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How might flukes and tapeworms maintain genome integrity without a canonical piRNA pathway?

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Abstract

Surveillance by RNA interference is central to controlling the mobilization of transposable elements (TEs). In stem cells, Piwi argonaute (Ago) proteins and associated proteins repress mobilization of TEs to maintain genome integrity. This defense mechanism targeting TEs is termed the Piwi-interacting RNA (Piwi-piRNA) pathway. In this Opinion, we draw attention to the situation that the genomes of cestodes and trematodes have lost the *piwi* and *vasa* genes that are hallmark characters of the germline multipotency program. This absence of Piwi-like Agos and Vasa helicases prompts the question: how does the germline of these flatworms withstand mobilization of TEs? Here we present an interpretation of mechanisms likely to defend the germline integrity of parasitic flatworms.

Keywords

Platyhelminthes; Cestoda; Trematoda; *piwi*; *vasa*; *argonaute*; transposable elements; germline; piRNA pathway

During the past decade draft genomes of several species of the phylum Platyhelminthes (see Glossary) have been reported and/or made available in public databases: (i) the freshwater planarian *Schmidtea mediterranea* (turbellarian); (ii) the blood flukes *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* (trematodes); and (iii) the cyclophyllidean tapeworms *Echinococcus multilocularis*, *Echinococcus granulosus*, *Taenia solium*, and *Hymenolepis microstoma* (cestodes) [1,2,3,4,5,6]. Closer scrutiny of these genomes revealed that *piwi*, *vasa*, and genes encoding group 4 Tudor homologues were absent from the cestode and trematode classes of the Platyhelminthes [7,8]. These otherwise conserved, post-transcriptional regulators are associated with stem cell maintenance and

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germ cell development in diverse taxa - pre-bilaterians, the platyhelminth order Tricladida, the free-living platyhelminth relatives of the parasitic flatworms, and many others.

Phylogenetic analysis of the Argonaute (Ago) protein family with six platyhelminth genomes and other informative species revealed the loss of the Piwi subfamily of Argonautes in cestodes and trematodes [3,8]. This contrasts strikingly with planarians, which express Piwi-like Agos in the somatic and germ stem cells [3,8]. Additionally, it has become apparent that cestodes and trematodes evolved a clade of Argonaute proteins exclusive to the parasitic flatworms. This clade is termed the group 4 Argonautes; it is discrete from the Ago-like clade, the Piwi-like clade, and the *C. elegans* group 3 Argonautes clade that traditionally constitute the Ago protein family [3,8] (Figure 1). It is also noteworthy that group 4 Tudor proteins, the interactive partners of Piwi, appear to be absent from these parasitic flatworms (flukes, tapeworms) [3]. In like fashion, phylograms of Vasa and related DEAD-box helicases further revealed the absence of orthologues of *vasa* in trematodes and cestodes [3,9]. Instead, these flatworms have evolved at least two *PL10*-like genes that cluster together and branch from the PL10 clade of DEAD-box helicases (Figure 2). Moreover, the flukes *S. mansoni* and *Clonorchis sinensis*, the cestodes noted above, and the monogenean, *Neobenedenia girellae*, encode a DEAD-box helicase that might be assignable to the PL10 family or the closely related p68 DEAD-box family [3,8,9,10]. Expression analysis of these latter DEAD-box helicases termed *S. mansoni vasa*-like gene 2 (*Smvlg2*) and *N. girellae vasa*-like gene 2 (*Ngvlg2*) revealed that they exhibit germline specific expression [9,10]. Silencing by RNA interference (RNAi) of *Ngvlg2* led to loss of germ cells, which indicated that the genes perform *vasa*-like roles during germline development [10]. *N. girellae* also has a third *N. girellae vasa*-like gene (*Ngvlg3*) that groups with *vasa* orthologues. This is noteworthy from a phylogenetic point of view since it has been proposed that monogeneans are basal to a clade composed of Classes Trematoda and Cestoda [11] and suggests that *vasa* orthologues had been lost in the common ancestor of flukes and tapeworms. However, transcripts encoding *Ngvlg3* mRNA were not detectable while silencing *Ngvlg3* did not affect gonad anatomy in the same fashion as silencing *Ngvlg1* (PL10-like cluster) and *Ngvlg2* [10].

Considered at large, the absence of Piwi, Vasa, and group 4 Tudor proteins indicates that parasitic flatworms (sub-phylum Neodermata, the cestodes, monogeneans and trematodes) have lost the ancestral components, classified traditionally as germline stem cell associated markers that are conserved in metazoans (Box 1). These enzymes and other factors establish and maintain *inter alia* the multipotency of progenitor germ cells (PGCs), germline stem cells (GSCs), and multipotent progenitor cells including the neoblasts (totipotent adult stem cells) of planarians, a hypothetical regulatory network collectively referred to as the germline multipotency program (GMP) [12,13]. Furthermore, it has been proposed that these factors are ancestrally linked in the GMP because together they participate in protection of the genome from the mobilization of endogenous and exogenous transposable elements (TEs), an ostensibly essential role in germline stem cells and somatic stem cells devoted to the production of progenitors for tissue renewal [14,15,16]. It is relevant to note here that approximately 30 to 50% of the genomes of the three major species of human schistosomes and other trematodes consists of TEs and other repetitive elements, although substantially less of the genome of (at least cyclophyllidean) cestodes is composed of these elements (~2 – 11%) [3,4,5,6,17] (Box 1).

Box 1

Outstanding questions

- Might the loss of Piwi/Vasa have been less crucial in evolution of the Cestoda and Trematoda if the canonical pathways in which proteins participate had already changed in their common ancestor?
- Given the marked dissimilarity of the TE content of the genomes of flukes (35%) and tapeworms (2%), might tapeworms and flukes have different mechanisms of combating TEs, or highly different efficiencies?
- Did flukes and tapeworms co-opt the stem cell specific siRNA pathway?

This opinion highlights the absence of *piwi*, the piRNA pathway, and *vasa* from flukes and tapeworms, and speculates on alternative mechanisms that might defend the integrity of the germline of the parasitic flatworms against TEs.

piRNA pathway - guardian of the germline

Piwi and associated proteins, including Vasa, are involved in the functions and synthesis of a novel class of small non-coding RNAs of 24–31 nucleotides in length termed Piwi-interacting RNAs (piRNAs). With model species, it became clear that the piRNA pathway was restricted to germ cells and, in some cases, gonadal somatic cells [13]. Deciphering the function of Piwi in the context of the silencing of TEs has mainly relied on findings in *Drosophila* and mammalian cell lines and, to a lesser extent, zebrafish and *C. elegans* [12,18]. The piRNAs associate with Piwi to establish an active piRNA-induced silencing complex (piRISC) that recognizes and silences TEs [15,19,20]. This process includes a Ping-Pong amplification cycle for piRNA biogenesis that amplifies piRNAs, increasing the quantity of piRISC available to silence TEs. The system functions to protect the integrity of the germline genome [15,16,19,21,22,23,24,25]. Piwi proteins and piRNAs may also play roles in epigenetic and post-transcriptional regulation of genes in fruit flies and mice [26,27,28,29,30,31,32,33]. Whereas most loci encoding piRNAs are located in introns and exons, similar to miRNA genes, ~20% of piRNAs map to genomic loci enriched with repetitive sequences and TEs [19,34,35].

A specific function for Vasa in the piRNA pathway remains to be determined but it has been linked to the biogenesis of piRNAs [36,37,38,39,40,41,42]. In *Drosophila*, Vasa is required for nuage (= chromatoid body) assembly, the site of piRNA biosynthesis, and in Vasa-deficient fruit flies, the Piwi proteins Agp3 and Aub fail to localize to the nuage particles, and synthesis of piRNAs by the Ping-Pong cycle is markedly diminished [43]. Moreover, TE activities increase in germ cells due to defective de novo methylation [41]. Immunoprecipitation studies demonstrated that primary transcripts of piRNAs clusters associate with Vasa, suggesting that Vasa is involved in transportation of precursor piRNAs from the nucleus to the cytoplasm [44,45,46]. *C. elegans* and mice exhibit similar phenomena [20,36,47,48,49]. In pull-down studies, the mouse orthologue of Vasa, Mouse Vasa-homologue (Mvh), binds with the Piwi proteins Mili, Miwi, and Miwi2. In addition, Mvh-deficient testes fail to silence TEs in germ cells, as do Vasa-deficient fruit flies, [20,36]. It is also notable that orthologues of *vasa* of *C. elegans*, *Drosophila*, and mice have been linked to Dicer. Although the specific mechanism(s) by which Vasa contributes to short interfering RNA (siRNA) rather than microRNA (miRNA) pathways remains unclear, it may facilitate export of precursor RNAs, in like fashion to its anticipated role with piRNAs [30,50,51,52,53,54].

The piRNA pathway likely arose early in the evolution of the Metazoa [55]. In pre-bilaterians such as poriferans and ctenophorans, and in the marine annelid *Platynereis* and some other lophotrochozoans, *piwi* is also expressed in multipotent somatic stem cells [12]. Functional data from species of lophotrochozoans are largely absent, except for some planarians. However, whereas it is likely that metazoans at large express piRNAs, the piRNA pathway is predominantly expressed in germ cells. Hence it is noteworthy that the planarian piRNA pathway occurs primarily in neoblasts, the adult totipotent stem cells, and is not restricted to germ cells [18]. Three Piwi orthologues, SMEDWI-1, SMEDWI-2, and SMEDWI-3 occur in *S. mediterranea* [18,56], and two, SMEDWI-2 and SMEDWI-3, are essential to neoblast function, regeneration, homeostasis, and piRNA expression [18,57,58]. Deep sequencing identified a diverse population of small RNAs of ~32 nt (range 25–35) in length in *S. mediterranea*th at resemble piRNAs of insects and vertebrates [18,59]. These small RNAs map to thousands of loci in the planarian genome; 20–30% mapped to TEs, with the remainder mapping to introns and exons of genes of unknown functions [18,58]. Deeper investigation is needed to clarify the piRNA pathway in planarians, such as pull-down experiments with SMEDWI protein specific antibodies to confirm physical interactions among the small RNAs and the SMEDWI proteins characteristic of *bona fide* piRNAs of the Ping-Pong piRNA biogenesis pathway. Similar observations have been made in *Dugesia japonica* that, like *S. mediterranea*, has inherited three *piwi* genes, *DjPiwi-1*, *-2*, and *-3* [60,61].

Have other Argonautes assumed Piwi roles in flukes and tapeworms?

Although Piwi is absent from the parasitic flatworms, schistosomes express several Argonautes including a protein termed Argonaute 2 (Ago2), which is a member of the group 4 Argonautes exclusive to flukes and tapeworms. Argonaute 2 of *S. mansoni* (*Sm*Ago2) is expressed in the ovary, vitelline gland, testes, the neoblast-like stem cells of the adult schistosomes, and germinal cells of the sporocyst [7,62,63]. The neoblast-like stem cells may be totipotent and exhibit a similar molecular signature to planarian neoblasts [7,62,63]. Gene silencing approaches indicate that *Sm*Ago2 is required for maintenance of germinal cells and the proliferation in sporocysts [63]. There are small non-coding RNAs (sncRNAs) of 20–21 nt that associate with Argonaute 2 (*Sj*Ago2) in *S. japonicum*, an sncRNA size reminiscent of endogenous short interfering RNAs (endo-siRNAs) rather than miRNAs or piRNAs [64]. Follow-up analysis of the sncRNA population in *S. japonicum* eggs ruled out piRNAs with the dominant species of small RNAs between 18 – 23 nt [64]. These endo-siRNAs arise from TEs, predominately non-long terminal repeat (LTR) retrotransposons and LTR retrotransposons [64]. These observations including the failure to identify a *piwi* gene indicate the absence of a canonical piRNA pathway in schistosomes. However, might there exist in its place, a non-canonical piRNA pathway? This would seem to be necessary given the need for a mechanism to regulate the mobility of TEs that comprise sizeable fractions of the genome [4] to guard the integrity of the genome of the germ cells of these parasitic flatworms. *Sm*Ago2/*Sj*Ago2 may perform as a guardian that suppresses the TE activities in germinal cells (Figure 3).

Scrutiny of the draft genomes of *E. multilocularis*, *E. granulosus*, *T. solium*, and *H. microstoma* supports the prediction that *piwi* does not occur in tapeworms; the *piwi* gene has not been identified in these genomes [3,6]. In the ancestor of *Echinococcus* and *Taenia*, the group 4 argonaute gene was subjected to two recent rounds of duplication, resulting in four copies with > 90% identity at the nucleotide level, and organized as two couples of tandemly arranged copies, but without identity in promoter regions. One copy is clearly a pseudogene with a highly fragmented coding region, and it is not clear yet how many of these genes are functional [3]. Nevertheless, a long non-coding RNA family, which is massively transcribed from repetitive elements in the genomes of *E. multilocularis* and *E. granulosus*, has been

identified as potential precursors of piRNA-like small RNAs [65]. These sncRNAs need to be investigated more fully as do a population of sncRNAs between 25 and 30 nt in length that has been identified, but not analyzed, in studies on miRNAs in *E. granulosus* [66]. Taken together, these findings indicate it is unlikely that a traditional piRNA pathway including Piwi is conserved in cestodes or trematodes.

Might other PL10-like helicases perform the role of Vasa?

Whereas *vasa* is absent from the genomes of cestodes and trematodes [3,7,9], three PL10-like genes, *Smvlg1*, *Smvlg2*, and *Smvlg3* have been characterized in *S. mansoni*. Expression profiles of these DEAD-box helicases suggest they perform roles similar to Vasa including functions related to the GMP and to stem cell maintenance [7,9,63]. Silencing of *Smvlg3* indicated that this PL10-like enzyme is necessary for proliferation and maintenance of germinal cells in the sporocyst, a population of cells that shares a molecular signature with neoblasts (adult totipotent stem cells) of planarians [63]. *Smvlg1*, *Smvlg2*, and *Smvlg3* may have assumed the role of *vasa* and display signatures similar to the GMP of other metazoans together with the neoblasts of planarians. In light of these observations, it is plausible that these PL10-like RNA helicases in schistosomes could perform the role of Vasa in the piRNA pathway (Box 1). Notably, unambiguous orthologues of *Smvlg1*, *Smvlg2*, and *Smvlg3* also evolved in the genomes of tapeworms [3] (Figure 1).

Concluding remarks: how do these pathogens flourish without Piwi and Vasa?

Accordingly, how might flukes and tapeworms maintain genome integrity without *piwi* and *vasa*? Perhaps these parasitic platyhelminths evolved a germline, stem cell-specific endogenous siRNA pathway to perform the roles of the piRNA pathway that is active in other metazoans, such as planarians (Figure 3A). We hypothesize the operation of a non-canonical TE silencing pathway in trematodes and cestodes. Here, long, single stranded RNA precursors [65] and/or long double stranded precursors might be cleaved by an unknown enzyme and/or Dicer. Small non-coding RNA, that is, endo-siRNA-like molecules interacting with Argonaute 2 (Ago 2), which belongs to group 4 of Argonaute proteins in parasitic flatworms, constitute the RNA induced silencing complex (RISC) [64] that would drive transcriptional gene silencing (TGS) and/or post-transcriptional gene silencing (PTGS) of TEs (Figure 3B). A summary of the admittedly scant information described so far concerning alternative piRNAs includes the 20–21 nt family in *S. japonicum* and the abundant piRNA-like RNA population of *E. granulosus* [65]; and both proteins and RNAs must be involved in such a pathway. Functional genomics analyses utilizing RNAi and transgenesis [67,68] to define pathways, enzymes and ncRNAs that participate in silencing of TEs represent potentially fruitful routes of investigation to address this enigma as do RNA-Seq and genome methylation status analyses of the endogenous TEs of these worms, and immunoprecipitations to characterize binding partners [64].

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Glossary

Ago clade of Argonautes	the Ago subfamily of Argonaute proteins interacts with miRNAs and siRNAs. Ago proteins, which are expressed in all tissues, exhibit three structural features: PAZ (Piwi, Argonaute, and Zwille) domain, the MID (middle) domain, and the C-terminal PIWI domain
Argonaute (Ago)	catalytic component in RNA-induced silencing complexes (RISC) responsible for coordinating downstream gene-silencing events. Argonaute proteins bind to different classes of small non-coding RNAs, i.e., microRNAs (miRNAs), small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs)
Argonaute protein family	includes the Ago and Piwi clades. *C. elegans* contain specific group 3 argonaute proteins, termed WAGO proteins, as well as the Ago and Piwi clade. Parasitic flatworms evolved a group 4 Ago clade
DEAD-box RNA helicases	member of Superfamily 2 (SF2) of helicases. Characterized by presence of 12 conserved motifs: Q-motif, Motif I, Motif Ia, motif Ib, motif Ic, motif II, motif III, motif IV, motif IVa, motif V, motif Va, and motif VI. Motif II contains the amino acid sequence DEAD (Asp-Glu-Ala-Asp), from which family name derived. The motifs are involved in ATP binding, hydrolysis, ATP-dependent RNA binding, and helicase activities. DEAD-box helicases occur in all cells; central to metabolism of RNA, from transcription to decay
Germline stem cells	germline stem cells have the capacity to generate all cells in the body. Under normal fate the germline stem cells are unipotent, producing gametes and self-renewing to maintain the generative population
Group 4 Tudor proteins	Tudor domains were initially identified as a common structural motif, approximately 60 amino acids, in the *Drosophila* Tudor protein. The motif has now been identified in a wide variety of organisms. These Tudor domain-containing proteins bind to proteins with methylated arginine and lysine residues and play a role in epigenetics, gene expression, and regulation of small RNAs. There are four different functional groups of Tudor proteins distinguished by the sequence flanking the Tudor domain. The group 4 Tudor proteins have been shown to interact with the methylated RG/RA box of Piwi proteins and are involved in piRNA biogenesis by promoting the formation of Piwi ribonucleoprotein (piRNP) complexes
microRNAs (miRNAs)	endogenous non-coding RNAs (~22 nt) that regulate gene expression at transcriptional and post-transcriptional level. Produced from genes or introns, processed from long primary transcripts. miRNAs target and bind complementary sequences in the 3′–untranslated region of mRNAs, leading to gene silencing via translation repression and/or mRNA degradation. Mature miRNAs associate with Ago clade of Argonautes
Neighbor-Joining Method	a method for construction of phylograms(evolutionary trees) that is distanced based and uses bottom-up clustering

Neoblasts	pluripotent adult stem cells with capacity for indefinite self-renewal, totipotency; drive long-term tissue homeostasis. Endow planarians with ability to regenerate. Neoblast-like stem cells occur in tapeworms and schistosomes. Characteristics include roundish-to-ovoid cells with a high nuclear-to-cytoplasmic ratio, large nucleolus, exhibit chromatoid bodies (RNA-protein bodies near nuclei), and numerous free ribosomes
Neodermata	the sub-phylum of the Platyhelminthes that includes the Classes Monogenea, Trematoda and Cestoda. All members of the Neodermata are parasites
Nuage	term used to describe the electron dense, ribonucleoprotein rich, perinuclear granule organelle-like structure in germ cells of *Drosophila* that plays a role in the piRNA pathway and piRNA-mediated silencing of transposons. The structure has been described in other animals and is termed chromatoid bodies, germinal granules, P granules, and mitochondrial cloud in other species. The nuage/chromatoid body has been described in the neoblasts of planarians
Ping-Pong amplification cycle	a mechanism for piRNA biogenesis hypothesized from observations in *Drosophila* and later in other organisms. This mechanism obviates the need for RNA-dependent RNA polymerase. The mechanism is initiated by mature sense primary piRNAs, derived from transposable elements and transcripts from genomic loci termed piRNA clusters, bound to proteins of the Piwi clade which recognize complementary sequences, antisense transcripts from the same piRNA cluster. The antisense piRNAs are cleaved, generating secondary piRNAs with new 5′-ends targeted by another Piwi protein. The 3′ end is then trimmed and then the mature antisense secondary piRNAs are now able to target sense transcripts from the piRNA clusters generating new sense piRNAs
Piwi clade of Argonautes	subfamily of Argonautes; typically expressed in germline and adult somatic stem cells of invertebrates; responsible for regeneration e.g. neoblasts in planarians. Does not occur in parasitic flatworms. Piwi, like Ago, has three main structural motifs: PAZ (Piwi, Argonaute, and Zwille) domain, the MID (middle) domain, and the C-terminal PIWI domain. Piwis associate with small non-coding RNAs termed piwi-interacting RNAs (piRNAs). Essential for biogenesis and/or stability of piRNAs, the piRNA biogenesis pathway, and the Ping-Pong amplification cycle
Piwi-interacting RNAs (piRNAs)	small RNAs of 24–30 nt in length thought to arise from long single stranded RNA precursors derived from transposable elements (TEs), intergenic regions, and protein-coding genes. piRNAs unction in preserving genome integrity and stability, and to play a role in antiviral immunity. piRNAs associated with specific Piwi proteins and together function in silencing TEs. Piwi proteins function in germline development, gametogenesis, germline stem cell maintenance, and meiosis

PL10	an ATP-dependent DEAD-box RNA helicase, also termed DEAD-box helicase 3 (DDX3) closely related to Vasa. PL10 is not restricted to the germline or somatic stem cells, Conserved in eukaryotes from yeast, plants to animals. Participates in RNA metabolism, tissue differentiation, embryogenesis, asexual reproduction, cell regeneration, tumorigenesis, apoptosis, cooption in viral pathology and innate immune
Platyhelminthes	Phylum Platyhelminthes includes parasitic clades Cestoda (tapeworms), Trematoda (flukes), and Monogenea (mostly ectoparasites of fish), and the mostly free-living planarian-like worms of order Tricladida
Small interfering RNAs (siRNAs)	class of small non-coding RNAs (~20–25 nucleotides) notable for their role in the RNA interference pathway. Derived from endogenous loci or foreign genetic material and involved in silencing of invading viruses, transposable element activity, and in some cases protein-coding genes
Transposable element (TE)	DNA elements that can move from location to another within the genome of a cell, these elements can move either by cut-and-paste mechanism (DNA transposons) or indirectly through an RNA intermediate, i.e. copy-and-paste mechanism (retrotransposons)
Vasa	ATP-dependent DEAD-box RNA helicase; archetypal germ cell marker; termed DEAD-box helicase 4 (Ddx4).

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Highlights

1. Flukes and cestodes do not have a piRNA pathway
2. How might these flatworm pathogens defend the germline genome?
3. Fluke genomes are replete with mobile genetic elements

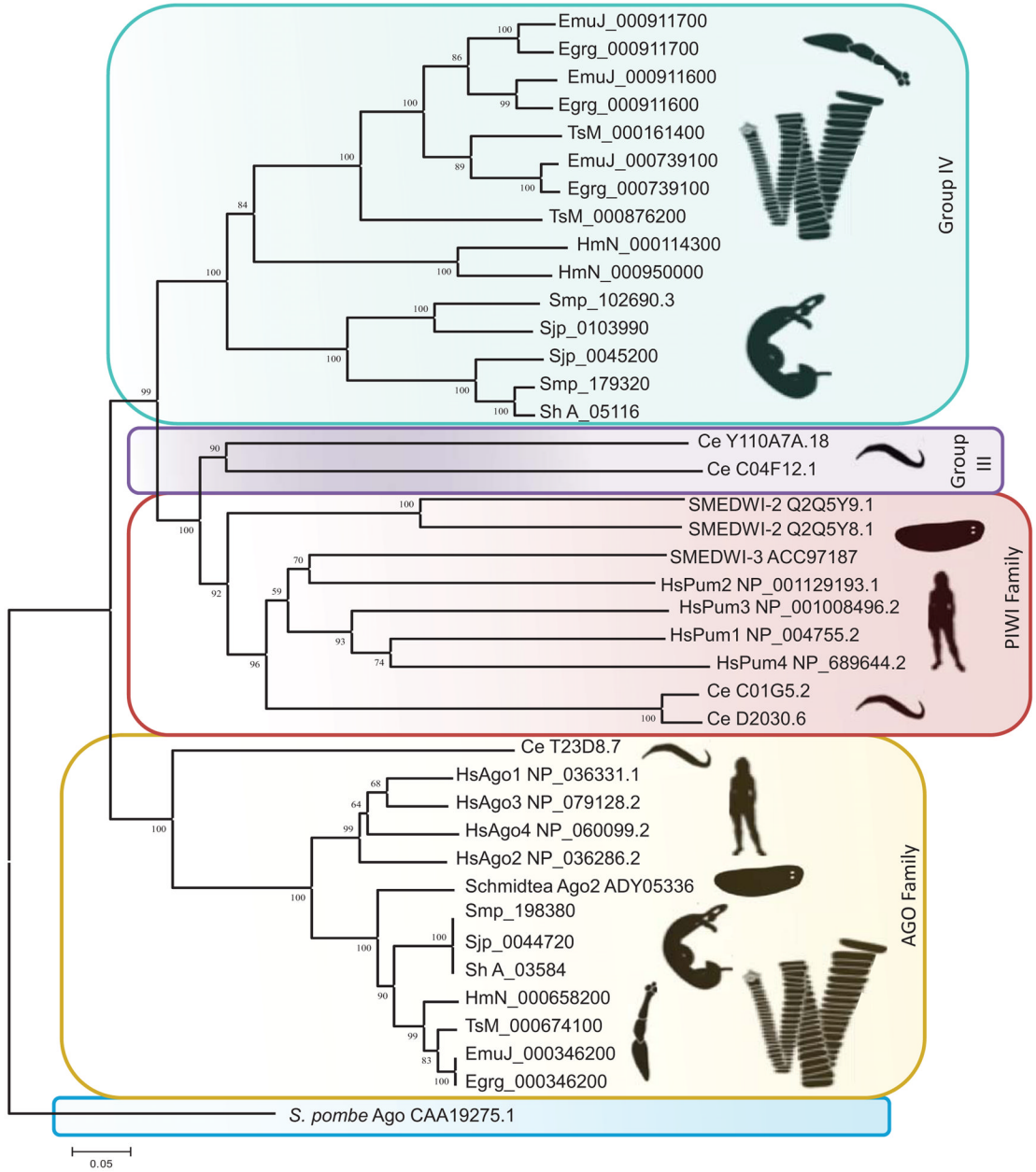

Figure 1.

Evolutionary relationships of platyhelminth Argonautes (AGO). The Neighbor-Joining method was employed to infer relationships using entire amino acid sequences from informative species, including trematodes, and cestodes. Phylogenetic analysis was conducted in MEGA5. Colored boxes highlight families of argonaute proteins, for example, the red region includes Piwi orthologues, and the green colored boxes include group 4 argonaute proteins of cestodes and trematodes. Adapted from [3]. Abbreviations: Egrg, *Echinococcus granulosus*; EmW, *E. multilocularis*; HmN, *Hymenolepis microstoma*; TsM, *Taenia solium*; Sjp, *Schistosoma japonicum*; Smp, *S. mansoni*; Sh, *Schistosoma haematobium*; Ce, *C. elegans*; SMED, *Schmidtea mediterranea*; Hs, *Homo sapiens; S. pombe, Schizosaccharomyces pombe.*

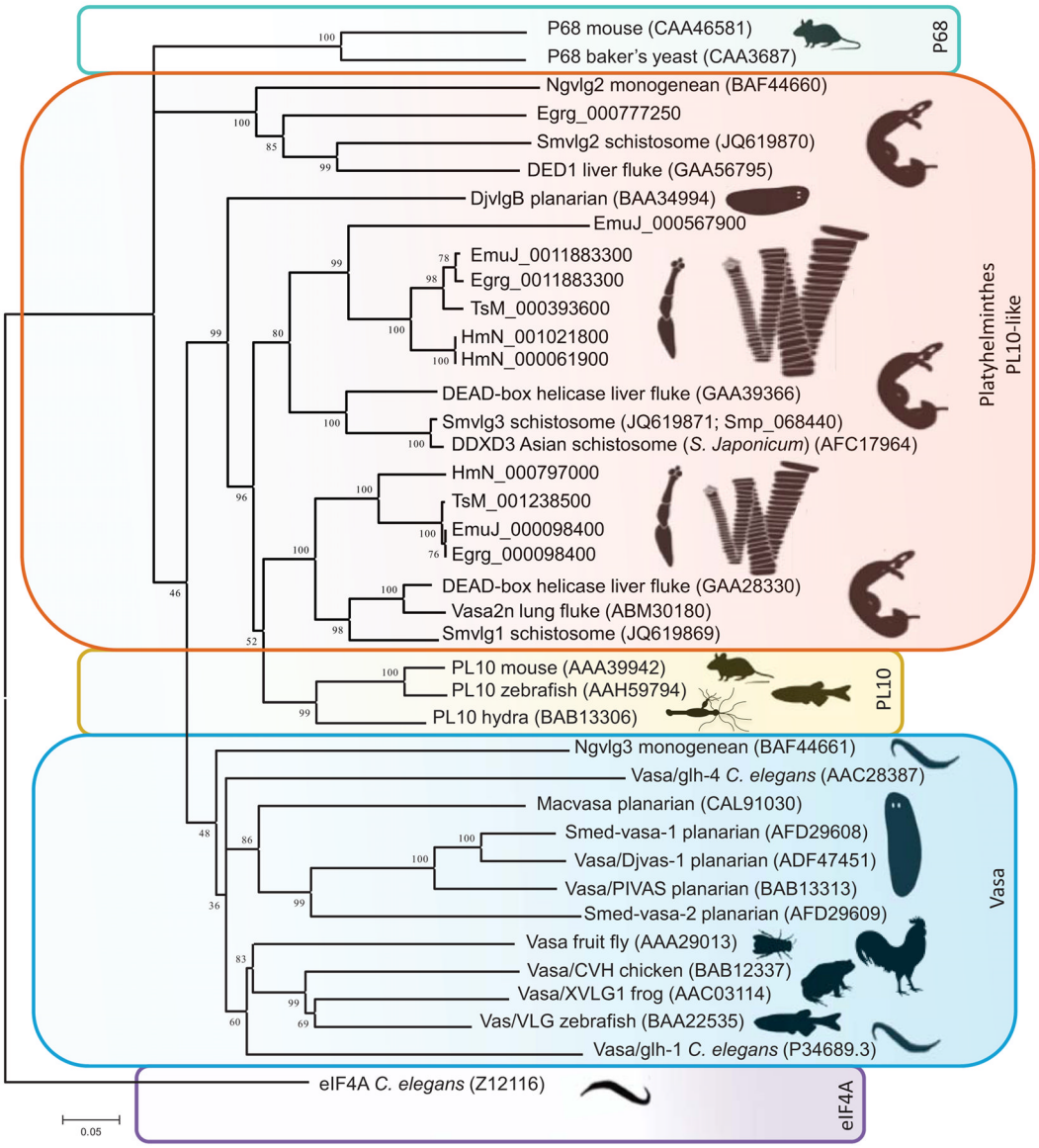

Figure 2.

Evolutionary relationships of Vasa, PL10, p68, and eIF4A. The Neighbor-Joining method was employed to infer relationships using entire amino acid sequences from informative species, including trematodes, and cestodes. Phylogenetic analysis was conducted in MEGA5. Among the DEAD-box helicases, p68 is closely related to Vasa and PL10. The eIF4A served as the out-group. Branch names indicate the common name of the species displayed; GenBank and GeneDB accessions are provided. Colored boxes highlight families of DEAD-box helicases, for example, the blue region includes vasa orthologues, and the peach colored boxes include platyhelminth PL10-like enzymes, etc. Adapted from [9] (with permission) to include PL10-like orthologues of cestodes.

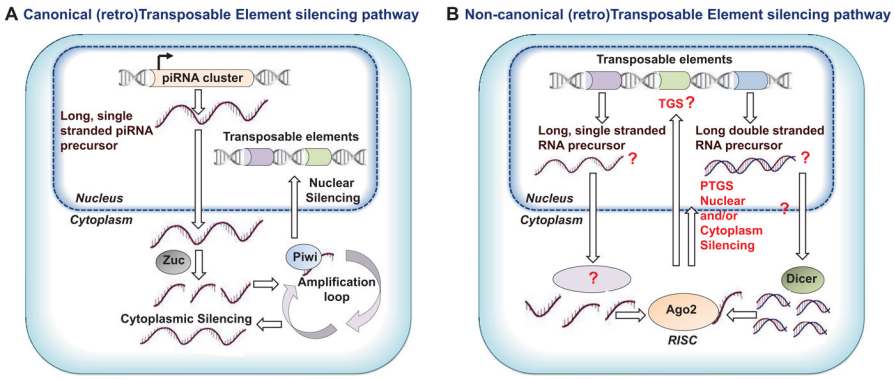

Figure 3.

(Retro)Transposable Element (TE) silencing pathways. **(A)** Canonical TE silencing pathway; piRNAs are transcribed from genomic loci termed piRNA clusters. Cleavage of these single strand transcripts by Zucchini (Zuc) generates an initial pool of primary piRNAs, which are loaded into Piwi. Secondary piRNAs are generated in a slicer-dependent amplification loop. Transposon transcripts are cleaved through this amplification cycle, accomplishing the silencing of cytoplasmic TEs. Piwi associated with piRNAs translocates to the nucleus and drives nuclear silencing. Adapted from [69] and [70]). **(B)** Hypothetical non-canonical TE silencing pathway in trematodes and cestodes. Long, single stranded RNA precursors [65] and/or long double stranded precursors are cleaved by an unknown enzyme and/or Dicer, respectively. Small non-coding RNA, that is, endo-siRNA-like molecules interacting with Argonaute 2 (Ago 2), which belongs to group 4 of Argonaute proteins in parasitic flatworms, constitute the RNA induced silencing complex (RISC) [64] that would drive transcriptional gene silencing (TGS) and/or post-transcriptional gene silencing (PTGS) of TEs.