Review

The piRNA pathway in *Drosophila* ovarian germ and somatic cells

By Kaoru SATO[*1] and Mikiko C. SIOMI[*1,†]

(Edited by Shigekazu NAGATA, M.J.A.)

Abstract: RNA silencing refers to gene silencing pathways mediated by small non-coding RNAs, including microRNAs. Piwi-interacting RNAs (piRNAs) constitute the largest class of small non-coding RNAs in animal gonads, which repress transposons to protect the germline genome from the selfish invasion of transposons. Deterioration of the system causes DNA damage, leading to severe defects in gametogenesis and infertility. Studies using *Drosophila* ovaries show that piRNAs originate from specific genomic loci, termed piRNA clusters, and that in piRNA biogenesis, cluster transcripts are processed into mature piRNAs via three distinct pathways: initiator or responder for ping-pong piRNAs and trailing for phased piRNAs. piRNAs then assemble with PIWI members of the Argonaute family of proteins to form piRNA-induced RNA silencing complexes (piRISCs), the core engine of the piRNA-mediated silencing pathway. Upon piRISC assembly, the PIWI member, Piwi, is translocated to the nucleus and represses transposons co-transcriptionally by inducing local heterochromatin formation at target transposon loci.

Keywords: piRNA, PIWI, transposon, non-coding RNA, RNA silencing, *Drosophila*

Introduction

Piwi-interacting RNAs (piRNAs) are a germline-specific class of small regulatory RNAs that repress transposons to maintain genome integrity.[1)–6)] Transposons move around the host genome through either copy-and-paste or cut-and-paste mechanisms.[5)] When this happens in the germline, it causes DNA damage, leading to defects in gametogenesis and infertility. Therefore, piRNA-mediated transposon silencing is crucial for the succession of life.[1)–6)]

piRNAs originate from intergenic loci known as piRNA clusters that are rich in transposon remnants.[7)–9)] Therefore, piRNAs arising from the clusters are able to act as antisense oligonucleotides to transposon transcripts.[7)–9)] piRNAs, however, have no enzyme activity, and instead assemble into piRNA-induced silencing complexes (piRISCs) with PIWI proteins, germline-specific members of the Argonaute family, to acquire silencing activity.[10)–16)]

piRISCs repress transposons co-transcriptionally or post-transcriptionally.[10)–20)] In the co-transcriptional silencing of transposons, piRISCs impact the chromatin state, inducing heterochromatinization at target loci.[18)–20)] Post-transcriptionally, transposon transcripts are cleaved by the endonuclease (Slicer) activity that PIWI possesses.[14)–16)] The cleaved products are then degraded in the cellular environment, resulting in transposon silencing that is similar to that in the RNAi pathway. However, in the piRNA pathway, PIWI cleavage products also serve as precursors to produce new sets of piRNAs.[7),15)] In this regard, piRNA-mediated post-transcriptional silencing can be considered an RNA recycling mechanism.[7),15)]

The piRNA pathway is highly conserved in animals, but the underlying mechanism has been most extensively studied in the *Drosophila* ovary. Detailed analysis showed that the piRNA pathway in ovarian germ cells and somatic cells is not identical mechanistically.[8),17)] For instance, the piRNA clus-

[*1] Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan.

[†] Correspondence should be addressed: M. C. Siomi, 2-11-16 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan (e-mail: siomim@bs.s.u-tokyo.ac.jp).

Abbreviations: Pandas: Panoramix-dNxf2 dependent TAP/p15 silencing; PICTS: Panx-induced co-transcriptional silencing; piRISC: piRNA-induced silencing complex; piRNA: Piwi-interacting RNA; Piwi: P-element induced wimpy testis; PPNP: Piwi-Panx-Nxf2-p15; SFiNX: Silencing factor interacting nuclear export variant.

doi: 10.2183/pjab.96.003

ters used in the two cells are different; ovarian somatic cells exclusively use so called uni-strand clusters, whereas germ cells predominantly use dual-strand clusters.[7)–9),13),17)] The number of PIWI members expressed is also different; of three PIWI members, Piwi is expressed in both cell types but two others, Aubergine (Aub) and AGO3, are specifically expressed in germ cells. In addition, piRNA biogenesis factors sometimes show cell specificity.[7),8),14)–17)] Nonetheless, the biogenesis products, namely piRNAs, are not easily distinguishable between germ cells and ovarian somatic cells; piRNAs in both cells are in a similar size range, 23–30 nucleotides (nt) long, and are phosphorylated and 2′-*O*-methylated at the 5′ and 3′ ends, respectively.[5),21)–23)]

In this review, we summarize the mechanisms of the piRNA pathway in *Drosophila* ovarian germ cells and somatic cells by discussing recent findings. We divide the pathway into "*piRNA biogenesis*" and "*mode of action of piRNA-mediated silencing*". *piRNA biogenesis* is further divided into three steps; *transcription of piRNA clusters*, *nuclear processing of piRNA precursors*, and *piRNA maturation*. The piRNA amplification pathway, also known as the ping-pong cycle, that operates in germ cells has well been documented in previous reviews and so is only briefly described in this review.

The piRNA pathway in ovarian germ cells

piRNA biogenesis: transcription of piRNA clusters. The piRNA clusters used in ovarian germ cells are predominantly dual-strand piRNA clusters.[7)–9),13),17)] Well-known clusters are *42AB*, *38C*, and *80F*. The factor that specifies clusters is Piwi. For cluster specification, Piwi deposits a repressive histone mark, histone H3 lysine 9 trimethyl (H3K9me3), on loci.[24),25)]

The dual-strand piRNA clusters lack their own promoters.[9),26)–28)] However, transcription occurs in both directions, as the name suggests, and starts internally from the sites where RNA polymerase II (RNA Pol II) is positioned. Rhino (Rhi, also known as HP1d), a paralog of heterochromatin protein, HP1a, plays a crucial role in determining transcription initiation sites by associating with H3K9me3.[26)–28)]

Rhi then associates with two co-factors, Deadlock (Del) and Cutoff (Cuff), to assemble a trimeric complex, termed the RDC.[26)] Del in the complex subsequently recruits Moonshiner (Moon), a paralog of transcription factor TFIIA-L, its co-factor TFIIA-S, and TRF2, a TATA box-binding protein-related

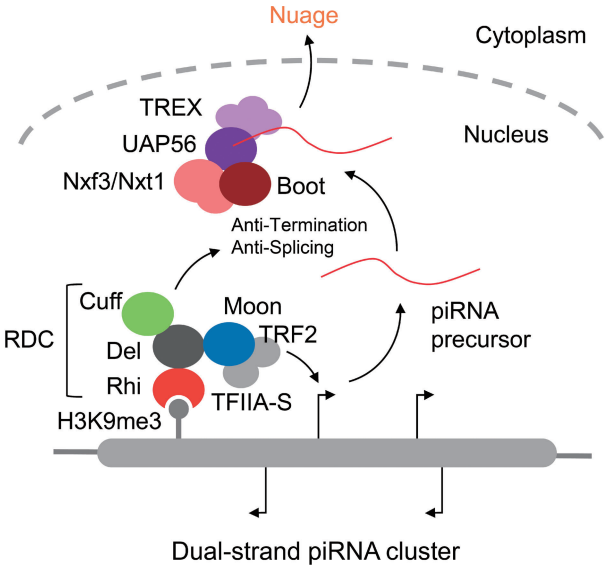

Fig. 1. piRNA production from dual-strand piRNA clusters in *Drosophila* germ cells. Rhi binds to H3K9me3 and forms the RDC complex with Del and Cuff, which then recruits a basic transcriptional factor consisting of Moon/TFIIA-S/TRF2 to initiate transcription of the cluster. Cuff regulates processing of the cluster transcripts by preventing splicing and termination of the precursors. Nxf3-Nxt1 heterodimer, Boot, and TREX/UAP56 regulate export of the cluster transcripts.

transcription factor IID core variant, to drive RNA Pol II-dependent transcription (Fig. 1).[29)] The transcription start sites may be rich in pyrimidine/purine (YR) dinucleotides. However, the high-ordered complex can form anywhere on a cluster; therefore, the RNA transcripts (*i.e.*, piRNA precursors) vary in length.[29)] The RNA transcripts lack 3′-poly (A) tailed, a unique feature of RNAs arising from dual-strand clusters.[29)]

piRNA biogenesis: nuclear processing of piRNA precursors. Cuff belongs to the Rai1/DXO family, which is characterized by members having a 5′-cap-binding pocket.[28)] Cuff is proposed to bind the 5′-end of dual-strand cluster-originated piRNA precursors for stabilization and splicing inhibition (Fig. 1).[28),30)–32)] In this context, UAP56, a component of the TRanscription-EXport (TREX) mRNA export complex,[33)] associates with Cuff to encourage splicing inhibition.[33)] 3′-end processing by the cleavage and polyadenylation specificity factor is also blocked by Cuff.[30)] Therefore, piRNA precursors are long, unspliced, and lack a poly(A) tail, thereby raising the sequence diversity in piRNA pools. The TREX complex binds piRNA precursors to export them to the cytoplasm and to accumulate them in

nuage, a perinuclear membrane-less organelle known as the piRNA biogenesis center in germ cells.[34)]

Germ-specific nuclear RNA export factor 3 (Nxf3) and its partner Nxt1/p15 also play important roles in nuclear processing and the export of piRNA precursors (Fig. 1).[35),36)] Nxf3 has an RNA-recognition motif and leucine-rich repeat (LRR) domain, an NTF2-like domain, and a leucine-rich nuclear export signal. Therefore, piRNA precursors bound to Nxf3-Nxt1/p15 are exported to the cytoplasm by Crm1, the nuclear export factor dedicated to leucine-rich nuclear localization signal (NLS)-containing cargos.[35)] The nucleo-cytoplasmic shuttling property of Nxf3 is used to ensure the translocation of piRNA precursors from the transcription site to nuage.[35),36)] The Nxf3-Nxt1/p15 heterodimer and the TREX component, UAP56, are recruited to the transcription sites on the clusters through physical interaction between Del and Bootlegger (Boot).[35),36)]

piRNA biogenesis: piRNA maturation. In germ cells, piRNA maturation occurs in nuage through three pathways; the *de novo* biogenesis pathway, the ping-pong cycle (*i.e.*, piRNA amplification pathway), and phasing.[7),8),17),37)–39)] The molecular mechanism underlying the *de novo* pathway remains obscure. The ping-pong cycle mechanism has been mainly studied using *Drosophila* ovaries and cultured BmN4 cells, which are ovarian germ cells originating from silkworm (*Bombyx mori*) ovaries.[40),41)] In BmN4 cells, two PIWI members, Siwi and AGO3, are expressed, which are the homologs of Aub and AGO3 in *Drosophila*, respectively. Because of this, in this review, the ping-pong mechanism will be described using "Aub" and "AGO3", although some findings were made in BmN4 cells.

The ping-pong pathway is initiated with Aub-loaded piRNAs produced through the *de novo* pathway.[7),15),40),41)] Because Aub-bound piRNAs are predominantly antisense to transposon mRNAs, Aub directs cleavage of transposon mRNAs and splits them into two fragments. The DEAD-box RNA helicase Vasa then displaces the products from Aub and helps to load the 3′ fragment of the two fragments onto AGO3.[41),42)] The 3′ end of the fragment is then processed further by 3′-to-5′ exoribonuclease to produce a mature piRNA (Fig. 2).[43)–45)] Subsequently, AGO3 directs cleavage of complementary (antisense) transposon transcripts.[7),15),40),41)] An as yet unidentified Vasa-like DEAD-box RNA helicase then loads the 3′ fragment of the two fragments produced by AGO3 cleavage onto Aub, leading to Aub-piRISC production. Aub and AGO3 then continue these reciprocal Slicer-dependent reactions, to realize a constant production of piRNAs from transposon transcripts.

The N-terminal regions of Aub and AGO3 contain symmetrical dimethylarginines (sDMAs). The factor responsible for this post-translational modification is the arginine methyltransferase PRMT5 (also known as Dart5 or Capsuléen).[46)–48)] Aub and AGO3 sDMA modification is necessary for piRNA production.[46)–48)] A Tudor-domain protein, Krimper, exclusively associates with piRNA-free AGO3 and promotes AGO3-sDMA modification and AGO3-piRNA production.[49),50)] In parallel, AGO3 triggers phased piRNA production.[38),39),51),52)] In this pathway, the trailer sequences of AGO3 cleavage products are processed by Zucchini (Zuc) and assemble into piRISCs with Piwi (Fig. 2).[38),39),51),52)] Piwi may bind *de novo* piRNAs as Aub does but the abundance of *de novo* piRNAs with Piwi is much lower than that of phased piRNAs.[51),52)] Zuc is an endonuclease that localizes to the outer membrane of mitochondria and defines both 5′ and 3′ ends of phased piRNAs through the enzyme reaction noted above.[38),39),53),54)] On the mitochondrial surface, the RNA helicase, Armi, relaxes piRNA precursors using its ATP-dependent, RNA unwinding activity to facilitate the Zuc reaction.[55)–58)]

Mode of action of piRNA-mediated silencing. As noted above, Aub and AGO3 silence transposons post-transcriptionally by cleaving transposon transcripts in sense and antisense orientations, respectively, in the ping-pong cycle. In this regard, the ping-pong cycle is considered to be a coupled event of piRNA biogenesis and transposon silencing.[51),52)]

Piwi does not exhibit Slicer activity and silences transposons co-transcriptionally in the nucleus.[14),18)–20),37)] The nuclear localization of Piwi is strictly regulated by its piRNA loading.[37),55),59)] Studies on Piwi-piRISC-mediated co-transcriptional silencing have been conducted using cultured ovarian somatic cells (OSCs). Therefore, the details of this silencing are described below in the section "*The piRNA pathway in ovarian somatic cells*".

The piRNA pathway in ovarian somatic cells

piRNA biogenesis: transcription of piRNA clusters. OSCs, both in ovaries and in culture, rely on uni-strand piRNA clusters to produce piRNAs.[8),38)] A representative cluster is *flamenco* (*flam*).[60)] The uni-strand piRNA clusters such as *flam* have their own promoters (Fig. 3).[9),28),61)] The

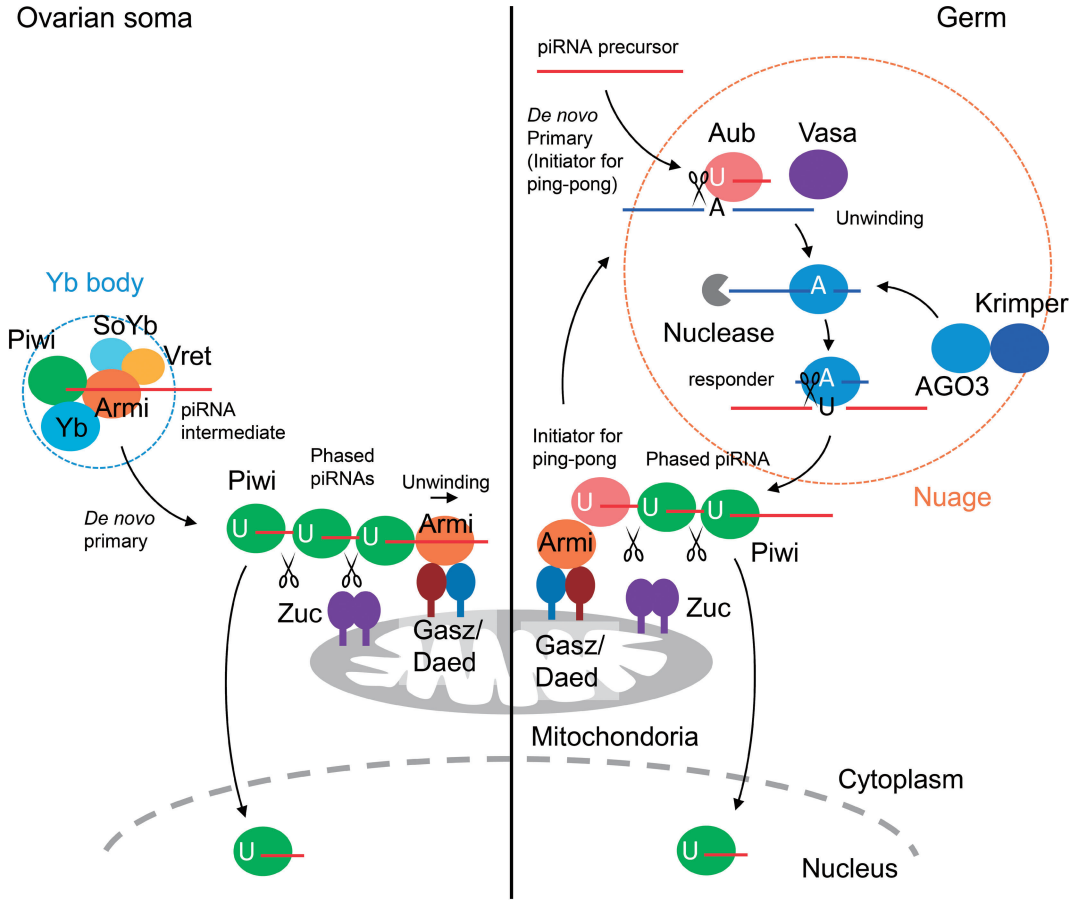

Fig. 2. piRNA biogenesis pathways in the *Drosophila* ovarian soma (left panel) and germ cell (right panel). In the soma, Yb assembles Piwi/Armi/SoYb/Vret in a Yb body. Piwi and Armi then translocate onto mitochondria, where Zuc/Gasz/Daed process the Piwi-bound piRNA production. In the germ cell, the ping-pong cycle operates in nuage. Upon slicing by Aub, piRNA precursors bound to AGO3 are unwound by Vasa and then trimmed by Nuclease. Phased piRNA production is induced by the ping-pong cycle.

cluster bodies are rich in H3K9me3 but the promoter regions are highly occupied by H3K4me2, a transcription-competent histone mark that enables RNA Pol II to transcribe the clusters.[9),26)–28)] Rhi is not expressed in OSCs and so does not contribute to transcription initiation.[26),27)] Because Rhi is absent, the dual-strand clusters might not be used in OSCs. The *flam* promoter contains an initiator motif (Inr) and a downstream promoter element (DPE) but lacks a TATA-box (Fig. 3).[61)] The *flam* promoter also harbors the binding site of Cubitus interruptus (Ci), a Zn-finger family transcription factor. The Ci binding region is −515 to −356 upstream of the transcription start site.[61)]

Uni-strand clusters are transcribed in one fixed direction. The direction of transcription opposes the direction of transposon fragments inserted in the clusters; thereby, mature piRNAs arising from the transcripts are mostly antisense to transposon mRNAs.

piRNA biogenesis: nuclear processing of piRNA precursors. The RNA transcripts of uni-strand piRNA clusters are 5′-capped and 3′-poly-adenylated and undergo alternative splicing, similar to canonical mRNAs produced by RNA Pol II.[61)] The first exon of *flam* transcripts is common to all splice variants[61),62)] but final spliced versions of *flam* RNAs have not been comprehensively determined. The mRNA export complex consisting of Nxf1/Tap and Nxt1/p15 then exports *flam* transcripts to the cytoplasm and accumulates them in Flam bodies (see below) (Fig. 3).[60)] Mago nashi (Mago) and Tsunagi (Tsu/Y14) in the exon junction complex (EJC), as well as the EJC accessory proteins, RNA-

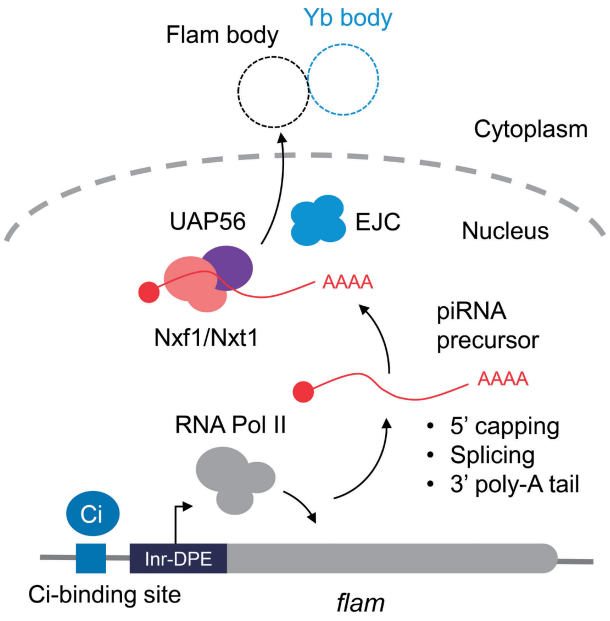

Fig. 3. piRNA production from a uni-strand piRNA cluster *flam*. The *flam* promoter harbors an Inr-DPE motif and a Ci-binding site. The *flam* transcripts are processed by 5′-capping, splicing, and 3′-poly-A tailing. Nxf1-Nxt1 heterodimer, EJC, and UAP56 regulate export of the cluster transcripts.

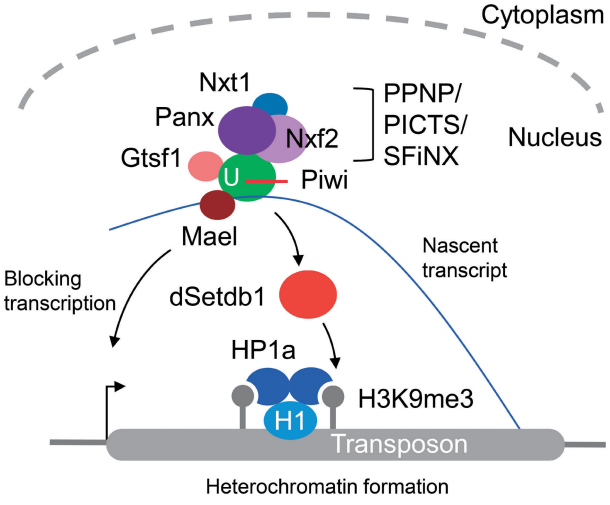

Fig. 4. Piwi-piRISCs trigger co-transcriptional gene silencing of transposons. Piwi-piRISCs trigger formation of PPNP/PICTS/SFiNX consisting of Panx/Nxf2/Nxt1 to recruit dSetdb1 for H3K9me3 deposition on targets. Gtsf1 is involved in the pathway. Mael blocks transcription in an H3K9me3-independent manner. HP1a and H1 lead to heterochromatin formation.

binding protein S1 (RnpS1) and Acinus (Acn), are required to release *flam* RNAs from the transcription sites of *flam*. Depletion of the EJC factors; however, has little effect on *flam* splicing and expression levels.[59),61)–63)] UAP56, an EJC interactor, is also required for *flam* RNA export.[60)]

piRNA biogenesis: piRNA maturation. *flam* RNAs accumulate at cytoplasmic Flam bodies upon export and serve as piRNA precursors for piRNA maturation (Fig. 2).[60),64)] The precursors are first processed at Yb bodies into intermediates, whose sizes are estimated to be 200–500 nt. Yb bodies are perinuclear membrane-less organelles located close to Flam bodies (Fig. 3).[37),55),64)] The piRNA factors localized to Yb bodies include three Tudor domain-containing proteins, female sterile (1) Yb (Yb), Vreteno (Vret), and Sister of Yb (SoYb), and a DEAD-box protein Armitage (Armi). Shutdown (Shu), a co-chaperone associated with HSP83, might also be localized to Yb bodies.[55)–58),65)–70)]

Yb body assembly depends on the RNA-binding activity and self-assembly of Yb (Fig. 2).[55),58),64)–66),71)] Yb has three functional domains, Helicase-C (Hel-C), RNA helicase, and extended Tudor (eTud) domains.[71)] The RNA-binding activity of Yb is conferred by the two C-terminal domains, RNA helicase and eTud, while self-assembly is conferred by the N-terminal Hel-C domain.[71)] Yb interacts with *flam* transcripts and other piRNA sources, including genic piRNA precursors, through *cis*-regulatory elements embedded in the RNAs.[72)] Yb body assembly requires both protein–protein (Yb-Yb) and protein–RNA (Yb-*flam* RNA) interactions.[64)] Furthermore, Yb bodies show sensitivity to 1,6-hexandiol, a small compound that disrupts intracellular structures formed through liquid–liquid phase separation.[71)] Thus, Yb bodies are considered to be multivalent condensates produced through liquid phase separation.[71)]

Armi is localized to Yb bodies by associating with Yb.[55),64),66)] At Yb bodies, Armi binds *flam* RNAs. Without Yb (*i.e.*, without Yb bodies), Armi can bind cellular RNAs in a random fashion in the cytosol and produce piRNAs from bound RNAs.[58),72)] This aberrant action greatly decreases the abundance of transposon-repressing *flam*-piRNAs. As a result, transposons are desilenced. Based on these findings, Yb is considered to be the determiner of *bona fide* piRNA precursors (as the *trans*-acting factor binding to *cis*-elements) whereas Armi is the inducer of piRNA production in OSCs.[58),71),72)]

Upon binding to *flam*-piRNA precursors at Yb bodies, Armi then translocates to mitochondria, where the RNAs are processed into mature piRNAs (see below) (Fig. 2).[53)–58),65)] During the inter-organelle translocation, piRNA intermediates are also

bound with Piwi.[54] The departure of Armi from Yb bodies depends on the Piwi-piRNA intermediate complex.[57),58] Of note, Yb body departure of the Piwi-piRNA intermediate complex also depends on Armi.[57),58] This mutual dependency between Armi and Piwi ensures piRNA maturation from *flam* RNAs but not from other cellular RNAs (excluding genic piRNA sources).[57),58]

Gasz, Minotaur (Mino), and Daedalus (Daed) are piRNA factors localized on the outer surface of mitochondria.[73)–75] Gasz and Daed interact with each other and act as the platform for Zuc-dependent piRNA maturation.[74] Armi, upon translocation from Yb bodies with the Piwi-piRNA intermediate, binds with mitochondrial Gasz and Daed, and unwinds the piRNA intermediate to facilitate Zuc cleavage.[74] Mino is a member of the glycerol-3-phosphate *O*-acetyltransferase (GPAT) family but the GPAT activity is dispensable for piRNA biogenesis. The function of Mino in piRNA biogenesis *per se* remains unknown.[73]

Zuc cleavage of piRNA intermediates gives rise to phased piRNAs, which are loaded onto Piwi.[38),39),51),52] Recombinant Zuc showed no strong sequence bias in RNA cleavage.[53] However, Piwi-bound phased piRNAs predominantly have uracil (U) at the 5′ end (1U).[7),37] This could be because of a property of Zuc to preferentially digest RNAs at U and a property of Piwi to preferentially bind RNAs harboring 1U.[38),39),72]

Mode of action of piRNA-mediated silencing. Piwi has a classical bipartite NLS at the N-terminus. However, Piwi does not localize to the nucleus before piRNA loading.[37),55),59] This regulation depends largely on a structural change to Piwi; the NLS is not available for the import factor, Importinα, to bind unless both 5′ and 3′ ends of a piRNA fit into the binding pockets of Piwi, through the MID and PAZ domains, respectively.[59]

Upon translocation to the nucleus, Piwi-piRISCs scan nascent transcripts in the nucleus, including protein-coding mRNAs.[76] Upon encountering genuine target transcripts, which depends on RNA–RNA complementary between piRNAs and target RNAs, Piwi induces co-transcriptional silencing of the target genes (Fig. 4).[77),78] Most transposons targeted by Piwi are inserted in euchromatic regions and are, therefore, transcriptionally active before silencing.[18] Even upon silencing, continuous Piwi supply is necessary to maintain the silenced status.[18] This means that even in conditions where Piwi represses transposons, weak transcription occurs from the target loci.[18]

Piwi binding to target RNAs is sufficient to induce transposon co-transcriptional silencing. The trimeric complex composed of Panoramix/Silencio (Panx), Nxf2, and Nxt1/p15, named PPNP, PICTS, Pandas, or SFiNX,[77)–82] should bind Piwi and target RNAs simultaneously to enforce the Piwi-target RNA binding, triggering co-transcriptional repression, prior to heterochromatin formation in the nuclear piRNA pathway (Fig. 4).[80),83] Panx has no known functional domains. Nxf2 has two LRR regions, an NTF2-like domain, and a UBA domain at the C-terminus.[80] Nxf2 within the complex interacts directly with target RNA transcripts via the first LRR.[80]

Asterix/Gametocyte-specific factor 1 (Gtsf1) can also be found in the vicinity of PPNP.[84),85] Gtsf1 has two CHHC zinc finger motifs known to function as RNA-binding modules.[84),85] Mutations introduced in the CHHC motifs of Gtsf1 abrogated piRNA-mediated transposon silencing but not piRNA biogenesis.[84),85] Thus, the RNA-binding activity of Gtsf1 is essential in piRNA-mediated co-transcriptional silencing although the function of the protein remains elusive.

Piwi-piRISCs deposit H3K9me3 marks on target loci by recruiting a H3K9 methyltransferase Eggless/SETDB1 (Egg) (Fig. 4).[18),78),86] Egg is post-translationally monoubiquitinated in the nucleus, which is required for its own catalytic activity and the piRNA-mediated transposon repression.[86)–88] Windei (Wde), the *Drosophila* homolog of mAM/MCAF1/ATF7IP, recruits Egg to the chromatin at target gene silencing loci.[86),87),89] Bidirectional spreading of H3K9me3 into neighboring genes involves Su(var)3-9, one of two other histone methyltransferases in *Drosophila*.[71] The deposition of H3K9me3 marks is followed by chromatin compaction involving heterochromatin protein HP1a and linker histone H1.[18),78),90]

Maelstrom (Mael) contains an HMG-box motif and an MAEL domain, the latter of which is required for Piwi-mediated co-transcriptional transposon silencing (Fig. 4).[57),91] The MAEL domain exhibits endonuclease activity for single-stranded RNAs *in vitro*.[91] Loss of Mael in OSCs increases RNA Pol II occupancy at target loci, leading to transposon derepression; however, the level of H3K9me3 was only mildly affected by the treatment[18] and the mechanism underlying this remains elusive. Notably, recent studies show that Mael represses canonical,

promoter-dependent transcription of transposons inserted in dual-strand piRNA clusters.[92] Mael may help Rhi drive transcription of piRNA clusters by preventing the production of mature transcripts from transposons potentially encoding active transposable proteins.[92]

Perspectives

Since the discovery of piRNAs in the early 2000s, they have been identified in both vertebrates and invertebrates, and it is estimated that there are many hundreds of thousands of different sequences; for example, more than 13,000 and 50,000 unique piRNA sequences have been discovered in fly and mouse, respectively.[1)–6),93)] The study of piRNA biology essentially started in 2003[11)] and has since revealed essential features, such as piRNA clusters, piRNA biogenesis pathways, and silencing modes. Importantly, most piRNA factors and features for host genome defense are evolutionally well conserved between species. Understanding the modes of piRNA actions has progressed greatly and has largely been established from the findings of genetic and high-performance computational experiments. However, many underlying molecular mechanisms remain elusive; *e.g.*, How does Rhi recognize the dual strand clusters? How does Piwi precisely find the target transposons and repress transcription by RNA Pol II prior to heterochromatin formation? And more importantly, a number of piRNA factor candidates has been found by genetic screening,[75),94),95)] but their functions in piRNA pathways still remain elusive. Therefore, biochemical dissection of the piRNA pathways is crucial to gain mechanistic insights into these mechanisms and to further progress piRNA biology.

Acknowledgements

We thank Kensaku Murano of Keio University for insightful comments on the manuscript. This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan to K.S. (KAKENHI grant number 18H04539) and M.C.S. (KAKENHI grant number 19H05466).

References

1) Ghildiyal, M. and Zamore, P.D. (2009) Small silencing RNAs: An expanding universe. Nat. Rev. Genet. **10**, 94–108.
2) Czech, B., Munafò, M., Ciabrelli, F., Eastwood, E.L., Fabry, M.H., Kneuss, E. *et al.* (2018) piRNA-guided genome defense: From biogenesis to silencing. Annu. Rev. Genet. **52**, 131–157.
3) Huang, X., Fejes Tóth, K. and Aravin, A.A. (2017) piRNA biogenesis in Drosophila melanogaster. Trends Genet. **33**, 882–894.
4) Iwasaki, Y.W., Siomi, M.C. and Siomi, H. (2015) PIWI-interacting RNA: Its biogenesis and functions. Annu. Rev. Biochem. **84**, 405–433.
5) Siomi, M.C., Sato, K., Pezic, D. and Aravin, A.A. (2011) PIWI-interacting small RNAs: The vanguard of genome defence. Nat. Rev. Mol. Cell Biol. **12**, 246–258.
6) Aravin, A.A., Hannon, G.J. and Brennecke, J. (2007) The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. Science **318**, 761–764.
7) Brennecke, J., Aravin, A.A., Stark, A., Dus, M., Kellis, M., Sachidanandam, R. *et al.* (2007) Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell **128**, 1089–1103.
8) Malone, C.D., Brennecke, J., Dus, M., Stark, A., McCombie, W.R., Sachidanandam, R. *et al.* (2009) Specialized piRNA pathways act in germline and somatic tissues of the Drosophila ovary. Cell **137**, 522–535.
9) Yamanaka, S., Siomi, M.C. and Siomi, H. (2014) piRNA clusters and open chromatin structure. Mob. DNA **5**, 22.
10) Vagin, V.V., Sigova, A., Li, C., Seitz, H., Gvozdev, V. and Zamore, P.D. (2006) A distinct small RNA pathway silences selfish genetic elements in the germline. Science **313**, 320–324.
11) Aravin, A.A., Lagos-Quintana, M., Yalcin, A., Zavolan, M., Marks, D., Snyder, B. *et al.* (2003) The small RNA profile during Drosophila melanogaster development. Dev. Cell **5**, 337–350.
12) Klattenhoff, C. and Theurkauf, W. (2008) Biogenesis and germline functions of piRNAs. Development **135**, 3–9.
13) Lau, N.C., Robine, N., Martin, R., Chung, W.J., Niki, Y., Berezikov, E. *et al.* (2009) Abundant primary piRNAs, endo-siRNAs, and microRNAs in a Drosophila ovary cell line. Genome Res. **19**, 1776–1785.
14) Saito, K., Nishida, K.M., Mori, T., Kawamura, Y., Miyoshi, K., Nagami, T. *et al.* (2006) Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the Drosophila genome. Genes Dev. **20**, 2214–2222.
15) Gunawardane, L.S., Saito, K., Nishida, K.M., Miyoshi, K., Kawamura, Y., Nagami, T. *et al.* (2007) A slicer-mediated mechanism for repeat-associated siRNA 5′ end formation in Drosophila. Science **315**, 1587–1590.
16) Nishida, K.M., Saito, K., Mori, T., Kawamura, Y., Nagami-Okada, T., Inagaki, S. *et al.* (2007) Gene silencing mechanisms mediated by Aubergine–piRNA complexes in Drosophila male gonad. RNA **13**, 1911–1922.
17) Li, C., Vagin, V.V., Lee, S., Xu, J., Ma, S., Xi, H.

et al. (2009) Collapse of germline piRNAs in the absence of Argonaute3 reveals somatic piRNAs in flies. Cell **137**, 509–521.
18) Sienski, G., Dönertas, D. and Brennecke, J. (2012) Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression. Cell **151**, 964–980.
19) Rozhkov, N.V., Hammell, M. and Hannon, G.J. (2013) Multiple roles for Piwi in silencing Drosophila transposons. Genes Dev. **27**, 400–412.
20) Klenov, M.S., Lavrov, S.A., Korbut, A.P., Stolyarenko, A.D., Yakushev, E.Y., Reuter, M. *et al.* (2014) Impact of nuclear Piwi elimination on chromatin state in Drosophila melanogaster ovaries. Nucleic Acids Res. **42**, 6208–6218.
21) Carmen, L., Michela, B., Rosaria, V. and Gabriella, M. (2009) Existence of snoRNA, microRNA, piRNA characteristics in a novel non-coding RNA: x-ncRNA and its biological implication in Homo sapiens. J. Bioinform. Seq. Anal. **1**, 031–040.
22) Houwing, S., Kamminga, L.M., Berezikov, E., Cronembold, D., Girard, A., van den Elst, H. *et al.* (2007) A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. Cell **129**, 69–82.
23) Faehnle, C.R. and Joshua-Tor, L. (2007) Argonautes confront new small RNAs. Curr. Opin. Chem. Biol. **11**, 569–577.
24) Akkouche, A., Mugat, B., Barckmann, B., Varela-Chavez, C., Li, B., Raffel, R. *et al.* (2017) Piwi is required during Drosophila embryogenesis to license dual-strand piRNA clusters for transposon repression in adult ovaries. Mol. Cell **66**, 411–419.e4.
25) Le Thomas, A., Stuwe, E., Li, S., Du, J., Marinov, G., Rozhkov, N. *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.
26) Mohn, F., Sienski, G., Handler, D. and Brennecke, J. (2014) The rhino-deadlock-cutoff complex licenses noncanonical transcription of dual-strand piRNA clusters in Drosophila. Cell **157**, 1364–1379.
27) Klattenhoff, C., Xi, H., Li, C., Lee, S., Xu, J., Khurana, J.S. *et al.* (2009) The Drosophila HP1 homolog Rhino is required for transposon silencing and piRNA production by dual-strand clusters. Cell **138**, 1137–1149.
28) Pane, A., Jiang, P., Zhao, D.Y., Singh, M. and Schüpbach, T. (2011) The cutoff protein regulates piRNA cluster expression and piRNA production in the Drosophila germline. EMBO J. **30**, 4601–4615.
29) Andersen, P.R., Tirian, L., Vunjak, M. and Brennecke, J. (2017) A heterochromatin-dependent transcription machinery drives piRNA expression. Nature **549**, 54–59.
30) Chen, Y.A., Stuwe, E., Luo, Y., Ninova, M., Le Thomas, A., Rozhavskaya, E. *et al.* (2016) Cutoff suppresses RNA polymerase II termination to ensure expression of piRNA precursors. Mol. Cell **63**, 97–109.
31) Pritykin, Y., Brito, T., Schupbach, T., Singh, M. and Pane, A. (2017) Integrative analysis unveils new functions for the Drosophila Cutoff protein in noncoding RNA biogenesis and gene regulation. RNA **23**, 1097–1109.
32) Zhang, Z., Wang, J., Schultz, N., Zhang, F., Parhad, S.S., Tu, S. *et al.* (2014) The HP1 homolog rhino anchors a nuclear complex that suppresses piRNA precursor splicing. Cell **157**, 1353–1363.
33) Zhang, F., Wang, J., Xu, J., Zhang, Z., Koppetsch, B.S., Schultz, N. *et al.* (2012) UAP56 couples piRNA clusters to the perinuclear transposon silencing machinery. Cell **151**, 871–884.
34) Hur, J.K., Luo, Y., Moon, S., Ninova, M., Marinov, G.K., Chung, Y.D. *et al.* (2016) Splicing-independent loading of TREX on nascent RNA is required for efficient expression of dual-strand piRNA clusters in Drosophila. Genes Dev. **30**, 840–855.
35) ElMaghraby, M.F., Andersen, P.R., Pühringer, F., Hohmann, U., Meixner, K., Lendl, T. *et al.* (2019) A heterochromatin-specific RNA export pathway facilitates piRNA production. Cell **178**, 964–979.
36) Kneuss, E., Munafò, M., Eastwood, E.L., Deumer, U.S., Preall, J.B., Hannon, G.J. *et al.* (2019) Specialization of the Drosophila nuclear export family protein Nxf3 for piRNA precursor export. Genes Dev. **33**, 1208–1220.
37) Saito, K., Inagaki, S., Mituyama, T., Kawamura, Y., Ono, Y., Sakota, E. *et al.* (2009) A regulatory circuit for piwi by the large Maf gene traffic jam in Drosophila. Nature **461**, 1296–1299.
38) Mohn, F., Handler, D. and Brennecke, J. (2015) Noncoding RNA. piRNA-guided slicing specifies transcripts for Zucchini-dependent, phased piRNA biogenesis. Science **348**, 812–817.
39) Han, B.W., Wang, W., Li, C., Weng, Z. and Zamore, P.D. (2015) Noncoding RNA. piRNA-guided transposon cleavage initiates Zucchini-dependent, phased piRNA production. Science **348**, 817–821.
40) Kawaoka, S., Hayashi, N., Suzuki, Y., Abe, H., Sugano, S., Tomari, Y. *et al.* (2009) The Bombyx ovary-derived cell line endogenously expresses PIWI/PIWI-interacting RNA complexes. RNA **15**, 1258–1264.
41) Nishida, K.M., Iwasaki, Y.W., Murota, Y., Nagao, A., Mannen, T., Kato, Y. *et al.* (2015) Respective functions of two distinct Siwi complexes assembled during PIWI-interacting RNA biogenesis in Bombyx germ cells. Cell Rep. **10**, 193–203.
42) Xiol, J., Spinelli, P., Laussmann, M.A., Homolka, D., Yang, Z., Cora, E. *et al.* (2014) RNA clamping by Vasa assembles a piRNA amplifier complex on transposon transcripts. Cell **157**, 1698–1711.
43) Hayashi, R., Schnabl, J., Handler, D., Mohn, F., Ameres, S.L. and Brennecke, J. (2016) Genetic and mechanistic diversity of piRNA 3′-end formation. Nature **539**, 588–592.
44) Izumi, N., Shoji, K., Sakaguchi, Y., Honda, S., Kirino, Y., Suzuki, T. *et al.* (2016) Identification

and functional analysis of the pre-piRNA 3′ trimmer in silkworms. Cell **164**, 962–973.
45) Nishida, K.M., Sakakibara, K., Iwasaki, Y.W., Yamada, H., Murakami, R., Murota, Y. *et al.* (2018) Hierarchical roles of mitochondrial Papi and Zucchini in Bombyx germline piRNA biogenesis. Nature **555**, 260–264.
46) Kirino, Y., Kim, N., de Planell-Saguer, M., Khandros, E., Chiorean, S., Klein, P.S. *et al.* (2009) Arginine methylation of Piwi proteins catalysed by dPRMT5 is required for Ago3 and Aub stability. Nat. Cell Biol. **11**, 652–658.
47) Kirino, Y., Vourekas, A., Kim, N., de Lima Alves, F., Rappsilber, J., Klein, P.S. *et al.* (2010) Arginine methylation of vasa protein is conserved across phyla. J. Biol. Chem. **285**, 8148–8154.
48) Nishida, K.M., Okada, T.N., Kawamura, T., Mituyama, T., Kawamura, Y., Inagaki, S. *et al.* (2009) Functional involvement of Tudor and dPRMT5 in the piRNA processing pathway in Drosophila germlines. EMBO J. **28**, 3820–3831.
49) Sato, K., Iwasaki, Y.W., Shibuya, A., Carninci, P., Tsuchizawa, Y., Ishizu, H. *et al.* (2015) Krimper enforces an antisense bias on piRNA pools by binding AGO3 in the Drosophila germline. Mol. Cell **59**, 553–563.
50) Webster, A., Li, S., Hur, J.K., Wachsmuth, M., Bois, J.S., Perkins, E.M. *et al.* (2015) Aub and Ago3 are recruited to nuage through two mechanisms to form a ping-pong complex assembled by Krimper. Mol. Cell **59**, 564–575.
51) Wang, W., Han, B.W., Tipping, C., Ge, D.T., Zhang, Z., Weng, Z. *et al.* (2015) Slicing and binding by Ago3 or Aub trigger piwi-bound piRNA production by distinct mechanisms. Mol. Cell **59**, 819–830.
52) Senti, K.A., Jurczak, D., Sachidanandam, R. and Brennecke, J. (2015) piRNA-guided slicing of transposon transcripts enforces their transcriptional silencing via specifying the nuclear piRNA repertoire. Genes Dev. **29**, 1747–1762.
53) Nishimasu, H., Ishizu, H., Saito, K., Fukuhara, S., Kamatani, M.K., Bonnefond, L. *et al.* (2012) Structure and function of Zucchini endoribonuclease in piRNA biogenesis. Nature **491**, 284–287.
54) Ipsaro, J.J., Haase, A.D., Knott, S.R., Joshua-Tor, L. and Hannon, G.J. (2012) The structural biochemistry of Zucchini implicates it as a nuclease in piRNA biogenesis. Nature **491**, 279–283.
55) Saito, K., Ishizu, H., Komai, M., Kotani, H., Kawamura, Y., Nishida, K.M. *et al.* (2010) Roles for the Yb body components Armitage and Yb in primary piRNA biogenesis in Drosophila. Genes Dev. **24**, 2493–2498.
56) Rogers, A.K., Situ, K., Perkins, E.M. and Toth, K.F. (2017) Zucchini-dependent piRNA processing is triggered by recruitment to the cytoplasmic processing machinery. Genes Dev. **31**, 1858–1869.
57) Ge, D.T., Wang, W., Tipping, C., Gainetdinov, I., Weng, Z. and Zamore, P.D. (2019) The RNA-binding ATPase, Armitage, couples piRNA amplification in nuage to phased piRNA production on mitochondria. Mol. Cell **74**, 982–995.e6.
58) Ishizu, H., Kinoshita, T., Hirakata, S., Komatsuzaki, C. and Siomi, M.C. (2019) Distinct and collaborative functions of Yb and Armitage in transposon-targeting piRNA biogenesis. Cell Rep. **27**, 1822–1835.e8.
59) Yashiro, R., Murota, Y., Nishida, K.M., Yamashiro, H., Fujii, K., Ogai, A. *et al.* (2018) Piwi nuclear localization and its regulatory mechanism in Drosophila ovarian somatic cells. Cell Rep. **23**, 3647–3657.
60) Dennis, C., Brasset, E., Sarkar, A. and Vaury, C. (2016) Export of piRNA precursors by EJC triggers assembly of cytoplasmic Yb-body in Drosophila. Nat. Commun. **7**, 13739.
61) Goriaux, C., Desset, S., Renaud, Y., Vaury, C. and Brasset, E. (2014) Transcriptional properties and splicing of the flamenco piRNA cluster. EMBO Rep. **15**, 411–418.
62) Hayashi, R., Handler, D., Ish-Horowicz, D. and Brennecke, J. (2014) The exon junction complex is required for definition and excision of neighboring introns in Drosophila. Genes Dev. **28**, 1772–1785.
63) Malone, C.D., Mestdagh, C., Akhtar, J., Kreim, N., Deinhard, P., Sachidanandam, R. *et al.* (2014) The exon junction complex controls transposable element activity by ensuring faithful splicing of the piwi transcript. Genes Dev. **28**, 1786–1799.
64) Murota, Y., Ishizu, H., Nakagawa, S., Iwasaki, Y.W., Shibata, S., Kamatani, M.K. *et al.* (2014) Yb integrates piRNA intermediates and processing factors into perinuclear bodies to enhance piRISC assembly. Cell Rep. **8**, 103–113.
65) Olivieri, D., Sykora, M.M., Sachidanandam, R., Mechtler, K. and Brennecke, J. (2010) An in vivo RNAi assay identifies major genetic and cellular requirements for primary piRNA biogenesis in Drosophila. EMBO J. **29**, 3301–3317.
66) Qi, H., Watanabe, T., Ku, H.Y., Liu, N., Zhong, M. and Lin, H. (2011) The Yb body, a major site for Piwi-associated RNA biogenesis and a gateway for Piwi expression and transport to the nucleus in somatic cells. J. Biol. Chem. **286**, 3789–3797.
67) Zamparini, A.L., Davis, M.Y., Malone, C.D., Vieira, E., Zavadil, J., Sachidanandam, R. *et al.* (2011) Vreteno, a gonad-specific protein, is essential for germline development and primary piRNA biogenesis in Drosophila. Development **138**, 4039–4050.
68) Handler, D., Olivieri, D., Novatchkova, M., Gruber, F.S., Meixner, K., Mechtler, K. *et al.* (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.
69) Olivieri, D., Senti, K.A., Subramanian, S., Sachidanandam, R. and Brennecke, J. (2012) The cochaperone shutdown defines a group of biogenesis factors essential for all piRNA populations in Drosophila. Mol. Cell **47**, 954–969.
70) Preall, J.B., Czech, B., Guzzardo, P.M., Muerdter, F.

and Hannon, G.J. (2012) Shutdown is a component of the Drosophila piRNA biogenesis machinery. RNA **18**, 1446–1457.
71) Hirakata, S., Ishizu, H., Fujita, A., Tomoe, Y. and Siomi, M.C. (2019) Requirements for multivalent Yb body assembly in transposon silencing in Drosophila. EMBO Rep. **20**, e47708.
72) Ishizu, H., Iwasaki, Y.W., Hirakata, S., Ozaki, H., Iwasaki, W., Siomi, H. *et al.* (2015) Somatic primary piRNA biogenesis driven by cis-acting RNA elements and trans-acting Yb. Cell Rep. **12**, 429–440.
73) Vagin, V.V., Yu, Y., Jankowska, A., Luo, Y., Wasik, K.A., Malone, C.D. *et al.* (2013) Minotaur is critical for primary piRNA biogenesis. RNA **19**, 1064–1077.
74) Munafò, M., Manelli, V., Falconio, F.A., Sawle, A., Kneuss, E., Eastwood, E.L. *et al.* (2019) Daedalus and Gasz recruit Armitage to mitochondria, bringing piRNA precursors to the biogenesis machinery. Genes Dev. **33**, 844–856.
75) Handler, D., Meixner, K., Pizka, M., Lauss, K., Schmied, C., Gruber, F.S. *et al.* (2013) The genetic makeup of the Drosophila piRNA pathway. Mol. Cell **50**, 762–777.
76) Ilyin, A.A., Ryazansky, S.S., Doronin, S.A., Olenkina, O.M., Mikhaleva, E.A., Yakushev, E.Y. *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.
77) Yu, Y., Gu, J., Jin, Y., Luo, Y., Preall, J.B., Ma, J. *et al.* (2015) Panoramix enforces piRNA-dependent cotranscriptional silencing. Science **350**, 339–342.
78) Sienski, G., Batki, J., Senti, K.A., Dönertas, D., Tirian, L., Meixner, K. *et al.* (2015) Silencio/CG9754 connects the Piwi-piRNA complex to the cellular heterochromatin machinery. Genes Dev. **29**, 2258–2271.
79) Fabry, M.H., Ciabrelli, F., Munafò, M., Eastwood, E.L., Kneuss, E., Falciatori, I. *et al.* (2019) piRNA-guided co-transcriptional silencing coopts nuclear export factors. eLife **8**, e47999.
80) Murano, K., Iwasaki, Y.W., Ishizu, H., Mashiko, A., Shibuya, A., Kondo, S. *et al.* (2019) Nuclear RNA export factor variant initiates piRNA-guided co-transcriptional silencing. EMBO J. **38**, e102870.
81) Batki, J., Schnabl, J., Wang, J., Handler, D., Andreev, V.I., Stieger, C.E. *et al.* (2019) The nascent RNA binding complex SFiNX licenses piRNA-guided heterochromatin formation. Nat. Struct. Mol. Biol. **26**, 720–731.
82) Zhao, K., Cheng, S., Miao, N., Xu, P., Lu, X., Zhang, Y. *et al.* (2019) A Pandas complex adapted for piRNA-guided transcriptional silencing and heterochromatin formation. Nat. Cell Biol. **21**, 1261–1272.
83) Clark, J.P., Rahman, R., Yang, N., Yang, L.H. and Lau, N.C. (2017) Drosophila PAF1 modulates PIWI/piRNA silencing capacity. Curr. Biol. **27**, 2718–2726.e4.
84) Ohtani, H., Iwasaki, Y.W., Shibuya, A., Siomi, H., Siomi, M.C. and Saito, K. (2013) DmGTSF1 is necessary for Piwi-piRISC-mediated transcriptional transposon silencing in the Drosophila ovary. Genes Dev. **27**, 1656–1661.
85) Dönertas, D., Sienski, G. and Brennecke, J. (2013) Drosophila Gtsf1 is an essential component of the Piwi-mediated transcriptional silencing complex. Genes Dev. **27**, 1693–1705.
86) Osumi, K., Sato, K., Murano, K., Siomi, H. and Siomi, M.C. (2019) Essential roles of Windei and nuclear monoubiquitination of Eggless/SETDB1 in transposon silencing. EMBO Rep. e48296.
87) Tsusaka, T., Shimura, C. and Shinkai, Y. (2019) ATF7IP regulates SETDB1 nuclear localization and increases its ubiquitination. EMBO Rep. e48297.
88) Sun, L. and Fang, J. (2016) E3-independent constitutive monoubiquitination complements histone methyltransferase activity of SETDB1. Mol. Cell **62**, 958–966.
89) Koch, C.M., Honemann-Capito, M., Egger-Adam, D. and Wodarz, A. (2009) Windei, the Drosophila homolog of mAM/MCAF1, is an essential cofactor of the H3K9 methyl transferase dSETDB1/Eggless in germ line development. PLoS Genet. **5**, e1000644.
90) Iwasaki, Y.W., Murano, K., Ishizu, H., Shibuya, A., Iyoda, Y., Siomi, M.C. *et al.* (2016) Piwi modulates chromatin accessibility by regulating multiple factors including histone H1 to repress transposons. Mol. Cell **63**, 408–419.
91) Matsumoto, N., Sato, K., Nishimasu, H., Namba, Y., Miyakubi, K., Dohmae, N. *et al.* (2015) Crystal structure and activity of the endoribonuclease domain of the piRNA pathway factor maelstrom. Cell Rep. **11**, 366–375.
92) Chang, T.H., Mattei, E., Gainetdinov, I., Colpan, C., Weng, Z. and Zamore, P.D. (2019) Maelstrom represses canonical polymerase II transcription within bi-directional piRNA clusters in Drosophila melanogaster. Mol. Cell **73**, 291–303.
93) Lin, H., Yin, H., Beyret, E., Findley, S. and Deng, W. (2008) The role of the piRNA pathway in stem cell self-renewal. Dev. Biol. **319**, 479.
94) Muerdter, F., Guzzardo, P.M., Gillis, J., Luo, Y., Yu, Y., Chen, C. *et al.* (2013) A genome-wide RNAi screen draws a genetic framework for transposon control and primary piRNA biogenesis in Drosophila. Mol. Cell **50**, 736–748.
95) Czech, B., Preall, J.B., McGinn, J. and Hannon, G.J. (2013) A transcriptome-wide RNAi screen in the Drosophila ovary reveals factors of the germline piRNA pathway. Mol. Cell **50**, 749–761.

(Received Oct. 1, 2019; accepted Oct. 28, 2019)

Profile

Kaoru Sato was born in Fukushima prefecture in 1981 and graduated from Tokyo University of Science in 2004. He then entered the Graduate School of Frontier Sciences, The University of Tokyo and received his Ph.D. degree under the supervision of Prof. Haruhiko Fujiwara in 2009. He worked as a postdoctoral fellow in Prof. Haruhiko Siomi's laboratory in the Keio University School of Medicine from 2009 to 2011, and became an Assistant Professor in 2012. In 2013, he joined Prof. Mikiko C. Siomi's laboratory in the Graduate School of Science at The University of Tokyo as an Assistant Professor. His research interests are on the roles of non-coding DNA, which is largely transcribed into non-coding RNAs, including piRNA, representing the "dark matter" of the genome. In 2009, he began research on piRNA biology and, to date, has contributed to the elucidation of the molecular mechanisms of piRNA biogenesis and the mode-of-action, mainly using *Drosophila* reproductive organs as a model system.

Profile

Mikiko C. Siomi was born in Aichi Prefecture in 1962 and graduated from Gifu University in 1984. She received two Ph.D. degrees, one from Kyoto University (in Agricultural Chemistry in 1994) and the other from Tokushima University (in Medical Science in 2003). During her postdoc training at the University of Pennsylvania School of Medicine, she studied the *fmr1* gene, the cause of fragile X syndrome, to understand how loss-of-function of *fmr1* causes mental retardation. She also studied the mechanisms underlying cytoplasmic-nuclear transport of macromolecules such as RNA-binding proteins and RNPs in human cells. After this postdoctoral research, Dr. Siomi's studies focused on small RNA-mediated gene silencing in *Drosophila*, which led to discoveries that microRNA- and siRNA-mediated gene silencing in flies occur in an independent manner, unlike in mammals, and that Ago2 endonuclease (Slicer) activity is essential for assembling the effector complex, RISC. Dr. Siomi's current study focuses particularly on piRNA-mediated transposon silencing and the gonad-specific RNA silencing pathway to maintain germline genome integrity. She became a Professor at The University of Tokyo, Graduate School of Sciences in 2012. She has served at the President of the RNA Society of Japan for from 2014 to 2018 and was elected as the Vice-President of the Molecular Biology Society of Japan in 2018. Dr. Siomi was awarded the Saruhashi Prize (by the Association for the Bright Future of Woman Scientists) in 2008 and was elected as an EMBO Associate Member in 2018.