and Hannon, G.J. (2009). Specialized piRNA pathways act in germline and somatic tissues of the *Drosophila* ovary. Cell *137*, 522–535.

Mi, S., Lee, X., Li, X., Veldman, G.M., Finnerty, H., Racie, L., LaVallie, E., Tang, X.Y., Edouard, P., Howes, S., et al. (2000). Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. Nature *403*, 785–789.

Miyoshi, K., Miyoshi, T., Hartig, J.V., Siomi, H., and Siomi, M.C. (2010). Molecular mechanisms that funnel RNA precursors into endogenous small-interfering RNA and microRNA biogenesis pathways in *Drosophila*. RNA *16*, 506–515.

Moshkovich, N., and Lei, E.P. (2010). HP1 recruitment in the absence of argonaute proteins in *Drosophila*. PLoS Genet. *6*, e1000880.

Murchison, E.P., Stein, P., Xuan, Z., Pan, H., Zhang, M.Q., Schultz, R.M., and Hannon, G.J. (2007). Critical roles for Dicer in the female germline. Genes Dev. *21*, 682–693.

Nishida, K.M., Saito, K., Mori, T., Kawamura, Y., Nagami-Okada, T., Inagaki, S., Siomi, H., and Siomi, M.C. (2007). Gene silencing mechanisms mediated by Aubergine piRNA complexes in *Drosophila* male gonad. RNA *13*, 1911–1922.

Nishida, K.M., Okada, T.N., Kawamura, T., Mituyama, T., Kawamura, Y., Inagaki, S., Huang, H., Chen, D., Kodama, T., Siomi, H., and Siomi, M.C. (2009). Functional involvement of Tudor and dPRMT5 in the piRNA processing pathway in *Drosophila* germlines. EMBO J. *28*, 3820–3831.

O'Donnell, K.A., and Boeke, J.D. (2007). Mighty Piwis defend the germline against genome intruders. Cell *129*, 37–44.

Okamura, K., and Lai, E.C. (2008). Endogenous small interfering RNAs in animals. Nat. Rev. Mol. Cell Biol. *9*, 673–678.

Okamura, K., Chung, W.J., Ruby, J.G., Guo, H., Bartel, D.P., and Lai, E.C. (2008). The *Drosophila* hairpin RNA pathway generates endogenous short interfering RNAs. Nature *453*, 803–806.

Olivieri, D., Sykora, M.M., Sachidanandam, R., Mechtler, K., and Brennecke, J. (2010). An in vivo RNAi assay identifies major genetic and cellular requirements for primary piRNA biogenesis in *Drosophila*. EMBO J. *29*, 3301–3317.

Pal-Bhadra, M., Leibovitch, B.A., Gandhi, S.G., Rao, M., Bhadra, U., Birchler, J.A., and Elgin, S.C. (2004). Heterochromatic silencing and HP1 localization in *Drosophila* are dependent on the RNAi machinery. Science *303*, 669–672.

Pane, A., Wehr, K., and Schüpbach, T. (2007). *zucchini* and *squash* encode two putative nucleases required for rasiRNA production in the *Drosophila* germline. Dev. Cell *12*, 851–862.

Pardue, M.L., Rashkova, S., Casacuberta, E., DeBaryshe, P.G., George, J.A., and Traverse, K.L. (2005). Two retrotransposons maintain telomeres in *Drosophila*. Chromosome Res. *13*, 443–453.

Patil, V.S., and Kai, T. (2010). Repression of retroelements in *Drosophila* germline via piRNA pathway by the tudor domain protein Tejas. Curr. Biol. *20*, 724–730.

Pélisson, A., Song, S.U., Prud'homme, N., Smith, P.A., Bucheton, A., and Corces, V.G. (1994). Gypsy transposition correlates with the production of a retroviral envelope-like protein under the tissue-specific control of the *Drosophila flamenco* gene. EMBO J. *13*, 4401–4411.

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

Rongo, C., and Lehmann, R. (1996). Regulated synthesis, transport and assembly of the *Drosophila* germ plasm. Trends Genet. *12*, 102–109.

Rouget, C., Papin, C., Boureux, A., Meunier, A.C., Franco, B., Robine, N., Lai, E.C., Pelisson, A., and Simonelig, M. (2010). Maternal mRNA deadenylation and decay by the piRNA pathway in the early *Drosophila* embryo. Nature. Published online October 17, 2010.

Rubin, G.M., Kidwell, M.G., and Bingham, P.M. (1982). The molecular basis of P-M hybrid dysgenesis: The nature of induced mutations. Cell *29*, 987–994.

Saito, K., Ishizuka, A., Siomi, H., and Siomi, M.C. (2005). Processing of pre-microRNAs by the Dicer-1-Loquacious complex in *Drosophila* cells. PLoS Biol. *3*, e235.

Saito, K., Nishida, K.M., Mori, T., Kawamura, Y., Miyoshi, K., Nagami, T., Siomi, H., and Siomi, M.C. (2006). Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the *Drosophila* genome. Genes Dev. *20*, 2214–2222.

Saito, K., Inagaki, S., Mituyama, T., Kawamura, Y., Ono, Y., Sakota, E., Kotani, H., Asai, K., Siomi, H., and Siomi, M.C. (2009). A regulatory circuit for *piwi* by the large Maf gene *traffic jam* in *Drosophila*. Nature *461*, 1296–1299.

Saito, K., Ishizu, H., Komai, M., Kotani, H., Kawamura, Y., Nishida, K.M., Siomi, H., and Siomi, M.C. (2010). Roles for the Yb body components Armitage and Yb in primary piRNA biogenesis in *Drosophila*. Genes Dev., in press. Published online October 24, 2010. 10.1101/gad.1989510.

Sarot, E., Payen-Groschêne, G., Bucheton, A., and Pélisson, A. (2004). Evidence for a piwi-dependent RNA silencing of the gypsy endogenous retrovirus by the Drosophila melanogaster flamenco gene. Genetics *166*, 1313–1321.

Savitsky, M., Kwon, D., Georgiev, P., Kalmykova, A., and Gvozdev, V. (2006). Telomere elongation is under the control of the RNAi-based mechanism in the *Drosophila* germline. Genes Dev. *20*, 345–354.

Schmidt, A., Palumbo, G., Bozzetti, M.P., Tritto, P., Pimpinelli, S., and Schäfer, U. (1999). Genetic and molecular characterization of sting, a gene involved in crystal formation and meiotic drive in the male germ line of *Drosophila melanogaster*. Genetics *151*, 749–760.

Senti, K., and Brennecke, J. (2010). The piRNA pathway: A fly's perspective on the guardian of the genome. Trends Genet., in press. Published online October 8, 2010. 10.1016/j.tig.2010.08.007.

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

Siomi, M.C., and Kuramochi-Miyagawa, S. (2009). RNA silencing in germlines—exquisite collaboration of Argonaute proteins with small RNAs for germline survival. Curr. Opin. Cell Biol. *21*, 426–434.

Siomi, H., and Siomi, M.C. (2009). On the road to reading the RNA-interference code. Nature *457*, 396–404.

Siomi, M.C., Saito, K., and Siomi, H. (2008). How selfish retrotransposons are silenced in *Drosophila* germline and somatic cells. FEBS Lett. *582*, 2473–2478.

Siomi, M.C., Mannen, T., and Siomi, H. (2010). How does the royal family of Tudor rule the PIWI-interacting RNA pathway? Genes Dev. *24*, 636–646.

Soper, S.F., van der Heijden, G.W., Hardiman, T.C., Goodheart, M., Martin, S.L., de Boer, P., and Bortvin, A. (2008). Mouse maelstrom, a component of nuage, is essential for spermatogenesis and transposon repression in meiosis. Dev. Cell *15*, 285–297.

Specchia, V., Piacentini, L., Tritto, P., Fanti, L., D'Alessandro, R., Palumbo, G., Pimpinelli, S., and Bozzetti, M.P. (2010). Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. Nature *463*, 662–665.

Tam, O.H., Aravin, A.A., Stein, P., Girard, A., Murchison, E.P., Cheloufi, S., Hodges, E., Anger, M., Sachidanandam, R., Schultz, R.M., and Hannon, G.J. (2008). Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes. Nature *453*, 534–538.

Thomson, T., and Lasko, P. (2004). *Drosophila tudor* is essential for polar granule assembly and pole cell specification, but not for posterior patterning. Genesis *40*, 164–170.

Thomson, T., and Lasko, P. (2005). Tudor and its domains: germ cell formation from a Tudor perspective. Cell Res. *15*, 281–291.

Thomson, T., and Lin, H. (2009). The biogenesis and function of PIWI proteins and piRNAs: Progress and prospect. Annu. Rev. Cell Dev. Biol. *25*, 355–376.

Vagin, V.V., Sigova, A., Li, C., Seitz, H., Gvozdev, V., and Zamore, P.D. (2006). A distinct small RNA pathway silences selfish genetic elements in the germline. Science *313*, 320–324.

Vagin, V.V., Wohlschlegel, J., Qu, J., Jonsson, Z., Huang, X., Chuma, S., Girard, A., Sachidanandam, R., Hannon, G.J., and Aravin, A.A. (2009). Proteomic analysis of murine Piwi proteins reveals a role for arginine methylation in specifying interaction with Tudor family members. Genes Dev. *23*, 1749–1762.

van Rij, R.P., Saleh, M.C., Berry, B., Foo, C., Houk, A., Antoniewski, C., and Andino, R. (2006). The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. Genes Dev. *20*, 2985–2995.

Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I., and Martienssen, R.A. (2002). Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. Science *297*, 1833–1837.

Wang, X.H., Aliyari, R., Li, W.X., Li, H.W., Kim, K., Carthew, R., Atkinson, P., and Ding, S.W. (2006). RNA interference directs innate immunity against viruses in adult *Drosophila*. Science *312*, 452–454.

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

Watanabe, T., Takeda, A., Tsukiyama, T., Mise, K., Okuno, T., Sasaki, H., Minami, N., and Imai, H. (2006). Identification and characterization of two novel classes of small RNAs in the mouse germline: Retrotransposon-derived siRNAs in oocytes and germline small RNAs in testes. Genes Dev. *20*, 1732–1743.

Watanabe, T., Totoki, Y., Toyoda, A., Kaneda, M., Kuramochi-Miyagawa, S., Obata, Y., Chiba, H., Kohara, Y., Kono, T., Nakano, T., et al. (2008). Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. Nature *453*, 539–543.

Wilczynska, A., Minshall, N., Armisen, J., Miska, E.A., and Standart, N. (2009). Two Piwi proteins, Xiwi and Xili, are expressed in the *Xenopus* female germline. RNA *15*, 337–345.

Williams, R.W., and Rubin, G.M. (2002). ARGONAUTE1 is required for efficient RNA interference in *Drosophila* embryos. Proc. Natl. Acad. Sci. USA *99*, 6889–6894.

Yin, H., and Lin, H. (2007). An epigenetic activation role of Piwi and a Piwi-associated piRNA in *Drosophila melanogaster*. Nature *450*, 304–308.

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

Zhou, R., Czech, B., Brennecke, J., Sachidanandam, R., Wohlschlegel, J.A., Perrimon, N., and Hannon, G.J. (2009). Processing of *Drosophila* endo-siRNAs depends on a specific Loquacious isoform. RNA *15*, 1886–1895.