Review

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piRNA- and siRNA-mediated transcriptional repression in *Drosophila*, mice, and yeast: new insights and biodiversity

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Abstract

The PIWI-interacting RNA (piRNA) pathway acts as a self-defense mechanism against transposons to maintain germline genome integrity. Failures in the piRNA pathway cause DNA damage in the germline genome, disturbing inheritance of "correct" genetic information by the next generations and leading to infertility. piRNAs execute transposon repression in two ways: degrading their RNA transcripts and compacting the genomic loci via heterochromatinization. The former event is mechanistically similar to siRNA-mediated RNA cleavage that occurs in the cytoplasm and has been investigated in many species including nematodes, fruit flies, and mammals. The latter event seems to be mechanistically parallel to siRNA-centered kinetochore assembly and subsequent chromosome segregation, which has so far been studied particularly in fission yeast. Despite the interspecies conservations, the overall schemes of the nuclear events show clear biodiversity across species. In this review, we summarize the recent progress regarding piRNA-mediated transcriptional silencing in *Drosophila* and discuss the biodiversity by comparing it with the equivalent piRNA-mediated system in mice and the siRNA-mediated system in fission yeast.

Keywords chromatin segregation; piRNA; siRNA; transcriptional repression
Subject Categories Chromatin, Transcription & Genomics; RNA Biology
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See the Glossary for abbreviations used in this article.

Introduction

PIWI-interacting RNAs (piRNAs) are small noncoding RNAs that are particularly abundant in animal reproductive tissues, where piRNAs protect the germline genome by repressing transposons (Iwasaki *et al*, 2015; Czech *et al*, 2018; Ozata *et al*, 2019). Dysfunction of piRNAs results in derepression of transposons, allowing them to move freely across the genome, leading to DNA damage, impaired gonadal development, and infertility (Klattenhoff *et al*, 2007).

Earlier studies in *Drosophila* uncovered an association between repetitive DNA sequences and repression of protein-coding genes (Livak, 1984; Palumbo *et al*, 1994). Later, small RNAs of 25–27 nucleotides (nt) in length derived from the *Suppressor of Stellate* [*Su* (*Ste*)] repeats located on the Y chromosome were found to be involved in repressing the *Stellate* (*Ste*) gene located on the X chromosome (Aravin *et al*, 2001; Stapleton *et al*, 2001). Deletion of *Su* (*Ste*) repeats derepressed the *Ste* gene. Consequently, Ste protein accumulated and crystallized in sperm cells, causing male infertility.

In 2003, comprehensive sequencing of small RNAs in *Drosophila* testes and embryos identified many repeat-associated small interfering RNAs (rasiRNAs), including those arising from *Su*(*Ste*) repeats and transposons (Aravin *et al*, 2003). Subsequent investigations using a number of organisms, including *Drosophila*, mice, and humans, revealed that rasiRNAs are present in their gonads and bind specifically to PIWI members, a subclade of the Argonaute family, but not to ubiquitous AGO (Girard *et al*, 2006; Grivna *et al*, 2006; Lau *et al*, 2006; Saito *et al*, 2006; Watanabe *et al*, 2006; Houwing *et al*, 2007; Nishida *et al*, 2007; Lau *et al*, 2009). On the basis of this cross-species observation, PIWI-bound rasiRNAs were collectively termed piRNAs.

PIWI proteins are defined by their greater similarity at the peptide sequence level to other PIWI subclade members than to AGO subclade members (Kim *et al*, 2009; Iwasaki *et al*, 2015). This simple definition of PIWI proteins (and their partner piRNAs) is confusing sometimes. For example, plants have no piRNAs. However, this interpretation is based on the fact that plants have no proteins defined as PIWIs. Some AGO members are present in the germline, bind repeat-derived small RNAs, and are required for fertility (Borges & Martienssen, 2015; Araki *et al*, 2020). In rare animals, such as mosquitoes, planarians, and Aplysia, some PIWI members are present outside of the germlines and are loaded with small RNAs that can map to transposons (Rajasethupathy *et al*, 2012; Shibata *et al*, 2016; Halbach *et al*, 2020; Kim *et al*, 2020). Tetrahymena expresses PIWI proteins, which bind to small RNAs that are produced in a Dicer-dependent manner (Mochizuki *et al*, 2002; Mochizuki, 2005). Dicer is otherwise known as a microRNA (miRNA) biogenesis factor (Kim *et al*, 2009). As piRNA research across species progresses further, such interspecies differences will become even more apparent.

piRNAs repress target genes at the transcriptional or post-transcriptional level. At which level piRNA-mediated silencing

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Glossary

Aub	Aubergine
Brm	Brahma
CLRC	Clr4 methyltransferase complex
Ctp	Cutup/LC8
DMR	differentially methylated region
DNMT	DNA methyltransferase
dNuRD	*Drosophila* Nucleosome Remodeling and Deacetylase
ds	double-stranded
Egg	Eggless
flam	*flamenco*
Gtsf1	Gametocyte-specific factor
H1	histone linker H1
H3K36	Histone 3 lysine 36
H3K4	Histone 3 lysine 4
H3K9me3	Histone 3 lysine 9 trimethylation
HP1a	Heterochromatin Protein 1a
IAP	intracisternal A-particle
LINE1	long interspersed nuclear element-1
LLPS	liquid–liquid phase separation
LTR	long terminal repeat
Mael	Maelstrom
miRNA	micro RNA
NLS	nuclear localization signal
nt	nucleotide
Nxf2	nuclear RNA export factor 2
OGC	ovarian germ cell
OSC	ovarian somatic cell
Pandas	Panoramix dNxf2-dependent TAP/p15 silencing
Panx	Panoramix
PGC	primordial germ cell
PICTS	Panoramix-induced co-transcriptional silencing
piRISC	RISC with piRNA
piRNA	PIWI-interacting RNA
PPNP	piwi-Panx-nxf2-p15
pre-piRISC	Piwi-piRISC precursor
RDRC	RNA-directed RNA polymerase complex
Rhi	Rhino
RISC	RNA-induced silencing complex
RITS	RNA-induced initiation of transcriptional gene silencing
RNA Pol II	RNA polymerase II
RNAi	RNA interference
S2	Schneider 2
SFiNX	silencing factor interacting nuclear export variant
SHREC	Snf2/Hdac-containing Repressor Complex
siRNA	small-interfering RNAs
ss	single-stranded
Sv210	Su(var)2–10
SWI/SNF	SWItch/Sucrose Non-Fermentable
TRAMP	Trf4/Air2/Mtr4p polyadenylation
Wde	Windei
Zuc	Zucchini

occurs is determined by the subcellular localization of PIWIs binding to piRNAs or the piRNA-induced silencing complex (piRISC). The post-transcriptional event is carried out by cytoplasmic piRISC with a mechanism similar to RNA interference (RNAi), i.e., by cleaving the target mRNAs. PIWI proteins involved in this event are *Drosophila* Aubergine (Aub) and mouse Mili (Piwil2), both of which have endonuclease (slicer) activity, similar to *Drosophila* Ago2, to cleave RNAs (Deng & Lin, 2002; Aravin *et al*, 2006; Saito *et al*, 2006; Vagin, 2006; Brennecke *et al*, 2007; Carmell *et al*, 2007; Aravin *et al*, 2008; Kuramochi-Miyagawa *et al*, 2008; Czech *et al*, 2018) .

In contrast, transcriptional silencing is carried out by nuclear piRISC that binds nascent RNA targets through piRNAs at the site of transcription. PIWI proteins involved in this event are *Drosophila* Piwi and mouse Miwi2 (Piwil4). These PIWIs have a nuclear import signal (NLS) and inactive slicer activity (Saito *et al*, 2009; De Fazio *et al*, 2011; Sienski *et al*, 2012; Yamaguchi *et al*, 2020). Thus, they associate with various co-factors, such as histone modifiers, chromatin mark readers, and DNA methyltransferases, to direct epigenetic changes at target loci via heterochromatin formation to repress transcription.

This nuclear silencing provides an additional benefit to the organism because repetitive sequences can disrupt the integrity of the genome through recombination, and heterochromatinization of these loci not only prevents the expression of harmful transposons that impair fertility but also minimizes undesirable recombination events. However, some animals, such as fish and silkworms, only have cytoplasmic PIWIs, despite being recombination positive.

Accumulating evidence shows that the overall framework of nuclear PIWI-mediated transposon silencing is similar to that of chromosomal compaction in fission yeast, which is mediated by Ago1 of the AGO subclade, although the biological purpose and meaning of the two events are distinct. In this review, we summarize mechanistic insights into Piwi-piRISC-mediated transcriptional silencing in *Drosophila* and compare its features with those of the Miwi2-piRISC-mediated mouse system and the Ago1/siRNA-mediated chromatin control in fission yeast.

Piwi-piRISC-mediated transcriptional silencing in *Drosophila*

This pathway has been well studied using cultured *Drosophila* ovary-derived somatic cells (OSSs/OSCs), which contain only mitotically active early follicle cells (Niki *et al*, 2006; Saito *et al*, 2009). Like follicular somatic cells in the ovary, OSCs express Piwi but not two other PIWI members, Aub and Ago3. Furthermore, Piwi binds piRNAs and represses transposons in OSCs as it does in the ovary. Thus, OSCs are suitable for studying the Piwi-piRNA pathway.

Cytoplasmic production and nuclear localization of Piwi-piRISC

Piwi-piRISC in OSCs is assembled sequentially via two cytoplasmic organelles, Yb bodies and mitochondria (Olivieri *et al*, 2010; Saito *et al*, 2010; Hirakata & Siomi, 2016; Yamashiro & Siomi, 2018). Yb bodies are non-membranous perinuclear granules formed through liquid–liquid phase separation (LLPS) (Hirakata *et al*, 2019). The piRNA precursors are mostly derived from the piRNA cluster *flamenco* (*flam*) on the X chromosome (Pélisson *et al*, 1994; Brennecke *et al*, 2007). The piRNA precursors contain *cis*-elements, to which Yb binds specifically as a *trans*-acting factor, and then multimerizes, inducing the LLPS-driven assembly of Yb bodies (Ishizu *et al*, 2015; Hirakata *et al*, 2019). Unbound Piwi and piRNA biogenesis factors, such as Armitage (Armi) and Vreteno, subsequently

localize to these bodies to initiate Piwi-piRISC production (Handler *et al*, 2011; Ishizu *et al*, 2019; Yamashiro *et al*, 2020).

In the Yb bodies, the piRNA precursors are converted into shorter intermediates by an unknown enzyme(s). This step generates multiple 5′-ends to which Piwi binds, becoming the Piwi-piRISC precursor (pre-piRISC) (Murota *et al*, 2014). This complex then heads to the mitochondrial surface along with the RNA helicase Armi, where the endonuclease Zucchini (Zuc) processes the Piwi-bound intermediates to mature piRNAs, thereby releasing Piwi-piRISC (Ipsaro *et al*, 2012; Nishimasu *et al*, 2012; Han *et al*, 2015; Mohn *et al*, 2015). The leftover RNA (i.e., the 3′ trailer) is used to produce phased piRNAs (Han *et al*, 2015; Mohn *et al*, 2015), which are also bound to nascent Piwi and become Piwi-piRISCs. Two mitochondrial factors, Gasz and Daedalus, serve together as the scaffold for Zuc processing (Munafò *et al*, 2019; Yamashiro *et al*, 2020).

Yb is unique to *Drosophila* OSCs, which indicates that Piwi-piRISCs in ovarian germ cells (OGCs) are produced in an Yb-independent manner (Handler *et al*, 2013). Piwi-piRISCs in OGCs are produced from RNA fragments cleaved by Ago3-piRISCs (Wang *et al*, 2015). The piRNAs within Piwi-piRISCs in OGCs are also phased (Han *et al*, 2015; Mohn *et al*, 2015; Pandey *et al*, 2017; Ge *et al*, 2019). Zuc is involved in the process, but other required factors may not be identical to those in OSCs.

Piwi has a bipartite nuclear localization signal (NLS) at the N-terminal end. The signal is hidden prior to piRISC assembly to ensure unbound Piwi does not enter the nucleus. However, upon piRISC assembly, the Piwi-NLS is exposed to the cytosol and binds to Importinα, which actively transports the piRISC into the nucleus (Yashiro *et al*, 2018). This "molecular gate" that depends on small RNA binding raises the efficiency of nuclear transposon regulation, because only piRNA-loaded Piwi, not unloaded Piwi, is competent to target transposons.

A Piwi mutant lacking the NLS was fully loaded with piRNAs, but never entered the nucleus. Consequently, transposons were desilenced, proving that Piwi-piRISC must enter the nucleus to exert the silencing effect (Saito *et al*, 2010; Klenov *et al*, 2011; Sienski *et al*, 2012; Yashiro *et al*, 2018).

Piwi-piRISC does not have slicer activity

It was previously thought that slicer activity was not necessary for Piwi, because mutations in the predicted slicer active site did not affect transposon repression (Saito *et al*, 2010; Sienski *et al*, 2012; Darricarrère *et al*, 2013). In fact, Piwi-piRISC in OSCs shows no slicer activity (Yamaguchi *et al*, 2020), because the canonical Asp–Glu–Asp–His (D–E–D–H) tetrad necessary for exerting slicer activity is substituted by Asp–Val–Asp–Lys in endogenous, wild type Piwi (Sheu-Gruttadauria & MacRae, 2017). Changing the peptide back to the D–E–D–H tetrad restored slicer activity (Yamaguchi *et al*, 2020).

Artificial tethering of Piwi to nascent reporter mRNAs via, for instance, the λN-boxB system did not repress the reporter genes (Sienski *et al*, 2015; Yu *et al*, 2015). This strongly supports the notion that Piwi must bind mRNA targets by itself via piRNAs, but not by other means such as the λN-boxB. Furthermore, Piwi must be continuously sustained on the target RNAs to maintain the repressive effect (Sarot *et al*, 2004; Sienski *et al*, 2012). The slicer activity given to Piwi by amino acid changes, which were introduced to wild type, naturally non-catalytic Piwi, hardly disturbed its RNA targeting effects, but Piwi was easily displaced from target RNAs upon cleavage (Yamaguchi *et al*, 2020). The displacement was more obvious when target RNAs were less complementary to Piwi-bound piRNAs. Such mismatches/-gaps in base-pairing between Piwi-bound piRNAs and transposon mRNAs are often found within the ovaries. Thus, Piwi lacking the slicer activity might be more effective in transposon repression in the tissue, due to sustained target RNA binding, which is perhaps the reason for waiving its slicer activity in evolution. Without the slicer activity, though, Piwi requires co-factors to accomplish gene silencing. In fact, a number of Piwi co-factors have been identified.

Piwi-piRISC attenuates target transcription via Maelstrom

Efficient gene silencing may require transcription attenuation right upon Piwi binding to the target RNA. If RNA synthesis continued at the regular speed, the transcripts would leave the genome before Piwi could trigger the silencing mode of action. A recent study has shown that Maelstrom (Mael) contributes to this process (Onishi *et al*, 2020) (Fig 1).

Mael was originally identified as a factor regulating anterior–posterior axis formation in early oocytes (Clegg *et al*, 1997; Sato *et al*, 2011). More recent studies revealed that Mael is indispensable for transposon silencing and that this Mael function is conserved in mice (Lim & Kai, 2007; Soper *et al*, 2008; Sienski *et al*, 2012; Castañeda *et al*, 2014; Chang *et al*, 2019). Mael's points of action in transposon silencing in *Drosophila* OSCs and OGCs are different: Mael in OSCs is not required for piRNA biogenesis, but it controls the chromatin accessibility of RNA Pol II, with little effect on the abundance of Histone 3 lysine 9 trimethylation (H3K9me3) around the transposon loci, resulting in transcriptional regulation of transposons (Sienski *et al*, 2012). Mael in OGCs controls piRNA biogenesis by attenuating canonical transcription of transposons in the piRNA clusters (Chang *et al*, 2019).

Mael in OSCs resides in the nuclear Piwi complex, and this complex further binds to Brahma (Brm), the core unit of the chromatin remodeler SWItch/Sucrose Non-Fermentable (SWI/SNF) complex (Onishi *et al*, 2020) (Fig 1). SWI/SNF relaxes chromatin structures around promoter regions, allowing RNA Pol II to initiate transcription (Wilson & Roberts, 2011). Piwi-targeted transposons in cultured OSCs are mostly controlled by SWI/SNF, and Piwi in collaboration with Mael reduces the level of SWI/SNF and RNA Pol II around long terminal repeats (LTRs) of target transposons, resulting in transcriptional attenuation (Onishi *et al*, 2020) (Fig 1). Piwi tethering by artificial piRNAs also repressed Brm-independent genes, but at a slower speed than Brm-dependent genes. Thus, it is likely that Piwi represses gene targets in a Mael-dependent and Mael-independent manner in slightly different ways: If transcription of the target genes is initiated under the control of SWI/SNF, Piwi first attenuates the transcription with help from Mael. If transcription is initiated in a SWI/SNF-independent manner, Piwi attenuates RNA synthesis by other unknown means. In both cases, heterochromatinization eventually takes place at the target loci. A similar Mael-mediated artificial tethering was conducted in germ cells within the ovary, but it failed to repress the reporter (Sienski *et al*, 2015). The dependency of Piwi-mediated transcriptional repression on Mael may not be identical in germ and somatic cells.

The PICTS/PPNP/SFiNX/Pandas complex anchors Piwi-piRISC to target RNAs

RNAi-based gene screening conducted within the ovaries identified numerous piRNA factors (Czech *et al*, 2013; Handler *et al*, 2013;

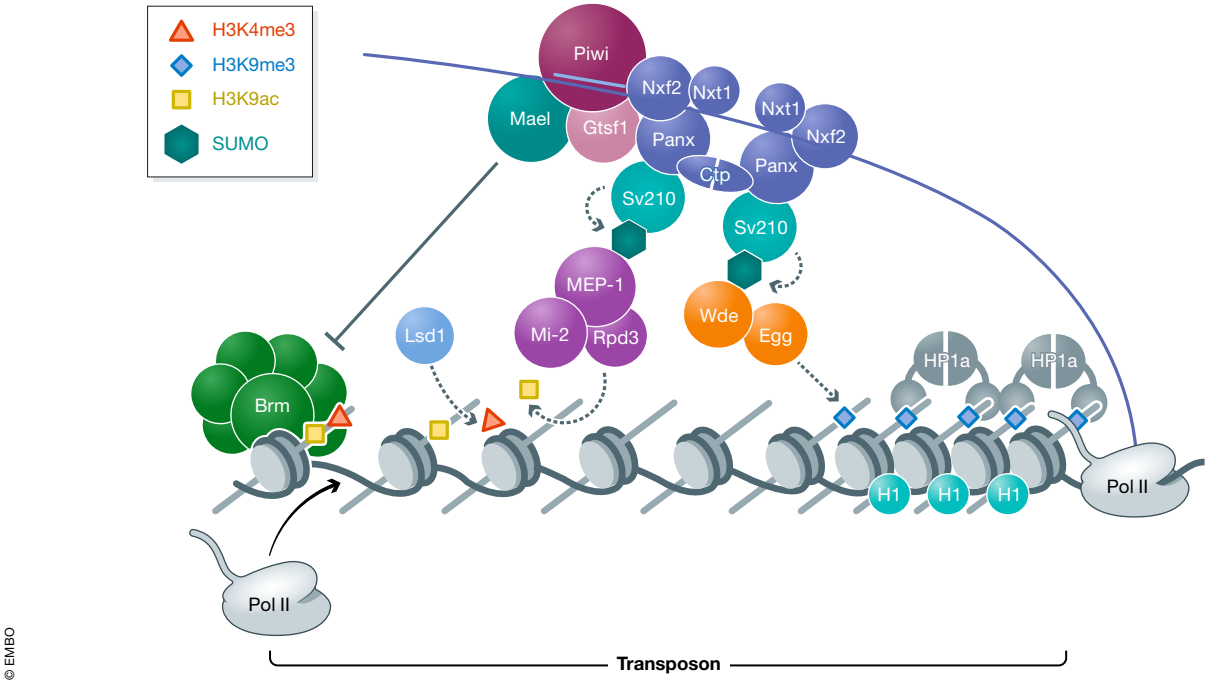

Figure 1. Piwi-mediated transcriptional silencing model.

Piwi-piRISC recognizes nascent transcripts of transposons. Piwi-piRISC recruits silencing factors, such as Mael, Panx, and Gtsf1. Gtsf1 interconnects Piwi-piRISC with Mael and Panx. Mael attenuates Brm-dependent transcription. Panx forms a PICTS/PPNP/SFiNX/Pandas complex with Nxf2 and Nxt1, and the complexes dimerize via Ctp. The PICTS/PPNP/SFiNX/Pandas complex dimer anchors Piwi-piRISC and co-factors to target RNAs. Sv210 interacts with the complex dimer and SUMOylates itself (and possibly other proteins) to provide a scaffold for histone modifiers. Mi-2, MEP-1, and Rpd3 deacetylate H3K9ac, and Egg and Wde induces H3K9me3 at the target loci. Lsd1 demethylates H3K4me3 and enhances the PICTS/PPNP/SFiNX/Pandas complex-mediated silencing. Finally, HP1a and H1 accomplish heterochromatin formation.

Muerdter *et al*, 2013). Of those, some factors were found necessary in both germ (nurse) cells and somatic (follicle) cells, which were presumed to co-function with Piwi, because Piwi is present in both cell types, whereas Aub and Ago3 are germ cell-specific (Handler *et al*, 2013). Besides Mael, Panoramix/Silencio (Panx) and nuclear RNA export factor 2 (Nxf2) were within this category, namely, necessary in both germ and somatic cells (Czech *et al*, 2013; Handler *et al*, 2013; Muerdter *et al*, 2013).

Artificial tethering of Panx to reporter RNAs aberrantly accumulated H3K9me3 at the gene locus and repressed expression even in the absence of Piwi, which indicates that Panx has an intrinsic ability to induce gene silencing independent of Piwi (Sienski *et al*, 2015; Yu *et al*, 2015). Unlike Nxf1, a Nxf2 homolog that functions in mRNA export, Nxf2 does not localize at the nuclear periphery. Structural data showed that the two putative nucleoporin-binding pockets in Nxf2 are restricted, supporting the idea that Nxf2 may have lost the ability to bind nucleoporins and therefore any role in mRNA export (Batki *et al*, 2019; Zhao *et al*, 2019).

Nxt1/p15 was originally reported as an Nxf1 co-factor (Suyama *et al*, 2000). Panx, Nxf2, and Nxt1 assemble a ternary complex, which was termed PICTS, PPNP, SFiNX, and Pandas in independent studies (Batki *et al*, 2019; Fabry *et al*, 2019; Murano *et al*, 2019; Zhao *et al*, 2019). Strictly speaking, the components of the protein complexes may be slightly different from each other, but in this review we call the complex PICTS/PPNP/SFiNX/Pandas. The three members of the complex stabilize each other through mutual protein–protein interactions (Batki *et al*, 2019; Fabry *et al*, 2019; Murano *et al*, 2019; Zhao *et al*, 2019).

Nxf2 binds RNAs through the LRR domain. Nxf2 lacking the LRR domain successfully induced silencing in tethering assays under normal conditions, but failed in the absence of endogenous Nxf2 (Batki *et al*, 2019; Murano *et al*, 2019). Thus, it was inferred that the PICTS/PPNP/SFiNX/Pandas complex anchors Piwi-piRISC to target RNAs through the LRR domain in Nxf2 (Fig 1). The complex was necessary prior to H3K9me3 accumulation at the Piwi target loci, supporting this notion (Murano *et al*, 2019).

Cutup/LC8 (Ctp) has recently been found to be the fourth component of the PICTS/PPNP/SFiNX/Pandas complex (Eastwood *et al*, 2021; Schnabl *et al*, 2021). Ctp is ubiquitous and functions in numerous biological processes by dimerizing its interacting complexes to stabilize them (Jespersen & Barbar, 2020). In the Piwi-piRNA pathway, the PICTS/PPNP/SFiNX/Pandas complex should be dimerized to support efficient transcriptional gene silencing and Ctp acts as the dimerization hub (Fig 1). Ctp, however, does not promote Nxf1 dimerization.

Gtsf1/Arx interconnects Piwi-piRISC with the PICTS/PPNP/SFiNX/Pandas complex

Gametocyte-specific factor/Asterix (Gtsf1/Arx) is a nuclear protein that interacts with Piwi in OSCs (Dönertas *et al*, 2013; Ohtani *et al*,

2013). Piwi-piRISC is produced and localized to the nucleus in Gtsf1/Arx-deficient OSCs, but the Piwi-dependent transposons were desilenced and the H3K9me3 level at the transposon loci was reduced. Thus, Gtsf1/Arx is not necessary for piRNA biogenesis, but it is necessary for Piwi-meditated silencing. In the nuclear step, Gtsf1/Arx interconnects Piwi-piRISC with the PICTS/PPNP/SFiNX/Pandas complex (Onishi *et al*, 2020) (Fig 1). Gtsf1/Arx has two CHHC-type zinc finger domains. Alteration of conserved residues in these domains desilenced transposons, although the precise function(s) of the domains remains unknown (Dönertas *et al*, 2013; Ohtani *et al*, 2013).

Factors co-functioning in Piwi-mediated heterochromatin formation

Drosophila has three H3K9-specific histone methyltransferases, Su(var)3–9, G9a, and Eggless/SetDB1 (Egg). Of these, only Egg functions in Piwi-mediated transcriptional silencing (Sienski *et al*, 2015) (Fig 1). Egg is ubiquitinated by Ubc2 and this modification is necessary for its methylation activity (Osumi *et al*, 2019). Windei (Wde), an Egg co-factor, retains Egg on chromatin (Osumi *et al*, 2019). The H3K4 demethylase, Lsd1, and its co-factor, CoREST, have also been shown to play important roles in Piwi-mediated transcriptional silencing (Yu *et al*, 2015) (Fig 1).

Mi-2, MEP-1, and Rpd3 assemble in a complex and are recruited together to the Piwi-targeted transposon loci, where they deacetylate H3K9 prior to its methylation by Egg (Mugat *et al*, 2020) (Fig 1). Mi-2, an ATP-dependent chromatin remodeler, and Rpd3 reside within the *Drosophila* nucleosome remodeling and deacetylase (dNuRD) complex (Bowen *et al*, 2004). Mi-2 is also present in the *Drosophila* MEP-1-containing (dMec) complex, but Rpd3 is not (Kunert *et al*, 2009). Thus, the complex containing Mi-2, MEP-1, and Rpd3 may be unique to the Piwi-mediated pathway (Mugat *et al*, 2020).

Su(var)2–10 (Sv210), a SUMO E3 ligase, is necessary for the physical link between Panx and effectors such as Egg, Wde, Mi-2, and MEP-1 (Mugat *et al*, 2020; Ninova *et al*, 2020). Sv210 repressed reporter transcription in artificial tethering assays, which required both Wde-Egg and the SUMOylation activity of Sv210 (Ninova *et al*, 2020). Given that many of the effectors have SUMO-interacting motifs, Sv210 SUMOylates proximal proteins, including itself, which may supply the scaffolding for recruiting Egg, Wde, Mi-2, and MEP-1 (Mugat *et al*, 2020; Ninova *et al*, 2020).

Heterochromatin Protein 1a (HP1a) specifically binds to H3K9me3 to maintain the heterochromatin and is necessary for transposon repression (Klenov *et al*, 2011; Wang & Elgin, 2011; Le Thomas *et al*, 2014). Histone H1 directly binds to Piwi and controls chromatin accessibility at the Piwi target loci without changing the levels of H3K9me3 and HP1a (Iwasaki *et al*, 2016) (Fig 1).

Miwi2-piRISC-mediated transcriptional silencing in mice

Drosophila Piwi functions in both ovaries and testes. However, Miwi2 (Piwil4) in mice, the Piwi paralog, is testis-specific (Carmell *et al*, 2007). The other mouse PIWI members, Miwi (Piwil1) and Mili (Piwil2), are also testis-specific but are cytoplasmic and silence transposons by cleaving the RNA transcripts as *Drosophila* Aub and Ago3 do (Kuramochi-Miyagawa *et al*, 2001; Deng & Lin, 2002; Kuramochi-Miyagawa, 2004; Aravin *et al*, 2006; De Fazio *et al*, 2011). All mouse PIWI members are necessary for spermatogenesis and fertility, but their expression timing during spermatogenesis differs: Miwi is expressed from the pachytene stage to round spermatid stage (Kuramochi-Miyagawa *et al*, 2001; Deng & Lin, 2002), Miwi2 is specific to progenitors of spermatogonial stem cells (SSCs) (Carmell *et al*, 2007), and Mili is expressed in all these cells (Kuramochi-Miyagawa *et al*, 2001; Kuramochi-Miyagawa, 2004; Aravin *et al*, 2006). Miwi2- and Mili-bound prepachytene piRNAs are rich in transposon sequences (Zheng *et al*, 2010). Conversely, Miwi- and Mili-bound pachytene piRNAs are rich in protein-coding gene sequences (Watanabe *et al*, 2015; Dai *et al*, 2019). Promoter deletion of pi6, one of the pachytene piRNA loci on chromosome 6, resulted in reduced male fertility, increased expression of several genes required for sperm function, and reduced production of pachytene piRNAs from other loci (Wu *et al*, 2020). However, the complete function of pachytene piRNAs is still unclear.

Miwi2-dependent de novo methylation in gonocytes

In the mammalian life cycle, major reprogramming occurs twice, first in the fertilized eggs and then in primordial germ cells (PGCs) (Reik, 2001; Sasaki & Matsui, 2008). During these periods, repressive epigenetic marks are erased to reset the epigenetic memory inherited from the parents (Reik, 2001; Sasaki & Matsui, 2008). In germ cells, after this reprogramming event, *de novo* DNA methylation occurs throughout the genome to ensure proper spermatogenesis (Popp *et al*, 2010; Seisenberger *et al*, 2012; Kobayashi *et al*, 2013; Molaro *et al*, 2014; Kubo *et al*, 2015). The male germ cells, in which *de novo* DNA methylation takes place, are called gonocytes, and they serve as progenitors of SSCs.

Gonocytes express Mili and Miwi2 (Kuramochi-Miyagawa, 2004; Aravin *et al*, 2006; Carmell *et al*, 2007). The Mili-piRISC cleaves target RNAs in the cytoplasm, from which Miwi2-bound piRNAs are produced (De Fazio *et al*, 2011). This resembles the situation in *Drosophila,* where Piwi- and Aub-bound piRNAs are produced from RNAs cleaved by Ago3-piRISC (Wang *et al*, 2015). The Miwi2-piRISC then enters the nucleus to repress transposon transcription via DNA methylation (Carmell *et al*, 2007; Kuramochi-Miyagawa *et al*, 2008). Evolutionarily young long interspersed nuclear element-1 (LINE1) and intracisternal A-particle (IAP) are the main targets of Miwi2 (Pezic *et al*, 2014; Kojima-Kita *et al*, 2016). Importantly, the region that gains DNA methylation is the target gene promoter (Pastor *et al*, 2014; Barau *et al*, 2016). This suggests that Miwi2 triggers *de novo* methylation specifically on the promoter region of certain types of transposons.

Mice express four Dnmt3 members, Dnmt3A, Dnmt3B, Dnmt3L, and Dnmt3C, and all these enzymes are required for spermatogenesis (Chedin *et al*, 2002; Bourc'his & Bestor, 2004; Kaneda *et al*, 2004; Suetake *et al*, 2004; Barau *et al*, 2016; Veland *et al*, 2019; Gao *et al*, 2020). Of those, Dnmt3C is gonocyte-specific and rodent-specific. Its depletion causes DNA demethylation of transposon promoter regions specifically, as seen in *mili* mutant cells (Barau *et al*, 2016). Dnmt3C-mediated *de novo* DNA methylation is specific for the Miwi2 pathway (Barau *et al*, 2016) (Fig 2).

Factors co-functioning in Miwi2-dependent de novo DNA methylation

Miwi2 interacts with multiple co-factors in gonocytes (Fig 2). One representative is TDRD9 (Shoji *et al*, 2009; Wenda *et al*, 2017).

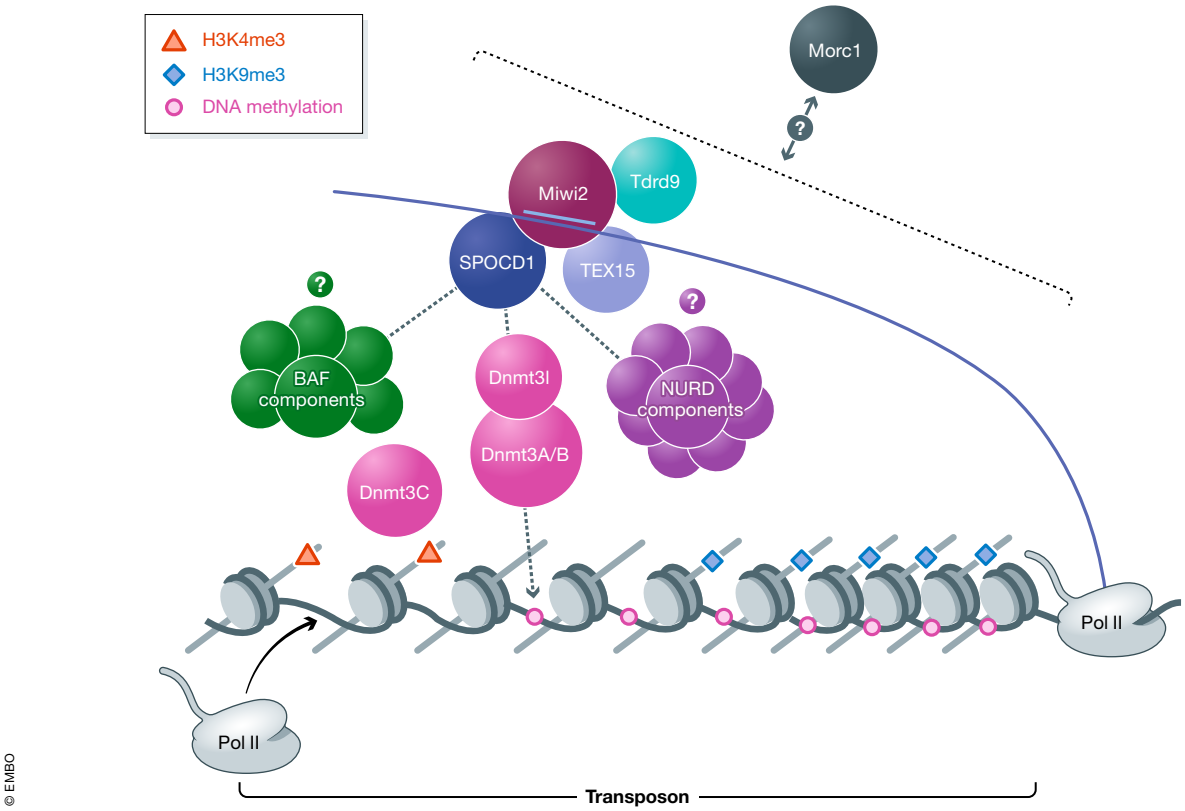

Figure 2. Miwi2-mediated transcriptional silencing model.

Miwi2-piRISC recognizes nascent transcripts of transposons. Miwi2 interacts with Tdrd9, TEX15, and SPOCD1. H3K4me3 marks are enriched in the Miwi2 target loci. The functions of Tdrd9 and TEX15 are still unknown. SPOCD1 recruits Dnmt3L and Dnmt3A to induce *de novo* DNA methylation of the target loci. H3K9me3 marks are also enriched along with DNA methylation. Components of BAF and NuRD also interact with SPOCD1 and Miwi2, but the involvement of these complexes is still unclear. Morc1 is an essential factor for *de novo* DNA methylation in gonocytes, but crosstalk with Miwi2-mediated pathways remains to be elucidated.

TDRD9 is a DExH-box helicase/ATPase containing a Tudor domain and is expressed in the testis of both embryos and adults. In gonocytes, TDRD9 is detected in cytoplasmic non-membranous granules and in the nucleus (Shoji *et al*, 2009; Wenda *et al*, 2017). *tdrd9* point mutation mice show defects in DNA methylation of LINE1 elements, leading to developmental arrest at the pachytene stage and infertility (Shoji *et al*, 2009; Wenda *et al*, 2017). However, these mutant mice produced piRNAs normally. This highlights the importance of TDRD9 in Miwi2-mediated transcriptional silencing (Shoji *et al*, 2009). However, its functional contribution remains undetermined. The fly homolog of TDRD9 is Spindle-E, which is essential in piRNA biogenesis, although its functional contribution also remains unknown (Lim *et al*, 2009).

SPOCD1 and TEX15 are necessary for *de novo* DNA methylation of transposons (Schöpp *et al*, 2020; Yang *et al*, 2020; Zoch *et al*, 2020) (Fig 2). SPOCD1 encodes a protein with a TFIIS-M and a SPOC domain. Tex15 harbors DUF3715 and two TEX15 domains and is expressed exclusively in both adult and fetal testis. *Tex15* deficiency leads to early meiotic arrest and complete infertility. Moreover, the DNA methylation level over young transposons decreases in the mutant, showing striking similarity with piRNA pathway mutants. Both SPOCD1 and TEX15 proteins are nuclear proteins whose expressions are restricted to testes, and interact with Miwi2 (Schöpp *et al*, 2020; Zoch *et al*, 2020). SPOCD1 recruits *de novo* DNA methyltransferases, Dmnt3A and Dmnt3L, to the sites where Miwi2 functions (Zoch *et al*, 2020). The SPOCD1 interacting factors include components of the NuRD and BAF complexes (Zoch *et al*, 2020). The BAF complex is one of the SWI/SNF complexes in mice (Wilson & Roberts, 2011).

Morc1 belongs to the Morc family of GHKL ATPases and its depletion causes male infertility (Watson *et al*, 1998; Inoue, 1999). Morc1 is abundant in blastocysts and gonocytes, but it is not present in differentiated cells (Hruz *et al*, 2008). In *morc1* mutant mice, male germ cells undergo transposon hypomethylation and derepression (Pastor *et al*, 2014). However, the abundance of piRNAs is maintained (Pastor *et al*, 2014). The differentially methylated regions (DMRs) in the mutant mice were concentrated around the transposon promoters (Pastor *et al*, 2014). This phenocopies *mili* and *dnmt3c*, but not *dnmt3l*, mutant mice (Barau *et al*, 2016). Morc1 may play an important role in Miwi2-dependent transposon silencing in gonocytes (Fig 2).

There is an intimate interplay between *de novo* DNA methylation and specific sets of histone modifications. In the early developmental stage of spermatogenesis and oogenesis, methylation of H3K9 at the young transposon loci by SetDB1 prior to *de novo* DNA methylation is important for subsequent proper *de novo* DNA methylation

(Liu *et al*, 2014). The molecular cascade of *de novo* DNA methylation is well characterized in mouse oocytes. Here, methylation at H3K36 by SetD2 and the subsequent removal of methylation from H3K4 is a prerequisite for the establishment of *de novo* DNA methylation (Stewart *et al*, 2015; Xu *et al*, 2019). In contrast, in gonocytes, Nsd1, another methyltransferase targeting H3K36, but not SetD2, is essential for *de novo* methylation (Shirane *et al*, 2020). Moreover, demethylated H3K4 (H3K4me2) was observed in gonocytes at the loci that undergo Miwi2-dependent DNA methylation, suggesting that the methylation of H3K36, followed by the demethylation at H3K4, is a common molecular cascade that leads to *de novo* DNA methylation in both oocytes and gonocytes.

Comparison of Piwi- and Miwi2-mediated transcriptional silencing

piRISC biogenesis Piwi- and Miwi2-piRISC bind nascent transposon mRNAs through RNA–RNA base-paring while transcription is still undergoing. Piwi- and Miwi2-bound piRNAs are mostly "antisense" to transposon mRNAs; thus, the two PIWI proteins target the mRNAs. Piwi- and Miwi2-bound piRNAs are produced mainly from piRNA cluster transcripts cleaved by Ago3- and Mili-piRISC, respectively (De Fazio *et al*, 2011; Wang *et al*, 2015) (Fig 3). It should be noted that *Drosophila* OSCs do not express Ago3; hence, Piwi-piRISC in the cells is produced in an Ago3-piRISC-independent manner (Saito *et al*, 2009) (Fig 3). Other aspects of the production of the Piwi- and Miwi2-piRISC have been summarized elsewhere (Ozata *et al*, 2019).

Silencing step The major difference between Piwi- and Miwi2-mediated RNA silencing is the involvement of DNA methylation (Fig 3). This is basically attributable to the fact that *Drosophila* lacks canonical DNMTs (Kunert *et al*, 2003). DNA methylation may occur weakly during fly embryonic development, but it is mediated by noncanonical Dnmt2 (Kunert *et al*, 2003). Dnmt3C, which plays a role in the Miwi2 system, is rodent-specific (Barau *et al*, 2016).

Although this DNA methylation issue is a major difference between *Drosophila* and mice, the piRNA pathway in these two organisms eventually forms heterochromatin at the target locus (Fig 3). However, it has been noted that only a few factors are commonly used in the two systems. For example, Panx, which is important for the Piwi-mediated system, is an orphan gene that only exists in *Drosophila* (Sienski *et al*, 2015; Yu *et al*, 2015). These differences may be due to convergent evolution of the piRNA systems in different species to counteract transposons. Gtsf1/Arx is one of the factors that appear in both the mouse (as Gtsf1) and fly systems as an interacting factor of Miwi2 and Piwi, respectively (Dönertas *et al*, 2013; Ohtani *et al*, 2013; Yoshimura *et al*, 2018). However, it shows distinct functions in these species. Mouse Gtsf1 is cytoplasmic and contributes to the production of Miwi2-bound piRNAs, while *Drosophila* Gtsf1/Arx localizes to the nucleus and co-functions with Piwi-piRISC to repress transposons (Dönertas *et al*, 2013; Ohtani *et al*, 2013; Yoshimura *et al*, 2018).

Histone modifiers, such as H3K4 demethylases and H3K9 methyltransferases, as well as the NuRD complex with the H3K9 deacetylation activity are likely to be used in both the mouse and *Drosophila* systems (Liu *et al*, 2014; Pezic *et al*, 2014; Sienski *et al*, 2015; Yu *et al*, 2015; Nagamori *et al*, 2018) (Figs 1 and 2). Furthermore, Piwi and Miwi2 interact with components of the BAF complex (Zoch *et al*, 2020) (Figs 1 and 2). Considering the Brm function in the Piwi-mediated system (shown above), Miwi2 may also regulate the Brm homolog to accomplish the task of transcriptional silencing.

Ago1/siRNA-mediated heterochromatin formation in *Schizosaccharomyces pombe*

In addition to the interspecies divergence of the molecular mechanism by which the nuclear Piwi-piRNA pathway represses transposons, there is another diversity in regard to how the small RNA-mediated silencing pathway in the nucleus affects the chromatin state. In fission yeast, Ago1/siRNA-mediated constitutive heterochromatin formation occurs at three genomic regions, the pericentromeric and sub-telomeric regions, and the mating-type locus, which we refer to as *cen*, *tel*, and *mat*, respectively. Each of the loci contains unique repetitive elements known as *dg/dh*, *cenH*, and *cenH*-like, respectively. The repetitive elements give rise to siRNAs

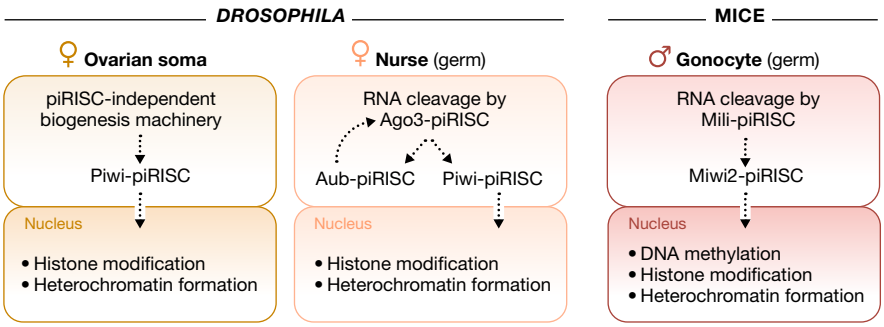

Figure 3. Comparison between the Piwi-piRISC and Miwi2-piRISC pathways.

In *Drosophila* ovaries, Piwi-interacting RNA production machineries in the cytoplasm are different in soma and germ cells. In soma, Piwi-piRNAs are produced by a piRISC-independent biogenesis machinery, whereas in germ cells, they are produced via Aub/Ago3-mediated cleavage of piRNA precursors. In the nucleus, Piwi-piRISC regulates histone modifications and subsequent heterochromatin formation. In mouse gonocytes, Miwi2-interacting piRNAs are produced via Mili-mediated precursor cleavage in the cytoplasm. In the nucleus, Miwi2-piRISC induces DNA methylation along with regulation of histone modification and then accomplishes heterochromatin formation.

that nucleate chromatin compaction with help from multiple co-factors (Reinhart & Bartel, 2002; Volpe *et al*, 2002; Verdel *et al*, 2004). The chromatin compaction is spread to flanking areas toward both sides to fully cover the loci (Motamedi *et al*, 2004; Noma *et al*, 2004). This siRNA-dependent constitutive heterochromatin formation has a broad effect on chromosome homeostasis, such as suppression of recombination, faithful segregation of chromosomes during cell division, and switching of mating-type information (Egel, 1984; Klar & Bonaduce, 1991; Thon & Klar, 1993; Allshire *et al*, 1995; Provost *et al*, 2002; Hall *et al*, 2003; Jia *et al*, 2004; Tuzon *et al*, 2004).

Key RNAi components involved in heterochromatin assembly in fission yeast

The repetitive elements are located within the constitutive heterochromatin regions, but they must be transcribed to a minor extent to produce siRNAs (Kato *et al*, 2005). Upon transcription, the nascent, single-stranded (ss) RNAs are converted to double-stranded (ds) RNAs by Rdp1, the core resident of the RNA-directed RNA polymerase complex (RDRC) (Motamedi *et al*, 2004; Sugiyama *et al*, 2005) (Fig 4). The RDRC contains two other components, the putative helicase, Hrr1, and noncanonical poly(A) polymerase family member Cid12 (Motamedi *et al*, 2004). The dsRNAs are subsequently processed into ~21-nt-long siRNA duplexes by Dcr1 (Fig 4), the fission yeast ortholog of human Dicer, and fly Dicer2 (Provost *et al*, 2002; Lee *et al*, 2004; Colmenares *et al*, 2007). The siRNA duplexes are loaded onto Ago1 in a stoichiometric manner, where the passenger strand of the siRNA duplex is then cleaved by the slicer activity of Ago1 and ejected into the nucleoplasm for degradation (Buker *et al*, 2007). The guide strand remains with Ago1, which assembles the RNA-induced initiation of transcriptional gene silencing (RITS) complex with chromodomain protein Chp1 and Gly-Trp (GW)-repeat protein Tas3, which serves as a platform for gene silencing by RITS (Verdel *et al*, 2004; Schalch *et al*, 2011) (Fig 4).

A mutation introduced into Rpb2, a subunit of RNA Pol II, reduced the levels of siRNAs derived from the repetitive elements, causing misregulation of the siRNA-mediated heterochromatin formation. Nonetheless, the cell growth of this *rbp2* mutant was comparable to that of the wild type strain (Kato *et al*, 2005). This indicates that the transcription state of repetitive elements is somewhat distinct from the transcription state of other euchromatic genes. For example, the *dg/dh* repeat within the *cen* locus is transcribed in both directions (Volpe *et al*, 2002). Furthermore, some splicing mutants disrupted the siRNA production from centromeric regions, although canonical splicing remained active. This also supports the notion that some features of RNA metabolism trigger RNAi-dependent heterochromatin formation (Bayne *et al*, 2008).

The RITS complex deposits repressive histone marks in the silencing loci

The RITS complex targets nascent RNAs and recruits the Clr4 methyltransferase complex (CLRC) to the loci (Horn *et al*, 2005; Jia *et al*, 2005; Zhang *et al*, 2008) (Fig 4). Clr4 is an H3K9 methyltransferase that shows similarities, both structurally and functionally, to mammalian Suv39h and *Drosophila* Su(var)3–9 (Rea *et al*, 2000; Nakayama *et al*, 2001; Schotta *et al*, 2002). The CLRC methylates H3K9 to H3K9me3, to which Chp1 within the RITS complex binds through the chromodomain, which stabilizes the RITS association to the heterochromatin (Noma *et al*, 2004; Cam *et al*, 2005; Petrie *et al*, 2005; Schalch *et al*, 2009). In this vicinity, Stc1 interconnects the CLRC to the RITS complex. The artificial tethering of Stc1 to arbitrary genomic regions recruits CLRC and induces heterochromatin formation in a RITS-independent manner, even in euchromatin regions, highlighting the role of Stc1 in CLRC recruitment to chromatin (Bayne *et al*, 2010) (Fig 4). It has been noted that Stc1 is structurally similar to Gtsf1/Arx; thus, its function may also be similar to that of Gtsf1/Arx (Bayne *et al*, 2010; Onishi *et al*, 2020) (Fig 5).

Chromosomal compaction spreading throughout the heterochromatin regions

siRNAs are derived mainly from repetitive elements embedded within the constitutive heterochromatin regions (Cam *et al*, 2005; Bühler *et al*, 2008). However, chromosomal compaction is not restricted to these elements. Rather, it spreads beyond these elements throughout the heterochromatin regions (Cam *et al*, 2005). Indeed, reporter genes artificially inserted very close to the *cen* locus, outside of *dg/dh*, were repressed depending on RITS, RDRC, CLRC, and Dcr1 (Volpe *et al*, 2002; Motamedi *et al*, 2004; Verdel *et al*, 2004; Jia *et al*, 2005). The key factor in this heterochromatin spreading is Swi6, an HP1 homolog in fission yeast (Hall *et al*, 2002; Noma *et al*, 2004). Swi6 binds H3K9me2 and H3K9me3 through its chromodomain and self-oligomerizes to build a platform for recruiting other chromatin proteins, such as histone deacetylases and chromatin remodelers (Nakayama *et al*, 2001; Fischer *et al*, 2009; Canzio *et al*, 2011) (Fig 4). In addition to Swi6, Tas3, one of the RITS complex components, is involved in *cis*-spreading of heterochromatin silencing (Li *et al*, 2009). Its C-terminal domain self-associates and forms a polymer structure in solution. *In vivo*, this domain is essential for RITS spreading over centromeric heterochromatin regions (Li *et al*, 2009).

Sir2, Clr3, and Clr6 are three major histone deacetylases in fission yeast, which play essential roles in suppressing genes located within heterochromatin regions (Grewal & Klar, 1997; Bjerling *et al*, 2002; Shankaranarayana *et al*, 2003; Hansen *et al*, 2005) (Fig 4). Sir2 is an NAD^+-dependent deacetylase and is required for the heterochromatic gene silencing at *cen, tel,* and *mat*. In *sir2* mutant cells, the acetylation level of H3K9 increases in these heterochromatic regions. In contrast, the methylation level of H3K9 as well as the abundance of Swi6 decreases. Clr3 assembles the Snf2/Hdac-containing repressor complex (SHREC) with the SNF2 remodeling factor Mit1 (Sugiyama *et al*, 2007). Clr3 maintains nucleosome occupancy at specific regions within the heterochromatin (Garcia *et al*, 2010; Yamane *et al*, 2011). Additionally, in *clr3* mutant cells, the nucleosome turnover rate increases all over the heterochromatin regions (Aygün *et al*, 2013). These nucleosome states are in favor of transcription, supporting direct involvement of Clr3 in transcriptional gene silencing over these regions. Histone chaperone Asf1/HIRA also plays a role in maintaining nucleosome occupancy over heterochromatin regions together with Clr6 (Yamane *et al*, 2011). The *clr3* and *asf1* double mutation causes a severer reduction in nucleosome occupancy within the heterochromatin regions, suggesting a redundant role of Clr3 and Clr6 in ensuring the chromatin state that suppresses transcription. Swi6 is involved in recruiting Clr3, Clr6, and Asf1 to heterochromatin regions (Motamedi *et al*, 2008). Overall, the Swi6-centered protein network ensures the repressed chromatin state within the heterochromatin regions.

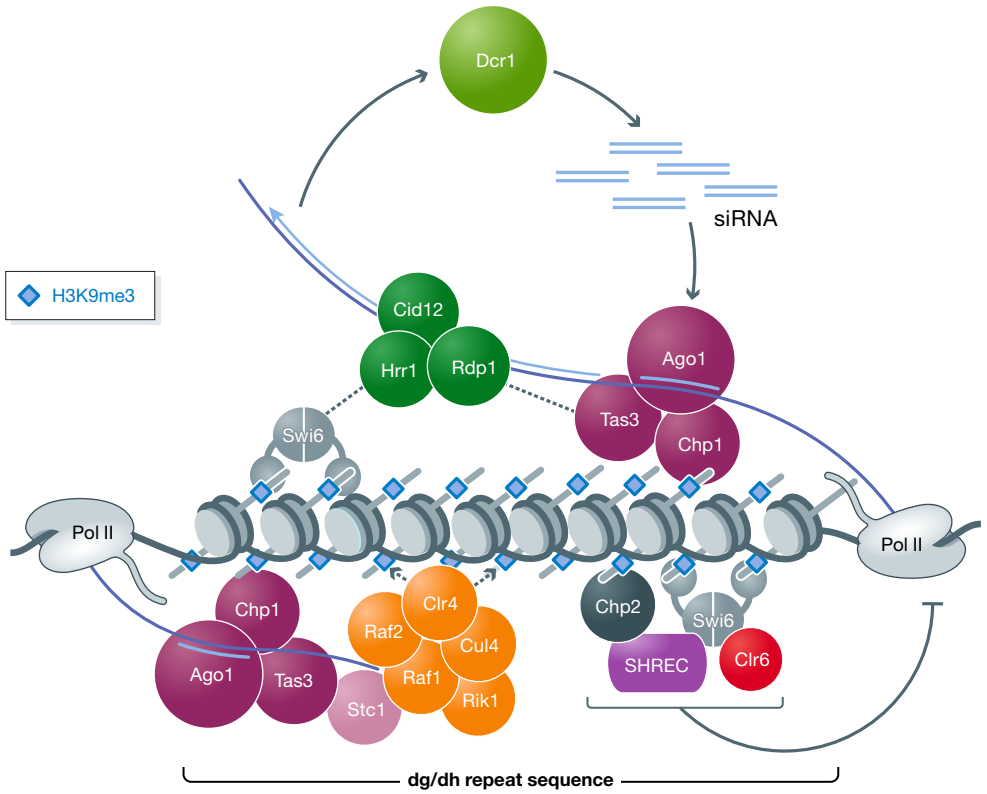

Figure 4. RITS-mediated heterochromatin formation model.

The RITS complex targets nascent RNA of *dg/dh* repeats. Chp1 in the RITS complex binds to H3K9me3 via its chromodomain and stabilizes RITS on the chromatin. RITS recruits RDRC and reverse transcribes *dg/dh* repeat sequences to produces double-stranded siRNA precursors. The precursors are processed by Dicer into secondary siRNAs. RITS also recruits CLRC and Stc1 interconnects the two complexes. Clr4 of CLRC interacts with H3K9me3 using its chromodomain and methylates neighboring H3K9. Chp2 and Swi6 recognize H3K9me3 and recruit SHREC and Clr6 to repress transcription and form heterochromatin.

Accessory proteins that modulate heterochromatin formation

Recent studies have revealed the link between RNA homeostasis and siRNA-dependent constitutive heterochromatin formation. One example involves the Mlo3 function in mRNA export in fission yeast (Thakurta *et al*, 2005). This protein is an RRM-containing protein homologous to Aly/REF. In *mlo3* mutant cells, the level of centromeric transcripts is increased, while the level of siRNAs derived from the transcripts is severely reduced (Zhang *et al*, 2008). Remarkably, Clr4 methyltransferase methylates Mlo3. However, alteration of Lys9 of histone H3 to arginine did not affect the level of siRNAs, highlighting the specific importance of Clr4-mediated Mlo3 methylation in siRNA production. The levels of H3K9me3 and Swi6 over centromeric heterochromatin regions are maintained in *mlo3* mutant cells, while siRNA production is attenuated. Thus, siRNA production can be uncoupled from the maintenance of heterochromatin marks as observed in the *mat* locus (Hall *et al*, 2002).

The Trf4/Air2/Mtr4p polyadenylation (TRAMP) complex, together with the nuclear exosome, plays an important role in processing ribosomal RNA, small nuclear RNA, and small nucleolar RNA (LaCava *et al*, 2005; Vanácová *et al*, 2005; Wyers *et al*, 2005). One subunit of TRAMP, Cid14, is a poly(A)-specific nucleotidyl transferase (Bühler *et al*, 2007). In *cid14* mutant cells, siRNAs from constitutive heterochromatin regions are mostly lost, while the precursors accumulate (Bühler *et al*, 2007). Similarly to the *mlo3* mutant cells, the levels of H3K9me3 and Swi6 in *cid14* mutant cells are hardly affected, further supporting the notion that siRNA production is uncoupled from the maintenance of heterochromatin marks (Bühler *et al*, 2007).

Conversely, Rrp6, a subunit of the nuclear exosome, has a distinct role in the formation of constitutive heterochromatin. In *ago1* mutant cells, the repetitive elements are transcriptionally upregulated, while the levels of H3K9me3 and Swi6 are decreased. Interestingly, the *ago1/rrp6* double mutant shows an even severer phenotype than the *ago1* mutant; consequently, the constitutive heterochromatin turns into the euchromatic state (Reyes-Turcu *et al*, 2011). This finding indicates that Ago1 and Rrp6

Figure 5. Domain structures of siRNA factors in fission yeast and piRNA factors in *Drosophila*.

Comparison of the domain structures of proteins involved in siRNA-mediated heterochromatin formation in fission yeast and piRNA-mediated transposon silencing in *Drosophila*, according to their roles. Data on domain structures were obtained from Uniprot, Pfam, and SMART.

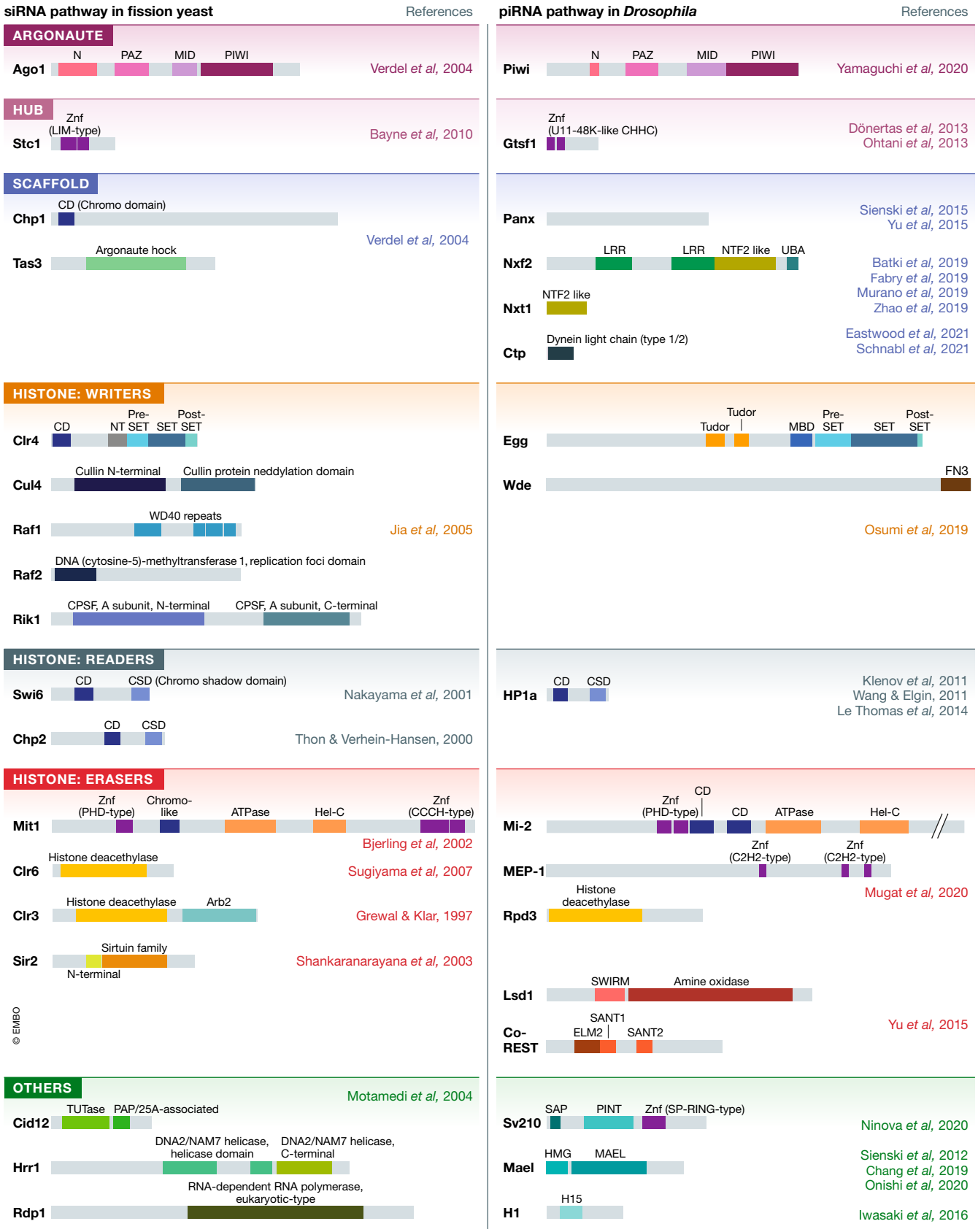

Figure 5.

independently trigger heterochromatin formation to suppress transcription. The redundancy in maintaining heterochromatin indicates the existence of an RNAi-independent heterochromatin formation pathway that includes Rrp6. This redundancy creates the additive effect on heterochromatin formation of the RNAi machinery and a protein that is involved in nuclear RNA processing. This phenotype was not observed in the *cid14/ago1* double mutant (Reyes-Turcu *et al*, 2011), because the *cid14* mutation suppresses the defect in heterochromatin formation caused by the Ago1 deletion. These data support the idea that TRAMP and the nuclear exosome play distinct roles in heterochromatin formation.

Transcription-independent, DNA motif-dependent heterochromatin assembly

Two members of the ATF/CREB family, Atf1 and Pcr1, induce heterochromatin formation at the *mat* locus in an siRNA-independent manner (Jia *et al*, 2005). The ATF/CREB family of transcription factors are leucine zipper proteins that bind to the cAMP response element (CRE). At the *mat* locus, in contrast to the *cen* locus, depletion of RNAi components hardly affects the heterochromatin status as well as the gene silencing status, although these components localize to the *mat* locus. Interestingly, depletion of the ATF/CREB-binding motif (CRE) in combination with the loss of RNAi machinery causes severe defects in heterochromatin formation. This shows that at the *mat* locus, the DNA elements (CRE) trigger an siRNA-independent pathway to ensure the heterochromatin status by recruiting Clr3 through Atf1/Pcr1. Similarly, at the *tel* locus, the DNA-binding protein, Taz1, induces siRNA-independent heterochromatin formation (Kanoh *et al*, 2005). Such DNA motif has not been found at the *cen* locus, although heterochromatin can be formed in an siRNA-independent but Rrp6-dependent manner.

Comparison of the Piwi-piRISC and RITS pathways

Chromodomain dependency In fission yeast, siRNAs are produced from repetitive elements within constitutive heterochromatin, which are rich in H3K9me3. Nonetheless, the elements are weakly transcribed to generate siRNAs only during S phase (Chen *et al*, 2008; Kloc *et al*, 2008). The siRNA production takes place within the vicinity and the siRNAs target the parental loci to maintain the heterochromatin state. Thus, it is reasonable that many key factors, such as Chp1, Chp2, Swi6, Mit1, and Clr4, possess the chromodomain H3K9me3-reading domain (Fig 5).

In contrast, the chromodomain is not common among Piwi co-factors, and only HP1 members and Mi-2 have this domain (Fig 5). The piRNA clusters used in OGCs are rich in H3K9me3 and are bound to the HP1 homolog, Rhino (Rhi), through the chromodomain. Rhi then recruits other factors to activate transcription internally within the clusters (Mohn *et al*, 2014; Zhang *et al*, 2014; Andersen *et al*, 2017). Rhi is unique to OGCs and absent in OSCs (Sumiyoshi *et al*, 2016). The main piRNA source in OSCs is *flam*, which is H3K9me3-free (Sienski *et al*, 2012). Transposons targeted by piRNAs are normally located within euchromatin, which is fundamentally H3K9me3-free before the action of Piwi (Sienski *et al*, 2012). Thus, Piwi co-factors do not have to rely on the chromodomain to function in this pathway. Clr4 in fission yeast contains a chromodomain together with a Pre-SET and SET domain (Fig 5). The *Drosophila* counterpart, Egg/SetDB1, has a TUDOR domain instead of the chromodomain (Fig 5). The TUDOR domain binds the active histone mark, H3K14ac, but not H3K9me3 (Jurkowska *et al*, 2017).

Determining the genomic sites where Piwi localizes is experimentally a difficult task. On the basis of this, the "target-engaged piRISC" model was proposed (Huang *et al*, 2013; Lin *et al*, 2015; Marinov *et al*, 2015; Ilyin *et al*, 2017). According to this model, nuclear Piwi-piRISCs scan nascent transcripts until they find landing sites, which should be highly complementary to the piRNAs. Once piRISCs bind the target RNAs, they transform to "target-engaged piRISCs" and start recruiting other co-factors (Sienski *et al*, 2015). This model is based on the structural change found in AGO-RISC before and after its binding to the target RNA (Wang *et al*, 2008). In fission yeast, the genomic sites to which Ago1, and even its co-factors, bind were easily determined experimentally. These findings prompted us to think that in *Drosophila*, the sequence specificity that is inherently buried in the nascent transcript triggers heterochromatin formation more than the chromatin status on such genomic regions does. Conversely, RNAi in fission yeast is forced to locate at heterochromatin regions and its activity is regulated by the chromatin status with the help of RNA recognition by the RITS complex. Thus, the difference in the number of chromodomain-containing proteins between the two pathways may reflect the mode of action that triggers heterochromatin formation.

Trans-silencing In most organisms, including *Drosophila*, siRNAs arising from dsRNAs expressed either exogenously or endogenously efficiently recognize the corresponding RNA sequences and silence them effectively. This indicates that siRNAs can target RNA transcripts *in trans* no matter where they originated from. Similarly, *Drosophila* piRNAs are produced from piRNA clusters (strictly speaking, this has only been demonstrated for the *flam* locus) and silence transposons located in euchromatin (Yamanaka *et al*, 2014; Sato & Siomi, 2018). In contrast to this fly system, siRNAs in fission

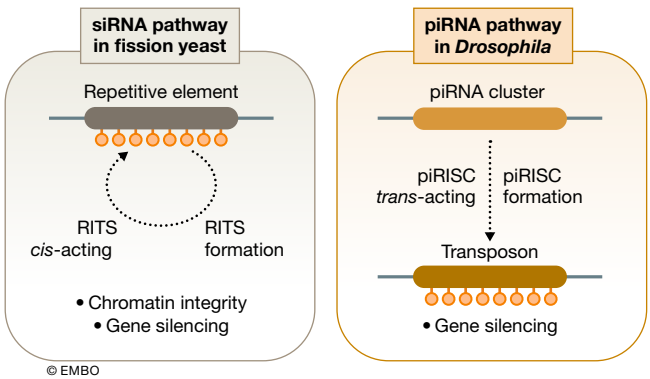

Figure 6. Comparison between the Piwi-piRISC and RITS pathways.

In fission yeast, siRNAs induce heterochromatin formation in *cis* by targeting the locus at which their own precursors are produced. RITS recognizes repressive chromatin and maintains chromatin in a compact state, thereby repressing incorrect gene expression and ensuring correct chromatin segregation. However, Piwi-piRNAs are produced from piRNA clusters and target intergenic active transposons *in trans*. Piwi-piRISC first attenuates transcription, then renders the transposon loci compact, and subsequently accomplishes gene silencing.

yeast do not induce heterochromatin assembly *in trans*, i.e., they avoid "*trans*-silencing". Namely, siRNAs in fission yeast are restricted to act on the parental elements from which they were produced (Fig 6). However, upon mutating Paf1, an RNA pol II interactor, the effect of *trans*-silencing was increased, and even genes located outside of the heterochromatin regions were repressed if their RNA transcripts were complementary to the siRNAs (Kowalik *et al*, 2015). In the *paf1* mutant cells, nascent transcripts are not efficiently released from the genes and the lingering RNAs become the target of the silencing machinery, leading to heterochromatin formation. This increase in the range of RNAi-targeting was also observed upon mutating Mst2, a histone acetyltransferase (Flury *et al*, 2017). The mutation may also retain nascent transcripts aberrantly long on the gene as seen in *paf1* mutant cells. Consistent with this notion, the defect in nuclear RNA processing at the transcription site caused by Rrp6 depletion led to efficient targeting of RNAi and subsequent heterochromatin formation on actively transcribed gene regions (Yamanaka *et al*, 2013).

In fission yeast, the length of introns within protein-coding genes is relatively small, and the average number of introns is smaller than that in humans (Mourier & Jeffares, 2003). Moreover, many genes in fission yeast do not even have introns. Conversely, pre-mRNAs in *Drosophila* are normally much longer than those in fission yeast and thus are theoretically retained on the genome for a longer period of time. This may contribute to the increased rate of "*trans*-targeting" in organisms other than fission yeast. Moreover, as discussed above, at least in *Drosophila*, the transcription rate of the Piwi targets is reduced by Mael, or other alternative means, before heterochromatin formation occurs at the target loci (Sienski *et al*, 2012; Chang *et al*, 2019; Onishi *et al*, 2020). Whether or not mice have a similar system remains undetermined.

piRNAs versus siRNAs Fission yeast are PIWI-free and piRNA-free, but *Drosophila* produce siRNAs and express AGO members in the germline. Endogenous siRNAs in *Drosophila* are the products of long dsRNAs, and the majority have transposon sequences. In cultured Schneider 2 (S2) cells, a *Drosophila* cell line of embryo origin, the loss of Dicer2 and Ago2 caused derepression of transposons, which indicates that RNAi contributes to transposon silencing at least in these non-gonadal somatic cells (Kawamura *et al*, 2008). Such endogenous RNAi may contribute to transposon silencing in the ovaries in addition to the piRNA pathway, but to a much lower, perhaps negligible, extent, because the *PIWI* mutants, but not the *ago2* mutant flies, show infertility (Cox *et al*, 1998; Kim *et al*, 2007).

Perspective

piRNA-mediated transcriptional transposon silencing in *Drosophila* and mice is essential for maintaining the genomic integrity of reproductive tissues. A more detailed understanding of the molecular mechanisms of piRNA-mediated transcriptional silencing has been gained in *Drosophila* (Iwasaki *et al*, 2015; Czech *et al*, 2018; Ozata *et al*, 2019). Recently, it has been reported that Piwi-piRISC efficiently represses transposon transcription by co-activating H3K9me3-independent and H3K9me3-dependent transcriptional repressive mechanisms (Murano *et al*, 2019; Onishi *et al*, 2020). Additionally, it has been suggested that H3K9 deacetylation and H3K4 demethylation are important for Piwi-mediated silencing (Yu *et al*, 2015; Mugat *et al*, 2020). The next challenge is to clarify the order of these inhibitory mechanisms and the detailed molecular functions of the co-factors.

In recent years, the detailed mechanism of Miwi2-mediated properties has become better understood. So far, phenotypic analysis has been the main method for analyzing Miwi2-mediated transcriptional repression systems. For more detailed mechanistic understanding, biochemical analysis of gonocytes is necessary. Recently, cell sorting technology and biochemical analysis using a small number of cells or a single cell have been developed, and it is expected that these technologies will be used to elucidate the molecular mechanism of Miwi2-mediated transcriptional silencing. Moreover, further progress will be made by comparing the Miwi2-mediated transcriptional silencing mechanism with that in *Drosophila*.

The model of Ago1-mediated heterochromatin formation in fission yeast has been used as a reference for a piRNA-mediated transcriptional repression model. However, as research on piRNA-mediated transcriptional silencing has progressed, differences between the two mechanisms have been revealed. First, piRNAs mainly target transposons integrated in euchromatic loci and convert them into a transcriptionally inert state, whereas siRNAs in fission yeast target repetitive elements transcribed from constitutive heterochromatin regions. It is interesting to note that the piRNA system seems to be specialized in the repression of active transcription (Aravin *et al*, 2008; Nagamori *et al*, 2018; Onishi *et al*, 2020). This unique feature of the piRNA system may be the result of an arms race between piRNAs and transposons. Therefore, clarifying the origin of piRNA-specific factors will shed light on the evolution of the piRNA system.

Box: In need of answers

- How do Piwi co-factors such as Gtsf1/Arx, Mael, PICTS/PPNP/SFiNX/Pandas, Egg, and other chromatin modification factors integrate the Piwi/piRNA-mediated transposon repression mechanisms? Artificial tethering of the silencing factors over time using drug-inducible expression systems may help to clarify this question.
- What is the molecular mechanism underlying Miwi2-mediated gene silencing? Biochemical analysis of Miwi2-interacting factors using FACS-sorted gonocytes may help to clarify this question. A cultured system suitable for biochemical analysis, equivalent to *Drosophila* OSCs, is desired.
- What are the molecular determinants and minimum requirements that limit *trans*-silencing by the RITS complex? *In vitro* reconstruction of the entire step of RNAi-mediated heterochromatin formation will help to answer this question.
- What accounts for the diversity and uniqueness of the piRNA system? Uncovering the origin of factors specific to the piRNA system may help to answer this question.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Allshire RC, Nimmo ER, Ekwall K, Javerzat JP, Cranston G (1995) Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation. *Genes Dev* 9: 218 – 233

Andersen PR, Tirian L, Vunjak M, Brennecke J (2017) A heterochromatin-dependent transcription machinery drives piRNA expression. *Nature* 549: 54 – 59

Araki S, Le NT, Koizumi K, Villar-Briones A, Nonomura K-I, Endo M, Inoue H, Saze H, Komiya R (2020) miR2118-dependent U-rich phasiRNA production in rice anther wall development. *Nat Commun* 11: 3115

Aravin AA, Naumova NM, Tulin AV, Vagin VV, Rozovsky YM, Gvozdev VA (2001) Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the *D. melanogaster* germline. *Curr Biol* 11: 1017 – 1027

Aravin AA, Lagos-Quintana M, Yalcin A, Zavolan M, Marks D, Snyder B, Gaasterland T, Meyer J, Tuschl T (2003) The small RNA profile during *Drosophila melanogaster* development. *Dev Cell* 5: 337 – 350

Aravin A, Gaidatzis D, Pfeffer S, Lagos-Quintana M, Landgraf P, Iovino N, Morris P, Brownstein MJ, 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 AA, Sachidanandam R, Bourc'his D, Schaefer C, Pezic D, Toth KF, Bestor T, Hannon GJ (2008) A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. *Mol Cell* 31: 785 – 799

Aygün O, Mehta S, Grewal SIS (2013) HDAC-mediated suppression of histone turnover promotes epigenetic stability of heterochromatin. *Nat Struct Mol Biol* 20: 547 – 554

Barau J, Teissandier A, Zamudio N, Roy S, Nalesso V, Hérault Y, Guillou F, Bourc'his D (2016) The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science* 354: 909 – 912

Batki J, Schnabl J, Wang J, Handler D, Andreev VI, Stieger CE, Novatchkova M, Lampersberger L, Kauneckaite K, Xie W *et al* (2019) The nascent RNA binding complex SFiNX licenses piRNA-guided heterochromatin formation. *Nat Struct Mol Biol* 26: 720 – 731

Bayne EH, Portoso M, Kagansky A, Kos-Braun IC, Urano T, Ekwall K, Alves F, Rappsilber J, Allshire RC (2008) Splicing factors facilitate RNAi-directed silencing in fission yeast. *Science* 322: 602 – 606

Bayne EH, White SA, Kagansky A, Bijos DA, Sanchez-Pulido L, Hoe K-L, Kim D-U, Park H-O, Ponting CP, Rappsilber J *et al* (2010) Stc1: a critical link between RNAi and chromatin modification required for heterochromatin integrity. *Cell* 140: 666 – 677

Bjerling P, Silverstein RA, Thon G, Caudy A, Grewal S, Ekwall K (2002) Functional divergence between histone deacetylases in fission yeast by distinct cellular localization and *in vivo* specificity. *Mol Cell Biol* 22: 2170 – 2181

Borges F, Martienssen RA (2015) The expanding world of small RNAs in plants. *Nat Rev Mol Cell Biol* 16: 727 – 741

Bourc'his D, Bestor TH (2004) Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature* 431: 96 – 99

Bowen NJ, Fujita N, Kajita M, Wade PA (2004) Mi-2/NuRD: multiple complexes for many purposes. *Biochim Biophys Acta* 1677: 52 – 57

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

Bühler M, Haas W, Gygi SP, Moazed D (2007) RNAi-dependent and -independent RNA turnover mechanisms contribute to heterochromatic gene silencing. *Cell* 129: 707 – 721

Bühler M, Spies N, Bartel DP, Moazed D (2008) TRAMP-mediated RNA surveillance prevents spurious entry of RNAs into the *Schizosaccharomyces pombe* siRNA pathway. *Nat Struct Mol Biol* 15: 1015 – 1023

Buker SM, Iida T, Bühler M, Villén J, Gygi SP, Nakayama J-I, Moazed D (2007) Two different Argonaute complexes are required for siRNA generation and heterochromatin assembly in fission yeast. *Nat Struct Mol Biol* 14: 200 – 207

Cam HP, Sugiyama T, Chen ES, Chen X, FitzGerald PC, Grewal SIS (2005) Comprehensive analysis of heterochromatin- and RNAi-mediated epigenetic control of the fission yeast genome. *Nat Genet* 37: 809 – 819

Canzio D, Chang EY, Shankar S, Kuchenbecker KM, Simon MD, Madhani HD, Narlikar GJ, Al-Sady B (2011) Chromodomain-mediated oligomerization of HP1 suggests a nucleosome-bridging mechanism for heterochromatin assembly. *Mol Cell* 41: 67 – 81

Carmell MA, Girard A, van de Kant HJG, Bourc'his D, Bestor TH, de Rooij DG, Hannon GJ (2007) MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline. *Dev Cell* 12: 503 – 514

Castañeda J, Genzor P, van der Heijden GW, Sarkeshik A, Yates JR, Ingolia NT, Bortvin A (2014) Reduced pachytene piRNAs and translation underlie spermiogenic arrest in Maelstrom mutant mice. *EMBO J* 33: 1999 – 2019

Chang TH, Mattei E, Gainetdinov I, Colpan C, Weng Z, Zamore PD (2019) Maelstrom represses canonical polymerase II transcription within Bi-directional piRNA clusters in *Drosophila melanogaster*. *Mol Cell* 73: 291 – 303.e6

Chedin F, Lieber MR, Hsieh C-L (2002) The DNA methyltransferase-like protein DNMT3L stimulates *de novo* methylation by Dnmt3a. *Proc Natl Acad Sci USA* 99: 16916 – 16921

Chen ES, Zhang K, Nicolas E, Cam HP, Zofall M, Grewal SIS (2008) Cell cycle control of centromeric repeat transcription and heterochromatin assembly. *Nature* 451: 734 – 737

Clegg NJ, Frost DM, Larkin MK, Subrahmanyan L, Bryant Z, Ruohola-Baker H (1997) maelstrom is required for an early step in the establishment of *Drosophila* oocyte polarity: posterior localization of grk mRNA. *Development* 124: 4661 – 4671

Colmenares SU, Buker SM, Buhler M, Dlakić M, Moazed D (2007) Coupling of double-stranded RNA synthesis and siRNA generation in fission yeast RNAi. *Mol Cell* 27: 449 – 461

Cox DN, Chao A, Baker J, Chang L, Qiao D, 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

Czech B, Munafò M, Ciabrelli F, Eastwood EL, Fabry MH, Kneuss E, Hannon GJ (2018) piRNA-guided genome defense: from biogenesis to silencing. *Annu Rev Genet* 52: 131 – 157

Czech B, Preall JB, McGinn J, Hannon GJ (2013) A transcriptome-wide RNAi screen in the *Drosophila* ovary reveals factors of the germline piRNA pathway. *Mol Cell* 50: 749 – 761

Dai P, Wang X, Gou L-T, Li Z-T, Wen ZE, Chen Z-G, Hua M-M, Zhong AI, Wang L, Su H *et al* (2019) A translation-activating function of MIWI/piRNA during mouse Spermiogenesis. *Cell* 179: 1566 – 1581.e16

Darricarrère N, Liu N, Watanabe T, Lin H (2013) Function of Piwi, a nuclear Piwi/Argonaute protein, is independent of its slicer activity. *Proc Natl Acad Sci USA* 110: 1297 – 1302

De Fazio S, Bartonicek N, Di Giacomo M, Abreu-Goodger C, Sankar A, Funaya C, Antony C, Moreira PN, Enright AJ, O'Carroll D (2011) The endonuclease activity of Mili fuels piRNA amplification that silences LINE1 elements. *Nature* 480: 259 – 263

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

Dönertas D, Sienski G, Brennecke J (2013) *Drosophila* Gtsf1 is an essential component of the Piwi-mediated transcriptional silencing complex. *Genes Dev* 27: 1693 – 1705

Eastwood EL, Jara KA, Bornelöv S, Munafò M, Frantzis V, Kneuss E, Barbar EJ, Czech B, Hannon GJ (2021) Dimerisation of the PICTS complex via LC8/Cut-up drives co-transcriptional transposon silencing in *Drosophila*. *Elife* 10: e65557

Egel R (1984) Two tightly linked silent cassettes in the mating-type region of *Schizosaccharomyces pombe*. *Curr Genet* 8: 199 – 203

Fabry MH, Ciabrelli F, Munafò M, Eastwood EL, Kneuss E, Falciatori I, Falconio FA, Hannon GJ, Czech B (2019) piRNA-guided co-transcriptional silencing coopts nuclear export factors. *Elife* 8: e47999

Fischer T, Cui B, Dhakshnamoorthy J, Zhou M, Rubin C, Zofall M, Veenstra TD, Grewal SIS (2009) Diverse roles of HP1 proteins in heterochromatin assembly and functions in fission yeast. *Proc Natl Acad Sci USA* 106: 8998 – 9003

Flury V, Georgescu PR, Iesmantavicius V, Shimada Y, Kuzdere T, Braun S, Bühler M (2017) The histone acetyltransferase Mst2 protects active chromatin from epigenetic silencing by acetylating the ubiquitin ligase Brl1. *Mol Cell* 67: 294 – 307.e9

Gao L, Emperle M, Guo Y, Grimm SA, Ren W, Adam S, Uryu H, Zhang Z-M, Chen D, Yin J *et al* (2020) Comprehensive structure-function characterization of DNMT3B and DNMT3A reveals distinctive *de novo* DNA methylation mechanisms. *Nat Commun* 11: 3355

Garcia JF, Dumesic PA, Hartley PD, El-Samad H, Madhani HD (2010) Combinatorial, site-specific requirement for heterochromatic silencing factors in the elimination of nucleosome-free regions. *Genes Dev* 24: 1758 – 1771

Ge DT, Wang W, Tipping C, Gainetdinov I, Weng Z, Zamore PD (2019) The RNA-binding ATPase, armitage, couples piRNA amplification in Nuage to phased piRNA production on mitochondria. *Mol Cell* 74: 982 – 995.e6

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

Grewal SI, Klar AJ (1997) A recombinationally repressed region between mat2 and mat3 loci shares homology to centromeric repeats and regulates directionality of mating-type switching in fission yeast. *Genetics* 146: 1221 – 1238

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

Halbach R, Miesen P, Joosten J, Taşköprü E, Rondeel I, Pennings B, Vogels CBF, Merkling SH, Koenraadt CJ, Lambrechts L *et al* (2020) A satellite repeat-derived piRNA controls embryonic development of Aedes. *Nature* 580: 274 – 277

Hall IM, Shankaranarayana GD, Noma K-I, Ayoub N, Cohen A, Grewal SIS (2002) Establishment and maintenance of a heterochromatin domain. *Science* 297: 2232 – 2237

Hall IM, Noma K-I, Grewal SIS (2003) RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. *Proc Natl Acad Sci USA* 100: 193 – 198

Han BW, Wang W, Li C, Weng Z, Zamore PD (2015) Noncoding RNA. piRNA-guided transposon cleavage initiates Zucchini-dependent, phased piRNA production. *Science* 348: 817 – 821

Handler D, Olivieri D, Novatchkova M, Gruber FS, Meixner K, Mechtler K, Stark A, Sachidanandam R, Brennecke J (2011) A systematic analysis of *Drosophila* TUDOR domain-containing proteins identifies Vreteno and the Tdrd12 family as essential primary piRNA pathway factors. *EMBO J* 30: 3977 – 3993

Handler D, Meixner K, Pizka M, Lauss K, Schmied C, Gruber FS, Brennecke J (2013) The genetic makeup of the *Drosophila* piRNA pathway. *Mol Cell* 50: 762 – 777

Hansen KR, Burns G, Mata J, Volpe TA, Martienssen RA, Bähler J, Thon G (2005) Global effects on gene expression in fission yeast by silencing and RNA interference machineries. *Mol Cell Biol* 25: 590 – 601

Hirakata S, Siomi MC (2016) piRNA biogenesis in the germline: from transcription of piRNA genomic sources to piRNA maturation. *Biochim Biophys Acta* 1859: 82 – 92

Hirakata S, Ishizu H, Fujita A, Tomoe Y, Siomi MC (2019) Requirements for multivalent Yb body assembly in transposon silencing in *Drosophila*. *EMBO Rep* 20: e47708

Horn PJ, Bastie J-N, Peterson CL (2005) A Rik1-associated, cullin-dependent E3 ubiquitin ligase is essential for heterochromatin formation. *Genes Dev* 19: 1705 – 1714

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

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P (2008) Genevestigator V3: a reference expression database for the meta-analysis of transcriptomes. *Adv Bioinform* 2008: 1 – 5

Huang XA, Yin H, Sweeney S, Raha D, Snyder M, Lin H (2013) A major epigenetic programming mechanism guided by piRNAs. *Dev Cell* 24: 502 – 516

Ilyin AA, Ryazansky SS, Doronin SA, Olenkina OM, Mikhaleva EA, Yakushev EY, Abramov YA, Belyakin SN, Ivankin AV, Pindyurin AV *et al* (2017) Piwi interacts with chromatin at nuclear pores and promiscuously binds nuclear transcripts in *Drosophila* ovarian somatic cells. *Nucleic Acids Res* 45: 7666 – 7680

Inoue N (1999) New gene family defined by MORC, a nuclear protein required for mouse spermatogenesis. *Hum Mol Genet* 8: 1201 – 1207

Ipsaro JJ, Haase AD, Knott SR, Joshua-Tor L, Hannon GJ (2012) The structural biochemistry of Zucchini implicates it as a nuclease in piRNA biogenesis. *Nature* 491: 279 – 283

Ishizu H, Iwasaki YW, Hirakata S, Ozaki H, Iwasaki W, Siomi H, Siomi MC (2015) Somatic primary piRNA biogenesis driven by cis-acting RNA elements and trans-acting Yb. *Cell Rep* 12: 429 – 440

Ishizu H, Kinoshita T, Hirakata S, Komatsuzaki C, Siomi MC (2019) Distinct and collaborative functions of yb and armitage in transposon-targeting piRNA biogenesis. *Cell Rep* 27: 1822 – 1835.e8

Iwasaki YW, Siomi MC, Siomi H (2015) PIWI-Interacting RNA: its biogenesis and functions. *Annu Rev Biochem* 84: 405 – 433

Iwasaki YW, Murano K, Ishizu H, Shibuya A, Iyoda Y, Siomi MC, Siomi H, Saito K (2016) Piwi modulates chromatin accessibility by regulating multiple factors including histone h1 to repress transposons. *Mol Cell* 63: 408 – 419

Jespersen N, Barbar E (2020) Emerging features of linear motif-binding hub proteins. *Trends Biochem Sci* 45: 375 – 384

Jia S, Noma K, Grewal SIS (2004) RNAi-independent heterochromatin nucleation by the stress-activated ATF/CREB family proteins. *Science* 304: 1971 – 1976

Jia S, Kobayashi R, Grewal SIS (2005) Ubiquitin ligase component Cul4 associates with Clr4 histone methyltransferase to assemble heterochromatin. *Nat Cell Biol* 7: 1007 – 1013

Jurkowska RZ, Qin SU, Kungulovski G, Tempel W, Liu Y, Bashtrykov P, Stiefelmaier J, Jurkowski TP, Kudithipudi S, Weirich S *et al* (2017) H3K14ac is linked to methylation of H3K9 by the triple Tudor domain of SETDB1. *Nat Commun* 8: 2057

Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E, Sasaki H (2004) Essential role for *de novo* DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* 429: 900 – 903

Kanoh J, Sadaie M, Urano T, Ishikawa F (2005) Telomere binding protein Taz1 establishes Swi6 heterochromatin independently of RNAi at telomeres. *Curr Biol* 15: 1808 – 1819

Kato H, Goto DB, Martienssen RA, Urano T, Furukawa K, Murakami Y (2005) RNA polymerase II is required for RNAi-dependent heterochromatin assembly. *Science* 309: 467 – 469

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

Kim K, Lee YS, Carthew RW (2007) Conversion of pre-RISC to holo-RISC by Ago2 during assembly of RNAi complexes. *RNA* 13: 22 – 29

Kim VN, Han J, Siomi MC (2009) Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 10: 126 – 139

Kim IV, Riedelbauch S, Kuhn C-D (2020) The piRNA pathway in planarian flatworms: new model, new insights. *Biol Chem* 401: 1123 – 1141

Klar AJ, Bonaduce MJ (1991) swi6, a gene required for mating-type switching, prohibits meiotic recombination in the mat2-mat3 'cold spot' of fission yeast. *Genetics* 129: 1033 – 1042

Klattenhoff C, Bratu DP, McGinnis-Schultz N, Koppetsch BS, Cook HA, Theurkauf WE (2007) *Drosophila* rasiRNA pathway mutations disrupt embryonic axis specification through activation of an ATR/Chk2 DNA damage response. *Dev Cell* 12: 45 – 55

Klenov MS, Sokolova OA, Yakushev EY, Stolyarenko AD, Mikhaleva EA, Lavrov SA, Gvozdev VA (2011) Separation of stem cell maintenance and transposon silencing functions of Piwi protein. *Proc Natl Acad Sci USA* 108: 18760 – 18765

Kloc A, Zaratiegui M, Nora E, Martienssen R (2008) RNA interference guides histone modification during the S phase of chromosomal replication. *Curr Biol* 18: 490 – 495

Kobayashi H, Sakurai T, Miura F, Imai M, Mochiduki K, Yanagisawa E, Sakashita A, Wakai T, Suzuki Y, Ito T *et al* (2013) High-resolution DNA methylome analysis of primordial germ cells identifies gender-specific reprogramming in mice. *Genome Res* 23: 616 – 627

Kojima-Kita K, Kuramochi-Miyagawa S, Nagamori I, Ogonuki N, Ogura A, Hasuwa H, Akazawa T, Inoue N, Nakano T (2016) MIWI2 as an effector of DNA methylation and gene silencing in embryonic male germ cells. *Cell Rep* 16: 2819 – 2828

Kowalik KM, Shimada Y, Flury V, Stadler MB, Batki J, Bühler M (2015) The Paf1 complex represses small-RNA-mediated epigenetic gene silencing. *Nature* 520: 248 – 252

Kubo N, Toh H, Shirane K, Shirakawa T, Kobayashi H, Sato T, Sone H, Sato Y, Tomizawa S-I, Tsurusaki Y *et al* (2015) DNA methylation and gene expression dynamics during spermatogonial stem cell differentiation in the early postnatal mouse testis. *BMC Genom* 16: 624

Kunert N, Marhold J, Stanke J, Stach D, Lyko F (2003) A Dnmt2-like protein mediates DNA methylation in *Drosophila*. *Development* 130: 5083 – 5090

Kunert N, Wagner E, Murawska M, Klinker H, Kremmer E, Brehm A (2009) dMec: a novel Mi-2 chromatin remodelling complex involved in transcriptional repression. *EMBO J* 28: 533 – 544

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

Kuramochi-Miyagawa S (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 TW *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

LaCava J, Houseley J, Saveanu C, Petfalski E, Thompson E, Jacquier A, Tollervey D (2005) RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. *Cell* 121: 713 – 724

Lau NC, Seto AG, Kim J, Kuramochi-Miyagawa S, Nakano T, Bartel DP, Kingston RE (2006) Characterization of the piRNA complex from rat testes. *Science* 313: 363 – 367

Lau NC, Robine N, Martin R, Chung W-J, Niki Y, Berezikov E, Lai EC (2009) Abundant primary piRNAs, endo-siRNAs, and microRNAs in a *Drosophila* ovary cell line. *Genome Res* 19: 1776 – 1785

Le Thomas A, Stuwe E, Li S, Du J, Marinov G, Rozhkov N, Chen Y-C, Luo Y, Sachidanandam R, Toth KF *et al* (2014) Transgenerationally inherited piRNAs trigger piRNA biogenesis by changing the chromatin of piRNA clusters and inducing precursor processing. *Genes Dev* 28: 1667 – 1680

Lee YS, Nakahara K, Pham JW, Kim K, He Z, Sontheimer EJ, Carthew RW (2004) Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* 117: 69 – 81

Li H, Motamedi MR, Yip CK, Wang Z, Walz T, Patel DJ, Moazed D (2009) An alpha motif at Tas3 C terminus mediates RITS cis spreading and promotes heterochromatic gene silencing. *Mol Cell* 34: 155 – 167

Lim AK, 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

Lim AK, Tao L, Kai T (2009) piRNAs mediate posttranscriptional retroelement silencing and localization to pi-bodies in the *Drosophila* germline. *J Cell Biol* 186: 333 – 342

Lin H, Chen M, Kundaje A, Valouev A, Yin H, Liu N, Neuenkirchen N, Zhong M, Snyder M (2015) Reassessment of Piwi binding to the genome and Piwi impact on RNA polymerase II distribution. *Dev Cell* 32: 772 – 774

Liu S, Brind'Amour J, Karimi MM, Shirane K, Bogutz A, Lefebvre L, Sasaki H, Shinkai Y, Lorincz MC (2014) Setdb1 is required for germline development and silencing of H3K9me3-marked endogenous retroviruses in primordial germ cells. *Genes Dev* 28: 2041 – 2055

Livak KJ (1984) Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. *Genetics* 107: 611 – 634

Marinov GK, Wang J, Handler D, Wold BJ, Weng Z, Hannon GJ, Aravin AA, Zamore PD, Brennecke J, Toth KF (2015) Pitfalls of mapping high-throughput sequencing data to repetitive sequences: Piwi's genomic targets still not identified. *Dev Cell* 32: 765 – 771

Mochizuki K (2005) A Dicer-like protein in Tetrahymena has distinct functions in genome rearrangement, chromosome segregation, and meiotic prophase. *Genes Dev* 19: 77 – 89

Mochizuki K, Fine NA, Fujisawa T, Gorovsky MA (2002) Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in tetrahymena. *Cell* 110: 689 – 699

Mohn F, Sienski G, Handler D, Brennecke J (2014) The rhino-deadlock-cutoff complex licenses noncanonical transcription of dual-strand piRNA clusters in *Drosophila*. *Cell* 157: 1364 – 1379

Mohn F, Handler D, Brennecke J (2015) Noncoding RNA. piRNA-guided slicing specifies transcripts for Zucchini-dependent, phased piRNA biogenesis. *Science* 348: 812 – 817

Molaro A, Falciatori I, Hodges E, Aravin AA, Marran K, Rafii S, McCombie WR, Smith AD, Hannon GJ (2014) Two waves of *de novo* methylation during mouse germ cell development. *Genes Dev* 28: 1544 – 1549

Motamedi MR, Verdel A, Colmenares SU, Gerber SA, Gygi SP, Moazed D (2004) Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. *Cell* 119: 789 – 802

Motamedi MR, Hong E-JE, Li X, Gerber S, Denison C, Gygi S, Moazed D (2008) HP1 proteins form distinct complexes and mediate heterochromatic gene silencing by nonoverlapping mechanisms. *Mol Cell* 32: 778 – 790

Mourier T, Jeffares DC (2003) Eukaryotic intron loss. *Science* 300: 1393

Muerdter F, Guzzardo PM, Gillis J, Luo Y, Yu Y, Chen C, Fekete R, Hannon GJ (2013) A genome-wide RNAi screen draws a genetic framework for transposon control and primary piRNA biogenesis in *Drosophila*. *Mol Cell* 50: 736 – 748

Mugat B, Nicot S, Varela-Chavez C, Jourdan C, Sato K, Basyuk E, Juge F, Siomi MC, Pélisson A, Chambeyron S (2020) The Mi-2 nucleosome remodeler and the Rpd3 histone deacetylase are involved in piRNA-guided heterochromatin formation. *Nat Commun* 11: 2818

Munafò M, Manelli V, Falconio FA, Sawle A, Kneuss E, Eastwood EL, Seah JWE, Czech B, Hannon GJ (2019) Daedalus and Gasz recruit Armitage to mitochondria, bringing piRNA precursors to the biogenesis machinery. *Genes Dev* 33: 844 – 856

Murano K, Iwasaki YW, Ishizu H, Mashiko A, Shibuya A, Kondo S, Adachi S, Suzuki S, Saito K, Natsume T *et al* (2019) Nuclear RNA export factor variant initiates piRNA-guided co-transcriptional silencing. *EMBO J* 38: e102870

Murota Y, Ishizu H, Nakagawa S, Iwasaki YW, Shibata S, Kamatani MK, Saito K, Okano H, Siomi H, Siomi MC (2014) Yb integrates piRNA intermediates and processing factors into perinuclear bodies to enhance piRISC assembly. *Cell Rep* 8: 103 – 113

Nagamori I, Kobayashi H, Nishimura T, Yamagishi R, Katahira J, Kuramochi-Miyagawa S, Kono T, Nakano T (2018) Relationship between PIWIL4-Mediated H3K4me2 demethylation and piRNA-dependent DNA methylation. *Cell Rep* 25: 350 – 356

Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI (2001) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* 292: 110 – 113

Niki Y, Yamaguchi T, Mahowald AP (2006) Establishment of stable cell lines of *Drosophila* germ-line stem cells. *Proc Natl Acad Sci USA* 103: 16325 – 16330

Ninova M, Chen Y-CA, Godneeva B, Rogers AK, Luo Y, Fejes Tóth K, Aravin AA (2020) Su(var)2–10 and the SUMO pathway link piRNA-guided target recognition to chromatin silencing. *Mol Cell* 77: 556 – 570.e6

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

Nishimasu H, Ishizu H, Saito K, Fukuhara S, Kamatani MK, Bonnefond L, Matsumoto N, Nishizawa T, Nakanaga K, Aoki J *et al* (2012) Structure and function of Zucchini endoribonuclease in piRNA biogenesis. *Nature* 491: 284 – 287

Noma K, Sugiyama T, Cam H, Verdel A, Zofall M, Jia S, Moazed D, Grewal SIS (2004) RITS acts in cis to promote RNA interference-mediated transcriptional and post-transcriptional silencing. *Nat Genet* 36: 1174 – 1180

Ohtani H, Iwasaki YW, Shibuya A, Siomi H, Siomi MC, Saito K (2013) DmGTSF1 is necessary for Piwi-piRISC-mediated transcriptional transposon silencing in the *Drosophila* ovary. *Genes Dev* 27: 1656 – 1661

Olivieri D, Sykora MM, Sachidanandam R, Mechtler K, 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

Onishi R, Sato K, Murano K, Negishi L, Siomi H, Siomi MC (2020) Piwi suppresses transcription of Brahma-dependent transposons via Maelstrom in ovarian somatic cells. *Sci Adv* 6

Osumi K, Sato K, Murano K, Siomi H, Siomi MC (2019) Essential roles of Windei and nuclear monoubiquitination of Eggless/SETDB1 in transposon silencing. *EMBO Rep* 20: e48296

Ozata DM, Gainetdinov I, Zoch A, O'Carroll D, Zamore PD (2019) PIWI-interacting RNAs: small RNAs with big functions. *Nat Rev Genet* 20: 89 – 108

Palumbo G, Bonaccorsi S, Robbins LG, Pimpinelli S (1994) Genetic analysis of Stellate elements of *Drosophila melanogaster*. *Genetics* 138: 1181 – 1197

Pandey RR, Homolka D, Chen K-M, Sachidanandam R, Fauvarque M-O, Pillai RS (2017) Recruitment of Armitage and Yb to a transcript triggers its phased processing into primary piRNAs in *Drosophila* ovaries. *PLoS Genet* 13: e1006956

Pastor WA, Stroud H, Nee K, Liu W, Pezic D, Manakov S, Lee SA, Moissiard G, Zamudio N, Bourc'his D *et al* (2014) MORC1 represses transposable elements in the mouse male germline. *Nat Commun* 5: 5795

Pélisson A, Song SU, Prud'homme N, Smith PA, Bucheton A, Corces VG (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

Petrie VJ, Wuitschick JD, Givens CD, Kosinski AM, Partridge JF (2005) RNA interference (RNAi)-dependent and RNAi-independent association of the Chp1 chromodomain protein with distinct heterochromatic loci in fission yeast. *Mol Cell Biol* 25: 2331 – 2346

Pezic D, Manakov SA, Sachidanandam R, Aravin AA (2014) piRNA pathway targets active LINE1 elements to establish the repressive H3K9me3 mark in germ cells. *Genes Dev* 28: 1410 – 1428

Popp C, Dean W, Feng S, Cokus SJ, Andrews S, Pellegrini M, Jacobsen SE, Reik W (2010) Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. *Nature* 463: 1101 – 1105

Provost P, Silverstein RA, Dishart D, Walfridsson J, Djupedal I, Kniola B, Wright A, Samuelsson B, Radmark O, Ekwall K (2002) Dicer is required for chromosome segregation and gene silencing in fission yeast cells. *Proc Natl Acad Sci USA* 99: 16648 – 16653

Rajasethupathy P, Antonov I, Sheridan R, Frey S, Sander C, Tuschl T, Kandel ER (2012) A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. *Cell* 149: 693 – 707

Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun Z-W, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD *et al* (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406: 593 – 599

Reik W (2001) Epigenetic reprogramming in mammalian development. *Science* 293: 1089 – 1093

Reinhart BJ, Bartel DP (2002) Small RNAs correspond to centromere heterochromatic repeats. *Science* 297: 1831

Reyes-Turcu FE, Zhang K, Zofall M, Chen E, Grewal SIS (2011) Defects in RNA quality control factors reveal RNAi-independent nucleation of heterochromatin. *Nat Struct Mol Biol* 18: 1132 – 1138

Saito K, Nishida KM, Mori T, Kawamura Y, Miyoshi K, Nagami T, Siomi H, Siomi MC (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, Siomi MC (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 KM, Siomi H, Siomi MC (2010) Roles for the Yb body components armitage and Yb in primary piRNA biogenesis in *Drosophila*. *Genes Dev* 24: 2493 – 2498

Sarot E, Payen-Groschêne G, Bucheton A, 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

Sasaki H, Matsui Y (2008) Epigenetic events in mammalian germ-cell development: reprogramming and beyond. *Nat Rev Genet* 9: 129 – 140

Sato K, Nishida KM, Shibuya A, Siomi MC, Siomi H (2011) Maelstrom coordinates microtubule organization during *Drosophila* oogenesis through interaction with components of the MTOC. *Genes Dev* 25: 2361 – 2373

Sato K, Siomi MC (2018) Two distinct transcriptional controls triggered by nuclear Piwi-piRISCs in the *Drosophila* piRNA pathway. *Curr Opin Struct Biol* 53: 69 – 76

Schalch T, Job G, Noffsinger VJ, Shanker S, Kuscu C, Joshua-Tor L, Partridge JF (2009) High-affinity binding of Chp1 chromodomain to K9 methylated histone H3 is required to establish centromeric heterochromatin. *Mol Cell* 34: 36 – 46

Schalch T, Job G, Shanker S, Partridge JF, Joshua-Tor L (2011) The Chp1-Tas3 core is a multifunctional platform critical for gene silencing by RITS. *Nat Struct Mol Biol* 18: 1351 – 1357

Schnabl J, Wang J, Hohmann U, Gehre M, Batki J, Andreev VI, Purkhauser K, Fasching N, Duchek P, Novatchkova M *et al* (2021) Molecular principles of Piwi-mediated cotranscriptional silencing through the dimeric SFiNX complex. *Genes Dev* 35: 392 – 409

Schöpp T, Zoch A, Berrens RV, Auchynnikava T, Kabayama Y, Vasiliauskaitė L, Rappsilber J, Allshire RC, O'Carroll D (2020) TEX15 is an essential executor of MIWI2-directed transposon DNA methylation and silencing. *Nat Commun* 11: 3739

Schotta G, Ebert A, Krauss V, Fischer A, Hoffmann J, Rea S, Jenuwein T, Dorn R, Reuter G (2002) Central role of *Drosophila* SU(VAR)3–9 in histone H3–K9 methylation and heterochromatic gene silencing. *EMBO J* 21: 1121 – 1131

Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, Popp C, Thienpont B, Dean W, Reik W (2012) The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. *Mol Cell* 48: 849 – 862

Shankaranarayana GD, Motamedi MR, Moazed D, Grewal SIS (2003) Sir2 regulates histone H3 lysine 9 methylation and heterochromatin assembly in fission yeast. *Curr Biol* 13: 1240 – 1246

Sheu-Gruttadauria J, MacRae IJ (2017) Structural foundations of RNA silencing by argonaute. *J Mol Biol* 429: 2619 – 2639

Shibata N, Kashima M, Ishiko T, Nishimura O, Rouhana L, Misaki K, Yonemura S, Saito K, Siomi H, Siomi M *et al* (2016) Inheritance of a nuclear PIWI from pluripotent stem cells by somatic descendants ensures differentiation by silencing transposons in planarian. *Dev Cell* 37: 226 – 237

Shirane K, Miura F, Ito T, Lorincz MC (2020) NSD1-deposited H3K36me2 directs *de novo* methylation in the mouse male germline and counteracts Polycomb-associated silencing. *Nat Genet* 52: 1088 – 1098

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

Sienski G, Dönertas D, Brennecke J (2012) Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression. *Cell* 151: 964 – 980

Sienski G, Batki J, Senti K-A, Dönertas D, Tirian L, Meixner K, Brennecke J (2015) Silencio/CG9754 connects the Piwi-piRNA complex to the cellular heterochromatin machinery. *Genes Dev* 29: 2258 – 2271

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

Stapleton W, Das S, McKee BD (2001) A role of the *Drosophila* homeless gene in repression of Stellate in male meiosis. *Chromosoma* 110: 228 – 240

Stewart KR, Veselovska L, Kim J, Huang J, Saadeh H, Tomizawa S, Smallwood SA, Chen T, Kelsey G (2015) Dynamic changes in histone modifications precede de novo DNA methylation in oocytes. *Genes Dev* 29: 2449 – 2462

Suetake I, Shinozaki F, Miyagawa J, Takeshima H, Tajima S (2004) DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction. *J Biol Chem* 279: 27816 – 27823

Sugiyama T, Cam H, Verdel A, Moazed D, Grewal SIS (2005) RNA-dependent RNA polymerase is an essential component of a self-enforcing loop coupling heterochromatin assembly to siRNA production. *Proc Natl Acad Sci USA* 102: 152 – 157

Sugiyama T, Cam HP, Sugiyama R, Noma K, Zofall M, Kobayashi R, Grewal SIS (2007) SHREC, an effector complex for heterochromatic transcriptional silencing. *Cell* 128: 491 – 504

Sumiyoshi T, Sato K, Yamamoto H, Iwasaki YW, Siomi H, Siomi MC (2016) Loss of l(3)mbt leads to acquisition of the ping-pong cycle in *Drosophila* ovarian somatic cells. *Genes Dev* 30: 1617 – 1622

Suyama M, Doerks T, Braun IC, Sattler M, Izaurralde E, Bork P (2000) Prediction of structural domains of TAP reveals details of its interaction with p15 and nucleoporins. *EMBO Rep* 1: 53 – 58

Thakurta AG, Gopal G, Yoon JH, Kozak L, Dhar R (2005) Homolog of BRCA2-interacting Dss1p and Uap56p link Mlo3p and Rae1p for mRNA export in fission yeast. *EMBO J* 24: 2512 – 2523

Thon G, Klar AJ (1993) Directionality of fission yeast mating-type interconversion is controlled by the location of the donor loci. *Genetics* 134: 1045 – 1054

Tuzon CT, Borgstrom B, Weilguny D, Egel R, Cooper JP, Nielsen O (2004) The fission yeast heterochromatin protein Rik1 is required for telomere clustering during meiosis. *J Cell Biol* 165: 759 – 765

Vagin VV (2006) A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* 313: 320 – 324

Vanácová S, Wolf J, Martin G, Blank D, Dettwiler S, Friedlein A, Langen H, Keith G, Keller W (2005) A new yeast poly(A) polymerase complex involved in RNA quality control. *PLoS Biol* 3: e189

Veland N, Lu Y, Hardikar S, Gaddis S, Zeng Y, Liu B, Estecio MR, Takata Y, Lin K, Tomida MW *et al* (2019) DNMT3L facilitates DNA methylation partly by maintaining DNMT3A stability in mouse embryonic stem cells. *Nucleic Acids Res* 47: 152 – 167

Verdel A, Jia S, Gerber S, Sugiyama T, Gygi S, Grewal SIS, Moazed D (2004) RNAi-mediated targeting of heterochromatin by the RITS complex. *Science* 303: 672 – 676

Volpe TA, Kidner C, Hall IM, Teng G, Grewal SIS, Martienssen RA (2002) Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* 297: 1833 – 1837

Wang Y, Juranek S, Li H, Sheng G, Tuschl T, Patel DJ (2008) Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex. *Nature* 456: 921 – 926

Wang SH, Elgin SCR (2011) *Drosophila* Piwi functions downstream of piRNA production mediating a chromatin-based transposon silencing mechanism in female germ line. *Proc Natl Acad Sci USA* 108: 21164 – 21169

Wang W, Han BW, Tipping C, Ge DT, Zhang Z, Weng Z, Zamore PD (2015) Slicing and binding by Ago3 or Aub trigger Piwi-bound piRNA production by distinct mechanisms. *Mol Cell* 59: 819 – 830

Watanabe T, Takeda A, Tsukiyama T, Mise K, Okuno T, Sasaki H, Minami N, 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, Cheng E, Zhong M, Lin H (2015) Retrotransposons and pseudogenes regulate mRNAs and lncRNAs via the piRNA pathway in the germline. *Genome Res* 25: 368 – 380

Watson ML, Zinn AR, Inoue N, Hess KD, Cobb J, Handel MA, Halaban R, Duchene CC, Albright GM, Moreadith RW (1998) Identification of morc (microrchidia), a mutation that results in arrest of spermatogenesis at an early meiotic stage in the mouse. *Proc Natl Acad Sci USA* 95: 14361 – 14366

Wenda JM, Homolka D, Yang Z, Spinelli P, Sachidanandam R, Pandey RR, Pillai RS (2017) Distinct roles of RNA helicases MVH and TDRD9 in PIWI slicing-triggered mammalian piRNA biogenesis and function. *Dev Cell* 41: 623 – 637.e9

Wilson BG, Roberts CWM (2011) SWI/SNF nucleosome remodellers and cancer. *Nat Rev Cancer* 11: 481 – 492

Wu P-H, Fu Y, Cecchini K, Özata DM, Arif A, Yu T, Colpan C, Gainetdinov I, Weng Z, Zamore PD (2020) The evolutionarily conserved piRNA-producing locus pi6 is required for male mouse fertility. *Nat Genet* 52: 728 – 739

Wyers F, Rougemaille M, Badis G, Rousselle J-C, Dufour M-E, Boulay J, Régnault B, Devaux F, Namane A, Séraphin B *et al* (2005) Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. *Cell* 121: 725 – 737

Xu Q, Xiang Y, Wang Q, Wang L, Brind'Amour J, Bogutz AB, Zhang Y, Zhang B, Yu G, Xia W *et al* (2019) SETD2 regulates the maternal epigenome, genomic imprinting and embryonic development. *Nat Genet* 51: 844 – 856

Yamaguchi S, Oe A, Nishida KM, Yamashita K, Kajiya A, Hirano S, Matsumoto N, Dohmae N, Ishitani R, Saito K *et al* (2020) Crystal structure of *Drosophila* Piwi. *Nat Commun* 11: 858

Yamanaka S, Mehta S, Reyes-Turcu FE, Zhuang F, Fuchs RT, Rong Y, Robb GB, Grewal SIS (2013) RNAi triggered by specialized machinery silences developmental genes and retrotransposons. *Nature* 493: 557 – 560

Yamanaka S, Siomi MC, Siomi H (2014) piRNA clusters and open chromatin structure. *Mob DNA* 5: 22

Yamane K, Mizuguchi T, Cui B, Zofall M, Noma K, Grewal SIS (2011) Asf1/HIRA facilitate global histone deacetylation and associate with HP1 to promote nucleosome occupancy at heterochromatic loci. *Mol Cell* 41: 56 – 66

Yamashiro H, Siomi MC (2018) PIWI-Interacting RNA in *Drosophila*: biogenesis, transposon regulation, and beyond. *Chem Rev* 118: 4404 – 4421

Yamashiro H, Negishi M, Kinoshita T, Ishizu H, Ohtani H, Siomi MC (2020) Armitage determines Piwi-piRISC processing from precursor formation and quality control to inter-organelle translocation. *EMBO Rep* 21: e48769

Yang F, Lan Y, Pandey RR, Homolka D, Berger SL, Pillai RS, Bartolomei MS, Wang PJ (2020) TEX15 associates with MILI and silences transposable elements in male germ cells. *Genes Dev* 34: 745 – 750

Yashiro R, Murota Y, Nishida KM, Yamashiro H, Fujii K, Ogai A, Yamanaka S, Negishi L, Siomi H, Siomi MC (2018) Piwi nuclear localization and its regulatory mechanism in *Drosophila* ovarian somatic cells. *Cell Rep* 23: 3647 – 3657

Yoshimura T, Watanabe T, Kuramochi-Miyagawa S, Takemoto N, Shiromoto Y, Kudo A, Kanai-Azuma M, Tashiro F, Miyazaki S, Katanaya A *et al* (2018) Mouse GTSF1 is an essential factor for secondary piRNA biogenesis. *EMBO Rep* 19: e42054

Yu Y, Gu J, Jin Y, Luo Y, Preall JB, Ma J, Czech B, Hannon GJ (2015) Panoramix enforces piRNA-dependent cotranscriptional silencing. *Science* 350: 339 – 342

Zhang K, Mosch K, Fischle W, Grewal SIS (2008) Roles of the Clr4 methyltransferase complex in nucleation, spreading and maintenance of heterochromatin. *Nat Struct Mol Biol* 15: 381 – 388

Zhang Z, Wang J, Schultz N, Zhang F, Parhad SS, Tu S, Vreven T, Zamore PD, Weng Z, Theurkauf WE (2014) The HP1 homolog rhino anchors a nuclear complex that suppresses piRNA precursor splicing. *Cell* 157: 1353 – 1363

Zhao K, Cheng S, Miao NA, Xu P, Lu X, Zhang Y, Wang M, Ouyang X, Yuan X, Liu W *et al* (2019) A Pandas complex adapted for piRNA-guided transcriptional silencing and heterochromatin formation. *Nat Cell Biol* 21: 1261 – 1272

Zheng K, Xiol J, Reuter M, Eckardt S, Leu NA, McLaughlin KJ, Stark A, Sachidanandam R, Pillai RS, Wang PJ (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

Zoch A, Auchynnikava T, Berrens RV, Kabayama Y, Schöpp T, Heep M, Vasiliauskaitė L, Pérez-Rico YA, Cook AG, Shkumatava A *et al* (2020) SPOCD1 is an essential executor of piRNA-directed *de novo* DNA methylation. *Nature* 584: 635 – 639