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PIWI proteins and PIWI–interacting RNAs in the soma

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Abstract

The discovery of millions of PIWI–interacting RNAs revealed a fascinating and unanticipated dimension of biology. The PIWI–piRNA pathway has been commonly perceived as germline–specific, even though the somatic function of PIWI proteins was documented when they were first discovered. Recent studies have begun to re–explore this pathway in somatic cells in diverse organisms, particularly lower eukaryotes. These studies have illustrated the multifaceted somatic functions of the pathway not only in transposon silencing but also in genome rearrangement and epigenetic programming, with biological roles in stem–cell function, whole–body regeneration, memory and possibly cancer.

The discoveries of small non-coding RNAs, including PIWI-interacting RNAs (piRNAs), have significantly expanded the RNA world. piRNAs are generally 24–32 nucleotides in length and bind specifically to the PIWI subfamily of Argonaute proteins. Piwi was originally discovered in *Drosophila*[1], in which it functions in germline stem-cell maintenance and self-renewal[2]. For clarity, we use PIWIs to refer collectively to PIWI proteins, whereas Piwi refers to the individual protein. Although piRNAs were discovered and formally defined in mammalian systems in 2006 as small non-coding RNAs that specifically interact with PIWIs[3-6], cloning of piRNAs in *Drosophila*[7-9] revealed that they include a previously discovered class of small non-coding RNAs called repeat-associated RNAs (rasiRNAs)[10,11]. Since 2006, the PIWI–piRNA field has rapidly advanced, with a focus on the germ line, in which PIWIs and piRNAs are enriched and PIWI mutations lead to a profound infertility phenotype[12-14]. Indeed, the name PIWI comes from the original mutant phenotype P-element-induced wimpy testis[1]. The best-known role of the PIWI–piRNA pathway in the germ line is in transposon silencing, because piRNAs map largely to transposable elements[9], with PIWI depletion leading to a drastic increase in transposon messenger RNA expression[15].

Despite the germline focus, since their discovery, PIWIs' somatic function has long been documented. Initial work on the *Drosophila* gene *piwi*, the first identified member that

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defines the *Argonaute* gene family, determined that its germline function depends on the somatic cells of the gonad[2]. Recently, significant insight into the somatic function of the PIWI–piRNA pathway has come from the ovarian somatic cells of *Drosophila*. In addition, groundbreaking work in lower eukaryotes has demonstrated a conserved function for PIWIs and their associated piRNAs in somatic tissues — particularly in stem cells. In this Review, we focus on the role of the PIWI–piRNA pathway in the soma of diverse organisms, from basal eukaryotes to humans. We begin by looking at PIWI expression and piRNA biogenesis in somatic tissues, and then illustrate how the PIWI–piRNA pathway exerts diverse functions, including epigenetic regulation, transposon silencing, genome rearrangement and developmental regulation. Through this Review we hope to illustrate a broader role for the PIWI–piRNA pathway in the soma.

Expression of PIWIs and piRNAs in somatic tissues

PIWIs are expressed in organisms from sponges to humans. This expression occurs in remarkably diverse cell types, ranging from naive pluripotent stem cells to differentiated somatic cells, with most somatic expression related to various totipotent and pluripotent stem cells[16-24] (Table 1). Several studies have demonstrated an essential stem-cell function for PIWIs. One interesting example comes from planarian stem cells. *piwi* genes are expressed in planarian totipotent stem cells, called neoblasts, that can repopulate all somatic and germline lineages. Planarians have an unusual regenerative capability, in which neoblasts are responsible for maintaining and regenerating all tissues. PIWIs play a crucial part in the neoblast lineage, as illustrated by the two phenotypic consequences of knockdown of mRNAs encoding PIWIs: first, animals are incapable of body-part regeneration; and second, failure of tissue maintenance ultimately results in death[21]. Furthermore, piRNA-like small RNAs, which depend on PIWIs for their biogenesis, have been identified in planaria. These RNAs are not germline-restricted, because ablation of the germ line by *nanos* mRNA knockdown does not affect piRNA-like RNA production[21,22]. In addition, RNA interference (RNAi) experiments in ascidians have demonstrated the requirement for PIWIs in whole-body regeneration (discussed later)[23,24].

Although substantial work has described PIWI expression beyond the germ line of lower eukaryotes, the somatic function of PIWIs was first characterized in somatic cells of the *Drosophila* ovary. The *Drosophila* Piwi protein is expressed in all somatic cells within the ovary and in early somatic cells of the testis[2,25]. Outside the gonad, Piwi binds to polytene chromosomes of the salivary gland[26]. Emerging and unexpected evidence shows that PIWIs function outside the gonad, particularly in the *Drosophila* head. The first genetic evidence showed that *piwi* mutation leads to position effect variegation in the expression of the eye colour gene *white* in a clonal fashion[8,26]. This suggests the possibility of cell-autonomous Piwi function in the eye. Direct molecular evidence came from a more recent study in which two *Drosophila* PIWIs, Argonaute-3 and Aubergine, which were once thought to be germline-specific, showed region-specific expression in the brain, with their mutation leading to transposon upregulation in fly heads[27]. Interestingly, piRNA-like small RNAs mapping to transposons and heterochromatin are detectable in the *Drosophila* head on depletion of the RNAi effector Argonaute-2 (ref. 28). A recent publication has shown that all *Drosophila* PIWIs are expressed during early embryogenesis and that depletion of

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maternal *Drosophila* PIWIs results in profound chromosomal and mitotic defects, thus establishing a crucial function for PIWIs in the earliest stage of somatic development[29]. Together with the work on basal eukaryotes, studies in *Drosophila* highlight the existence and function of PIWIs in somatic tissues. Although somatic PIWIs clearly function in *Drosophila*, it remains a mystery whether piRNAs are generated outside the gonad, because some of the most studied piRNA biogenesis factors are not robustly expressed in non-gonadal somatic tissue (discussed later).

In light of the above findings, an important question remains: are PIWIs present in mammalian somatic tissues? There are four human PIWIs: HIWI (also known as PIWIL1), HILI (also known as PIWIL2), HIWI2 (also known as PIWIL4) and HIWI3 (also known as PIWIL3). HIWI is expressed in haematopoietic stem cells but not in their differentiated progeny. Although this once led to the suggestion that HIWI might be involved in the stemness of these stem cells[30], a powerful genetic experiment in which all three mouse *Piwi* genes were knocked out showed no detectable effect on haematopoiesis. Thus, PIWI expression in haematopoietic stem cells may not have any functional implication or PIWI function is redundant with other proteins, possibly Argonaute subfamily proteins[31]. With regards to human health, many studies demonstrate PIWI expression in a wide variety of human cancers; however, these data are at best correlative and it is too early to tell whether PIWIs have any role in cancer (discussed later)[32-36].

The expression of PIWIs in mammalian somatic tissues implies the potential existence of somatic piRNAs. One study provided evidence of piRNA expression in diverse somatic tissues of both the mouse and macaque[37]. However, the lack of appropriate negative controls combined with a small library size make it difficult to differentiate between true piRNA expression and contamination during library preparation. Indeed, this study might be a cautionary tale describing the challenges in the search for mammalian somatic piRNAs. Further work will help to determine whether piRNAs are expressed outside the germ line in mammals. Perhaps malignant tissues are good sites to begin the search given the expression of PIWIs in cancer.

piRNA biogenesis in the *Drosophila* ovarian soma

Our mechanistic understanding of somatic piRNA biogenesis comes from recent work in the somatic follicle cells of the *Drosophila* ovary. piRNAs in the ovarian soma are generated by a Piwi-dependent mechanism through the primary piRNA biogenesis pathway[38,39]. This process is independent of the other two *Drosophila* PIWIs, Aubergine and Argonaute-3, which function as a piRNA amplification loop in the germline cells (known as secondary piRNA biogenesis). In the primary piRNA biogenesis pathway, long piRNA precursors are transcribed from specific genomic loci known as piRNA clusters, cleaved and modified in the cytoplasm, and then transported into the nucleus in complex with Piwi (Fig. 1). Ovarian soma-specific piRNAs are transcribed from two main loci. The *flamenco* locus contains transposon remnants and encodes a long single-stranded piRNA precursor that is antisense to *flamenco*'s component transposons. Precursors generated from the *flamenco* locus target transposons of the gypsy family of long terminal repeat (LTR) transposons, including *gypsy, ZAM* and *idefix*[38]. Another ovarian soma-specific locus, *traffic jam*, has two functions: the 3′

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UTR of its transcript is a substrate for somatic piRNA production, whereas its protein product drives Piwi expression in ovarian somatic cells[39].

It is unclear how long single-stranded piRNA precursors are exported from the nucleus and how these precursors are initially processed into smaller fragments in the cytoplasm. One proposed, but unproven, candidate for piRNA 5′ end determination is Zucchini, an outer mitochondrial membrane protein with single-strand-specific endonuclease activity *in vitro*[40]. In *Drosophila*, the maturing precursor then enters the perinuclear Yb body[41-43], an ovarian soma-specific cytoplasmic structure named after the Yb protein[44]. Although it is not yet clear what occurs in the Yb body, several of its components are crucial for the processing of piRNA precursors into mature piRNAs, and for subsequent Piwi nuclear localization. These components include the helicase Armitage[41-43] and the TUDOR-domain-containing protein Vreteno[44]. Both Vreteno[45] and Yb[41,43] are needed for Piwi expression and/or stability. Zucchini also functions in piRNA maturation. Knockdown of the mRNA encoding Zucchini in a *Drosophila* ovarian somatic cell line leads to cytoplasmic accumulation of piRNA-intermediate-like molecules[46] and accumulation of Piwi in the Yb body; correspondingly, nuclear Piwi is absent[42]. The mouse homologues of the above piRNA biogenesis factors, including Zucchini[47], Armitage[48,49], Shutdown[50,51] and Vreteno[52] are crucial for piRNA biogenesis in the testes, and male mutants are infertile, thus implying conservation of these factors in piRNA biogenesis.

Next, piRNAs are loaded onto Piwi in the cytoplasm through an uncharacterized step that is independent of Piwi slicer activity and nuclear localization signal[39,53]. The co-chaperone Shutdown, which is essential to piRNA biogenesis in both the ovarian soma and germ line, may function in piRNA loading[54]. Ovarian somatic piRNAs contain a characteristic 5′ U, but the mechanism generating this 5′ U bias is unknown. It is possible that Piwi itself preferentially binds RNAs with 5′ U, as is the case for silkworm Piwi *in vitro*[55]. This might authenticate and stabilize piRNA intermediates with 5′ U for further processing. An unknown protein then trims the 3′ ends of the maturing piRNAs. The shared structure of Argonaute family proteins suggests the possibility that piRNA length may be determined by the number of bases protected within Piwi[56-58]. This also explains the characteristic size profile of piRNAs bound by each of the three *Drosophila* PIWIs[9]. Mature piRNAs are then 2′-*O*-methylated by a conserved methyltransferase, Pimet[59], perhaps to ensure their stability. Finally, in an uncharacterized step, the Piwi–piRNA complex is imported into the nucleus to exert its regulatory function.

Epigenetic regulation by the PIWI–piRNA pathway

Strong evidence indicates that Piwi and piRNAs play a crucial part in epigenetic regulation. Pioneering work has shown that Piwi functions in the transcriptional silencing of *Adh* transgene arrays, and small RNAs have been proposed to have a role in Piwi-mediated silencing[60]. Piwi is a predominantly nuclear protein[25] that localizes to salivary gland polytene chromosomes in an RNA-dependent manner[26], and co-localizes with known epigenetic modifiers such as the Polycomb group proteins[61]. There is general agreement that Piwi is required for appropriate histone methylation and transcriptional gene silencing, as loss of Piwi in both germ line and soma leads to a decrease in the repressive methylation

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mark on histone 3 lysine 9 (H3K9)[62-66], an increase in RNA Pol II occupancy[63-65] and an increase in nascent transcript[60,63,66]. Indeed, tissue-specific knockdown of Piwi in somatic ovarian follicle cells implicates transcriptional repression as the dominant mode of transposable element control in the soma[66].

What is the mechanism by which Piwi directs epigenetic modification? The answer to this lies in piRNA, which probably guides Piwi to specific target sequences in the genome by sequence complementarity[63-66]. Chromatin immunoprecipitation (ChIP) data from our laboratory suggest that the Piwi–piRNA complex binds its genomic target in euchromatin through a nascent transcript (often a long non-coding RNA), and in heterochromatin predominantly through a direct piRNA-DNA interaction[26,64]. According to these results, which await reproduction by other labs, the Piwi–piRNA complex then recruits epigenetic factors such as HP1a and the histone methyltransferase Su(var)3-9 to exert their function (Fig. 2). HP1a is a highly conserved chromatin factor whose *N*-terminal chromodomain binds trimethylated H3K9 whereas the *C*-terminal chromoshadow domain dimerizes with the same domain in another HP1a molecule and interacts with other proteins[64]. Piwi may directly recruit HP1a[26]. HP1a may then recruit one of its well-characterized interactors, Su(var)3-9, which is responsible for most H3K9 methylation in *Drosophila*[64]. Alternatively, others have proposed that Piwi may first recruit a histone methyltransferase such as Su(var)3-9 or Setdb1. The methyltransferase could establish H3K9 methylation and in turn recruit HP1a; this possibility is supported by work in fission yeast[67]. In addition to these core epigenetic factors, Asterix (also known as DmGTSF1), an upstream nuclear factor, is required for Piwi-directed H3K9 methylation[68-70]. A recently identified downstream effector of Piwi, Maelstrom, is not required for the establishment of H3K9 methylation but is required for the transcriptional silencing of transposable elements[63].

Several studies have revealed unanticipated epigenetic gene regulation carried out by Piwi. In *Drosophila simulans*, maternally deposited piRNAs against the retrotransposon *tirant* initiated H3K9-mediated transcriptional gene silencing of this element in the somatic tissues of developing embryos[71]. This study shows that maternal germline-derived piRNAs are required for somatic epigenetic programming. A recent study expanded on this idea by showing that both maternal and zygotic Piwi are required for establishment of heterochromatin in non-gonadal somatic cells of the early embryo[72]. Interestingly, Piwi-targeting of transposable elements for silencing means that those genes containing or in proximity to transposable element sequences may be piRNA pathway targets. The presence of a transposon or its remnants in an intron or in proximity to a gene correlates with significant transcriptional repression[63,69]. Surprisingly, Piwi can also act as an epigenetic activator. In *Drosophila*, Piwi establishes euchromatic features of chromosome 3R telomere-associated sequence (3R-TAS)[8], and whole-genome studies have shown that Piwi binding may enhance transcriptional activation marks in multiple regions[64]. In support of this unexpected epigenetic function, a recent independent study confirmed that *piwi* mutant flies have increased HP1a enrichment in regions, including 3R-TAS, in which Piwi is implicated as an epigenetic activator[72]. How can Piwi-binding lead to transcriptional silencing in most target sites, but activation in a small number of target sites? Perhaps Piwi interacts with different partners and/or is influenced by the local chromatin micro-environment or its

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sublocalization within the nucleus. Clearly, Piwi has many different roles in a variety of cellular processes, and one challenge in the field is to unite or to further distinguish between these many functions.

PIWI–piRNA pathway in genome rearrangement

Ciliates are single-celled organisms that possess remarkable nuclear dimorphism. They have two distinct genomes: a somatic macronucleus that functions in vegetative growth and is actively transcribed, and a transcriptionally silent germline micronucleus that functions in the exchange of genetic information during sexual reproduction, known as conjugation. Following conjugation and mitosis, the zygotic genome in one of the two micronuclei in each daughter cell is extensively edited to create a new and partial somatic genome that replaces the parental germline genome, in a process known as genome rearrangement or, more appropriately, somatic elimination. During somatic elimination, repetitive sequences are removed from the genome before polyploidization, thus preventing their transcription in the resultant macronuclei[73]. Although other organisms have complex mechanisms for transcriptional and post-transcriptional silencing of transposable elements, the ciliates simply delete such sequences from the somatic macronuclear genome.

Early studies in the ciliate *Tetrahymena* revealed that their Piwi orthologues are essential for proper genome rearrangement and piRNA production. These piRNAs, termed scan RNAs in the original literature, are derived from the micronucleus during conjugation. Importantly, *Tetrahymena* piRNAs are distinct from those in other species as they are produced through a Dicer-dependent mechanism from double-stranded RNA precursors[74]. In this sense, they are more like endogenous small interfering RNAs[75] (endo-siRNAs). After mating, the piRNAs provide sequence-specificity to target germline-restricted regions in the developing somatic macronucleus for elimination (Fig. 3)[76]; consequent histone methylation then groups these sequences for collective deletion[77]. In the current model, for which there is strong evidence in both *Tetrahymena* and *Paramecium*, long non-coding RNAs transcribed from the parental somatic macronucleus act as sponges for germline-derived piRNAs. Thus, unbound piRNAs specifically mark any non-somatic regions in the genome for elimination in the developing daughter macronucleus[78].

By contrast, piRNAs in the ciliate *Oxytricha* complex with a Piwi orthologue, Otiwi1, to mark somatic genes for retention during development of the somatic macronucleus. PIWIs are so crucial to *Oxytricha* that on knockdown of the mRNA encoding Otiwi1 the organisms do not survive after mating. Furthermore, injection of RNAs that target normally deleted genes leads to their retention through multiple sexual generations[79]. This suggests that genomic composition changes of the somatic macronucleus are heritable across multiple generations. In *Tetrahymena*, the minority of the developing somatic genome is directed for deletion, whereas in *Oxytricha* the minority of the developing somatic genome is directed for retention. Although it is not known why these distantly related species solve this puzzle of genome rearrangement in opposite ways, it is clear that each has evolved the most efficient system with the fewest possible piRNA targets to direct the assembly of a complete somatic macronuclear genome[79].

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PIWI–piRNA pathway in somatic development

The PIWI–piRNA pathway has broad developmental functions in diverse organisms, from memory in the sea slug *Aplysia* to whole-body regeneration in botryllids. In *Aplysia*, both synaptic plasticity and associative memory formation require the PIWI–piRNA pathway. *Aplysia* Piwi, in complex with piRNAs, responds to the neurotransmitter serotonin by directing CpG methylation of the CREB2 promoter. CREB2 is a major inhibitor of memory in *Aplysia*, so Piwi-mediated transcriptional silencing of CREB2 results in memory through long-lasting, cell-wide enhancement of synaptic transmission[80]. The capacity of the PIWI–piRNA pathway to epigenetically regulate genes is thus exerted in mature neurons to promote cellular memory, emphasizing PIWI function in differentiated somatic tissue.

Colonial ascidians are chordates that are capable of whole-body regeneration. Remarkably, the ascidian *Botrylloides leachii* can regenerate its entire body from any blood vessel fragment containing only a few cells. This phenomenon depends on a population of Piwi-positive cells present on the luminal side of the vascular epithelium. On RNAi knockdown of the mRNA encoding Piwi, organisms cannot undergo whole-body regeneration[23]. In the closely related *Botryllus schlosseri*, Piwi is also crucial for whole-body regeneration. Piwi positive-cells, which contribute to both the germline and somatic lineages of future generations, reside within the endostyle niche. *B. schlosseri* undergoes weekly asexual growth, during which a population of Piwi-positive stem cells vacates the original endostyle niche and migrates to the niche within the growing daughter organism before the parent niche undergoes massive apoptosis. In this way, the stem-cell population efficiently preserves itself over the course of the colony's lifetime through migration to the offspring[24].

Piwi suppresses phenotypic variation

The PIWI–piRNA pathway has a direct role in buffering against phenotypic variation, and Piwi depletion in *Drosophila* results in new somatic defects in a random fashion and at low frequency. Canalization, a term coined by Conrad Waddington in 1942 (ref. 81), describes developmental robustness: the ability of a system to generate a single phenotype regardless of genetic or environmental perturbations. The heat-shock protein Hsp90 is a crucial chaperone in the suppression of phenotypic variation, that is, in canalization. Depletion of Hsp90 generates diverse somatic phenotypic variants in species ranging from *Arabidopsis*[82] to *Drosophila*[83]. These variants are heritable even in the absence of heat shock or other forms of stress, and even on repletion of the wild-type Hsp90 levels in progeny, indicating that the effect is not simply due to the chaperone function of Hsp90 during stress. *Drosophila* Piwi forms a complex with Hsp90 and the heat-shock organizing protein, Hop, *in vivo* to suppress phenotypic variation[84].

This buffering of variation is probably accomplished through both genetic and epigenetic mechanisms. Evidence for a genetic mechanism stems from a study in which Hsp90 depletion in *Drosophila* leads to defective transposable element silencing by the PIWI–piRNA pathway. The resultant transposon-mediated mutagenesis may generate new phenotypes[85]. However, the transposon mutagenesis hypothesis cannot explain the full spectrum of canalization phenotypes. Although one copy of maternal *piwi* is sufficient for

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transposon silencing, it is insufficient to suppress phenotypic variation[84]. In addition, maternal epigenetic factors, such as the *trithorax* group of genes that maintain active chromatin, also buffer against phenotypic variation in *Drosophila*[86]. These observations indicate the involvement of an epigenetic mechanism in suppressing phenotypic variation. With regards to the inheritance of such phenotypes, work in the *Caenorhabditis elegans* germ line has shown that piRNAs induce transgenerational epigenetic inheritance[87]. Regardless of the mechanism, the PIWI–piRNA pathway clearly functions to suppress expression of new phenotypes and to maintain developmental robustness in *Drosophila*.

The uncertain meaning of PIWI in cancer

Cancer stem cells may help to explain resistance to cancer treatment and relapse after treatment in certain forms of cancer. Thus, great interest exists in developing our understanding of the basic biology of cancer stem cells and in identifying factors that drive their stemness. A large number of studies document the ectopic expression of PIWIs in cancers. This was first reported in seminoma, a testicular germ-cell tumour in which HIWI was drastically overexpressed[32]. Related to this overexpression, HIWI mapped to a genomic region linked to seminomas and non-seminomas. Since then, PIWI expression has been shown in a variety of somatic cancers: HIWI is expressed in gastric cancer[36,88], whereas HILI is expressed in breast cancer, colon cancer, gastrointestinal stromal tumours, renal cell carcinoma and endometrial carcinoma[33]. Some early studies also suggested that PIWI expression could be used as a prognostic marker[35]. In both hepatocellular carcinoma[34] and soft-tissue sarcoma[35], tumour HIWI expression is associated with increased risk of tumour-related death. The discovery of PIWI expression in diverse forms of human somatic cancers opens up a promising area of research, especially given the well-established role of PIWIs in stem-cell maintenance and self-renewal.

Consistent with this notion, HILI enrichment was reported in a cancer cell subpopulation expressing the stemness factors OCT4 and NANOG[89]. In addition, a large variety of embryonic and developmental genes are expressed in cancers. However, this does not necessarily imply that they have a causative role in tumorigenesis. Such a conclusion will need to be based on functional studies.

At present, such functional studies are scarce. HILI overexpression in a fibroblast cell line activates STAT3 and the antiapoptotic factor BCLX, suggesting that HILI might function as an oncogene[33]. Genetic studies in *Drosophila* revealed that *piwi* mutation attenuates tumour growth in a sensitized *lethal (3) malignant brain tumour* (*l(3)mbt*)-mutant background. Other piRNA pathway genes were also upregulated in *l(3)mbt* tumours. These findings are perhaps the strongest data available at present in correlating ectopic PIWI expression with tumour growth[90].

These preliminary mechanistic data are promising, but research on PIWIs in cancer remains at an early stage and is primarily correlative. It is known that insertional mutagenesis by LINE1 elements is common in human epithelial cancers[91]. Therefore, it is possible that PIWIs are expressed in reaction to increased transposon activity in cancer, and thus act to protect the genome. Perhaps an *in vivo* overexpression model would be a good starting point

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to determine whether ectopic or overexpression of a PIWI protein can actually cause cancer, or is merely a consequence of tumorigenesis. Without a doubt, more large-scale, systematic research is needed before we can conclude whether or not human PIWIs have any role in cancer.

Outstanding questions

PIWIs occupy the interface between stem-cell and small RNA biology. They serve diverse roles in diverse tissues, from totipotent stem cells to totally differentiated cell types, from the germ line to the soma. Tantalizing questions remain, including how broadly are PIWIs and piRNAs expressed in mammalian somatic tissues? And, what is their function there, if any? The mammalian soma awaits our rigorous interrogation. At a more fundamental level, what are the molecular mechanisms by which Piwi regulates stemness? Conversely, what exactly is Piwi doing in differentiated tissues? The lower eukaryotes, in which we are making rapid progress on this topic, provide an excellent arena for these investigations.

The reason underlying ectopic expression of PIWIs in many human cancers is still a mystery. Do PIWIs have a role in cancer? If so, do PIWIs provoke dedifferentiation or lend a competitive advantage by enhancing stemness or even as a reactive mechanism to suppress transposition? Finally, are piRNAs ectopically expressed in cancers? If so, what is their function? Answers to these questions will not only shed light on the function of PIWIs and piRNAs but could also mark paths that are ripe for cancer research.

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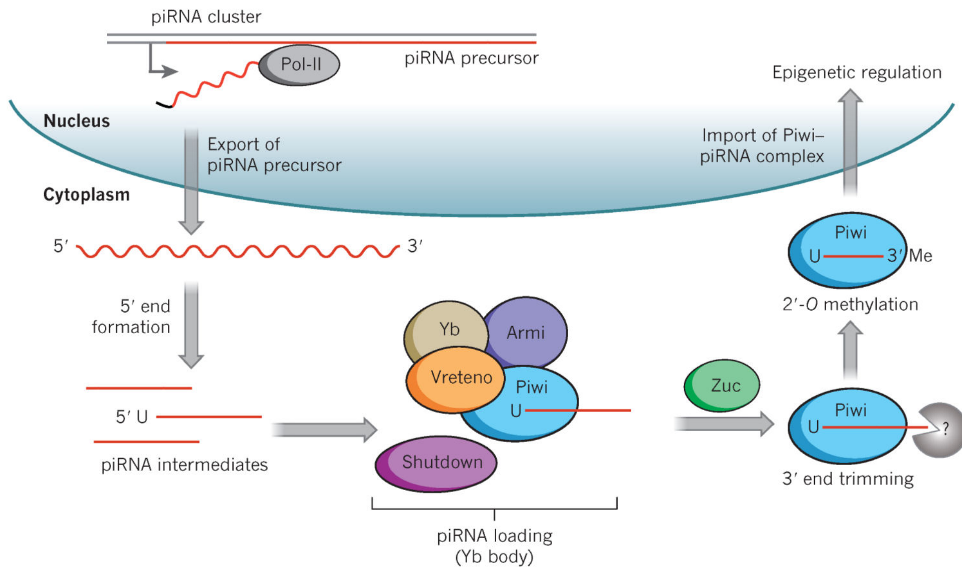

Figure 1. piRNA biogenesis in the *Drosophila* ovarian soma

piRNAs are generated from specific genomic loci known as piRNA clusters, which include *flamenco*, the 5′ UTRs of mRNAs and *traffic jam* in the soma. The long single-stranded piRNA precursor (red) is then exported from the nucleus. In the cytoplasm, the precursors are processed into mature piRNAs. The precursors are cleaved by an unknown endonuclease to generate their 5′ end, and transported into the perinuclear Yb body for further processing. The precursors are loaded onto Piwi in a process that is dependent on Yb, Armitage (Armi) and Vreteno. Overlapping proteins indicate protein–protein interaction. The co-chaperone Shutdown plays an uncharacterized, but crucial, part in piRNA loading. The putative endonuclease Zucchini (Zuc) is required for piRNA maturation and for nuclear localization of Piwi. Subsequently, piRNAs are trimmed to the appropriate length by an unidentified exonuclease and 2′-*O*-methylated at the piRNA 3′ end, rendering them more stable. The Piwi–piRNA complex is then transported into the nucleus, where it modulates chromatin state.

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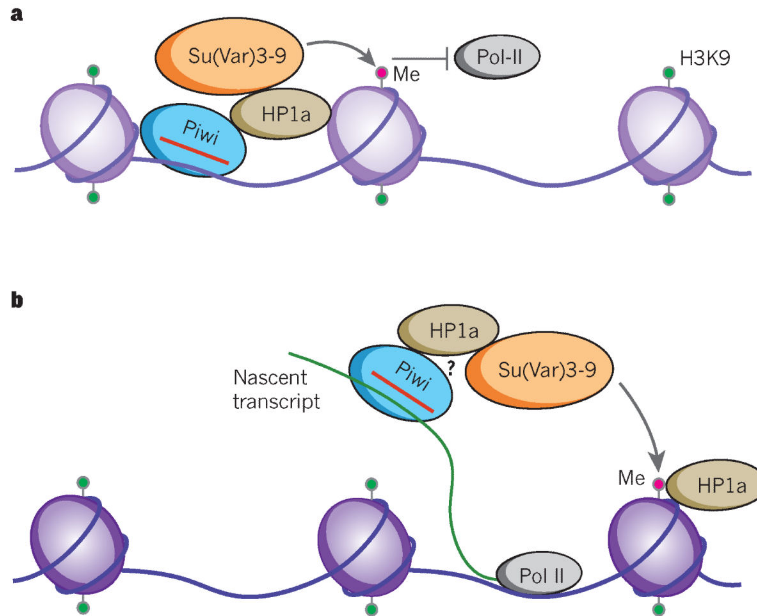

Figure 2. Piwi–piRNA mediated epigenetic regulation

Simplified illustrations of the currently proposed models of Piwi-mediated transcriptional gene silencing. **a**, In heterochromatin, Piwi may be guided to its target sequences by the complementarity of its bound piRNA (red) to genomic DNA. On binding, Piwi recruits the epigenetic modifier HP1a that then recruits the major *Drosophila* histone methyltransferase Su(var)3-9, which then deposits a methyl group on the unmethylated histone 3 lysine 9 (H3K9). Through an unknown mechanism, a critical mass of the H3K9 repressive chromatin marks inhibits Pol II transcription, effectively silencing the Piwi–piRNA target. **b**, In euchromatin, Piwi targets nascent transcripts (green) by piRNA sequence complementarity. Subsequent to this, there are multiple working models. Piwi may directly recruit HP1a, which in turn recruits a histone methyltransferase (Su(var)3-9 is shown) that then deposits methyl groups on the unmethylated H3K9. Alternatively, Piwi may directly recruit the histone methyltransferase, and subsequently HP1a may then bind the methylated H3K9. Regardless of the mechanism, the net effect is that these repressive marks, in concert with Maelstrom (not shown), inhibit RNA Pol II transcription.

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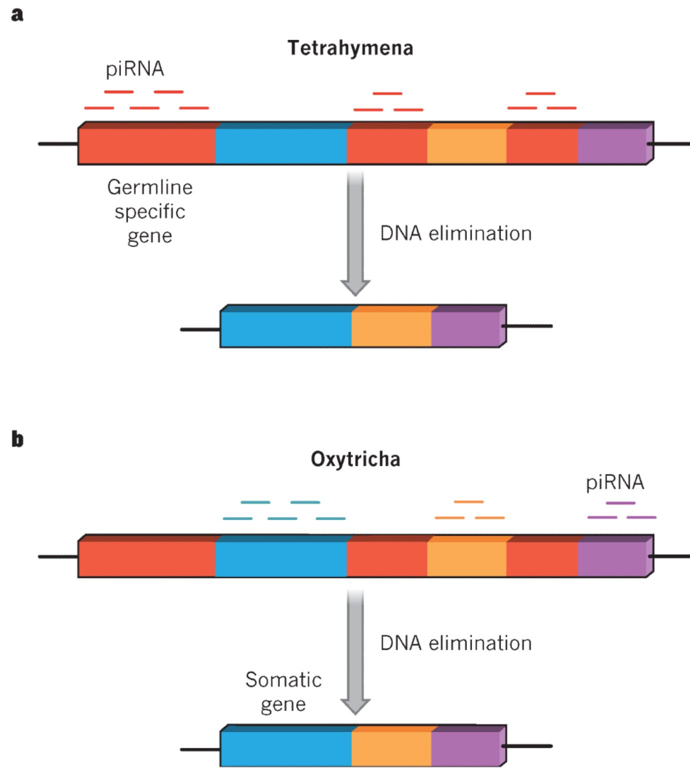

Figure 3. Somatic genome rearrangement in ciliates

Unicellular ciliates posses two nuclei: the germline micronucleus and the somatic macronucleus. After mating, the developing macronucleus is extensively edited to remove germline-specific sequences. The new mature somatic nucleus contains only genes needed for somatic (vegetative) growth. The rest of the germline-specific genome (red boxes) undergoes DNA elimination, an extraordinary method to purge the somatic genome of repetitive sequences and transposons. This process, called somatic genome rearrangement, differs between *Tetrahymena* and *Oxytricha*. **a**, *Tetrahymena* piRNAs (red lines) are generated by the germ line, and target germline-specific sequences of the developing somatic macronucleus for elimination. **b**, *Oxytricha* piRNAs (blue, orange and purple lines) are generated by the parent somatic macronucleus, and direct the retention of somatic genes in the mature somatic macronucleus (blue, orange and purple boxes).

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Table 1

Piwi orthologue expression

Phylum	Common name	Species	Known Piwi genes	PIWI protein expression	Reference
Porifera	Sponge	*Ephydatia fluviatilis*	*EfPiwiA* and *EfPiwiB*	Archeocytes (stem cells that differentiate into both somatic and germ cells)	16
Cnidaria	Jellyfish	*Clytia hemisphaerica*	*Piwi*	Somatic stem cells of the tentacle bulb (produce stinging cells characteristic of the cnidarians)	17
	Jellyfish	*Podocoryne carnea*	*Cniwi*	Somatic stem cells of the tentacle bulb (see above); striated muscle cells capable of transdifferentiation	18
Ctenophora	Comb jellyfish	*Pleurobrachia pileus*	*PpiPiwi1* and *PpiPiwi2*	Actively dividing adult somatic cells; germ line	19
Platyhelminthes	Planaria	*Schmidtea mediterranea*	*smedwi-1*, *smedwi-2* and *smedwi-3*	Neoblasts (totipotent stem cells that can repopulate all somatic and germline lineages)	21, 22
	Saltwater flatworm	*Macrostomum lignano*	*macpiwi*	Neoblasts (see above)	20
Mollusca	Sea slug	*Aplysia californica*	*Piwi*	Nervous system, heart and germ line	80
Arthropoda	Fruitfly	*Drosophila melanogaster*	*piwi, aub* and *AGO3*	Gonad, brain, salivary gland	2, 26, 92, 93
Chordata	Sea squirt (ascidian)	*Botrylloides leachii* and *Botryllus schlosseri*	*Piwi*	Stem cell population (capable of whole-body regeneration)	23, 24
	Mouse	*Mus musculus*	*Miwi, Mili* and *Miwi2*	Diverse cancers (breast cancer, rhabdomyosarcoma, medulloblastoma); male germ line	12-14, 33
	Human	*Homo sapiens*	*HIWI, HILI, HIWI2* and *HIWI3*	Diverse cancers (breast cancer, cervical cancer, endometrial carcinoma, seminomas, hepatocellular carcinoma, gastric cancer, pancreatic adenocarcinoma, gastrointestinal stromal tumours, colon cancer, renal cell carcinoma); haematopoietic stem cells; and male germ line	30, 32-36